Biofortification of Pulses: Strategies and Challenges

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Abstract

Pulses are the prime source of dietary proteins of the vegetarian people inhabiting mostly in the developing countries. In general, pulses provides around 18-28% protein and one third of all dietary protein nitrogen. Besides dietary proteins, these also offer many minerals required essentially by the human beings. The production of pulses in the country over the years especially during the last decade has tremendously improved as a record pulses production of 18.34 million tonnes was realized during 2012-13. For 2013-14, the 4thadvance estimates of DAC have predicted the production to touch 19.27 million tonnes-a significant milestone in the pulses scenario. Biofortification, the process of integrating nutrients into food crops, provides a sustainable and economic way of increasing the density of minerals or micronutrients in important staple crops. This approach will help to control the volume of malnourished people worldwide. Moreover, biofortification presents an easily accessible means especially concerned to the malnourished population in rural areas who normally have either no or very poor accessibility to market places. Therefore, biofortification strategy aims to incorporate the nutrient accumulation and related plant attributes in those commercially accepted and superior cultivars that are already in food chain due to their good agronomic performances, primarily the seed yield.

Keywords: **B**iofortification, hidden hunger, nutrients, pulsed

1. Introduction

Pulses are the prime source of dietary proteins of the vegetarian people inhabiting mostly in the developing countries. In general, pulses provides around 18−28% protein and one third of all dietary protein nitrogen. Besides dietary proteins, these also offer many minerals required essentially by the human beings.The major pulses grown in India includes chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), mungbean (*Vigna radiata*), urdbean (*Vigna mungo*), cowpea (*V.unguiculata*), lentil (*Lens culinaris* ssp. *Culinaris*), lathyrus (*Lathyrus sativus* L.), French bean (*Phaseolus vulgaris*), horse gram (*Macrotyloma uniflorum*), field pea (*Pisum sativum*) and moth bean (*V. aconitifolium*). Amongst these, the most important pulse crops grown on acreage basis comprises of chickpea (48%), pigeonpea (15%), mungbean (7%), urdbean (7%), lentil (5%) and field pea (5%). The production of pulses in the country over the years especially during the last decade has tremendously improved as a record pulses production of 18.34 million tonnes was realized during 2012−13. For 2013-14, the 4thadvance estimates of DAC have predicted the production to touch 19.27mt -a significant milestone in the pulses scenario.

Biofortification, the process of integrating nutrients into food crops, provides a sustainable and economic way of increasing the density of minerals or micronutrients in important staple crops. This approach will help to control the volume of malnourished people worldwide. Moreover, biofortification presents an easily accessible means especially concerned to the malnourished population in rural areas who normally have either no or very poor accessibility to market places. Therefore, biofortification strategy aims to incorporate the nutrient accumulation and related plant attributes in those commercially accepted and superior cultivars that are already in food chain due to their good agronomic performances, primarily the seed yield. Marketed surpluses of these crops may make their way into retail outlets, reaching consumers in first rural and then urban areas, in contrast to complementary interventions, such as fortification and supplementation, that begin in urban centres (UNSCN, 2004). Although biofortified food developed from crop biofortication may not be able to supply the level of minerals or vitamins per day as is usually achieved through supplements or fortified foods, yet these can indeed facilitate increasing the daily adequacy of micronutrient intakes among resources-poor individuals (Bouis et al., 2011).

2. Managing the Problem of Malnutrition Worldwide

The term 'hidden hunger' describes the acute deficiency of micronutrient elements inherent in human diets that are adequate in terms of energy; however lack vitamins and/or mineral elements (Pfeiffer and McClafferty, 2007). The diets of a large proportion of the world's population is deficient in a broad range of mineral elements including Fe, Zn, Ca, Mg, Cu, Se or I, which ultimately affect human health and longevity. The implications of malnutrition have also been evident in national economies (Gomez-Galera et al., 2010). Variety of ways can be used to tackle the mineral malnutrition like increasing the amount of aquatic and animal products in diets, mineral supplementation, food fortification and/or increasing the bioavailability of mineral elements in edible crops. For however, biofortification of staple crops that constitutes the predominant portion of the human diets is the most appropriate approach, and the biofortification of food crops can be achieved through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, has been advocated (White and Broadley, 2009).

Given the limitations of conventional interventions, biofortification has been introduced an effective long-term approach for nutritional enhancement of crop plants (Zhu et al., 2007). Biofortification focuses on enhancing the mineral nutritional qualities through enhancing both mineral levels and their bioavailability in the edible part of staple crops. The various methods that aim to biofortifying crops include agronomic intervention, breeding practices or genetic modification and microbiological changes. Most importantly, only plant breeding and genetic engineering can influence mineral bioavailability. Plant breeding harnesses the natural genetic variation, whereas genetic engineering relies on transferring a gene that does not reside within the crop gene pool.

