Review Article

Bio-Fortification in Crops, Recent Approaches, Challenges and Future Strategies: A Review

Abstract

Micronutrient deficiencies such as iron (Fe), zinc (Zn), selenium (Se), iodine (I), carotenoids

and folic acid affect individuals globally, posing significant challenges to human health and development. Biofortification through plant breeding and genetic engineering has emerged as a cost-effective and sustainable solution to address these deficiencies. This strategy is particularly beneficial for health of low-income people with limited access to commercially fortified foods. With a one-time investment and the ability for farmers to propagate seeds at minimal cost, biofortification offers a promising avenue for long-term nutritional improvement. Recent advancements have seen the introduction of biofortified crop varieties through targeting various macro and micronutrients, antioxidants and other bioavailable components. Despite its potential, biofortified crops encounter obstacles related to development, distribution, and consumer acceptance. Overcoming these challenges is crucial for optimizing the utilization of biofortified foods and achieving widespread impact in combating malnutrition and starvation worldwide.

Keyword: Biofortification, Genetic engineering, Hidden hunger, Micronutrient, Plant breeding

Introduction:

Micronutrients, such as iron (Fe), zinc (Zn), selenium (Se), iodine (I), carotenoids and folic acid are crucial for human growth and development because they support several metabolic processes. A large portion of the global population relies on plant-based meals, which are frequently deficient in essential micronutrients (Waters & Grusak., 2008) and frequently fall short of the recommended daily requirements (RDA) for certain micronutrients. One in three individuals worldwide struggles with micronutrient deficiency also known as "hidden hunger" (FAO, 2013). The definition of hidden hunger is more like an elucidation. The Challenge of Hidden Hunger, the 2014 Global Hunger Index, asserts that: “Micronutrient deficiency, usually known as hidden hunger, is a type of undernutrition that develops when vitamin and mineral intake or absorption are inadequate to support adults' normal mental and physical wellness and development as well as children's excellent health and development. Poor diet, illness or increased micronutrient requirements not satisfied during both stages of pregnancy and lactation are some of the causes.” **(**Von Grebmar et al., 2014). However, incorporating health-related elements and minerals into a meal could be viewed as a tactic to combat malnutrition or address a particular nutritional need (Vlaic, *et al*., 2019). The only way to increase the nutritional value of Agricultural products before harvest in the case of unprocessed food, such as fruits and vegetables, is to adopt superior genotypes or certain agronomical practices (Kyriacou & Rouphael., 2018). Enhancing the nutritional value of grains through a variety of techniques, such as altering crop agronomic practices, traditional plant breeding and cutting-edge biotechnology alternatives. Since the Sustainable development goals' targets were established, it has been of the uttermost importance. Malnutrition manifests itself in a variety of ways including undernourishment, obesity and overweighing, and dietary-related, non-contagious conditions. The increasing demand for the addition of mineral elements to fresh food articles for consumption by humans has sparked active research with a focus on developing appropriate application techniques. This article encompasses advancements in the biofortification of vegetables, fruits and grains for number of essential minerals, which include magnesium (Mg), calcium (Ca), iodine (I), zinc (Zn), selenium (Se), iron (Fe), copper (Cu) and silicon (Si) which are frequently absent or insufficiently present in human diets. This review intends to describe and bring to light, the most effective agronomic ways to increase the quantity of the evaluated minerals in the cooked form of food articles (grains, fruits, and vegetables) after their perceptive role in human nutrition and physiology of plants.

What is bio-fortification and why is it needed:

Different strategies are used to alleviate micronutrient malnutrition; the most prevalent are product fortification and medical supplementation (Buturi *et al.,* 2021). Biofortification, which involves using plant breeding to improve micronutrients, is one approach to addressing hidden hunger and deficiencies in micronutrients. We use the term "biofortification" to describe a genetic modification of a crop variety to increase the level of specific micronutrients in the edible part of the crop, in contrast to "fortification," which is the addition of nutrients, such as folic acid to wheat flour or iodine to salt. Agronomic biofortification, which involves adding nutrients to soil or leaves to boost their concentration in food, is also not included (De Valença *et al*., 2017). Conventional crop breeding and selection have been mostly responsible for biofortification, whilst direct genome modification has also been used, as in the case of Golden Rice (Ginkel & Cherfas, 2023).

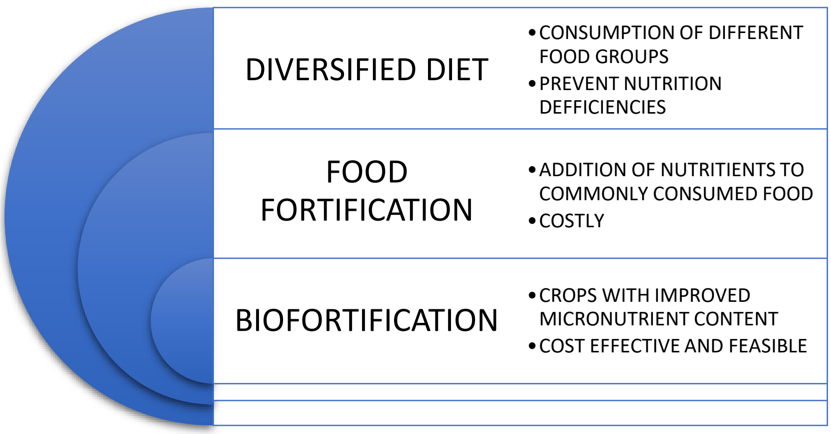


Fig .1 Three strategies to address micronutrient malnutrition

This type of biofortification is centred on a straightforward set of guidelines. There are more than two billion undernourished people. Particularly the poor cannot afford the varied diets that would provide sufficient micronutrients. Increase the micronutrient content of staple crops by biofortifying them. The HarvestPlus research programme of the CGIAR, which concentrated on enhancing vitamin A, iron, and zinc in several starchy cereals, root, and tuber crops, has been largely responsible for the worldwide research effort on biofortification. The development of iron-rich beans, cowpeas, and millets, zinc-rich maize, rice, and wheat, as well as vitamin A-rich bananas, plantains, maize, cassava, and sweet potatoes by HarvestPlus and its affiliates, is to date (HarvestPlus, 2019).

