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# Bio-fortification: A Promising Tool to Combat Malnutrition- A Review

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**ABSTRACT: The majority of the staple food crops are poor sources of nutrients, which limits access to diverse diets and other micronutrients for normal human nourishment. Previously, researchers were more focused on obtaining a high yield from crops per unit area to feed the whole world and attain food security. But now more emphasis has been given to research related to the production of quality foods containing nutrients and vitamins to eradicate malnutrition problems, and there comes the importance of fortification in crops. Biofortification is a novel technique that is considered an economical and sustainable method of increasing the concentration and bioavailability of essential elements in edible parts of plants through various means. Several biofortified food crops, mainly grains, legumes, oil seeds, fruits, and vegetables, have been released, and some are under research. It is proven that such crops could provide a sufficient level of nutrients to an undernourished population and alleviate hidden hunger. Despite the high success rate and acceptability of biofortification techniques, there are some limitations to these methods, such as the high initial cost involved in production and the time-consuming regulatory process. Nevertheless, in the coming future, biofortified crops have a great potential to tackle the problems caused by malnutrition.**

**Keywords:** Biofortification, micronutrients, vitamins, bioavailability.

## INTRODUCTION

The world population is expanding in a geometric fashion, and there is a global threat to nutritional security, necessitating the implementation of practical and affordable methods to manage the world food system. The lack of essential nutrients affects more than 2 billion people worldwide (Huang *et al.,* 2020). Nutrient supplementation programmes have traditionally been used to administer vitamins and minerals in case of malnutrition; however, this approach falls short of the objectives established by the international health organizations, and such nutrient supplements won't be accessible to those below the poverty line (Gilani and Nasim 2007; Massot *et al.*, 2013). Therefore, biofortification of various crops enables a long-term and sustainable solution for providing people with crops that are high in nutrients. Field crops that are used as staple foods all over the world have been the main focus of biofortification research. Biofortification, or "biological fortification," can be promoted as a promising tool, as it permits the insertion of necessary nutrients into the edible component of a specific crop by selective breeding, genetic alteration, or the use of enhanced fertilisers. So far, cereal crops, which include wheat, rice, maize, oilseeds, pulse crops, and fodder crops that can absorb nutrients supplied externally, have all been biofortified. The development of biofortified crops eliminates the need to use fortifying agents every time during the processing of food items, making biofortification, from an economic perspective, a one-time operation that provides a cost-efficient, long-lasting, and consistent method of tackling hidden hunger (Nestel *et al.,* 2006; Pfeiffer *et al.,* 2007).

## NEED FOR BIOFORTIFICATION RESEARCH

For a healthy and productive life, the human body needs around 40 essential nutrients in adequate quantity (Garg *et al.,* 2018). The body needs moderate amounts of the mineral's sodium, potassium, calcium, magnesium, phosphorus, chlorine, and sulphur, which are classified as essential macronutrients. Micronutrients are another category of essential nutrients that the human body needs in very minute quantities. These include iron, zinc, copper, manganese, iodine, selenium, molybdenum, cobalt and nickel (Prashanth *et al.,* 2015). Micronutrients serve as cofactors for a number of enzymes in the human body, controlling key metabolic processes (Welch and Graham 2004). Cereals such as rice, wheat, and maize, then vegetables and fruits, are the primary sources of these nutrients in human diet and we depend on agriculture for food. There will be only limited supply of these agricultural commodities in developing countries, and they may contain an insufficient amount of nutrients to meet the daily requirements**.** These food materials with insufficient nutrients cannot support healthy diets and may instead lead to ill health, increased morbidity and disability, stunted mental and physical development, weakened livelihoods, and a decline in the socioeconomic development of the country (Chizuru *et al.*, 2003). Around the world, 43% of infants and 38% of pregnant women suffer from severe micronutrient deficiency disorders (Stevens *et al.,* 2013). So, in many developing nations, nutrient deficiency in food produce is a main issue, and uneven nutrient distribution among various plant parts is another crucial factor to take into account (Zhu *et al.*, 2007). Rice leaves, for example, contain more iron than polished rice grains. Biofortified crops contain all the necessary nutrients for better health as well as sufficient calories to meet energy demands. Hence, biofortification tries to boost the amount of required nutrients in plants' edible parts by various means (Welch and Graham 2004). In comparison to many other methods for enhancing a person's nutritional condition, biofortification has an advantage because it targets the whole population via staple foods.

