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1. Biofortification: Process to Overcome the Nutrient Deficiency

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Abstract:

Nearly half of the world's population is known to suffer from micronutrient malnutrition, which is one of the greatest threats to mankind. Since plant breeding has aimed to increase yield or other major agronomic traits mainly rather than nutritional quality, other attempts to address the issue have mainly focused on industrial fortification or pharmaceutical supplementation. Women and young children are particularly prone to micronutrient malnutrition, also known as the "hidden hunger," which is primarily caused by inadequate dietary intake of micronutrients. Malnutrition or hidden hunger mitigation may be achieved through biofortification, the process of increasing the bioavailable amounts of key micronutrients in consumable parts of crop plants, agronomically or genetically. Crops are being targeted for greater micronutrient content by traditional and transgenic breeding techniques. Many cultivars have been released, and more traditional and transgenic varieties are in the breeding pipeline. Biofortification is a potential intervention for *addressing hidden hunger, as shown by the findings of efficacy and effectiveness studies. This chapter highlights different interventions to mitigate micronutrient malnutrition and future challenges of biofortification.*

Keywords:

Hidden hunger, Biofortification, Genetic improvement, Genetic engineering, micronutrients

1.1 Introduction:

Earlier, agricultural research in the lower income countries concentrated on boosting cereal production, which resulted in increased food grain production by manifold. In current scenario, agriculture must now produce more nutrient rich food grains in addition to more calories (Kennedy et al., 2003). Micronutrient malnutrition affects nearly half of the world's population as the majority of world's population relies on plant-based food for daily calory intake. Plant based foods contains generally lower level of key micronutrients, hence plant-based diets are unable to meet the recommended daily allowance (RDA). Since living beings are unable to synthesize these key micronutrients, they needed to be supplied through the diet (Bouis et al., 2011; Dutta et al., 2020; Sharma et al., 2021). Iron (Fe) and zinc (Zn) deficiency are two key micronutrient deficiency. Nearly 2 billion people are affected by zinc deficiency. Children and expectant mothers are more risk prone to Zn inadequacy. About 25% children and 37% pregnant women are anemic due to Fe deficiency (www.harvestplus.org). Such deficiency results in poor growth and development and affects longevity of life (Yadava et al., 2018). Biofortification is a process of breeding micronutrients into food crops, by which more micronutrients can be delivered over a long period of time in relatively inexpensive and sustainable way (Bouis, 1999; Heck et al., 2020). Though biofortified food crops cannot provide as high level of key micronutrients as provided by industrially fortified food, still daily intake can provide adequate amount of nutrients (Bouis et al., 2011). However, biofortification alone is not expected to eradicate micronutrient malnutritions in all population groups, so the issue of micronutrient malnutrition cannot be resolved by a single intervention, but biofortification complements well with other existing approaches to provide the most vulnerable populations with micronutrients in a cost-effective and sustainable manner (Meenakshi et al., 2010; Qaim et al., 2007). Rural populations that are undernourished due to inaccessibility to a variety of diets, supplemented foods, and industrially fortified foods may benefit from biofortification. The goal of the biofortification approach is to introduce the nutrient-rich traits into popular cultivars having desirable agronomic and dietary traits, like better yield. Contrary to existing interventions like industrial fortification and food supplementation, which start in urban areas, surplus production of these biofortified food crops may reach at markets, thus becoming available first to rural people and then urban people. An initial and one-time investment in a crop biofortification programme can result in micronutrient-dense popular cultivars available to the farmers to cultivate, in contrast to industrial fortification, where the continue flow of financial resources is necessary. The advantages of the initial investment can be multiplied by evaluating the performance of varieties bred for one country in other countries or locations and adapting them to those conditions. Agronomic interventions, conventional plant breeding, molecular plant breeding and transgenic biofortification are currently the most popular approaches in addition to industrial fortification interventions. Through increased application of fertilizers, agronomic biofortification can temporarily increase micronutrient levels. Though this can complement the plant breeding interventions, still further insight is needed. Like foliar application of Zn fertilizer resulted into increment of Z content in wheat grain up to 20 ppm, but only for the one season (Zou et al., 2012). Through conventional plant breeding, biofortification involves crossing of vitamins and mineral rich elite parents' generation after generation to create agronomically superior plants with optimum level of nutrients. When a crop lacks the genetic variability for the nutrient naturally or when it is impossible to successfully breed in sufficient quantities of bioavailable micronutrients, transgenic approaches are advantageous. Once a transgenic event has been created, it takes years of backcrossing and traditional breeding to transfer transgene ito elite parents and ensuring the stable inheritance of transgene. While transgenic breeding can occasionally provide micronutrient increments other than conventionally available cultivars, many countries do not have the necessary legal frameworks in place to permit the release and cultivation of such genetically modified varieties.