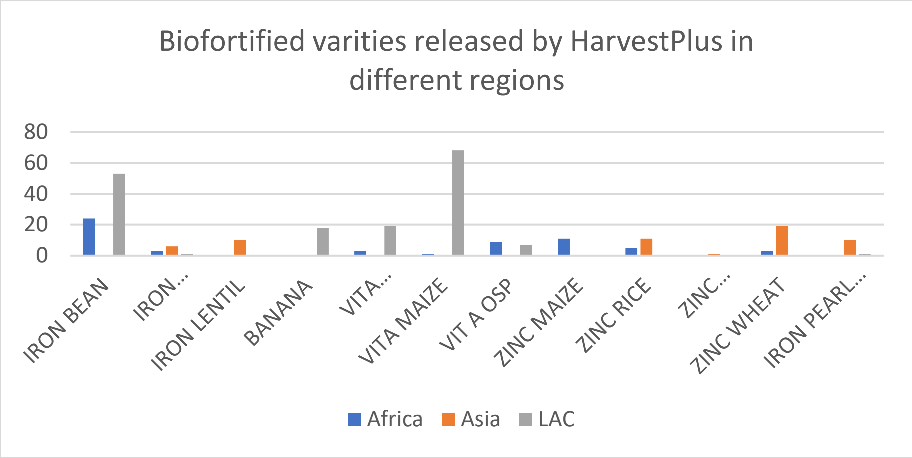


Fig 2. Graph- biofortified varieties released by HarvestPlus in different regions.

Agronomic and genetic techniques, which can be used via conventional breeding or transgenic techniques, are two of the various ways to produce biofortified crops (White & Broadley, 2009 and Siwela et al., 2020). The primary objectives are to boost the amount of minerals or other specific health-related components in the edible portion. Transgenic programmes involve biotechnology research which allows the genetic modification of a species to produce a plant with desired characteristics (such as a higher concentration of specific nutrients). Despite the potential for long-term cost savings, this methodology is now the least used because the research and development process is still so sluggish and expensive. In any event, the development of a premium product with greater nutritional content, able to satisfy the new consumers' demand, ready to pay for a healthy way of eating, offsets the higher costs associated with the cultivation of biofortified vegetables in developed countries (Timpanaro *et al*., 2020). Furthermore, several nations have strong legal restrictions that prohibit genetically modified organisms (GMOs). In a similar line, it is possible to cross various genotypes in an effort to introduce desired qualities that are present in plants naturally in new cultivars. Traditional breeding using a genetic approach has been done for many years and can produce new kinds with a greater amount of specific nutrients. Finding the needed traits among the genetic resources at hand is the restriction in this instance (Gómez-Galera et al., 2010).

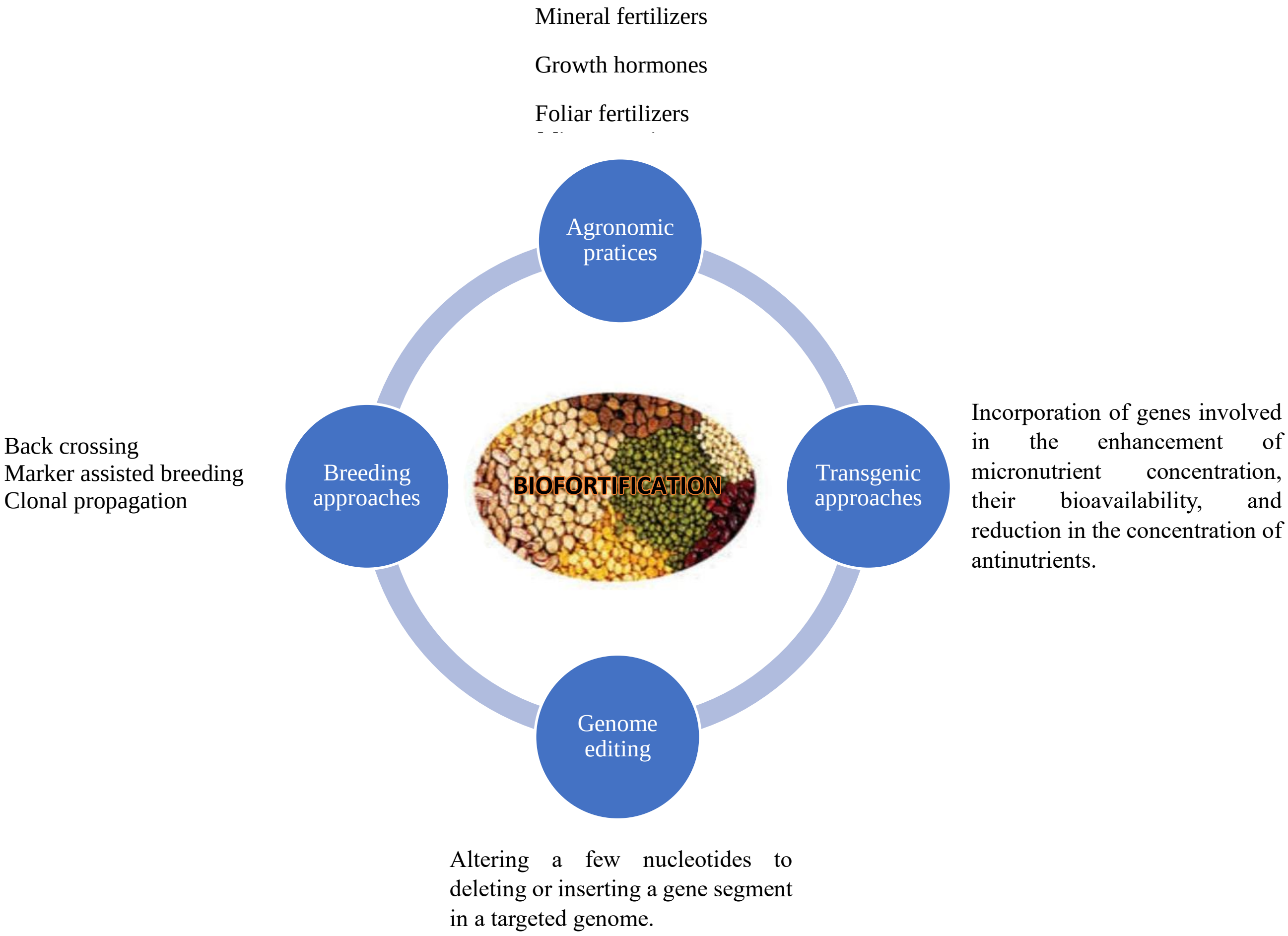
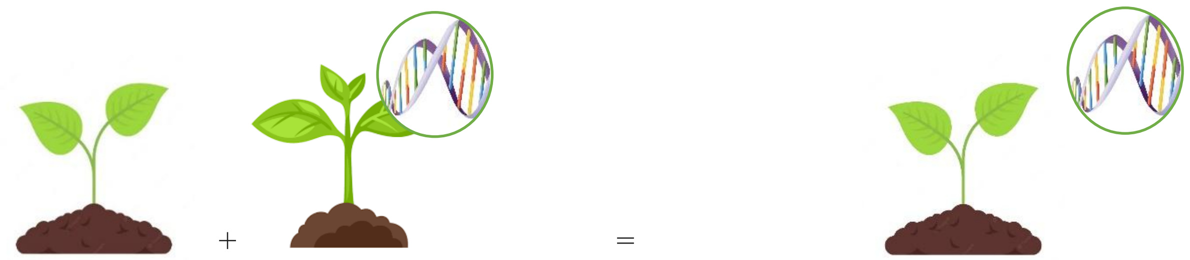


Fig 3: Various approaches to Crop Bio-fortification.