## BIOFORTIFICATION APPROACHES

Essential nutrients are biofortified into crop plants mainly by means of agronomic and genetic methods. An agronomical approach includes improved soil management practices, increased fertiliser application, and using plant growth-promoting microorganisms. where the genetic approach represents plant breeding and transgenic means (DNA technology). Crops targeted by agronomic practice, plant breeding, and transgenic methods include wheat, rice, sorghum, maize, common beans, lupine, sweet potatoes, and tomatoes**.** Since cereals are considered staple food crops, they have been biofortified by all these strategies. Most of the crops have been improved by agronomic practices, whereas oil seed biofortification has been achieved through transgenic means; however, the effectiveness of biofortification is much higher with a breeding approach.

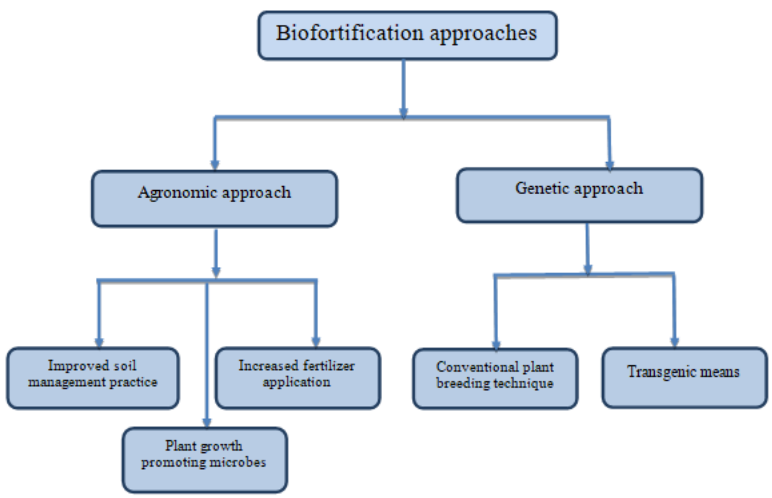


**Fig. 1.** Classification of different biofortification approaches.

**Improved soil management practices.** Nutrient use efficacy of a plant depends on several factors such as physical, chemical and biological properties of the soil. Sometimes plants won't be able to absorb the nutrients directly from the soil even in the presence of sufficient nutrients due to variation in pH, soil moisture deficits, improper aeration of the soil. Therefore, soil properties have to be improved first before incorporating nutrients in soil. Soil management practises will improve the availability of micronutrients for plant uptake. Soil management practises include lime application for increasing the pH of acidic soil (pH <7), amending the soil with gypsum when the soil is alkaline (pH > 7). Soil pH plays an important role in improving the growth of the plant and nutrient availability; even the slightest variation in soil pH may affect the availability of nutrients. Another management practice is incorporating crop residues back to soil, it can not only improve the aeration of soil but also the moisture holding capacity of soil. As most of the nutrient uptake is done by mass flow and diffusion, soil moisture is the main factor that affects the nutrient concentration in plants. Optimum moisture helps in better root growth, and improves the availability and mobility of nutrients. Water deficit at the time of grain filling may reduce the lipid content in wheat grains, while mild water stress during grain filling will improve the starch content in grains (Zhao *et al.,* 2009). So proper water management is crucial in all the crops during the critical stage for improving the quality of the food product. Intercropping and crop rotation with soil exhausting crop is another way of soil management. Crop rotation will improve the soil's physical properties. Studies have reported that organic matter content and nutrients lost from the soil through intensively tilled row crops can be regained by crop rotation and intercropping with perennial sod crops.

**Increased fertilizer application.** An ideal soil where the plant could grow rarely exists in world, soil micronutrient deficiency was reported in many parts of the world, which limit plant nutrient availability and subsequently effect human nourishment. Inadequate intake of the nutrients has pronouncedly detrimental effects, since they are essential to human body. Due to the production of insufficient and poor-quality fodder, there is a significant gap in the supply and demand of cattle feeds. Therefore, in terms of human and livestock health, nutritional safety is a subject of great importance (Kennedy *et al.,* 2020). In order to increase crop yield, farmers are applying mineral fertilisers to soil, but within specific limit, the same approach can also be used to increase the nutrient accumulation of cereal grains (White and Broadley 2009).

