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Techniques for the Enrichment of Micronutrients in Crops through Biofortification: A Review

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Deficiency of micronutrients such as iron, zinc, selenium and vitamin A affect human health globally. This deficiency is being controlled by supplementation and nutrient fortification, however, new alternatives are needed to fulfill the optimum nutrient requirement especially in rural poor areas. Biofortification, enrichment of micronutrients in staple food by plant breeding, is an alternative which looks promising. Laboratory experiments show that biofortification is possible without damaging agronomic productivity. Analysis of predictive cost-benefit also support the benefits of biofortification and emphasize on its importance in the armamentarium for managing deficiency of micronutrient. The challenge to overcome is to convince consumer and producers to accept biofortified crops. This study focuses on the techniques that can be used to produced biofortified crops.

Keywords: Biofortified crops; iron; vitamin A; zinc.

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1. INTRODUCTION

Malnutrition also is known as "the hidden hunger" is one of the major problems and a rising issue in developing countries. After the green revolution, growing massive amounts of cash crops has become a trend resulting in a lower production of mineral-rich legumes and fruits in the world. This decrease in the production has not only lead to sole dependency upon cereal crops but has also increased prices of fruits, legumes and other foods in the world food market. The cereal crops deficient in essential nutrients has become more affordable and economical in turn giving rise to malnutrition [1,2].

Human body requires almost 22 essential mineral elements to work efficiently. Some are required in large amounts while other minerals such as Fe, Zn, Cu, I and Se are required in trace amounts. The high amount of these minerals in the body is harmful [3,4]. These elements enter the food chain through plants. Some important mineral elements such as sodium and potassium occur only as inorganic soluble ions in the plant. On the other hand, other minerals can be present either in inorganic salts or organic compounds in both soluble and insoluble form. The elements frequently lacking in human food are Zn, I and Fe, although other elements like Se, Mg, Ca and Cu are also considered highly deficient in human diet but the problem is limited only to some populations [3] [5-10]. It is reported that out of 6 billion people of the world 30% are Zn deficient, about 15% are Se deficient, 60–80% are Fe deficient and about 30% are Iodine deficient [9,11]. The diseases caused by the deficiency and excess of Zn, Fe and Selenium is summarized in Table 1 and Table 2.

The root cause of this deficiency is not one, rather it is due to a collection of factors that leads to malnutrition in the population. High consumption of staple food such as cereals and comparatively low intake of fruits, vegetables, fish and animal products which provide a high source of essential minerals, sowing of crops in areas with low mineral phyto-availability and consumption of processed food are the key factors that leads to a deficiency of essential minerals in the diet. In addition to all these factors, another problem is the availability of these minerals in the human consumable tissues of plants. An element's chemical state determines the human's ability to absorb and assimilate it [3,12-14]. The plant contains both the absorption stimulators and the anti-nutrient compounds in them. The absorption stimulating compounds known as promoters allows efficient uptake and assimilation of minerals through the gut. Whereas, the anti-nutrients such as phytate (IP_6) or polyphenolics (tannins) hinder their absorption [15].

The problem of malnutrition is not only growing amongst the human population but is also an issue of concern for the plants. Plants require about 14 minerals for their growth among which are nitrogen, phosphorous, potassium, calcium, magnesium, zinc, iron, selenium etc. These minerals are naturally obtained from the soil. Low

Table 1. Diseases caused due to micronutrient deficiency

Micronutrient deficiency	Disease caused by the deficiency of micronutrients
Iron	• Anemia • Neurodegenerative disorders
Zinc	• Neuropsychiatric disorders • Neurosensory disorders • Mental lethargy • Delayed wound healing • Infertility • Hypogonadism • Cancer • Dwarfism
Selenium	• Cardiovascular disorders • Oxidative stress • Inflammation • Keshan disease • Keshin- beck disease • Crohn's disease

Table 2. Diseases caused by an excess of micronutrient

Excess of Micronutrients	Diseases caused by excess of micronutrients
Iron	• Hemochromatosis • Arthritis • Liver diseases
Zinc	• Lethargy • Respiratory tract disorders • Nausea and vomiting • Diarrhea • Elevated risk of prostate cancer
Selenium	• Selenosis • Lesion of skin • Lesion of the nervous system • Nausea • Diarrhea • Fatigue

availability of these minerals in the soil limits the plant's growth and effects its metabolic activities such as germination rate, seedling vigor and the natural life cycle [16]. The plants are deficient in minerals because they are grown on mineral deficient soils. The minerals are lost in soil from prolonged waterlogging, salinity and low amount of organic matter. Other important properties determining the mineral content of plant are the soil's pH, its redox condition, cation exchange capacity, richness in microbes and soil structure [16].

The problem of mineral deficiency is more significant among the cereal crops. Cereals are a major source of nutrition in the developing countries and amongst the food crops wheat is an important source of energy in the world and is considered a staple food especially in rural and underdeveloped areas. In Central and West Asian countries, it is observed that about 50% of daily calorie intake on average is obtained from wheat [17].