I. Genetic Engineering Approach: When a particular micronutrient is not produced naturally in the crops, changes in the necessary characters is not accessible commonly in the readily accessible germplasm, and/or changes cannot be acquired through conventional breeding. Hence biofortification by genetic engineering provides a second approach (Perez-Massot et al., 2013). This method can be used to concurrently target the elimination of antinutrients and the incorporation of promoters that can increase the bioavailability of micronutrients in addition to boosting the concentration of microelements (White & Broadley, 2005) (Garg et al., 2018). This method employed genes from bacteria as well as other species in addition to those connected to different metabolic pathways used by plants (Christou & Twyman, 2004). A transgenic strategy has been used to successfully modify several crops to address a micronutrient scarcity.

II. Conventional Plant Breeding Approach: The method of Plant breeding is one among the sustainable and cost-effective approaches of biofortification which can enhance the health of impoverished people worldwide (Bouis et al., 2011). This strategy has been utilized for treating micronutrient deficiencies, such as those involving carotenoids, iron, and zinc (White & Broadley, 2005). For the plant breeding approach to biofortification to be successful, there must be genetic variety in the gene pool. Various studies have divulged that the quantity of minerals and vitamins in diverse crops varies significantly (White & Broadley, 2009). Screening a large variety of germplasms can identify parental genotypes that have elevated micronutrient concentration, and they could be used in crosses and also for the creation of molecular markers to aid marker-assisted selection in breeding and genetic research. To find about the genotype and environment interaction (GXE), promising lines might be examined in several different places (Bouie & Saltzman, 2017). Through conventional plant breeding techniques, research has been undertaken on crops to increase essential micronutrients like carotenoids, Fe, Zn, Se, I, and folates.



Targeted plant   Plant with gene of interest   Gene incorporated on the targeted plant

Fig 4: Incorporation of gene of interest on selected plant

III. Agronomic Approach: The supplementation of essential nutrients as inorganic compounds through fertilization has proven effective in elevating the mineral content of crops such as rice, wheat, and maize (Bouis et al., 2010). Nonetheless, implementing this strategy universally poses challenges due to the associated costs and variable crop responses contingent upon the properties of the nutrient and the specific characteristics of the crop**.**

3. Micronutrients involved in biofortification and their sources:

Sources of micronutrients: In general, the macronutrients (proteins, carbs, and fats) and micronutrients (vitamins, minerals, and other elements needed in precise quantities) present in the foods ingested as part of an eclectic diet vary. When accessible, staple meals provide nearly all the diet's macronutrients and are eaten once or more during the day; however, which foods are considered staples depends on culture. These social standards, religious perspectives, and socioeconomic practices differ widely between nations and also express how much influence women have (Pingali and Sunder, 2017). Animal-based meals can be excellent sources of calories, protein, and minerals, but they are frequently inaccessible to the most vulnerable households. Overall, the world's meals have become more uniform, relying more on fewer varieties of grain crops that are crucial to the international economy (Khoury et al., 2014).

Table 1*:* Micronutrient Fortification in Common Crops

Micronutrient	Crops	Reference
Calcium (Ca)	Finger millet Basil Lettuce	Knez et al.,2021 D'Imperio et al., 2016 Borgeshi et al., 2013
Magnesium (Mg)	Onion	Kleiber et.al., 2012
Iodine(I)	Chinese cabbage Carrot Cowpea	Buturi et al 2021 Signore et.al.,2018 Buturi et al.,2021

Zinc (Zn)	Kale Lettuce	Sousa Lima et al.,2015 Barrameda-Medina et.al.,2017
Selenium (Se)	Radish Tomato	Silva et al.2020 Castillo et al., 2016
Iron (Fe)	Potato Tomato	Kormann et al.,2017 Carrasco-Gil et al., 2016
Silicon (Si)	Chard Green bean Kale	De Souza et al.,2019 Montesano et al.,2016 De Souza et al.,2019
Copper (Cu)	Spinach	Obrador et al.,2013

I. Micronutrients commonly involved with the Bio-fortification of various crops:

a. Iron: Life forms require iron (Fe), which is essential for several metabolic activities like electron transport and the production of deoxyribonucleic acid (DNA) (Abbaspour et al., 2014). The human body needs iron to create the oxygen-transporting proteins haemoglobin and myoglobin as well as the enzymes needed for electron transfer as well as oxidation-reduction reactions (Hurrell, 1997; McDowell, 2003). The Recommended dietary allowance (RDA) for Fe is 8 mg for adult men and 18 mg for adult females, as determined by the Food and Nutrition Board (The Institute of Medicine), National Academy of Sciences (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). Lack of iron can result in fatigue, light cephalalgia, and unfavourable pregnancy outcomes including early delivery, babies with low birth weights, delayed baby growth and development, and impaired cognitive abilities (Bailey et al., 2015).

b. Selenium: Se a micronutrient, which is crucial for growth and development, also shields the body from disease, oxidative stress, and the spread of cancer (Zeng & Comb, 2008). The recommended daily intake **(RDI**) for selenium is 55 g/day for males as well as females (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). Selenium deficiency in humans is connected to various conditions, including Keshin-Beck, Keshan, and myxedematous cretinism (Coppinger & Diamond, 2001).

c. Zinc: Another essential mineral that humans require is zinc (Zn). It plays a role in various biological processes, including cell growth and proliferation, wound healing, membrane signalling systems (Prasad, 1996; MacDonald, 2000), quenching reactive oxygen radicals to prevent oxidative damage to cells (Rostan et al., 2002; Prasad et al., 2004), and lowering the risk of malignancies such as pancreatic and prostate cancer (Costello & Franklin, 2017). Zn's recommended daily allowance for adults is 8 mg for females and 11 mg for males (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). A poor immune system, frequent infections, mental disease, stunted growth, and infertility are among the effects of Zn deficiency (Roohani *et al*., 2013).