When crops are raised in an environment where the specific mineral applied may get immediately unavailable in the soil, targeted application of nutrients to the root zone or direct foliar application will be required. Foliar application of soluble inorganic fertilisers are made if the critical nutrients required are not readily absorbed to edible part of the plant. Biofortification of rice plants by foliar spray of iron and zinc was an effective way to promote its concentration in rice grains (Shohag *et al.,* 2013). In case of wheat, the inclusion of zinc in fertilizer program has reduced human zinc deficiency in regions with potential zinc-deficient soil and also improved its bioavailability by reducing antinutrient factors like phytic acid (Yang *et al.,* 2011). Selenium-enriched soybean has been produced by the foliar application of selenium complex salts as fertilizers (Yang *et al.,* 2003). In developing countries, the application of nutrients through fertigation and foliar applications are not economical and these chemicals applied to soil may leach down to water bodies and leads to eutrophication. Therefore, incorporating organic manures and composts will be a better option since it is more economical, environment friendly and raw materials for making compost are easily available in rural areas as compared to urban cities. Ulm *et al.* (2018), reported that application of compost is more effective and economical in rural parts of the country. Long-term compost application increased soil pH, CEC and organic carbon.

**Plant growth promoting micro-organisms.** In addition to fertilizers, soil microorganisms that are plant growth-promoting (bio fertilizers) can also be used to mobilise nutritionally important elements from non-available to available forms through nitrogen fixation, phosphorus solubilization, and plant growth promoting substances like siderophores, enzymes capable of decomposing organic compounds, and other organic acids. Soil microorganisms can also be used to increase the phytoavailability of mineral elements (Heijden *et al.,* 2008). Some leguminous crops are associated with mycorrhizal fungi that improve plant growth under nutrient deficient conditions. Mycorrhizal roots can take up much more phosphorus and increase the uptake of other nutrients like K, S, Cu, and Zn. The symbiotic fungus can alter the mineral composition of the plant directly or indirectly. There are six types of mycorrhiza classified based on their morphological features (Wang and Qiu 2006). Arbuscular mycorrhiza (AM) is the most common type of mycorrhiza, fungal structures can be found inside the cortical cells of the root, which function as the main site of plant-fungal nutrient exchange. Arbuscular mycorrhiza can be considered as an agriculturally important fungus since it is closely related to human nutrition and has external hyphae that have a large surface area for nutrient absorption from soils. Studies are now concentrated on boosting the uptake of nutrients and metabolic activity of sorghum by utilising the interaction between plant growth-promoting bacteria and arbuscular mycorrhizal fungi (Dhawi *et al.,* 2015).

Another group of plant growth promoting micro-organisms are rhizobacteria, which have a beneficial effect on plant growth by colonizing the root system. The PGPRs belong to several genera, *viz*., *Agrobacterium, Arthrobacter, Basillus, Pseudomonas, Xanthomonas, Rhiziobium*etc. The use of PGPR is steadily increasing in agriculture, as it reduces the use of chemical fertilizers, pesticides, and related agrochemicals (Rana *et al.,* 2012). The $N_2$-fixing bacteria also play an important role in increasing nitrogen content in plant parts under nitrogen limited conditions (Hardarson *et al.,* 2004). Different crops have been targeted through agronomical biofortification to improve the human nutritional status. For example, canola cropinoculated with growth-promoting rhizobacteria such as *Azotobacter vinelandii and Azospirillumbrasilense*, along with inorganic fertilizers led to increased production of oleic acid, linoleic acid and protein content in canola seed, which indicate, rhizobacteria are good at increasing nutrient content in canola oil (Nosheen *et al.,* 2011). Wheat grains have been biofortified with iron on adopting integrated organic fertilisers, and zinc using Bacillus aryabhattai (Ramzani *et al.,* 2016).

