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Different approaches for Biofortification: A review

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Abstract

Modern agriculture has been largely successful in meeting the energy needs of poor populations in developing countries. In the past 40 years, agricultural research has placed increased cereal production at its center. Recently, however, there has been a shift: agriculture must now not only produce more calories to reduce hunger, but also more nutrient-rich food to reduce hidden hunger. One in three people in the world suffer from hidden hunger, caused by a lack of minerals and vitamins in their diets, which leads to negative health consequences. Biofortification is a process of improvement of nutritional profile of plant-based foods through agronomic interventions, genetic engineering, and conventional plant breeding. Biofortification through agronomic approaches can be achieved by applying mineral fertilizers to the soil, foliar fertilization and soil inoculation with beneficial microorganisms. Biofortification through genetic engineering is an alternative approach when variation in the desired traits is not available naturally in the available germplasm, a specific micronutrient does not naturally exist in crops, and/or modifications cannot be achieved by conventional breeding.

Keywords: Biofortification, agronomic, genetic engineering, plant breeding, micro nutrients

Introduction

Biofortification is a process of increasing the density of vitamins and minerals in a crop through plant breeding, transgenic techniques, or agronomic practices. Biofortified staple crops, when consumed regularly, will generate measureable improvements in human health and nutrition. Humans require at least 49 nutrients to meet their metabolic needs and to be healthy (Grantham *et al.*, 1999) [18]. Some are required in large amounts such as carbohydrates, fats, proteins, vitamins, minerals such as N, K, P, Ca, Mg, S others such as Fe, Zn, Cu, I and Se, are required in trace amounts because higher concentrations can be harmful. Inadequate consumption will result in adverse metabolic disturbances leading to Sickness, Poor health, lower worker productivity, learning disabilities in children Increased morbidity, Increased mortality rates, High healthcare costs, Diminishing human potential, Diminishing national economy. FAO recommendation of nutrient intakes for males and females between ages of 25 and 50 years: Ultimately, these mineral elements enter the food chain through plants. The mineral elements most frequently lacking in human diets are Fe, Zn and I. It is estimated that, of the world’s 6 billion people, 60–80% are Fe deficient, 30% are Zn deficient, 30% are I deficient and about 15% are Se deficient (Frossard *et al.*, 2000).

The reasons for malnutrition are: Crop production in low mineral Phyto available areas and consumption of staple crops with inherently low tissue mineral concentration. These deficiencies are caused by diets characterized by high intakes of staple foods but low intakes of vegetables, fruits, and animal and fish products, which are rich sources of minerals. Traditional interventions to address mineral malnutrition have focused on supplementation, food fortification and dietary diversification. For various reasons, none of these have been universally successful. They require safe delivery systems, stable political policies, appropriate social infrastructures and continued investment. Recently, a complimentary solution to mineral malnutrition termed ‘bio-fortification’ has been proposed.

Bio-fortification has been defined as the process of increasing the bio-available concentrations of essential elements in edible portions of crop plants through agronomic intervention or genetic selection.

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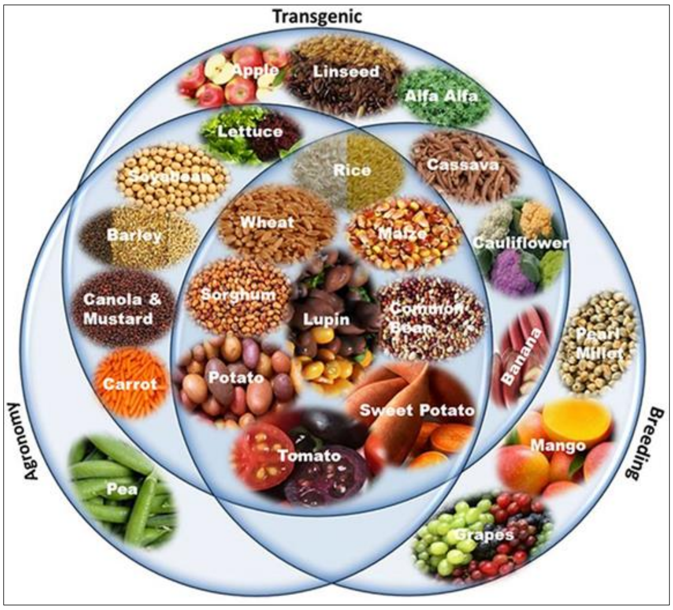