d. Carotenoids: Plants naturally synthesize coloured pigments called carotenoids. Since both humans and animals are unable to synthesise carotenoids, carotenoids must be obtained through diets originating from plants (Fraser & Bramley, 2004). In the human body, carotenoids function as significant antioxidants and are crucial to many physiological functions. In nature, more than 600 carotenoids are identified. Age-related degeneration is prevented by lutein and zeaxanthin (Fraser & Bramley, 2004). Lutein lowers the likelihood of cataracts and has a link to lowering the cardiovascular disease (Moeller et al., 2000). Mammals need vit-A for healthy cell division, development of bone and eyesight (Stephens et al., 1996). B-Cryptoxanthin suppresses osteoclastic bone resorption while stimulating osteoblastic bone growth (Yamaguchi & Uchiyama, 2004).

e. Iodine: Thyroxine (T4) and triiodothyronine (T3), the thyroid hormones, are dependent on iodine, which is also necessary for healthy growth, development, and metabolism. As to the Food and Nutrition Board of the Institute of Medicine, the recommended daily intake (RDI) of I_2 for adult males and females is 150 µg (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). Iodine deficiency results in hypothyroidism, goitre, cretinism, mental retardation, decreased fertility and higher rates of neonatal and perinatal mortality (Delange, 1994). It is essential for brain development, deficiency during pregnancy might result in cognitive problems in the offspring (Skeaff, 2011).

f. Magnesium: The mineral is crucial for assisting over 300 different enzymes in carrying out numerous chemical processes in the body, including those that produce proteins and robust bones, control blood sugar and blood pressure, and maintain healthy muscle and nerve function. Magnesium also functions as an electrical conductor, which causes the heart to beat steadily and contract muscles. For individuals aged 19 to 51 and over, the Recommended Dietary Allowance (RDA) is 400–420 mg for men and 310–320 mg for women per day. About 350–360 mg per day is needed for pregnancy, while 310–320 mg is needed for lactation. Our bodies store over half of all the magnesium we contain, in our bones, with the remainder stored in different body tissues. Deficiency symptoms include Fatigue, weakness, Poor appetite, Nausea, vomiting, Numbness or tingling in skin, Muscle cramps, Seizures and Abnormal heart rate.

g. Calcium: The mineral calcium is most frequently linked to strong bones and teeth, but it also has a significant impact on blood clotting, muscle contraction, regular heart rhythms, and neuron function. The recommended daily allowance (RDA) for calcium is 1,000 mg for women aged 19 to 50 and 1,200 mg for women aged 51 and beyond. The RDA for women who are pregnant, or nursing is 1,000 mg. The RDA is 1,000 mg for men aged 19 to 70 and 1,200 mg for men aged 71 and beyond. The body stores 99% of its calcium in the bones, with the remaining 1% being found in other tissues such as muscle, blood, and other tissues. Deficiency symptoms include Muscle cramps or weakness, Numbness or tingling in fingers, Abnormal heart rate and Poor appetite.

h. Silicon: The manufacture of collagen and elastin depends on silicon, which is also critical for the health of bones, cartilage, tendons, joints, and connective tissues. While elastin gives tissues, skin, hair, and blood vessels their elasticity, collagen serves as a structure to support the tissues. In actuality, bone is a unique kind of connective tissue. The concentration of silicon decreases as the bone ages, while deposits of calcium and phosphorus are simultaneously created. Consequently, it may be said that silicon regulates the amount of calcium and phosphorus that accumulates in bone tissue. Apart from its involvement in bone and connective tissue health, silicon also contributes to other health benefits like preventing aluminium toxicity and shielding vascular tissue. The recommended daily requirement for silicon is between 20-50 mg. A lack of silicon can harm the skin and hair, dry out the nails, and cause them to break easily. Furthermore, a low silicon diet is the consequence of decreased stomach acidity brought on by disease or ageing, which also reduces the body's capacity to metabolise silicon from dietary sources. Silicone supplements should not be taken by expectant or nursing women since they may affect the mothers and their unborn children.

i. Copper: Copper supports several enzymes involved in the body's energy production, iron absorption and breakdown, red blood cell formation, collagen synthesis, connective tissue, and neurotransmitter production in the brain. In addition, copper is necessary for healthy immunological and brain development. It is also a part of the antioxidant enzyme superoxide dismutase, which breaks down dangerous oxygen molecules known as free radicals. For individuals 19 years of age and older, the Recommended Dietary Allowance (RDA) is 900 micrograms per day for men and women. 1,300 micrograms per day are needed for pregnancy and breastfeeding in adults 19 years of age and older, and 1,000 micrograms per day in younger people 14 to 18 years of age. Deficiency symptoms include Anaemia, High cholesterol, Osteoporosis, bone fractures, Increased infections, and Loss of skin pigment.

Via conventional plant breeding

a. Rice: For the poor throughout most of the Asian countries, rice accounts for up to 80% of their daily energy uptake. The Bangladesh Rice Research Institute (BRRI) and the International Rice Research Institute (IRRI) created rice cultivars with high zinc for India and Bangladesh. The first breeding objective for polished rice was established as 24 ppm zinc, which is 8 ppm more than the initial zinc concentration sold commercially. Trials for Official registration is underway in India and Bangladesh for high-yielding cultivars that have achieved over 75% of the aim; release is anticipated in 2013. An iron-rich rice cultivar was released in China in 2011, and a high-zinc rice variety was discovered in Brazil and registered for distribution by Embrapa in 2012. According to retention studies, parboiling and milling do not significantly lower the zinc concentration of rice compared to iron because zine is more evenly distributed across brown rice grains (Liang et al., 2008). The Bangladesh Rice Research Institute measured the amount of zinc that was lost from rice throughout milling and washing process before cooking. Before cooking, washing the milled grain caused about 10% of its zinc to be lost (Juliano, 1985). An additional 10-14% of the zinc in rice may be lost if it is boiled in too much water which is then discarded before consumption. Yet, there is no proof of the bioavailability or effectiveness of rice that has been biofortified with zinc. The low responsiveness of concentration of zinc in blood to relatively small quantities of extra zinc consumption make it difficult to demonstrate the effectiveness of a food-based strategy to upgrade zinc intake. More investigation is required to determine the effects of zinc therapies on human health, as well as more sensitive biochemical markers of zinc levels (Saltzman et al., 2013) the zinc in rice may be lost if it is boiled in too much water which is then discarded before consumption. As of yet, there is no proof of the bioavailability or effectiveness of rice that has been biofortified with zinc. The low sensitivity of blood zinc concentration to relatively small quantities of extra zinc consumption make it difficult to demonstrate the effectiveness of a food-based strategy to improve zinc intake. More investigation is required to determine the effects of zinc therapies on human health, as well as more sensitive biochemical markers of zinc levels (Saltzman et al., 2013).

