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Engineered Biofortification: A Sustainable Solution to Malnutrition

Muhammad Sarwar Khan*, Rimsha Riaz and Saher Qadeer

Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Pakistan

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***Corresponding author:** Muhammad Sarwar Khan, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan

Abstract

Vitamins and minerals are essential nutrients that are required for normal functioning and growth of the body. Plants are a major source of these nutrients. Grains are consumed as staple food in developing countries, and these are lacking in essential micronutrients, leading to health problems. To address such inadequacies, biofortification using transgenic approaches has long been on the wish-list of biotechnologists whereby essential micronutrients are incorporated into plants. In addition to adding nutritional elements in crops, transgenic technology has ensured the bioavailability of these micronutrients. This review summarizes the current status of nutritional deficiencies, nutritional deficiencies disorders and strategies for the biofortification of cereals, and briefly describes how novel approaches facilitate bioavailability of these micronutrients in food.

Keywords: Biofortification; Essential micronutrients; Minerals; Vitamins; Transgenic technology

Abbreviations: MNM: Micronutrient Malnutrition; DALYs: Disability-Adjusted Life Years; YLD: Years Lived With Disability; YLL: Years of Life Lost; TALENs: Transcription Activator-Like Effector Nucleases; CRISPR : Clustered Regularly-Interspaced Short Palindromic Repeats; LCY-B: Lycopene β cyclase; CHY: β-Carotene Hydroxylase; CRTI: Phytoene Desaturase; CRTW: β- Carotene Ketolase; GMO: Genetically Modified Organism

Global Status of Hidden Hunger

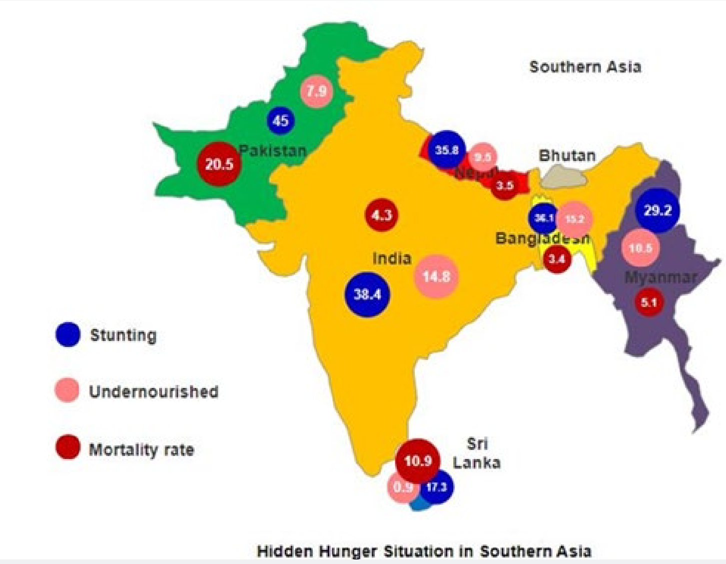

Figure 1: Map of the hidden hunger index across South-Asian countries [7].

Micronutrients vitamins and minerals - though required by the body in very small amounts, are vital for development, disease prevention, and general well-being. Micronutrient Malnutrition (MNM) is causing serious health issues, including increased mortality, a defective immune system, anemia, frailty, retarded mental and physical growth [1]. Lack of vitamin A, iron, zinc and iodine are prevalent nutrient deficiencies which together with various micronutrient deficiencies account for 7% of global disease burden annually [2,3]. Two billion people are reported to suffer from hidden hunger across the planet. This proportion is increasing with the rise in the world's population, posing a negative impact on economies and people's health status. About 88% of countries face a severe problem of multiple forms of malnutrition and the world is striving to fulfill global nutritional challenges [4]. According to a survey, low iodine intake among school children was 43.9% in Europe and 39.3% in Africa. This percentage is elevated in Southeast Asia [5]. About 32.8% women between the ages of 15 and 49 were afflicted with iron deficiency in 2017, increasing from 31.6% in 2000 [6,7]. Currently, hidden hunger is predominant in low and middle-income countries of Africa and South Asia (Figure 1).

MNM has been well addressed in developed countries through dietary diversification and food supplementation but, in developing countries, these strategies are often expensive and difficult to sustain, especially in rural areas. Cereals are naturally deficient in micronutrients, that are further exaggerated during industrial processing, storage and cooking [8,9]. Consumption of only a few staple crops is a prime source of Micronutrient Malnutrition (MNM). Therefore, the biofortification of staple crops using modern technologies is the most cost-effective and sustainable solution to this global health problem.