Fig 1: Show the transgenic agronomy and breeding

Biofortified crops generated by different approaches: Genetic engineering (transgenic), agronomic and breeding. Staple cereals, most common vegetables, beans, and fruits have been targeted by all three approaches. Some crops have been targeted by only one or two approaches depending on its significance and prevalence in the daily human diet.

Cereals have been biofortified in largest number by all three biofortification approaches. Legumes and vegetables have also been targeted by all the approaches in almost equal percentage. Transgenic approach covers highest number of crops. Oilseed crops have been mainly targeted by transgenic approaches due to limited genetic variability.

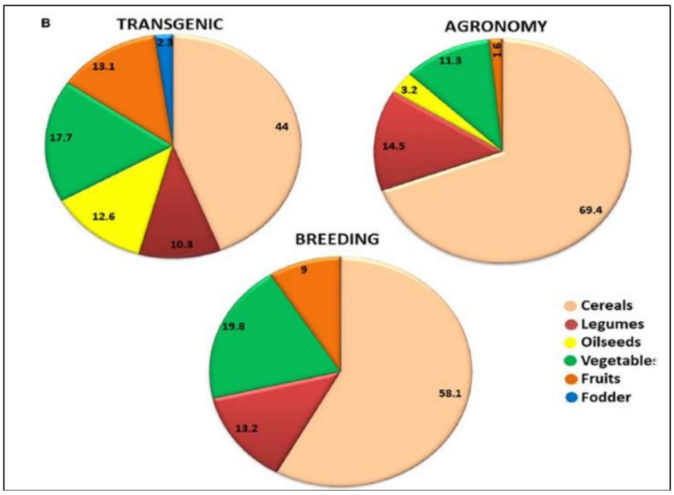

Fig 2: Percentage of different crops biofortified by different approaches

Biofortification through Agronomic approach

Given the limitations of conventional nutritional interventions, biofortification has been introduced an effective long-term approach for nutritional enhancement of crop plants (Zhu *et al.*, 2007). Breeding varieties with an increased ability to acquire mineral elements is being seriously attempted (Zhu *et al*., 2007; White and Broadley, 2009) [48]. On the other hand, biofortification of food crops can be achieved easily and rapidly through the application of mineral fertilizers (Prasad *et al.*, 2014) [36]. In reality, breeding and agronomic approaches can be complementary to each other. There are 4 major ways of achieving this, namely seed priming, seed coating, soil applications of micronutrient fertilizers and foliar application of fertilizers. The available information is briefly reviewed.

Seed priming

Seed priming is the practice of treating the seeds with micronutrients by soaking in nutrient solution of a specific concentration for a specific time or duration. Seed priming of chickpea seeds in a 0.05% solution of zinc sulphate heptahydrate ($ZnSO4.7H2\ O$) was found quite effective and on an average enhanced chickpea yield by 19% compared to non-primed seeds (Harris *et al.*, 2008) [19]. Additionally, seed priming also enhanced Zn concentration in chickpea seed by 29%, which is a fairly sizeable increase. Seed priming with zinc is helpful in improving crop emergence, stand establishment, plant growth, yield and nutrient concentration (IIPR, 2014–15) [23]. In moderately Zn deficient soils, zinc priming is an effective tool, whereas under severe deficiency it may not fulfil the zinc requirement of the plant. For example, seed priming alone of kidney bean is not sufficient to fulfill its requirement (Harris *et al.*, 2008) [19]

Seeds coating

Seed coatings with trace elements, *viz*. molybdenum, iron, zinc, manganese and boron, have been found more effective. In alkaline soil, application of iron, zinc and manganese have considerable significance, where availability of these elements is decreased. Further, molybdenum is commonly used in legumes with lime especially when sown in acid soils. Varieties of chelated and mineral forms of trace elements have been used in seed coatings. The effectiveness of seed coating depends largely on chemical used, soil type, soil health or fertility status, coating time, coating agent, ratio of chemical to seed etc. Application of zinc through seed coating improves zinc concentration in seeds (Singh, 2007; Masuthi *et al.*, 2009; IIPR, 2014–15) [40, 41, 23] besides improving seed emergence, plant growth and leaf area.