b. Wheat: CIMMYT is in charge of developing high-zinc wheat for Pakistan and India. For whole wheat, the initial breeding target was set at 33 ppm zinc, which is 8 ppm higher than the original baseline zinc concentration. High-zinc wheat's enhanced agronomic qualities over popular varieties are anticipated to propel adoption, and breeding efforts have concentrated on increasing zinc content as well as resistance to emerging strains of stem rust and yellow rust. Both India and Pakistan are conducting multilocation trials; the first release with 75% of the desired level of zinc is anticipated in India in 2013. In 2011, a wheat variety was introduced in China that had a zinc concentration of 44 ppm, significantly higher than the desired level (www.harvestplus-china.org).

c. Maize: Provitamin A maize breeding is driven by International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) in relation with NARES in southern Africa. Germplasm evaluation found genetic variation for the objective level (15 ppm) of provitamin A carotenoids in temperate maize, which was then reproduced into the tropical varieties. Ongoing improvements in marker-assisted selection technology have sped up and precision of recognizing genes controlling the characters of interest in maize. Genotypes that can provide 25% of the EAR for women and preschool children were released in Zambia (three varieties) and Nigeria (two varieties) in 2012. A maize variety with comparable provitamin A levels has been registered for release by Embrapa of Brazil in South America (www.biofort.com.br). Studies on the storage stability of the 2012-released varieties are still ongoing; however, a prior investigation involving several varieties revealed a 25-60% reduction in provitamin A following drying along with four months of dark storage with 251 degrees Celsius (Burt et al., 2010).

Via transgenics development:

a. Golden rice: Golden rice was the first to developed by The Swiss Federal Institute of Technology, and Syngenta continued the research as part of their then-commercial pipeline. After Syngenta settled not to go after the character as a commercial product, transgenic events with greater levels of provitamin A, up to 37 ppm in a US variety-was created and contributed to be utilized by the Golden Rice Network (Al-Babili &Beyer, 2005). IRRI is currently in charge of Golden Rice research. The GR2 events had been backcrossed into varieties for Bangladesh, India, the Philippines and Indonesia beginning in 2006. Golden rice has been shown through bioavailability testing to be a useful source of vitamin A for humans, with a projected 3.8:1 beta-carotene to retinol conversion rate (Tang et al., 2009).

b. African bio-fortified sorghum: Transgenic sorghum has reduced relative bio-accessibility, but the grain's higher carotenoids increased total and provitamin A carotenoids' overall levels of accessibility. Further research on bioavailability has demonstrated that lowering phytate levels increases iron absorption by 20–30% and zinc absorption by 30–40% (Saltzman et al., 2013).

c**. Iodine potato -** Fortified potatoes are a nutritionally enhanced variety of potatoes that have been augmented with additional vitamins and minerals to address nutritional deficiencies in certain populations. The fortification process involves adding essential nutrients such as vitamin A, vitamin C, and iron to the potato, thereby increasing its overall nutritional value. This approach aims to combat micronutrient deficiencies and improve the health outcomes of individuals who rely heavily on potatoes as a staple food. Fortified potatoes can play a crucial role in enhancing dietary diversity and addressing specific nutrient needs, contributing to overall public health. (Bouis.et.al.,2017). Potato has been majorly incorporated with PSY gene and by the incorporation of three other genes; PSY, lycopene β -cyclase and phytoene desaturase (Diretto et al., 2006).

Difficulties with Bio-fortification:

a. Dietary diversity: The fundamental rationale for biofortification fails to acknowledge the significance of dietary diversity in providing sufficient nourishment. It doesn't think to diversify production or diets before jumping straight to biofortified staples (Pingali & Sunder, 2017; Fanzo, 2021; Siddique et al., 2021). When taken as a whole, diet is heavily influenced by culture and is based on historical cultural practices as well as regional farming, seed, and market conditions that is, on what is suitable, readily available, reasonably priced, and accessible (Fanzo, 2021). Numerous recent research in rich and developing nations have cast doubt on the notion that varied, sustained healthy meals are invariably more expensive than existing diets (Batis et al., 2021). Harvest Plus has claimed that it will compare biofortification to industrial fortification and supplementation between 2020 and 2030, as well as evaluate health results in addition to micronutrient insufficiency status (Bouis et al., 2019).

b. Impact on health: A committee was established by the World Health Organization in 2016 to examine data regarding the nutritional effects of biofortification (CGIAR, 2018). The World Health Organization (WHO) classified biofortification as a "Category 3 Intervention" on its website in January 2022, stating that "systematic reviews have not yet been conducted" and that "available evidence is limited". "More research is required before specific recommendations can be made," the conclusion states (World Health Organisation, 2022).

c. Yield penalty: There are numerous instances of biofortification that blend high yield with elevated micronutrient levels. High-Zinc Maize (Kawikhonliu *et al.*, 2022), Zinc and Iron enriched wheat (Velu *et al.*, 2019), etc. Furthermore, new studies show that the yield penalty is still an obstacle. Zinc content in rice can be diluted by increasing yield, and combination selection for both qualities yield less genetic gain compared to either feature considered alone

(Calayugan et al., 2021). In cassava and maize, there have been observed negative correlations between yield and beta-carotene (Chavez *et al*., 2005).

d. Genetic uniformity: There are certain risks to genetic uniformity which are well known (Late blight of potato, Southern corn blight, Fusarium in banana), also the benefits of genetic diversity are well received in the field of agriculture (Wolfe & Barrett, 1980; Wolfe, 1985; Bocci *et al*., 2020). By continuously backcrossing into a single adapted parent and introducing elevated micronutrient levels into several new types using the exact same genetic package, bio-fortification helps maintain genetic homogeneity. Breeders can now choose offspring that have the beneficial traits of the recipient (high-yielding) parent through marker-assisted backcrossing, which has emerged as the preferred technique for bio-fortification (Natesan *et al*., 2020). Near clones of commonly cultivated types are the final result, improving genetic consistency and susceptibility to abiotic and biotic stresses.