**Table 1: Agronomic biofortification in different crops for improving nutrient status (Garg *et al.*, 2018).**

| Crop | Different types of biofortification | Status | Source |
|---|---|---|---|
| Rice | Zinc<br>Selenium<br>Iron | Research | Boonchuay *et al.* (2013)<br>Fang *et al.* (2008)<br>He *et al.* (2013); Yuan *et al.* (2013) |
| Wheat | Iron<br>Zinc<br>P fertilizer + mycorrhiza<br>Bacillus aryabhattai (zinc) | Research | Aciksoz *et al.* (2011)<br>Cakmak *et al.* (2010)<br>Noori *et al.* (2014)<br>Ramesh *et al.* (2014) |
| Maize | Plant growth-promoting rhizobacteria + Cyanobacteria (zinc) | Research | Prasanna *et al.* (2015) |
| Chickpea | (iron, zinc, calcium, copper, manganese, Mg) Actinobacteria | Research | Sathya *et al.* (2013) |
| Mustard | rhizosphere bacteria, Se | Research | Yasin *et al.* (2015) |
| Sweet potato | Beta-carotene | Research | Laurie *et al.* (2012) |
| Lettuce | Iodine, Se | Released | Smolen *et al.* (2013) |

**Limitations of Agronomic strategies**

— Agronomic biofortification involves physical application of mineral nutrients to crops which could improve the nutritional and health status of the plant but only on a temporary basis (Cakmak and Kutman 2017).

— Using fertilisers may most likely to make food more expensive and less accessible to those who are most in need of it and it is labour intensive as it involves incorporation of bulky fertilizers to soil.

— Even though plants are capable of absorbing minerals from the soil, they may store these minerals in non-edible parts like leaves rather than fruits or seeds, or they may store the mineral in an unavailable form that has no influence on nutrition (Frossard *et al*., 2000).

— Fertilizer applied to soil may leach into water bodies and contribute to enhanced eutrophication and toxicity problems. Sometimes applied fertilizers may negatively influence the native microbial population in soil and higher level of NPK fertilizers may hinder the uptake of micronutrients from soil.

— Excessive tillage for intensive farming, soil amendment using sulphates and other chemicals applied to soil on a long-term basis may affect the soil health and reduce the productivity of the soil.

— Fertilizers applied to soil has an impact on the environment also, synthetic and organic fertilizers applied to soil is the major source of nitrous oxide in the atmosphere. In the year 2020 almost 74% of total $N_2O$ emission in U.S was contributed by the agricultural soil management practices.

— When compared to inorganic fertilizers, biofertilizers are environment friendly and sustainable but they are non-available to all, are very costly. Farmers in developing and under developed countries cannot afford it.

**Factors to consider before implementing agronomic biofortification**

— Screening of present varieties for their response to micronutrient fertilizer application (Shahane and Shivay 2022)

— Conducting soil test prior to fertilizer application for knowing the initial nutrient status.

— Study of naturally occurring plant growth promoting micro fauna in soil and their use in micronutrient (especially Fe and Zn) solubilisation and mobilization.

— Identification of crop and soil specific management practices and fertilizer application at the right place, right time, using right method and right quantity.

— Development of affordable customized fertilizers for micronutrient application.

— Research on enhancing the efficiency of applied fertilizers.

**Genetic approach.** The genetic composition of the nutrient content in food grains has already been studied to increase the crop yield and nutrient content in grains. It is possible to increase the concentration of nutrients in edible plant portions by means of genetic biofortification through plant breeding methods and targeted genetic manipulation.

**Plant breeding technique.** The method of biofortification that is most widely accepted is biofortification through conventional breeding. It is an effective and affordable alternative for agronomic and transgenic based techniques. The conventional breeding strategy involves choosing crop variety which is high yielding that naturally contain excessive proportion of desirable nutrients and cross-breeding those under traditional methods to develop crops with required nutritional and agronomic characteristics. Numerous international organisations have started breeding programmes to increase the nutritional value of crops.

Wheat is a staple food in majority of nations, biofortification research is focused mostly on this crop. Wheat and its closely related wild species have a wide range of grain iron and zinc contents that have been used to produce modern elite cultivars (Monasterio and Graham 2000). In 2014, six wheat varieties with high zinc content (BHU 1, 3, 5, 6, 7, and 18) were released in India using this variation. Maize is a valuable crop that is not just grown for human consumption but also a source of animal feed and used in industries. Breeding programmes have mostly focussed on the wide genetic variability of maize. Researchers have found wild relatives of maize with high amount of provitamin A content. Provitamin A maize is one of the most significant biofortification breakthroughs (Pixley *et al.*, 2013). Potatoesare the main source of antioxidants in human body. On breeding diploid potatoes containing high iron and zinc with disease-resistant tetraploid clones, the International Potato Centre (CIP) has developed a variety of potato that is resistant to disease and highin iron and zinc content (Haynes *et al.*, 2012). Sweet potato is known as food security crop but most varieties grown are high dry matter white-fleshed types, lacking beta-carotene. Orange-fleshed sweet potato (OFSP) is a biofortified verity of sweet potato developed by CGIAR researchers at (CIP) isrich plant-based source of beta-carotene (Low *et al.*, 2017).