Table 1: Biofortified cereal crops acquired by transgenic means.

Nutrient	RDA (Male & Female)	Target Crops	Introduced Genes	Efficiency	References
Iron	8mg & 18 mg	Rice	OsGluB1 promoter+SoyferH1	2-4 times (brown &polished seeds)	[11-13]
			OsGlbpromoter+Pvferritin	>6 times (polished seeds)	[14]
			35S promoter+AtNAS1		
			OsGlb promoter+Afphytase		
			OsGluB1 promoter+SoyferH2	3 times (polished)	[15]
			OsGlb1 promoter+SoyferH2		
			OsGluB1 promoter+SoyferH2	6 times (greenhouse- polished seeds)	
			OsGlb1 promoter+SoyferH2		[16]
			OsActin1 promoter+HvNAS1		
			OsSUT1 promoter+OsYSL2	4.4 times (paddy field-polished seeds)	
			OsGlb1 promoter+OsYSL2		
			OsGluB1 promoter+SoyferH2	4 times (greenhouse-polished seeds)	
			OsGlb1 promoter+SoyferH2		[17]
			HvNAS1, HvNAAT-A,-B	2.5 times (calcareous soil-polished seeds)	
			and IDS3 genome fragments		
			OsGluB1 promoter+SoyferH2		
			OsGlb1 promoter+SoyferH2		
			OsActin1 promoter+HvNAS1	>3 times (polished seeds)	[18]
			OsSUT1 promoter+OsYSL2		
			OsGlb1 promoter+OsYSL2		
		Banana	Soybean Ferritin cDNA	6.32 times (leaves)	[19]
		Cassava	CrFEA1 (Chlamydomonas reinhardtii)	10-36 ppm (roots)	[20]
			Patatin promoter+AtVIT1	3-4 times (roots)	[21]
		Wheat	HMW-GS 1Dx5 promoter+ Soybean ferritin	1.5 times (seeds)	[22]
			OsNAS2	2 times (seeds), 2.5 times (flour)	[23]
			TaFerritin1-A	50-85% increase (flour)	[24]
		Lettuce	GmFerritin	1.5 times (leaves)	[25]
		Maize	Soybean ferritin+PhyA (Aspergillus niger)	20-70% increase (seeds)	[26]

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		Rice	Phytoene synthase, Lycopene cyclase (Daffodil) & Carotene desaturase (E. Uredovora)		[27]
			Phytoene synthase (Maize) & Carotene desaturase (E. Uredovora)	23 times	[28]
Vitamin A	900µg & 700 µg retinol activity equivalents (RAE)/day	Wheat	Bacterial carotenoid biosynthetic genes (CrtB, CrtI)	65 times	[29]
			Phytoene synthase and Carotene desaturase	10.8 folds	[30]
		Maize	Overexpression of crtB and crtI under super γ-zein promoter	34 folds	[31]
			Wheat glutenin promoter- Zmpsy1+ barley hordein promoter- PacrtI	36.73%	[32]
		Soybean	Phytoene synthase gene (crtB)	1500 folds (Seeds)	[33]
			Phytoene synthase and Ketolase genes (crtB + crtW, crtB + bkt1)		[34]
			β-conglycinin (β) promoter + PAC (Phytoene synthase-2ACarotene desaturase)	175 folds	[35]
		Sorghum	Phytoene synthase (PSY-1), Carotene desaturase (CRT-I), Phosphomanose isomerase (PMI), Low Phytic acid (LPA-1)	4-8 folds	[36]
		Potato	GBSS Promoter + cauliflower or gene		[37]
			Over expression of StLCYb gene	1.5-1.9 times	[38]
		Cassava	crtB and DXS gene		[39]
		Flaxseeds	35S Promoter + Phytoene synthase (crtB)	7.8-18.6%	[40]
		Canola	Overexpression of Phytoene synthase (crtB)	50 folds	[41]
			Geranylgeranyl diphosphate synthase (crtE),		[42]
			phytoene desaturase (crtI)		
			and lycopene cyclase (crtY		
			and the plant B. napus		
			lycopene β-cyclase) + crtB		

Folate	400µg/day	Rice	GTP-cyclohydrolase-I	100 times	[43]
			Aminodeoxychorismate synthase (Arabidopsis thaliana)	150 folds	[44]
		Tomato	GTP cyclohydrolase I (GCHI)	2-3 folds	[45]
			Over expression of Arabidopsis aminodeoxychorismate synthase (AtADCS)	Up to 15 folds	[46]
Zinc	11mg, 8mg	Barley	Arabidopsis zinc transporter	1.5-3 times	[47]
		Rice	HvNAAT-B or IDS3	1.35 times	[48]