Delivery and commercialization of bio-fortified crops:

The design of a delivery strategy is influenced by two factors: the visibility or unsightliness of the bio-fortified feature, and the presence or absence of a robust infrastructure for seed dispersion, such as seed markets and sectors. Crops biofortified with zinc or iron are similar in appearance to their non-biofortified counterparts, whereas crops lacking provitamin A are clearly differentiated (Saltzman et al., 2013**).** Harvest Plus and its different NGO companions provided OSP to almost 24,000 homes in Mozambique and Uganda between 2007 and 2009 (Harvestplus, 2010). Although the pilot delivery project was unique to Uganda, it expanded upon two earlier CIP initiatives in Mozambique, Eat Orange and Towards Sustainable Nutrition Improvement. Planting supplies were sent through NGO partners since sweet potato vines were not sold in Uganda or Mozambique. While a team dedicated to effect evaluation conducted a randomized control study in parallel, an operations research component kept an eye on implementation activities. The two delivery strategies that were examined in the effect evaluation component were an intense way that involved planting material delivery and training for two years, and a mild method that involved planting material delivery and instruction for just one year. It was demonstrated that the less intense strategy was just as successful as the more intensive one, and the experiment increased farm households' acceptance and consumption of OSP in both nations. Consequently, the main target groups of this intervention children and women saw a two fold increase in their vitamin A intake (Saltzman *et al*., 2013).

Effectiveness of bio-fortification:

The main source of proof for the efficacy of biofortification is orange sweet potatoes (OSP). In both nations, the effectiveness was evaluated using a randomized control study. The aforementioned pilot delivery effort increased the likelihood of OSP adoption in Mozambique by 68% and in Uganda by 61% (Hotz *et al.*, 2012a, 2012b). Adoption of OSP led to a significant area under cultivation replacement of other sweet potato varieties; in Uganda and Mozambique, the project raised the percentage of OSP in total sweet potato areas by 44% and 59%, respectively. By the project's completion, vitamin A intake in Mozambique had doubled for each of the age/gender groups compared to baseline intakes; in Uganda, it had improved by nearly doubled for women and two-thirds for older and younger children. In Uganda and Mozambique, OSP accounted for 52% and 74% of the total vitamin A consumption for children aged 6 to 35 months, which is the age group most concerned. As an outcome of the project, the substantial incidence of suboptimal vitamin A intake amongst a subset of breastfeeding children aged 12 to 35 months in Uganda decreased from almost 50 per cent to only 12%. In children aged 5-7 who had reduced blood levels of vitamin A at the beginning of the study, researchers additionally were able to evaluate a slight but significant effect of eating OSP on their intake of vitamin A. Upon experiment completion, the researchers also discovered that women with lower risk of marginal vitamin A deficiency were those who received higher vitamin A via OSP consumption (Hotz et al., 2012a).

Challenges and future strategies for bio-fortification:

Effective biofortification requires large yields and higher micronutrient densities; these crops also need to be embraced by farmers and eaten by the intended population (Bouis et al., 2011). By 2030, Harvestplus has three major obstacles to overcome as stated by Saltzman and Bouis (Bouis & Saltzman, 2016): increasing consumer demand; mainstreaming biofortified features into private and public plant breeding programs; and integrating biofortification into private and public policy. The success of biofortification strategies depends on a number of factors, including genetic variation in the gene pool, the decrease of antinutrients (particularly phytate and polyphenols), and an increase in the concentration of promoter substances like ascorbic acid (vitamin C) and select amino acids (cysteine, lysine, and methionine), which improve the uptake of essential minerals and/or high yield (White & Broadley, 2009). The goal of effective biofortification should be to raise the concentration of micronutrients while also boosting their bioavailability. This can be accomplished by lowering the levels of antinutrients that obstruct absorption and raising the concentrations of promoters, which increase the absorption of minerals (White & Broadley, 2009). Since milling, polishing, and boiling can remove a significant quantity of minerals from the diet, postharvest processing can also be crucial to the effective use of biofortified crops (Gregorio *et al*., 2000). Consequently, after processing and heating, efforts should be taken to preserve the micronutrient levels in consumable seeds as well as the consumer's ability to absorb them (Haas *et al*., 2005). The nutritional makeup of the harvested seeds can be dramatically changed by these stressors. As previously indicated, the micronutrients that are being targeted are either antioxidants or components of enzymes that are indulged in different metabolic processes, such as electron transfer and oxidation reductions. As a result, they shield cells from damage due to oxidation by scavenging reactive oxygen species that are produced in response to environmental stress (Hurrell, 1997; McDowell., 2003). More micronutrient-concentrated biofortified crops are more able to tolerate harsh environmental conditions and show increased adaptation to them.

Conclusion:

One in three people worldwide suffer from a micronutrient deficiency, which is a serious concern because micronutrients are critical for human advancement and growth. When it comes to addressing micronutrient deficiencies, biofortification via plant breeding is thought to be the most cost-effective and long-lasting method. This strategy is widely acknowledged and has the ability to assist those residing in rural, somewhat isolated places with limited access to fortified foods sold for a profit. Additionally, it needs a single expenditure, and farmers can multiply seeds at almost zero marginal cost over the course of several years. The introduction of multiple crop types that are biofortified and aiding in the target populations' recovery from micronutrient deficiencies has resulted in notable advancements in recent years. Bio-fortification is conducted for various macro, micro-nutrients, antioxidants and other components that increase bioavailability of other nutrients. Currently a wide range of crops have been fortified thus making optimum nutrition a reality for people all over the world and help fight starvation and malnutrition as well. But such crops face many difficulties pertaining to creation, delivery and acceptance. In order to effectively optimize the usage of biofortified foods, a number of obstacles must be overcome.

References

Al-Babili, S. and Beyer, P., 2005. Golden Rice–five years on the road–five years to go. *Trends in plant science*, *10*(12), pp.565-573.

Barrameda-Medina, Y.; Blasco, B.; Lentini, M.; Esposito, S.; Baenas, N.; Moreno, D.A.; Ruiz, J.M. Zinc 2017. Biofortification improves phytochemicals and amino-acidic profile in Brassica oleracea cv. Bronco. Plant Sci., 258, 45–51.

Barrett, J.A. and Wolfe, M.S., 1980. Pathogen Response to Host Resistance and its Implication in Breeding Programmes1. *EPPO Bulletin*, *10*(3), pp.341-347.

Batis, C., Marron-Ponce, J.A., Stern, D., Vandevijvere, S., Barquera, S. and Rivera, J.A., 2021. Adoption of healthy and sustainable diets in Mexico does not imply higher expenditure on food. *Nature food*, *2*(10), pp.792-801.