Wheat (*Triticum* spp.) is a self-pollinating annual crop plant that belongs to the grasses family *Poaceae,* tribe *Triticeae* and genus *Triticum.* The crop has initially originated in Asia and parts of Africa. All the species belonging to the genus *Triticum* can be divided into three basic groups, each distinguished by the number of chromosomes in the generative and vegetative cells [18]. It is usually grown in temperate climates with an optimum growing temperature of 25ºC. It is one of the most widely sown cash crops as it has a shorter growing season and an excellent yield. The wheat is regularly studied for crop improvement against diseases, pests and for improved quality and yield. The grounded seed of the wheat forms basic ingredient as flour in bakery food items, bread and pasta and is a rich source of proteins, carbohydrates, lipids, fiber, minerals and vitamins. With new varieties in the market now after conventional or genetic breeding, wheat now faces deficiency in mineral contents, especially in zinc, iron and selenium. Various strategies have been applied to overcome the problem of mineral deficiency however, success has only been seen for zinc, iron and selenium amongst many another mineral that lacks in the crop. Moreover, wheat is rich in phytic acid and phenols which chelates zinc and iron and forms insoluble salts making these minerals less accessible for human consumption. Generally, grain Zn and Fe concentrations in commercial wheat cultivars are 20-35 mg/kg [1,2]. This concentration is not adequate for daily consumption by human and long-term dependency on these crops can lead to disorders such as stunting, anemia, susceptibility to infectious diseases and disrupted physical and mental development [1,2].

A vast variety of strategies have been planned to overcome the problem of nutrition deprival in the world. These include fortification of food, food supplements intake, fertilization of crops and biofortification of food crops. Fertilization of the crops although increases the mineral concentrations in the leaves but does not give promising results in fruits, seeds or grain [6,8,19-23]. Moreover, fertilizers application has to be done on regular terms which are not cost effective and may also lead towards increased pollution and eutrophication.

The use of food supplements such as tablets for minerals and multivitamins also require a vast amount of money for production, supply and projects that can reach to the rural and urban areas. Fortification of food such as fortified milk with iron and fortified margarine with vitamin A is the processing of food just before consumption and relies greatly upon industrial processing. In contrast, to all these techniques biofortification is a natural process that can give a long-term solution for malnutrition both in plants and animals. This is also a slogan for the international biofortification research and development. Although the strategy for biofortification is unproven yet, it has the potential to become sustainable, cost-effective and reach remote rural populations [7]. Biofortification can be achieved by conventional breeding and by using modern biotechnology.

2. METHODS OF BIOFORTIFICATION

Biofortification can be achieved via mineral fertilization also known as agronomic biofortification, conventional breeding and or by the production of transgenic plants.

2.1 Agronomic Biofortification

Mineral fertilization or agronomic biofortification is a rapid and simple method to overcome the mineral deficiency in plants. It can be used as an immediate measure to solve the increasing problem of mineral deficiency. However, this technique is not suitable for iron or immobile minerals and is not cost effective. In addition, this technique does not solve the problem of unavailability of minerals in human edible portions of the plant. In fact, the solution seems to be an easy application has many complications along with it. Many factors such as the method of application of the fertilizer (foliar spray or soil application), type of the mineral that requires biofortification, its mobility and the soil composition are to be undertaken. If a high amount of mineral is applied it turns out to be harmful to the plant and humans, let alone solve the problem of malnutrition.

So far, agronomic biofortification has been promising only for selective minerals and has not been declared a universal solution for all minerals. Good results have been achieved so far in selenium, zinc and iodine in various crops as these minerals are mobile in the soil in contrast to iron which is bound to the soil particles and converted to Fe(III). Fe also has limited mobility in the phloem. Therefore, application of iron-fortified fertilizers is not effective to various plants as its immobility cause it to be unavailable in the soil through adsorption, precipitation and oxidation reactions. However, use of iron chelates can be used as iron fertilizers as an alternative [20,24].

Agronomic biofortification applied through soil fertilizers or foliar spray is not environment-friendly technique as it may lead to eutrophication and increased pollution. It is not economical as regular application of fertilizers is needed in order to achieve the desired concentration of the minerals. However, as a temporary security of the minerals restoration, it is a fast and rapid approach that can be used for a short time but is not effective as a long-term solution.

2.2 Conventional Breeding

Breeding for enhancing the micronutrients in plants is a constitute of genetic methods of biofortification. Many useful genes can be utilized by scrutinizing the germplasm variation and by using molecular markers techniques for tracking the target genes. New techniques such as TILLING that is, saturating metabolic pathways with mutations and subsequently identifying the genes involved can be used for breeding and produce high yielding and nutrient-rich varieties of crops [25].