Nutritional enrichment of pulse crops could be accomplished by several ways. Some of these potential ways are outlined here:

2.1. Agronomic interventions

2.2. Breeding approaches

2.3. Genetic modification

2.4. Microbiological approaches

2.1. Agronomic interventions

Applying mineral fertilizers to the soil for maintaining soil health and improving plant quality is age old practice for hundreds of years, but within certain limits the same strategy can also be used to increase mineral accumulation within grains for nutritional purposes (Rengel et al., 1999). The response to the applied nutrient in the grain reflected more pronounced when deficiency of that particular element is there in the soil or the element is mobilizes faster and easily. Also, even if plants can absorb minerals efficiently from the soil, they may store the mineral in leaves but not fruits or seeds, or they may accumulate the mineral in a form that is not bioavailable, thus having no impact on nutrition (Frossard et al., 2000).

The agronomic strategies like, improving solubilisation and mobilization of the elements in the soil helps in enhancing concentration of mineral elements in the edible part of the plant or grain. (White and Broadley, 2009). The practice of applying easily soluble inorganic fertilizers directly to the roots or leaves or to the crops is more effective under the situations where mineral elements becomes unavailable to the plant immediately after application. Likewise, foliar fertilization is more practical and effective under the conditions where mineral elements are not readily translocated to edible tissues. Therefore, agronomic biofortification is the easiest and fastest way for biofortification of pulse grains with Fe, Zn, or other desirable micro mineral nutrients. In the changing global economy the purchasing power of the rural poor is getting low, because of this they may not afford expensive mineral supplements and animal products. Agronomic biofortification is the only way to reach the poorest of the poor rural masses to enhance the composition of desired nutrient in their diet. Hence, the role of agronomic biofortification in solving hidden hunger or micronutrient malnutrition is tremendous.

From the standpoint of application of biofortification agronomically, foliar application is cheap, efficient as it requires lesser amount of Fe and Zn fertilizers than their soil application. In addition, when cultivars or GM crops with grains denser in Fe and Zn are developed, adequate Fe and Zn fertilization will be necessary. The genetic and agronomic approaches are therefore, complementary to each other and should progress in tandem. The possible agronomic interventions include the following approaches.

2.1.1. Foliar fertilization

2.1.2. Seed priming

2.1.3. Soil application of fertilizers

2.1.4. Seed coating

Amongst these, the foliar fertilization is the most common and handy in changing the micronutrient status of edible plant parts to an appreciable level. Soluble fertilizers of N, P, K, S, Zn and Fe can be dissolved in potable water or pesticides for *in situ* application onto green leaves or plant parts of crops especially at fruiting or seed formation. Sometimes spraying of foliar fertilizers along with lime as a neutralizing agent is useful for rapid or higher translocation and accumulation. To the contrary, in soil applied fertilization, the fertilizers or such other fortifying materials are added to soil for subsequent absorption by plants roots and translocation in tissues within the plants. Here the fortification inputs are subjected to various losses in soil system. Seed priming is an act of priming the viable seed material or propagules with a solvent or plain water to imbibe and absorb which later disable the seed against incipient deficiency and make use of micronutrient nutrition effectively and efficiently. Seed coating is a treatment accommodating micronutrient(s) and such other materials with seed embryo proceeding for germination of seed and later growth and development of plants later. Here initial boost will enable the seed to fight against hidden hunger and act like that of vaccination in animals. Besides these there are some uncommon agronomic fortification approaches benefitting both plant and seeds such as injecting micronutrients into plant tissues, use of nanoparticles and other micromolecules impregnated with nutrients, other novel methods involving biofortification of final or finished products through processing or value addition.

2.2. Breeding approaches

Wide range of natural genetic variation is the main driving force in nutritional enhancement of pulse crops though crop breeding (Welch and Graham, 2005). Breeding approaches include surveying the vast germplasm for nutrient variation, estimating the impact of interactions of genotype with the diverse external environmental conditions and searching the possibilities of increasing the content or concentration of bioavailable nutrient in the edible part. In terms of sustainability, the nutrional breeding of crop plants has several advantages. However, breeding, by and large, relies on long and repetitive cycle of hybridization and selection, thereby are time-consuming and labour-intensive. Nevertheless, in recent years, modern molecular tools like DNA markers and marker assisted selection (MAS) schemes have become available to expedite the development of nutrient-rich genotypes, howeversimultaneously attention also needs to be directed towardsother factors like soil properties (e.g. pH, organic composition) which often interfere with mineral uptake and accumulation (Cakmak, 2008).

The possible breeding strategies include:

2.2.1. Conventional plant breeding approaches

2.2.2. Mutation breeding

2.2.3. Molecular breeding or marker assisted breeding

2.3. Genetic modification

The successful application of transgenic technologies to enhance the nutritional value of crops has been demonstrated in several crops like, that of golden rice. Transgenic technology facilitates incorporation of novel genetic variation or foreign gene into the candidate crop from the outside of its entire gene pool *i.e.* plants can be tailored to *green factories* for the synthesis of desired compounds. However, implementation of this approach relies upon the manner the nutritional compound is synthesized viz., *de novo* (by the plant itself) or accessed from the outside the plant body. Metabolic engineeringmay be required to modulate the organic molecules (amino acids, fatty acids and vitamins) which are manufactured by the plant and increasing the nutritional value requires some form of with the aim of increasing the amount of these desirable compounds (Ye et al., 2000). By contrast, strategies need to be in place that managesnutrient uptake, transport and/or accumulation in edible tissues which are generally received by the plant from outside. The following sections highlights reports of transgenic approaches utilized to enhance the nutrient content of crops.