Among the major agronomic strategies highlighted as key solution to Zn and Fe deficiency, foliar-fertilization strategies may be considered more sustainable and economically viable strategies for micronutrient enrichment of the grains (Cakmak, 2008; Prasad *et al.*, 2014) [10, 36].

Mineral Fertilizer

Mineral fertilizers are inorganic substances containing essential minerals and can be applied to the soil to improve the micronutrient status of soil and thus plant quality. The phytoavailability of minerals in the soil is often low; thus, to improve the concentration of minerals in the edible plant tissues, the application of mineral fertilizers with improved solubility and mobility of the minerals is required (White, 2009) [48]. This method can be used to fortify plants with mineral elements, but not organic nutrients, such as vitamins, which are synthesized by the plant itself. This method was successfully implemented for Se, I, and Zn, as these elements had good mobility in the soil as well as in the plant. Plants were successfully enriched with I and Zn in China and Thailand using inorganic fertilizers, respectively (Winkler, 2011) [49]. However, Fe fertilization was not successful due to a low mobility of Fe in soil (Grusak, 1999) [18] The concentration of Zn was increased in field pea grains by either soil application of Zn fertilizer alone or combined with foliar treatments; thus, these methods could be potentially used for the biofortification of field peas (Poblaciones, 2016) [35]

Pulse crops were biofortified with micronutrients, Fe, Zn, and Se, through foliar application in various studies that resulted in increased levels of these micronutrients in the harvested grain. Márquez-Quiroz *et al*. reported increased concentration of Fe (29–32%) in seeds of cowpeas. Ali *et al*. reported increased Fe concentration (46%) in mungbeans upon foliar application of Fe. Similarly, foliar application of Fe and Zn significantly increased the concentration of these minerals along with protein in seeds of cowpeas (Salih, 2013) [37] and chickpeas (Nandan *et al.*, 2018) [31] Shivay *et al*. observed a correlation between Zn uptake and the grain yield of chickpeas following foliar application of Zn, and reported that this approach was better than soil application.

Soil application of micronutrient fertilizers

Soil application of zinc in chickpea also had significant variations among genotypes. Among chickpea genotypes the order of grain zinc concentration was 'Pusa 372' > 'Pusa 2024' > 'Pusa 5028' during the first year, while it was differed in the second year being 'Pusa 5028' > 'Pusa 372' > 'Pusa 2024'. These findings indicate that genotypic variations as well as environmental factors govern the concentration and uptake of zinc by the grain and straw (Shivay *et al*., 2014b; Shivay *et al*., 2014c) [36, 39]. The range of variation in Zn concentration in chickpea grain 36.0 to 44.9 mg/kg and in straw 29.5 to 43.3 mg/kg. Each successive level (2.5 kg/ha) of Zn application significantly increased Zn concentration in chickpea grain and straw and the highest Zn concentration of 50.1 mg Zn/kg grain was obtained at 7.5 kg Zn/ha. Likewise, each successive level (2.5 kg Zn/ha) of Zn application increased Zn concentration in chickpea straw up to 7.5 kg Zn/ha. The Zn concentration in chickpea grain increased from 33.6–38.6 mg/kg in no-Zn (check) to 45.9–48.4 mg/kg with the application of 7.5 kg Zn/ha over the years (Shivay *et al*., 2014b; Shivay *et al.,* 2014c) [36, 39].