Current Trends in Biofortification

The premise behind biofortification is a well-known axiom 'health comes from the farm, not the pharmacy'. Biofortification is an important practice, employing advanced genetic techniques to express additional amounts of nutrients that are otherwise scarce. Special emphasis is given to inorganic nutrients and vitamins such as iron, zinc and vitamin A [10]. Confronting undernutrition, biofortification is the first agriculture-based concept involved in enhancing vitamin and mineral content of food crops using various, either plant breeding, or transgenic techniques. Cereal crops, including rice, maize, wheat, and sorghum, are the promising targets to vanquish malnutrition in countries where these are the staple crops (Table 1). A biofortified crop must fulfill three criteria: first; it must be lucrative for farmers, second; it must ensure an effective bioavailability of nutrients, and third, it must be amenable to consumers in afflicted communities [11-20]. Traditional methods that were once applied to supplement inorganic nutrient availability show limited improvement. Such methods included fertilizing crops via irrigation or foliar sprays to increase the inorganic nutrients (mostly zinc, selenium, and iodine) contained in the plant [21-30]. Breeding crops by conventional means produces a breeding line with higher intrinsic nutrient content, an alternative to the aforementioned technique. Various strategies involving exotic germplasm integration, heterosis breeding, genotype-environment interaction, and mutation breeding are used for the biofortification of grains via genetic refinement [31]. However, loss of hybrid vigor, reduced expression of desired traits, extremely long timespan and species-barriers are the main hurdles of conventional breeding methods [32,33]. Taxonomic constraints are not a problem for genetic engineering, as heritable traits can be transferred among diverse species through transgenic techniques. A valuable biofortified crop can be engineered using transgenic techniques to incorporate multiple genes that directly encode desired nutrients or culminate the expression of certain factors that enhance uptake efficiency or decrease anti-nutrient factors. Construction of synthetic genes, efficient regeneration methods, and transformation techniques are all involved in developing a valuable transgenic crop [34]. Recently biotechnology based on the genome-editing principle has provided new insight into improving the nutrient content of plants. These include TALENs and CRISPR/Cas9 which allow specific additions, modifications, and mutations in plants and other eukaryotic systems [35,36]. These genome editing tools can develop such biofortified crops that, in theory, will not be subjected to stringent regulation and easily accepted by the consumers who are uncomfortable with the idea of GMOs [37]. These techniques have already experimented in rice, *Arabidopsis*, tobacco, maize, barley yielding satisfactory results [38-39]. As far as genetic engineering is concerned, there is a dire need to broaden our understanding of endogenous metabolic pathways pivotal for biofortification, accompanied by maintaining natural reservoirs, resolving biosafety issues and intellectual property rights [40]. High provitamin A and iron-rich cassava, high provitamin A Golden rice, high unsaturated fatty acid soybean, and high lysine maize are some nutrient-enriched crops to combat against malnutrition [41].