1.2 Interventions to Address Micronutrient Deficiencies:

The malnutrition caused by inadequacy of micronutrient in calorie-dense but vitamins and/or minerals deficient human diets is termed as "hidden hunger." A significant portion of the global population consumes diets that are deficient in key micronutrients such as iron, zinc, iodine, calcium etc., which has an impact on human health and longevity and, consequently, on national economies (White and Broadley, 2009). A brief description of different biofortification interventions is provided below –

1.2.1 Dietary Diversification:

The requirements of all essential nutrients cannot be satisfied by a one type of food. To meet all of the nutritional needs of our body, a balanced diet is necessary. Balanced diet refers to a selection of such food combinations that fulfills requirements of every nutrient needed for overall growth and development of human.

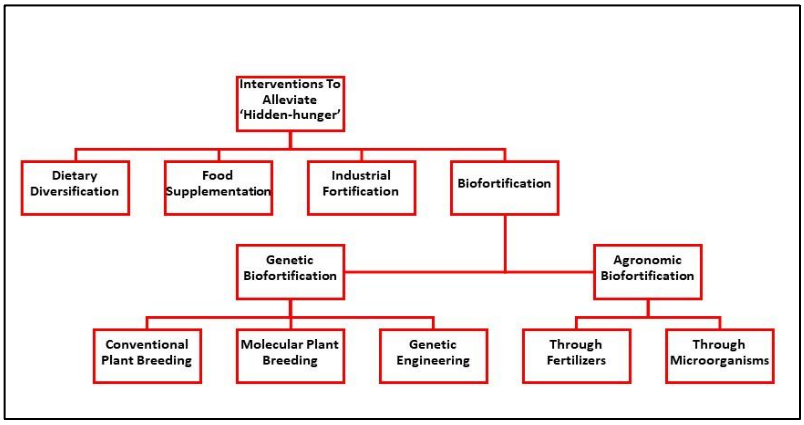

Figure 1.1: Different Interventions to Address Micronutrient Malnutrition

The importance of micronutrients is increased by the fact that availability of micronutrients in soil, genetic variability, fertilizer application, and package and practices to rear a crop all affect how much of micronutrients foods contain. Dietary diversification over the long term provides a healthy diet with an adequate balance of energy sources, necessary minerals, and dietary fiber content. Despite the fact that dietary diversity is an effective and sustainable method of preventing hidden hunger, different cultivation methods may affect the micronutrient concentration in a given food product (Thompson and Amoroso, 2010). While most people can get enough nutrition from foods like pulses, food grains, leafy vegetables, fruits, and animal products, some population groups, like pregnant women, require supplementation because their needs are higher (FAO, 2013). Children may need different amounts of nutrients at different stages of their growth. Therefore, for typical human nutrition and health, a mix of various types of nutritionally balanced foods is needed.