Foliar application of micronutrients and urea

The practice of applying easily-soluble inorganic fertilizers directly to the leaves of the crop plants is more effective under the situations where mineral elements becomes unavailable to the plant immediately after application during later stage of growth. Likewise, foliar fertilization is more practical and effective under the conditions where mineral elements are not readily translocated to edible tissues. Therefore, foliar fertilization is the easiest and fastest way of biofortification of pulses grains with Fe, Zn, or other desirable micro-mineral nutrients. As majority of pulses are grown under rainfed/ dryland conditions wherein foliar fertilization has a greater advantage.

Ferrandon and Chamel (1988) [14] found that applying Zn, Fe and Mn either in chelated or sulphate salt form are extensively fixed by the leaf cuticle. They also reported reduced absorption of Zn, Fe and Mn in chelated form compared with inorganic salt. However, the translocation within the plant system was high when applied in chelated form. It is well established that upward transport of the nutrients may take place in either phloem or xylem, whereas export of plant nutrients from leaves and downward transport in the stem may take place solely in phloem. The transport of nutrients (*e.g.* zinc) from source (leaves) to the sink (grain) also not takes place uniformly, it occurs at variable rate (Pearson and Rengel, 1995) [35]. The effectiveness of the foliar fertilization is also governed by the factors, *viz*. concentration of solution, timing of spray, stages of crop development, form of fertilizers etc. Foliar fertilization with Zn-EDTA, Fe-EDTA and other chelates has been used in majority of cereals and pulses. Chelates are more effective than sulphate salt. Three sprays of ZnEDTA 0.5% solution spray @ 500 litres/ha at maximum vegetative growth + flowering + grain-filling stages was better than ZnSO4.7H2 O in increasing the productivity and Zn concentration in grain as well as straw of chickpea (Shivay *et al.*, 2014a) [39]. The effectiveness of the form of fertilizer is also affected by the stage of crop development and timing of spray. Foliar fertilization with urea is reported to improve yield attributes, yield and chlorophyll content of chickpea. The physiological traits of chickpea, *viz*. NRA, RWC and chlorophyll content, in leaves attained higher values with 1% urea spray (Verma *et al.*, 2009) [43].