Factors Affecting Nutrient Bioavailability

Increasing the nutrient concentration in crops is the first step in producing nutrient-rich food. Staple crops (especially cereals) are deficient in micronutrients and also contain anti-nutrient factors that further lower the bioavailability of dietary iron, zinc, carotenoids and other key micronutrients [42,43]. While developing biofortified crops, factors like chemical and physical properties of the crops, food processing, food matrix, gut microbes, environmental factors [44] or even dietary practices, which could potentially end up affecting the bioavailability of micronutrients, should be considered. Food is processed not only for food safety but also for a better taste. Cooking, drying and fermentation are methods generally used in food processing, but food nutrient content is decreased in all such preparation activities, for example, vitamin B_9, thiamine, and vitamin C are more affected during processing than vitamin K, D, B_3, B_5 and B_7. Milling and dehusking of the cereals reduces vitamin B derivatives, minerals, and dietary fibers while blanching and dehydration are responsible for leaching out of water-soluble vitamins such as vitamin C and B complex. Freezing, pasteurization and high-pressure processing maintain the nutritive quality of food, but nutritional value of vegetables is reduced by excessive peeling as vitamins are concentrated close to the periphery [45]. The artificial addition of nutrients at the cost of their natural, intrinsic content is, therefore, not a beneficial trade-off, because the artificial nutrient addition is very limited as compared to the loss of minerals during the processing of food. Hence, the healthiest state of food is indeed the one closest to its natural one [46]. Contrarily, bioavailability of nutrients, in some cases, is increased during processing as it stimulates catalytic activation of certain enzymes, notably alliinase and polyphenol oxidase [47]. After cooking the food grains, the enhancing effect of both β-carotene and sulfur-augmented vegetables was increased in comparison to uncooked grains [48,49]. Although cooking generally decreases the nutrients, at the same time some nutrients are released from the food matrix thus enhancing their bioavailability [50].Nutrients may also affect the bioavailability of each other, for example, vitamin C augments non-heme iron assimilation, high zinc content decreases iron and copper accessibility and an increase in calcium, magnesium and phosphorus absorption is brought about by vitamin D. Some fat and oil soluble minerals enhance the bio absorption of nutrients such as carotenoids. In contrast, phosphate-rich chelators decrease nutrient accessibility [37]. Phytate is present in cereals and is reported to direct a decline in zinc bioavailability and the situation is further aggravated with dietary calcium [51-70]. Nutrients counter -interfere, by modifying the meal habits accordingly, they become more digestible and more available to the body [71-74]. Gut micro-floral interference in fabrication and bioavailability enhances the absorption of vitamins through membrane transporters although; there is a configuration difference between microbe-derived vitamins and nutritionally derived vitamins. The presence of gastrointestinal membrane transporters maintains the metabolic equilibrium of the body by increasing microbe-derived nutrients such as the vitamin B complex [75].

People should change their eating habits and food preparation methods to get maximum nutrients from their diet [74]. For instance, to curb carotenoids deficiency (vitamin), one should take fruits in the form of shakes and leafy vegetables after shallow frying. Carotenoids are available in chloroplasts as oil droplets in fruits, making their availability easier. While these are present as protein complexes in chloroplast of leafy vegetables, their intake with fats, especially vegetable oil, assists in a significant increase in bio-absorption. To enhance the bioavailability of beta-carotene, vegetables should be cooked with food acidulants and spices [76-78]. Though simple, these suggestions will also increase financial burden by resorting to food recipes that aren't common. Further, it is difficult to change people's behavior towards their meal habits due to social traditions. Globally, all worldwide organizations should participate in recreating a favorable environment in terms of effective food systems in order to change the behavior of society [79].

Biofortification provides a solution for desired improvements in diet to combat malnourishment. At present, genetic engineering along with plant breeding methodologies facilitates either down-regulating or abolishing anti-nutrients or enhancing promoter elements in fortified crops [80-86]. Vitamin A enriched golden rice, vitamin A and iron-enriched bananas, iron-fortified wheat and Bio cassava plus containing iron and provitamin A are developed by transgenic technology, have been successfully boosting the nutritional status of their target populations. However, misuse of these approaches may cause risk as many anti-nutrients are crucial for plant metabolism, biotic and abiotic stress tolerance, and their balance is significant for both bioavailability of nutrients and crop yield [87]. Furthermore, polyphenols and phytates are also valuable for humans due to their anti-diabetic and anti-carcinogenic properties as well as their ability to reduce the incidence of cardiovascular disease [88-90]. Therefore, one should be aware of possible side effects associated with these factors before changing the crop status [91].

Strategies for Biofortification of Grains

Cereal crops are indispensable in the framework of any developing country. For this reason, better strategies for the biofortification of grains should be introduced that curtail the undernutrition problem. Genes involved in translocation of micronutrients to seeds are inadequate with respect to biofortification with increased bioavailability, but localization of nutrients, specific developmental stage and chelators are also of great interest [92]. ZIP gene family (ZIP1, ZIP2, ZIP3, ZIP4) are metal ion transporters that maintain the metal ion level. OsZIP3 reported for rice is responsible for balancing zinc level in cells particularly in leaves but in meristematic cells OsZIP4 may cause zinc translocation. The presence of co-transported chelators is critical for enhanced micronutrient expression; constitutive expression of rice *ZIP4* gene resulted in anomalous circulation and lower zinc concentration in seeds [93]. Such problems can be mitigated by controlled overexpression [92].