**Table 2: Crops bio-fortified for improving nutrient status by means of plant breeding (Garg *et al.*, 2018).**

| Crop | Different types of biofortification | Status | Verity | Source |
|---|---|---|---|---|
| Rice | Zinc<br>Iron | Released | BRRIdhan- 62 and 72 BRRIdhan- 64 (Bangladesh) | CIAT, Harvest Plus |
| Wheat | Zinc and iron<br><br>Lutein<br>Anthocyanins (colored wheat) | Released<br><br>Research<br>Registered | WB2 (India)-<br>NABIMG-9, NABIMG-10 (India) | Indian Institute of Wheat and Barley Research<br>Digesu *et al.* (2009)<br>Garg *et al.* (2016) |
| Maize | Vitamin A | Released | GV-662A, 664A and 665A (Zambia) | CIMMYT and IITA |
| Cow Pea | Iron | Released | Pant Lobia-1 and 2 (India) | G.B. P. Agri. University |
| Potato | Fe, Zn, Cu and Mn | Research | - | Haynes *et al.* (2012) |
| Sweet potato | Vitamin A | Released | Kakamega and Ejumula (Uganda) | International Potato Centre (CIP), Low *et al.* (2017) |

**Transgenic means.** If there is only little or no genetic variability in case of nutrient composition of different crops, the transgenic technique could be an effective alternative for the production of biofortified crops (Zhu *et al.*, 2007). To transfer and express adesirable gene from one plant species to another, availability of the unlimited genetic pool has a part to play. Furthermore, Transgenic methods will remain the only possible way to fortify a crop when a specific micronutrient does not naturally exist in that crop (Perez *et al.*, 2013). A major factor for producing transgenic crops has been the ability to characterise and define gene function and using these genes to develop modified crops (Christoum and Twyman 2004). Transgenic approaches can not only be used for enhancing micronutrient content but also to reduce the amount of antinutrient factors that restrict the bioavailability of essential nutrients in plants. Additionally, genetic modifications might be directed to increase the concentration of micronutrients in the edible parts of commercial crops, redistribute micronutrients among tissues, increasing the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways (Agrawal *et al.*, 2005). On contrary to nutrition-based agronomic biofortification programmes, it requires a great deal of effort, time, and investment to develop transgenically biofortified crops at the onset of the research and development phase. On the other hand, it is an economical and sustainable long-term strategy (Meenakshi *et al.*, 2009; White and Broadley 2005).

Various crops have had genetic modification to increase the concentration of micronutrients in their edible parts. Golden Rice was a remarkable advancement in this area, beta-carotene, a precursor to provitamin A, was significantly increased by expressing the PSY and carotene desaturase genes and reduced disease burden (Beyer *et al.*, 2002; Datta *et al.*, 2003). By targeted gene encoding, phytoene, a precursor to beta-carotene, has been enhanced in rice up to 23-fold (Burkhardt *et al.*, 1997). Wheat phytase activity was boosted by the expression of the phytochrome gene to increase iron bioavailability (Brinch *et al.*, 2000). In amaranths albumin gene was used to increase the protein content of wheat grains, particularly the essential amino acids lysine, methionine, cysteine, and tyrosine. Kim *et al.* (2012) has showed that over expression of PSY and carotene desaturase in soybeans results in the production of high provitamin A (beta-carotene).Linseed oil is prone to auto-oxidation and may produce harmful by-products, on inhibiting the CHS gene, hydrolysable tannin will accumulate and the resulting genetically engineered flax plants have stable oil production and increased antioxidant capacity (Lorenc *et al.*, 2007). Sulfur-containing amino acids like cysteine and methionine are deficient in alfalfa, on expressing cystathionine y-synthase methionine content was enhanced (Avaram *et al.*, 2005). Dhara Mustard Hybrid-11 (DMH-11) might become India's first genetically modified (GM) food crop released for cultivation, developed by Centre for genetic manipulation of crop plants at Delhi university using three genes *viz.*, barstar, barnase and bar from *Bacillus amyloliquefaciens* which is responsible for tolerance to gluphosinate in plants (Kaushik, 2018).