Borghesi, E.; Carmassi, G.; Uguccioni, M.C.; Vernieri, P.; Malorgio, F. 2013. Effects of calcium and salinity stress on quality of lettuce in soilless culture. J. Plant Nutr..

Bouis HE, Welch RM. 2010. Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the Global South. Crop Sci.;50: pp.20- 32

Bouis, H. E., & Saltzman, A. 2017. Improving nutrition through biofortification: A review of evidence from Harvest Plus, 2003 through. Global Food Security, 12, 49-58.

Bouis, H.E., Hotz, C., McClafferty, B., Meenakshi, J.V. and Pfeiffer, W.H., 2011. Biofortification: a new tool to reduce micronutrient malnutrition. *Food and nutrition bulletin*, *32*(1_suppl1), pp.S31-S40. DOI: 10.1177/15648265110321S105

Bouis, H.E., Saltzman, A. and Birol, E., 2019. Improving nutrition through biofortification. *Agriculture for improved nutrition: seizing the momentum*, *47*.

Buturi, C.V., Mauro, R.P., Fogliano, V., Leonardi, C. and Giuffrida, F., 2021. Mineral biofortification of vegetables as a tool to improve human diet. *Foods*, *10*(2), p.223.

Buturi.C.V., Leonardi. V. F. C.& Giuffrida.F. 2021. Mineral Biofortification of Vegetables as a tool to Improve Human Diet. Foods,10(2),223, https://doi.org/10.3390/foods10020223

Calayugan, M.I.C., Swamy, B.M., Nha, C.T., Palanog, A.D., Biswas, P.S., Descalsota-Empleo, G.I., Min, Y.M.M. and Inabangan-Asilo, M.A., 2021. Zinc-biofortified rice: A sustainable food-based product for fighting zinc malnutrition. In *Rice improvement: Physiological, molecular breeding and genetic perspectives* (pp. 449-470). Cham: Springer International Publishing.

Castillo-Godina, R.G.; Foroughbakhch-Pournavab, R.; 2016. Benavides-Mendoza, A. Effect of selenium on elemental concentration and antioxidant enzymatic activity of tomato plants. J. Agric. Sci. Technol. 18, 233–244.

Chavez, A.L., Sánchez, T., Jaramillo, G., Bedoya, J.M., Echeverry, J., Bolaños, E.A., Ceballos, H. and Iglesias, C.A., 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica*, *143*(1), pp.125-133.

Christou, P. and Twyman, R.M., 2004. The potential of genetically enhanced plants to address food insecurity. *Nutrition research reviews*, *17*(1), pp.23-42.

D'Imperio, M.; Renna, M.; Cardinali, A.; Buttaro, D.; Serio, F.; Santamaria, P. Calcium 2016. Biofortification and bioaccessibility in soilless "baby leaf" vegetable production. Food Chem., 213, 149–156.

da Silva, D.F., Cipriano, P.E., de Souza, R.R., Júnior, M.S., da Silva, R.F., Faquin, V., de Souza Silva, M.L. and Guilherme, L.R.G., 2020. Anatomical and physiological characteristics of Raphanus sativus L. submitted to different selenium sources and forms application. *Scientia Horticulturae*, 260, p.108839.

De Sousa Lima, F.; Nascimento, C.W.A.; Da Silva Sousa, C. 2015. Zinc fertilization as an alternative to increase the concentration of micronutrients in edible parts of vegetables. Rev. Bras. Ciencias Agrar., 10, 403–408.

De Souza, J.Z.; De Mello Prado, R.; Silva, S.L.; Farias, T.P.; Neto, J.G.; Souza Junior, J.P. 2019. Silicon Leaf Fertilization Promotes Biofortification and Increases Dry Matter, Ascorbate Content, and Decreases Post-Harvest Leaf Water Loss of Chard and Kale. Commun. *Soil Sci. Plant Anal.* 2019, 50, 164–172

De Valença, A.W., Bake, A., Brouwer, I.D. and Giller, K.E., 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global food security*, *12*, pp.8-14.

Diretto, G., Tavazza, R., Welsch, R., Pizzichini, D., Mourgues, F., Papacchioli, V., Beyer, P. and Giuliano, G., 2006. Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. BMC plant biology, 6, pp.1-11.

Diretto, G., Tavazza, R., Welsch, R., Pizzichini, D., Mourgues, F., Papacchioli, V., Beyer, P. and Giuliano, G., 2006. Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. BMC plant biology, 6, pp.1-11.

Fanzo, J., Bellows, A.L., Spiker, M.L., Thorne-Lyman, A.L. and Bloem, M.W., 2021. The importance of food systems and the environment for nutrition. *The American Journal of Clinical Nutrition*, *113*(1), pp.7-16.

Gómez-Galera, S., Rojas, E., Sudhakar, D., Zhu, C., Pelacho, A.M., Capell, T. and Christou, P., 2010. Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic research*, *19*, pp.165-180.

Gregorio, G.B., Senadhira, D., Htut, H. and Graham, R.D., 2000. Breeding for trace mineral density in rice. *Food and Nutrition Bulletin*, *21*(4), pp.382-386. https://doi.org/10.1177/156482650002100407

Haas, D. and Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature reviews microbiology*, *3*(4), pp.307-319.

HarvestPlus, 2010. Disseminating Orange-fleshed Sweet Potato: Findings From a HarvestPlus Project in Mozambique and Uganda. HarvestPlus, Washington, DC

HarvestPlus. 2019. Biofortification: The Evidence. A Summary of Research Informing Scaling Up of Biofortification to Improve Nutrition and Health Globally. Washington, DC: HarvestPlus.https://www.harvestplus.org/sites/default/fles/publications/Biofortifcation%20Evidence.pdf.

Hotz, C., Abdelrahman, L., Sison, C., Moursi, M. and Loechl, C., 2012. A food composition table for Central and Eastern Uganda. *Washington, DC: International Food Policy Research Institute and International Center for Tropical Agriculture*, *2*.

https://www.harvestplus.org/.

https://www.ifpri.org/publication/analysis-cgiar-2018-policy-contributions-overview-and-country-level-insights

Hurrell, R.M., 1997. Factors associated with regular exercise. *Perceptual and motor skills*, *84*(3), pp.871-874.

Juliano, B.O., 1985. Factors affecting nutritional properties of rice protein. *Trans Natl Acad Sci Technol*, *7*, pp.205-216.