Effective biofortification of certain cereal species, such as wheat, only requires enhanced micronutrient concentration whereas increased micronutrient uptake in rice causes low seed nutrients [94-96]. Micronutrient uptake in plants is not the reason for low nutrient concentrations in some species. Rather there is a downstream step responsible for the low levels. For this reason, just increasing a micronutrient is not feasible for biofortification and consequently, the sink strength of plants needs to be raised to pull-in micronutrients [92]. Using this strategy, the soybean iron storage protein ferritin has been overexpressed in rice resulting in an up to threefold increase in iron concentration in some transgenic plants whereas in maize the increase is 20-70%

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of iron in grains using an endosperm specific promoter [11,26]. An increase in the translocation ability of micronutrients in grains is due to increase in number of transporters. Constitutive expression of NAS produces an elevated micronutrient quantity in transgenic tobacco whereas overexpression of NAS3 in rice leads to high iron, copper, and zinc [97,98]. Transgenic iron biofortified rice was successfully developed by increasing grain sink potency and translocation capability [92].

Metabolic processes of various vitamins can be genetically manipulated to increase them simultaneously in crops. Biofortified maize varieties have been developed using the gene stacking technique by DNA transformation [99]. Multivitamin enhancement in maize is targeted by modifying vitamin's metabolic pathways through genetic engineering. Transgenic maize encompassing ZMPSY1 cDNA with the wheat LMW glutenin promoter and *CRTI* gene regulated by the Dhordein promoter of barley was engineered to enhance β-carotene. Besides β-carotene, vitamin D and C genes were also incorporated in corn-producing an elite transgenic with high level of vitamins [100]. Maize produced by traditional crossing methods contains less vitamin A content in comparison to transgenic lines. A carotenoid enriched rice variety was developed by adding the PSY1 gene of maize crops [27]. Genes of bacterial origin regulated by super γ-zein promoters, including CRTB (Phytoene synthase) and CRTI (Phytoene desaturase), were integrated into maize boosting carotenoid levels [31]. Plant-based PSY1, LCY-B (Lycopene β cyclase) and CHY (β-carotene hydroxylase) genes, while CRTI (Phytoene desaturase) and CRTW (β-carotene ketolase) from bacteria were placed under an endosperm-specific promoter and gave rise to different kernel phenotypes [32]. Both α-carotene and β-carotene production competes for a single precursor 'lycopene' that is produced by geranyl diphosphate with phytoene as an intermediary product in the presence of phytoene synthase and phytoene desaturase, thus by reducing the α-carotene and increasing the β-carotene leads to a more specific approach than by incrementing the geranyl-geranyl pyrophosphate synthesis. Increasing β-carotene by blocking the factors that reduce its production is another avenue for nutritional enrichment of crop plants [51].

Conclusion and Perspectives

Despite rigorous international efforts, MNM remains a global threat to public health. Plant-based strategies to boost nutrient uptake from the diet are productive. Although it can be attained by food diversification, owing to geopolitical issues, it is highly unlikely to be achieved in near future. For this reason, an economical solution is the need of the hour. Biofortification offers an enduring and highly supportive plant-based solution to raise the nutritional status of the undernourished around the globe [58]. Biofortification methodologies are based on foliar and soil application of fertilizers, breeding superior crops and genetic manipulation of crop plants. Genetic manipulation of crop plants in attaining nutritional security presents a viable stratagem against malnutrition, especially among the rural populace of developing countries. Several crops have been genetically biofortified with essential minerals, vitamins and amino acids using multiple genes from a myriad of sources to ensure the enhancement and bioavailability of nutrients after digestion [50].

For grain nutrient enhancement, biological processes underlying uptake of nutrients from the soil and their localization from roots to different plant parts should be studied in detail. Though considerable progress has been made in this regard, there is much that has yet to be investigated. Simultaneous targeting of multiple genes driven by developmentally inducible promoters provides a promising approach in developing biofortified cereals [92]. However, for the biofortification of staple cereal crops it remains a challenge because to accomplish this, it is necessary for plant breeders, molecular scientists and nutritionists to communicate with each other. Conventional breeding strategies are being exercised and are also appreciated by the community to address nutritional deficiencies. Though transgenic ally-biofortified foods are more economical and beneficial many hurdles remain regarding their acceptance and laborious regulatory sanctioning procedures. Health issues related to these modified crops, such as intolerance and allergies, lack of biodiversity and cross-contamination are also mattered of great concern [10]. Despite these obstacles, biofortified crops achieved through genetic engineering promise a bright future as it complements other interventions to overcome the problem of malnutrition among the people of developing countries.

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Author's Contribution

Muhammad Sarwar Khan, Rimsha Riaz and Saher Qadeer contributed equally in the write-up and making corrections. All authors have read and approved the final manuscript for submission.

Conflicts of Interest

Authors; Muhammad Sarwar Khan, Rimsha Riaz and Saher Qadeer declare that they do not have any conflict of interest with other authors.

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