Enhancing Nutritional Quality in Crops

Abstract

Micronutrient deficiency, called as hidden hunger, has devastating effects on mental health, productivity, economic losses and even death as an extremity. This form of malnutrition is highly complex. Developing countries are finding alternatives to traditional diets, in the forms of nutritional supplementation and transitions, to fight hidden hunger. Biofortification involves the breeding or genetic modification of crops to increase their nutritional value. Crop biofortification offer the most effective and sustainable approach to alleviate all forms of malnutrition, in economical terms as well. The implementation of breeding based biofortified crops, with enhanced micronutrient content, has the potential to assist current interventions to formal health systems and markets. The Sustainable Development Goals (SDGs) are internationally accepted universal call to end poverty, improve livelihood and protect the earth in sustainable ways. Crop biofortification have direct links to twelve of the seventeen SDGs. Biofortification of staple crops pose a significant achievement in providing food security with appropriate commercialization of the target consumers. Major crops like maize, rice, wheat, soybean, pearl millet and mustard have been biofortified. Maize has been biofortified for protein quality, provitamin A, and vitamin E.

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Wheat and rice were biofortified for protein content, iron and zinc, and Pearl millet for iron and zinc. Similarly, soybean has been recently biofortified with reduced trypsin inhibitor and lipoxygenase. The special efforts to popularize and commercialization of these biofortified crops have already been achieved and are currently underway among masses.

Keywords: *Biofortification, Crops, Nutrition, Breeding, Hidden hunger.*

I. INTRODUCTION

The increasing global population asserts a great challenge to food grain production to meet daily food consumption needs. The role of climate change in the present scenario could put 30% of the population at risk of hunger by 2050 (van Dijk *et al.,* 2021; Jayhoon *et al.,* 2023). The prevalent form of hunger is related to micronutrient malnutrition, also called 'hidden hunger' observed in both resource-poor and wealthy families, and takes a toll on the country's GDP (Steur *et al.,* 2015). Moreover, the majorly consumed staples are low in key micronutrients that are deficient in millions of undernourished. Biofortification of food sources appears to be a promising approach to this challenging scenario. 'Biofortification' is a sustainable and low-cost effective mode wherein breeding and biotechnical interventions develop micronutrient-enriched plant foods.

II. CROP BIOFORTIFICATION

Biofortification, a sustainable and cost-effective approach, aims to improve the nutritional quality of staple crops to combat micronutrient deficiencies. In recent years, biofortification efforts have focused on enhancing the nutritional content of major crops like wheat, rice, maize, pearl millet, soybean, groundnut etc. to address deficiencies in essential micronutrients. Biofortification involves the breeding and selection of crops to increase their nutrient content. This process can be achieved through conventional breeding techniques or modern biotechnological approaches. By focusing on specific traits, such as increased iron, zinc, or vitamin content, scientists strive to develop biofortified wheat varieties with enhanced nutritional profiles.

2.1 Targeting Micronutrient Deficiencies

Micronutrient deficiencies, also known as hidden hunger, affect billions of people worldwide. Iron and zinc deficiencies, in particular, can lead to serious health issues, including impaired cognitive development, compromised immune function, and increased susceptibility to diseases. Crop biofortification presents an opportunity to alleviate these deficiencies and improve overall health outcomes.

2.2 Impact on Human Health

The consumption of biofortified foodgrains has the potential to significantly impact human health, especially in regions where wheat is a dietary staple. By alleviating micronutrient deficiencies, it can contribute to improved cognitive function, strengthened immune systems, and reduced vulnerability to diseases. The enhanced nutritional profile of biofortified wheat also supports maternal and child health, promoting proper growth and development.

2.3 Agricultural Challenges and Successes

The successful implementation of biofortification faces several challenges, including breeding high-yielding, disease-resistant varieties with improved nutritional traits. However, ongoing research and collaborative efforts have resulted in promising outcomes. For example, biofortified varieties of wheat, maize and rice have been successfully introduced and adopted in various countries, and in India as well, demonstrating their potential for impact. Biofortification of crops has significant social and economic implications. By targeting staple crops, which are accessible and affordable for many populations, biofortification can reach a large number of individuals, particularly those in low-income communities. Improving the nutritional content of such widely consumed crops helps combat malnutrition and improve public health at a broader scale.

III. METHODS TO BIOFORTIFY CROPS

3.1 Breeding Methods

Breeding-based biofortification is the most widely applied in achieving enhanced nutritional quality of crops. These methods are relatively cheaper, easily applicable in low-cost facilities, scalable to extremely large and low-scale methods, and results in a relatively reliable product with minimal technical expertise as compared to state-of-the-art genetic engineering aspects. The first step in proceeding with the breeding-facilitated biofortification is the identification of nutrient deficiency and its mode of impact on human values and health. Consequently, a germplasm line, or pool is identified in the form of landrace, mutant, exotic lines or wild forms to be transferred into the target population, or line, most preferably an elite variety. Some of the examples and achievements of breeding-based biofortification are provided in Table 1.

The most widely utilized approach for breeding-based biofortification is marker-assisted backcross breeding (MABB). In this approach, the donor line or germplasm with a favorable allele for the trait of nutritional quality is identified. The F1 generation is generated by crossing this line with the target recipient line, to transfer the allele into heterozygous form. Subsequently, the F1 is backcrossed to the recipient line for maximum genome recovery. The number of backcrosses is dependent on the extent of genome recovery achieved. This approach is extremely applicable and utilized in improving a line or elite variety for conversion into a biofortified line. Another approach involved the development of new variety by inter-crossing donor lines with several elite inbreed lines and then identifying the superior hybrids with better combining ability. The identified lines are then crossed in all random mated; the hybrids are combined and tested for adaptability to target environments. Examples of such populations developed by CIMMYT are Susuma, Obatampa, Thai o2 Composite, Composite K (H.E. o2), etc. for o2 in maize. These biofortified populations provide broad base for vast utilization in breeding programs across diverse locations and environmental conditions.