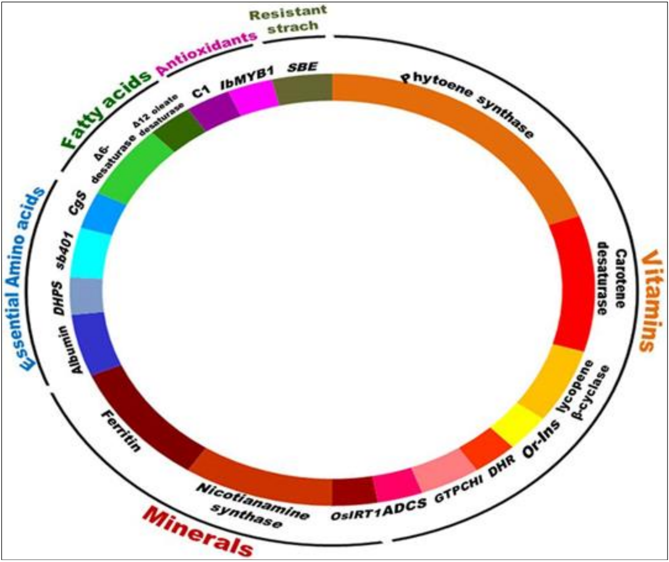

Fig 3: Biofortification through genetic engineering

Biofortification through genetic engineering is an alternative approach when variation in the desired traits is not available naturally in the available germplasm, a specific micronutrient does not naturally exist in crops, and/or modifications cannot be achieved by conventional breeding (Mayer *et al.,* 2008; Perez-Massot *et al*., 2013) [30, 34]. This approach was supported by the availability of fully sequenced genomes in various crops in recent years. Along with increasing the concentration of micronutrients, this approach can also be targeted simultaneously for removal of antinutrients or inclusion of promoters that can enhance the bioavailability of micronutrients (White, 2009; Garg, 2018; Carvalho, 2013) [48, 15, 11]. This approach had not only utilized genes associated with various metabolic pathways operated in plants, but also from bacteria and other organisms (Christou, 2004 and Newell-McGloughlin, 2008) [12, 33]. Development of transgenic crops requires a substantial investment during the initial stage, but this could be a sustainable approach that has the potential to target large populations, especially in developing countries (White, 2005; Hirschi, 2009) [21]. Several crops have been successfully modified using a transgenic approach to overcome a micronutrient deficiency. For example, enhanced accumulation (3 to 4 times) of Fe was noted in rice via expression of the iron-storage protein, ferritin (Goto *et al.*, 2000 and Vasconcelos, 2003) [16, 42]

Recently, transgenic multivitamin corn was produced by the simultaneous modification of three distinct metabolic pathways to increase the levels of three vitamins, i.e., -carotene (169-fold), ascorbate (6-fold), and folate (2-fold), in the endosperm, and this could pave the way to develop nutritionally complete cereals (Naqvi, 2009) [32]. Using metabolic engineering, the folate concentration was increased in tomato and rice (Blancquaert, 2013, 2014) [3, 2]. Storozhenko *et al*. (2007) [3] reported more than 100-fold increase in folate concentration in rice by overexpression of Arabidopsis thaliana pterin and para-aminobenzoate genes, precursors of the folate biosynthesis pathway, whereas Hossain *et al.* reported a two- to four-fold increase in Arabidopsis by overexpression of the gene involved in pterin biosynthesis.

In recent years, targeted gene editing technologies using artificial nucleases, zinc finger nucleases (ZFNs), transcription activator – like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system (CRISPR/Cas9) have given rise to the possibility to precisely modify genes of interest, and thus have potential application for crop improvement (Bortesi, 2015 and Jaganathan, 2018) [5, 25]. These technologies have been used in various crops including rice (Li, 2012; Zhang, 2014) wheat (Wang, 2014) [25] and tomatoes (Brooks, 2014). Recently, CRISPR/Cas9 and TALENs technologies were used to generate mutant lines for genes involved in small RNA processing of Glycine max and Medicago truncatula. Similarly, CRISPR/Cas9-mediated genome editing technology was used in cowpeas to successfully disrupt symbiotic nitrogen fixation (SNF) gene activation (Ji *et al.*, 2019) [26] These findings pave the way for applicability of use of gene editing technologies for various traits of interest in legumes.

Biofortification through Plant breeding

This can be an effective approach for crop improvement; however, political opposition to GMOs in many countries, a complex legal framework for the acceptance and commercialization of transgenic crops, along with expensive and time-consuming regulatory processes are the major limitations of this method (Winkler 2011; Inaba 2004 and Watanabe *et al.*, 2005) [49, 24, 45] For example, golden rice has been available since the early 2000s and has the potential to deliver more than 50 per cent of the estimated average requirement for vitamin A, but unfortunately it has not been commercially introduced in any country to date due to risk factors involved in the regulatory approval processes (Wesseler 2014; Bouis and Saltzman, 2017) [47, 7]. Restrictions on the use of genetically modified crops in many countries prompted Harvest Plus to take the initiative to address micronutrient deficiencies through conventional plant breeding (Nestel *et al.*, 2006 and Saltzman *et al.*, 2013) Biofortification through plant breeding is a cost-effective and sustainable approach that can improve the health status of low-income people globally (Bouis, 2002; Bouis 2011: Blancquaert *et al*., 2014) [8, 6]. This approach has been used to control deficiencies of micronutrients including carotenoids, Fe, and Zn (White and Broadley, 2005; Welch *et al.*, 2005) [45]

Conclusion

Awareness of dietary diversity must be followed up to alleviate micronutrient malnutrition. As people of under developed nations cannot afford to supplemented and diversified foods, research and development of nutrient enriched biofortified crops should carried out to face this problem. There are several aspects of biofortification but agronomic aspect (Ferti-fortification) is simpler one and is mostly followed.

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