1.2.2 Food Supplementation:

The term "supplementation" refers to including additional micronutrients in a person's diet to make up for nutritional deficiencies. Pills or mineral tablets distribution schemes are one among the promising short-term approaches which has helped in improving nutritional health. This aids in easing severe mineral deficiencies, but it is not feasible for large populations thus must be replaced as soon as possible with biofortification. In developed nations with few cases of nutrient deficiencies, supplementation is concentrated on a relatively small group affected with certain insufficiencies. Supplementation is strongly advised in low-income nations which are highly malnutritional (Nantel and Tontisirin 2002). However, because people live in isolated, rural areas with difficult accessibility, supplementation programmes can be expensive and challenging to sustain in developing countries. Vitamin A supplementation was one of the most economical approaches globally which helped in increased child survival (Edejer et al., 2005; Shrimpton and Schultink, 2002). Because vitamin A supplementation is linked to a lower risk of death from all causes, it is frequently incorporated into national health policies (Imdad et al., 2010). Supplemental vit A was generally given to children at risk between the ages of 6 months and 5 years (UNICEF, 2007). Supplementation of other micronutrients like amino acids, folic acid, zinc, iron, and others that are deficient in the body is less common. Iron-folate supplementation is sometimes required to expectant mothers, but due to a lack of resources, this is uncommon in developing nations. However, to avoid maternal anaemia, low birth weights, and immature births, the WHO advises pregnant women to take 400 mg of folic acid daily in addition to 30-60 mg of iron (WHO, 2016b).

1.2.3 Industrial Fortification:

The process of food fortification involves adding nutrients to food in order to maintain or raise the standard of a population's diet. Food fortification is needed since people suffer from insufficiency of vitamins and minerals as they mostly rely on processed foods. Consumers can obtain the necessary amount of nutrients in their food thanks to industrial fortification, where certain amounts of nutrients are added to foods while processing. One sustainable and comparatively affordable public health measure is the augmentation of table salt with iodine. The number of countries with an iodine deficiency has decreased from 54 to 32 since 2003, and nearly three-fourth of the population has iodized salt in their reach globally (Andersson et al., 2012). Vitamin B-complex, Fe and Zn rich wheat flour and vitamin A-fortified edible oil are two additional examples of fortification. Since urban people have more access to industrially processed and fortified edibles, fortification may be more effective; however, it is challenging for rural consumers to get benefited from food fortification. There are a number of drawbacks associated with food fortification. It may be hard to find out the proper micronutrient amounts, consumers preference and others such as cooking may decrease nutrient content and nutrient bioavailability. To use food fortification strategies effectively and sustainably, stakeholders including policymakers, researchers, economists, etc. must actively coordinate.

In the recent years, traditional food fortification programmes are being supports by new public sector initiatives such as: Micronutrient Initiative (Canada), The Flour processing approach (Emory University), The “Mid-Day Meal Scheme” (India) and the “Global Alliance for Improved Nutrition” (GAIN, Geneva).

The International Zinc Nutrition Consultative Group and the “Network for Sustained Elimination of Iodine Deficiency” are two more significant international initiatives. Additionally, initiatives are being made to establish geographical standards for fortification. According to the “Flour Fortification Initiative”, which assesses global development, 26% of the world's marketed wheat flour is processed, helping 1.8 billion people globally.

Industrial processing only applies to marketed products, so it may not benefit in rural communities, where people obtain their food through noncommercialized channels. With such restrictions, it is obvious that industrial food fortification cannot, in the medium term, solve the issue of micronutrient deficiencies completely.

1.2.4 Biofortification:

To enhance the levels of micronutrients in edible parts, this can be achieved by traditional breeding or molecular breeding or by genetic engineering. Mineral element deficiencies in food crops may result from a variety of factors, including mineral-deficient soils, lowered mineral availability for uptake due to factors like high pH, limited distribution/translocation of ions, and mineral accumulation in parts of food crops that are not edible. Biofortification has been suggested as a long-term alternative to conventional interventions because their effectiveness in enhancing mineral nutrition is limited (Zhu et al., 2007). Biofortification focuses to raise not only the nutrient levels but also increased bioavailability in the consumable part of food crops and thus improving the mineral nutritional qualities of crops at the source. The only way to increase bioavailability is by breeding, whereas the former can be accomplished agronomically as well as genetically.