Plants can be bred for increased acquisition and accumulation of minerals in their edible tissues. However, as far as wheat is considered it does not have a much genetic variation for zinc and iron concentration in its grain. It is seen that the wild-type wheat species possess a considerable amount of genetic variation and should be considered for molecular breeding [16]. The wild-type species and the modern cultivars of wheat have been studied for their differences in zinc and iron concentrations. Allelic variation was brought at a chromosomal locus that promoted early senescence and remobilization of iron, zinc and manganese from senescing leaves to seeds, from a tetraploid wheat (*Triticum turgidum* ssp. *dicoccoides*) into a cultivated wheat (*Triticum durum*). A high concentration of iron and zinc was seen in the grain of the modern variety after breeding [26]. Similarly, it has been seen that wild varieties of wheat (*Triticum dicoccum* and *Triticum spelta*) contains a higher amount of selenium as compared to cultivated varieties [8,15,27].

The breeding technique although a good measure but is very time consuming and the outcome is always uncertain. Moreover, the wheat before utilization undergoes milling and processing resulting in the loss of high mineral content to waste. The question now stands whether genetic variation brought about for enhanced mineral content is a successful biofortification technique or not. Minerals in the plants seed are not confined to a specific place rather it is non-homogeneously spread along the seed and is concentrated in the aleurone layer or the husk. This is dependent upon the seed morphology, its size and the number of layers [16].

Plant seeds and grains also contain varying amount of various anti-nutrients such as phytic acid and tannins that cause poor absorption of Ca, Zn and Fe in the gut and reduces the bioavailability of these minerals [3,7,13]. In addition, plants also have promoter compounds that facilitate the availability of these minerals. These promoters including vitamin C, beta-carotene and many other organic compounds. The expression of both compounds is controlled by genes as well as environmental factors [7].

Variations in the amounts of promoters and anti-nutrients to make minerals readily available in human consumable portions can be brought about by breeding. The concentration of promoters can be increased and anti-nutrients concentration can either be lowered or completely eliminated [7]. Several low phytic acid mutants have been produced by breeding methods in rice [12], wheat [28] and several other crops. However, the useful characteristics of these anti-nutrients cannot be overlooked such as phytic acid acts as an anti-cancer agent for human. Many anti-nutrient compounds also determine the plant's resistance to pests and abiotic stress. In contrast, the promoters are controlled by a relatively small number of genes and can be manipulated by breeding for increased bioavailability of minerals [7]. Ascorbic acid, a strong antioxidant, can be used to serve this purpose but it is easily degraded by heat [11]. Cysteine also works towards increasing bioavailability of particularly zinc and iron. However, heat has similar effects on cysteine as that for ascorbic acid [16,29]. More studies need to be carried out to find specific promoter expressions for specific crops.

2.3 Transgenic Crop Approach

The transgenic approach of biofortification focuses on modifying a plant to efficiently adsorb minerals from roots and translocate them to edible portions. It is a rapid method that is not dependent upon the existing germ pool and can be applied directly to the cultivated varieties of crops. This approach also extends to increase and decrease of promoters and anti-nutrients respectively [16].

Several ways have been applied to various plants to increase the mineral value. For example, in non graminaceous plants the iron uptake can be increased by overexpressing iron reductases genes whereas, in graminaceous species, it can be acquired by increasing the synthesis and exudation of phytosiderophores [15]. Transgenic approaches also focus on increasing the concentration of promoter compounds in the plant that can translocate minerals to fruits and seeds. It increases the availability of metal binding compounds such as ferritin and lactoferritin and compounds like vitamin C and beta-carotene [14]. Success has been achieved in rice endosperm by expressing ferritin or lactoferritin that significantly increases the concentration of zinc and copper. Also, overexpression of ferritin in lettuce increased iron levels in the plant [14,29]. Various approaches have also been laid down for increasing beta-carotene, lysine and cysteine in plants.

Another approach is to reduce phytic acid concentrations in the seeds and grains. Two strategies have experimented; these include knocking out of IP_6 biosynthetic pathway enzymes and overexpressing phytase enzyme in human consumable tissues of plant [14].

3. CONCLUSION AND FUTURE PERSPECTIVE

Malnutrition is a serious problem and has severe consequences. The problem needs to reviewed extensively and steps need to be taken on an immediate basis in order to overcome the problem of hidden hunger faced by the world at the moment. Wheat being among most important cash crops and staple crops needs to be given much importance and new applicable approaches should be taken to modify it and those techniques should be adopted that are economical and gives successful outputs in a shorter time. Much research and study are still required in the field of biofortification to practically utilize it for mass production of crop plants. It should be given immediate attention and incentives should be given to promote research in this field. The issue of acceptance of genetically modified plants is still in the process of argument. Measures should be taken to promote awareness among the population about biotechnology and molecular techniques and products. The study should be broadened to many other minerals that are still lacking in the soil and plants and in turn causing diseases in the organisms consuming them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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