Tissue culture techniques enable regeneration of entire plant from a single cell. These techniques are now used extensively to produce disease-free planting material of clonally propagated crops such as bananas (Ducreux et al., 2005). With the availability of efficient protocols, tissue culture facilitate utilization of the crop wild relative in routine breeding program which are otherwise not crossable with the cultivated types. The incorporation of wild relatives in breeding program in turn helps in expanding the genetic base of a particular crop. These techniques are particularly relevant to the pulse crops as these crops suffer from the presence of a very narrow genetic base which can also be credited to the domestication bottlenecks or syndromes and the breeding history of these crops. Tissue culture stands to be crucial to the improvement of root and tuber crops.

2.4. Microbiological approaches

Plant Growth Promoting Rhizobacteria (PGPR) include beneficial bacteria that colonize plant roots and augment plant growth by ample variety of mechanisms. Application of PGPR in the soil has multifaceted advantages which reduces the use of fertilizers and other agrochemicals in agriculture (Rana et al., 2012). Uptake of iron, zinc and other micronutrients by the plant roots is improved due to secretion of phytosiderophores by microorganisms present in the soil rhizosphere. Microorganisms present in the soil are also involved in the mechanisms like transformation and sequestration of acids and alkalis. Among bacteria, siderophore-mediated iron uptake by fluorescent pseudomonads is getting attractions. PGPR constitutes a significant part of the protective flora that benefit plants by enhancing root function, suppressing disease and accelerating growth and development (Glick, 1995). The competition for micronutrients also varied with the plant species and microorganisms present in the soil. Species of *Azotobacter* differed in their competitiveness with crops in extracting Fe and Zn from the soil (Shivay et al., 2010). Biofortification of pulse crops through application of PGPRs can be therefore considered as a possible supplementary technique.

All pulses are associated with mycorrhizal fungi that improves uptake of nutrients from the soil. Thereby, concentration of mineral elements is improved in the grain. However, role of mycorrhizas on element biofortification may be piloted through improved agricultural practices. Mycorrhizas can potentially offer a more effective and sustainable element biofortification to curb global human malnutrition (Wang and Qiu, 2006). In soil AM is the most important mycorrhiza and closely relates to human nutrition. The widespread AM mycelia explore soil substrates and acquire soil inorganic elements including major (N, P, K) and micro-nutrients (Fe, Zn, Cu) efficiently and effectively (Caris et al., 1998; Koide and Kabir, 2000).

3. Challenges in Biofortification

3.1. Antinutrients

The absorption of Fe, Zn and Ca by the gut is hindered by some limiting factors like phytate and tannins. Phytate concentration is more pronounced in seed or grain part of the plant. Phytate concentration in edible portions of the plant is varied intra-specifically (Glahn et al., 2002; Coelho et al., 2005) independent of differences in Fe and Zn concentrations. Non-transgenic techniques helped in developing low phytic acid (LPA) mutants (Banziger and Long, 2000). Unexpectedly, plants with LPA mutations often show higher levels of grain Fe, Zn and Mg (or similar levels to those in found in wild type), although they do have reduced concentrations of seed Ca. Tannin concentration in edible tissues also varies greatly between varieties (Lin et al., 2005). Hence, breeding for reduced concentrations of these antinutrients appears feasible.

3.2. Other future challenges

➢ Consumer preference-due to colour changes (e.g. Golden Rice) biofortified crops may not be preferred by the consumers.

➢ Production of crops for human nutrition with increased iron concentration. Detailed knowledge on mechanisms regulating iron compartmentalisation in various plant organs will offer a major contribution for reaching such goal.

➢ Extending research on prebiotics and micronutrient absorption.

➢ Promoting large-scale prospective studies on assessing the effects of nutrient enhancement in major crops in relieving malnutrition and other associated health problems

➢ Improving the efficiency with which minerals are mobilized in the soil

➢ Enhancing the mineral uptake efficiency of the important crops

➢ Expanding the understanding of mineral accumulation and the transport within the plant body

4. Conclusion

Biofortification have multiple advantages that may complement other ways and techniques of improving micro-nutrient nutrition in food crops including pulses. Evidences emerges that biofortified pulses have the potential to nourish malnourished population. Biofortified pulses are having huge potential to combat hidden hunger as the edible portions are denser in bioavailable, micro-nutrient, minerals and vitamins. Thus, biofortification will emerge as an agricultural based cheaper strategy in mitigating nutritional needs of malnourished population throughout the world.

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