Plant breeding or genetic engineering, both are comparable, yet from agronomic approaches where the former approaches involve the change in genetic architecture of target crop. Although their scopes are different, both genetic engineering and plant breeding aim to develop such genotypes having alleles/combination of alleles that effectively accumulates bioavailable nutrients, but genetic engineering provides ways to introduce alleles from any source, whereas breeding attains this by combining the nutrient-dense high yielding lines and selecting those with favourable traits generation after generation.

Genetic engineering and plant breeding, in contrast to conventional intervention strategies, are both environmentally and economically feasible (Stein et al., 2008). On long terms, biofortification is also likely to be more feasible than traditional approaches because it avoids dependence on infrastructure for industrial fortification. In addition, plants absorb nutrients that are bioavailable *per se* and add to the food's flavour and test. Economic studies have demonstrated numerous promising health benefits of biofortification programmes, particularly when used in collaboration with traditional techniques (Buois 2002; Stein et al., 2008).

A. Conventional Plant Breeding Approaches:

By utilizing the natural genetic variation, plant breeding programmes aim to increase the amount and bioavailability of minerals in staple crops (Welch and Graham 2005). Discovering genetic variation for micronutrient traits, evaluating their stable inheritance under various environment and determining economic viability of breeding for higher nutrients availability in consumable parts keeping yield and quality parameters intact are some conventional breeding approaches. Conventional breeding, however, has its limitations since it relies on genetic diversity that is already present and usable in the crop to add nutritionally important traits or, rarely uses wild relatives with cross compatibility. One such successful event is quality protein maize (QPM), where years of traditional breeding efforts created cultivars with wide acceptance.

It has been anticipated that more Fe and Zn in the plant may, according to some reports, also lead to increased yield. This is the case with Fe and Zn in rice and wheat. Other biofortified crops have been released for both minerals and yield traits, such as the orange-fleshed sweet potatoes in Africa (Unnevehr et al., 2007). The majority of cereal varities have little variation in their kernel Fe and Zn contents (Bouis, 2003; Rawat et al., 2009). At CIMMYT, germplasm screening programmes in wheat for iron and zinc under various environments has provided insights into genetic variation. From maxica wheat accessions, Graham et al., (1999) found a range of 25.2-53.3 µg/g for zinc and 28.8-56.5 µg/g for iron. Wheat germplasm contains enough genetic diversity to significantly raise Fe and Zn concentrations in wheat grain. The concentrations of kernel Fe and Zn in the examined wheat lines showed a strong correlation.

These results suggested scope of simultaneous improvement of Fe and Zn content in wheat via plant breeding (Chhuneja et al., 2006). It has been reported that wild relatives have grain Fe and Zn content that is three to four times more than that of modern hexaploidy wheat cultivars (Rawat et al., 2009).

B. Molecular Plant Breeding Approaches:

Recent advances in plant breeding have enabled breeder to achieve the breeding targets in a faster, though more reliable way. There are several reports available on QTLs for grain micronutrient traits in cereals, particularly in wheat and rice. According to reports, wild wheat and *Aegilops* have genes for high grain Fe and Zn content on chromosomes 2 and 7. Three quantitative trait loci (QTLs) for grain Fe and Zn content have already been identified on chromosomes 2A and 7A in recombinant inbred lines (RIL) population of diploid wheat

(Tiwari et al., 2009, 2010). RIL population from tetraploid wheat *T. durum-T. dicoccoides*, Peleg et al., (2009) also identified three significant quantitative trait loci (QTLs) for kernel Fe and Zn concentrations. In a Hanxuan10 x Lumai14 double haploid wheat population, Shi et al., (2008) identified number of QTLs on chromosomes 1A, 2D, 3A, 4A, 4D, 5A, and 7A for Zn content. In rice a number of QTLs have been mapped on chromosome 1, 3, 5, 7 and 12 (Anuradha et al., 2012).