**Table 3: Crops biofortified for improving nutrient status by transgenic means (Garg *et al.*, 2018).**

| Crop | Different types of biofortification | Status | Verity/ country | Source |
|---|---|---|---|---|
| Rice | Beta-carotene<br>Folate<br>Iron<br>Phytic acid<br>Flavonoids | Research | - | Ye *et al.* (2000)<br>Beyer *et al.* (2002)<br>Storozhenko *et al.*(2007)<br>Takahashi *et al.* (2001)<br>Lee and An(2009) |
| Wheat | Provitamin A<br>Iron<br>phytic acid<br>Anthocyanin | Research | - | Wang *et al.* (2014)<br>Borg *et al.* (2012)<br>Bhati *et al.* (2016)<br>Doshi *et al.* (2006) |
| Maize | Vitamin E<br>Lysine | Research | - | Cahoon *et al.* (2003)<br>Tang *et al.* (2013) |
| Soybean | Oleic acid | Released | G94-1, 16 and 19, (USA) | Dupon |
| Potato | Increased amylopectin and reduced amylose in starch | Released | Starch Potato (AM 04-1020) USA | BASF |
| Sweet potato | Beta-carotene<br>Antioxidants | Research | - | Kim *et al.* (2013)<br>Park *et al.* (2015) |
| Linseed/flax | Amino acids | Released | (FP967) Canada | University of Saskatchewan |
| Tomato | Ascorbate | Research | - | Haroldsen *et al.* (2010) |
| Indian Mustard | Gluphosinate tolerance | Released | Dhara Mustard Hybrid-11 (DMH-11) | Kaushik (2018) |

**Limitations of genetic strategies**

— Long-term biofortification through conventional breeding is clearly successful but the genetic diversity with respect to the micronutrient content in plant gene pool is very limited.

— Transgenic crops are not widely accepted by the people.

— Regulatory processes for the commercialization of transgenic crops are time consuming (Watanabe *et al.*, 2005)

- The transgenic technique requires more work to identify, modify and express a particular target gene and to understand its effects, mostly it has very poor success rate.

— Cost involved in developing a transgenic crop is very high when compared to other approaches however after its release cultivation of such verities will be more sustainable and economical.

## CONCLUSION

In this paper, we have discussed several applications, benefits, concepts, limitation and future prospects of biofortification in various crops for enhancing the bioavailability of minerals and vitamins in food products and thereby we could reduce health problems caused by malnutrition in developing and under developed countries. It has been accepted that biofortification is an effective and promising agricultural approach for boosting the nutritional status of undernourished people worldwide. Human malnutrition can be addressed with the help of biofortification techniques that include plant breeding, targeted genetic modification, and by using mineral fertilisers. In order to boost the amount and bioavailability of key mineral components in human diets, researches are being conducted worldwide for enrichment of nutrients in agricultural produce, particularly in case of staple cereal crops, millets, pulses and tuber crops. Future prospects for biofortified crops are quite promising since they have the capability to reduce malnutrition among billions of poor people, especially in developing nations.

## FUTURE SCOPE

- Expand research on prebiotics by bio-fortification of crops with prebiotics which have the potential to boost the gut associated immune defence, by improving and restoring the growth of good microbes in the gut since this prebiotic serve as the food source for probiotics.
- Studies has to be focused more on reducing the level of antinutritional compounds such as phytic acid in food produce which inhibit the absorption of minerals in the gut.
- In case of biofortified products, market orientation will play an important role in the future, either the biofortified crops must be incorporated into existing marketing chains or new market opportunities must be generated by linking producers and consumers through product and market development.
- Crops which are developed through biofortification should be tested for concentration of various heavy metals, in laboratory as in open-field trials, before releasing to the public.
- Development of mineral packed seeds that are rich in trace elements to resist against biotic and abiotic stresses including diseases and environmental stresses (Bouis, 2003).

• Nanotechnology in the field of agriculture is an emerging technique that could reduce accumulation of macronutrients in soil plant system and minimize the environmental impact, Applications of nano fertilizersis a preferable approach for agronomic biofortification due to their features, which include large surface area, enhanced penetrability, target delivery and controlled release of the required nutrient to the plant system.

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