Kawikhonliu, Z., 2022. *Zinc biofortification of maize (Zea mays L.) and integrated nutrient management in foothill condition of Nagaland* (Doctoral dissertation, Nagaland University).

Kenz. M & Stangoulis. 2021. Calcium Biofortification of Crops – Challenges and Projected Benefits. *Front Plant Sci*.,12,669053.

Kleiber, T.; Golcz, A.; Krzesiński, W. 2012. Effect of magnesium nutrition of onion (Allium cepa L.). Part I. Yielding and nutrient status. Ecol. Chem. Eng. S, 19, 97–105.

Kromann, P., Valverde, F., Alvarado, S., Vélez, R., Pisuña, J., Potosí, B., Taipe, A., Caballero, D., Cabezas, A. and Devaux, A., 2017. Can Andean potatoes be agronomically biofortified with iron and zinc fertilizers?. Plant and Soil, 411, pp.121-138.

Kyriacou, M.C. and Rouphael, Y., 2018. Towards a new definition of quality for fresh fruits and vegetables. *Scientia Horticulturae*, *234*, pp.463-469.

Liang, J., Li, Z., Tsuji, K., Nakano, K., Nout, M.R. and Hamer, R.J., 2008. Milling characteristics and distribution of phytic acid and zinc in long-, medium-and short-grain rice. *Journal of Cereal Science*, *48*(1), pp.83-91.

McDowell, L.R., 2003. *Minerals in animal and human nutrition* (No. Ed. 2). *Elsevier Science* BV.

Montesano, F.F.; D'Imperio, M.; Parente, A.; 2016. Cardinali, A.; Renna, M.; Serio, F. Green bean biofortification for Si through soilless cultivation: Plant response and Si bioaccessibility in pods. *Sci. Rep*., 6, 1–9

Natesan, S., Duraisamy, T., Pukalenthy, B., Chandran, S., Nallathambi, J., Adhimoolam, K., Manickam, D., Sampathrajan, V., Muniyandi, S.J., Meitei, L.J. and Thirunavukkarasu, N., 2020. Enhancing β-carotene concentration in parental lines of CO6 maize hybrid through marker-assisted backcross breeding (MABB). *Frontiers in Nutrition*, *7*, p.134.

Obrador, A.; Gonzalez, D.; Alvarez, J.M. 2013. Effect of inorganic and organic copper fertilizers on copper nutrition in Spinacia oleracea and on labile copper in soil. *J. Agric. Food Chem.* 2013, 61, 4692–4701

Pérez-Massot, E., Banakar, R., Gómez-Galera, S., Zorrilla-López, U., Sanahuja, G., Arjó, G., Miralpeix, B., Vamvaka, E., Farré, G., Rivera, S.M. and Dashevskaya, S., 2013. The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes & nutrition*, *8*, pp.29-41.

Pingali, P. and Sunder, N., 2017. Transitioning toward nutrition-sensitive food systems in developing countries. *Annual Review of Resource Economics*, *9*, pp.439-459.

Roohani, N., Hurrell, R., Kelishadi, R. and Schulin, R., 2013. Zinc and its importance for human health: An integrative review. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, *18*(2), p.144.

Saltzman, A., Birol, E., Bouis, H.E., Boy, E., De Moura, F.F., Islam, Y. and Pfeiffer, W.H., 2013. Biofortification: progress toward a more nourishing future. *Global food security*, *2*(1), pp.9-17.

Siddique, K.H., Li, X. and Gruber, K., 2021. Rediscovering Asia's forgotten crops to fight chronic and hidden hunger. *Nature Plants*, *7*(2), pp.116-122.

Signore, A.; Renna, M.; D'Imperio, M.; Serio, F.; 2018. Santamaria, P. Preliminary evidences of biofortification with iodine of "carota di polignano", an Italian carrot landrace. *Front. Plant Sci*., 9, 1–8.

Siwela, M., Pillay, K., Govender, L., Lottering, S., Mudau, F.N., Modi, A.T. and Mabhaudhi, T., 2020. Biofortified crops for combating hidden hunger in South Africa: availability, acceptability, micronutrient retention and bioavailability. *Foods*, *9*(6), p.815.

Skeaff, S.A., 2011. Iodine deficiency in pregnancy: the effect on neurodevelopment in the child. *Nutrients*, *3*(2), pp.265-273.

Tang, T., Xie, H., Wang, Y., Lü, B. and Liang, J., 2009. The effect of sucrose and abscisic acid interaction on sucrose synthase and its relationship to grain filling of rice (Oryza sativa L.). *Journal of experimental botany*, *60*(9), pp.2641-2652.

Timpanaro, G., Bellia, C., Foti, V.T. and Scuderi, A., 2020. Consumer behaviour of purchasing biofortified food products. *Sustainability*, *12*(16), p.6297.

van Ginkel, M. and Cherfas, J., 2023. What is wrong with biofortification. *Global Food Security*, *37*, p.100689.

Velu, G., Crespo Herrera, L., Guzman, C., Huerta, J., Payne, T. and Singh, R.P., 2019. Assessing genetic diversity to breed competitive biofortified wheat with enhanced grain Zn and Fe concentrations. *Frontiers in Plant Science*, *9*, p.413924.

Vlaic, R.A., Mureșan, C.C., Muste, S., Mureșan, V., Pop, A. and MUREȘAN, G.P.A., 2019. Boletus edulis mushroom flour-based wheat bread as innovative fortified bakery product. *Bulletin UASVM Food Science and Technology*, *76*(1), pp.52-62.

Von Grebmer, K., Saltzman, A., Birol, E., Wiesman, D., Prasai, N., Yin, S., Yohannes, Y., Menon, P., Thompson, J. and Sonntag, A., 2014. *Synopsis: 2014 global hunger index: The challenge of hidden hunger* (Vol. 83). *Intl Food Policy Res Inst*.

Waters, B.M. and Grusak, M.A., 2008. Quantitative trait locus mapping for seed mineral concentrations in two Arabidopsis thaliana recombinant inbred populations. *New Phytologist*, *179*(4), pp.1033-1047.

White, P.J. and Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. *Trends in plant science*, *10*(12), pp.586-593.

White, P.J. and Broadley, M.R., 2009. Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, *182*(1), pp.49-84. https://doi.org/10.1111/j.1469-8137.2008.02738.x

White, P.J. and Broadley, M.R., 2009. Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, *182*(1), pp.49-84.

Wolfe, M.S., 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual review of phytopathology*, *23*(1), pp.251-273.

World Health Organization, 2022. WHO European regional obesity report 2022. World Health Organization. Regional Office for Europe.