C. Genetic Engineering Approaches:

It has been successfully shown that genetic engineering has the potential to enhance the nutrient level in edible crops. Increased provitamin A concentrations have been developed in genetically modified (GM) tomato (De Steur et al., 2015). Biofortification goals such as increase soil uptake, improve micronutrient transport to shoots and grains, increase the sequestration of ions in endosperm, and decrease anti-nutritional factors in kernels can be achieved by genetic engineering. Suzuki et al., (2003) reported that human lactoferrin, a primary Fe-binding protein in milk, is expressed at very high levels in rice. The recombined lactoferrin was demonstrated to be completely iron saturated.

Similar to this, Goto et al., (1999) inserted ferritin from soybean into rice, and it was discovered that some transformants had iron contents twice as much as wild-type rice. Naqvi et al., (2009) created transgenic maize inbred and found simultaneous alteration of three distinct metabolic pathways specifically increased vitamin level in the endosperm. The transgenic kernels had 169 times as much beta-carotene, six times as much ascorbate, and twice as much folate.

D. Agronomic Approaches:

There are a number of non-genetic strategies that can be taken to enhance nutrient level in the consumable parts. Greater minerals availability in the soil for roots to uptake, these include management techniques, fertilizer application of key micronutrients, and enhancing soil organic matter.

Applying fertilizers and/or enhancing the solubility and mobility of minerals in the soil are two common agronomic practices to enhance the concentrations of elements in edible parts (White and Broadley 2009). The former is achieved by foliar or soil application of fertilizers, while later is achieved by use of micro-organisms. It is common practice to apply concentrated doses of fertilizers to the soil or the leaves of plants when growing crops cannot utilize nutrient available in the soil immediately. Foliar applications of soluble fertilizers are used when mineral elements are difficult to transfer to edible tissues.

1.3 Future Challenges for Biofortified Crops

Success of a crop biofortification programme is assessed on three parameters - if the raise in micronutrient level has significant impact on nutritional status; if the increased amount of micronutrient is bioavailable or not; if the farmers will adopt biofortified varieties or consumers will buy or consume it. Thus, crop biofortification faces following challenges –

1.3.1 Performance of Biofortified Crop:

High yields, pest and disease resistance, and other desirable agronomic traits can all be maintained while incorporating high nutrient and vitamins into the consumable foods. Farmers won't use biofortified staple crops if they lack desirable agronomic traits, or they are inferior in yielding ability, so every released cultivar must be competent enough with currently cultivated or already popular varieties to be successfully adopted by farmers

1.3.2 Acceptance of Genetically Modified Crops:

Several transgenic biofortified crops with novel agronomic traits are currently being developed (e.g., disease resistance). Although transgenic methods may shorten time to market, there is still a lot of opposition to transgenic crops. Also, many of the developing nations are not having regulations for testing and release of genetically modified biofortified crops.

1.4 Conclusion:

There are significant gaps in our understanding of biofortification; additional efficiency trials and studies are required to support and supplement the promising evidence we have so far. Transgenic methods have greater potential in achieving this than traditional breeding in the breeding multi-nutrients and vitamins into single variety, making breeding more cost-effective. Additionally, biofortification is secondary to current interventions, but each target country must be taken into account to determine the best combination of biofortification, supplementation, industrial fortification, and dietary diversification. Additionally, there needs to be better coordination between these programmes. The assessment of potential risks associated with excessive intake must be taken into consideration, along with food safety and quality assurance. Possible allergies and ill-effects linked to increased nutrient intake still need to be thoroughly researched while keeping environmental changes in mind. On food content information and health claims, it is important to highlight international standards and national regulations. The biofortification strategy needs to be modified to work within the country's regulatory framework. Approaches to biofortification should be balanced, and coordination with other public strategies needs to be prioritized. Although conventional breeding improvements are not regulated, the transgenic approach needs the proper regulatory framework to be adapted.

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