Table 1: Examples of Significant Biofortification in Crops.

Sl.No.	Crop	Trait	Varieties Developed	References
1	Maize	Lysine, tryptophan, provitamin A and vitamin E	Vivek QPM 9, Pusa HM 4 Improved, Pusa HM 8 Improved, Pusa HM 9 Improved, Pusa Vivek QPM 9 Improved, IQMH 202, IQMH 203, Malviya Swarn Makka 1, Pusa Biofortified Maize Hybrid 1	Hossain *et al.*, (2022); Yadava *et al.*, (2022)
2	Wheat	Protein, iron and zinc	WB 02, HPBW 01, MACS 4028, DDW 47, HI 8802, HD 3249	
3	Rice	Protein, iron and zinc	CR Dhan 310, DRR Dhan 48, Zinco Rice MS, CR Dhan 315	
4	Pearl millet	Protein, iron and zinc	HHB 299, AHB 1269Fe, Phule Mahashakti, HHB 311, HHB 67 Improved 2	
5	Mustard	Erucic acid and Glucosinolates	Pusa Mustard 30, Pusa Mustard 32, Pusa Double Zero Mustard 31, RCH 1, PGHS 1699	
6	Soybean	Trypsin inhibitor, Lipoxygenase and Oleic acid	NRC 127, NRC 147, MACSNRC 1667	

IV. BIOFORTIFICATION IN MAIZE

Humans are currently dependent on 15 of 50,000 edible plants as sources of major food energy intake. Maize is one of the three crops that together meet 75% of this demand besides being 3rd highly produced crop next to rice and wheat. It is utilized as both animal feed and human consumption at nearly 59% and 23%, respectively (Yadav *et al.,* 2015). Given the pivotal role of crops in human and animal nutrition, the enrichment of maize with essential nutrients holds significant importance.

4.1 Protein Enhancement of Maize

Maize has a deficiency of two crucial amino acids, lysine and tryptophan, which significantly affect the quality of its protein. The insufficient presence of these essential amino acids leads to a condition known as protein energy malnutrition. 'Kwashiorkor' and 'Marasmus' are commonly observed diseases resulting from a lack of adequate protein intake (Bain *et al.,* 2013). Maize protein contains 1.5-2% lysine in the endosperm flour (Vasal *et al.,* 1980; Sarika *et al.,* 2017). The presence of an excessive amount of lysine-deficient zeins and a scarcity of lysine-rich non-zeins contributes to this issue. However, a solution has been identified in the form of mutant opaque2 (o2), which enhances the lysine and tryptophan content in maize. This improvement is achieved by reducing the levels of 19- and 22-kDa α-zeins and minimizing the degradation of lysine (Mertz *et al.,* 1964; Schmidt *et al.,* 1990) however, pleiotropic effects of the mutation expressed as soft and opaque endosperm results in yield losses, posing challenges and majorly storage pest susceptibility in an unmodified form (Vasal *et al.,* 1980). The development of Quality Protein Maize (QPM) involves the combination of endosperm and amino acid modifiers, resulting in increased levels of lysine and tryptophan. This modification also leads to the formation of vitreous endosperm in maize kernels. These advancements have been proven to offer significant nutritional benefits. (Bjarnason and Vasal 1992; Prasanna *et al.,* 2001; Akalu *et al.,* 2010; Gunaratna *et al.,* 2010). As a result of these efforts, numerous conversions to Quality Protein Maize (QPM) have been achieved, leading to the successful commercialization of QPM inbreds and hybrids in dent corn, flint corn, and waxy corn varieties. This progress has enabled the availability of QPM maize across different types of corn, offering improved nutritional value (Yang *et al.,* 2013; Surender *et al.,* 2017; Hossain *et al.,* 2018; Talukder *et al.,* 2022). Marker-assisted backcross breeding (MABB) has played a crucial role in the release and commercialization of several maize varieties. Some of these varieties include HQPM-1, HQPM-4, HQPM-5, HQPM-7, Pusa HM4 improved, Pusa HM8 improved, and Pusa HM9 improved.

MABB has facilitated the selection and incorporation of desirable traits in these varieties, contributing to their enhanced performance and quality (Gupta *et al.,* 2015; Hossain *et al.,* 2018). Apart from the opaque2 (o2) mutation, another beneficial mutation called opaque16 (o16) has been identified. This mutation is closely linked to SSR markers umc1149 and umc1141. When the o16 mutation is stacked with o2 in maize, it results in a significant increase of 30% in lysine content compared to the effects observed with o2 mutation alone (o2o2). This stacking of mutations offers a promising approach to further enhance the lysine levels in maize varieties (Yang *et al.,* 2005). Genotypes with o16o16 alone recorded over 2-fold more lysine and tryptophan than normal maize (Sarika *et al.,* 2017). Unlike o2, the o16 lines have vitreous endosperm, grain hardness, protein-starch matrix and zein profiles similar to normal maize endosperm (Sarika *et al.,* 2018).

4.2 Provitamin A and Vitamin E-Rich Maize

Vitamin A deficiencies can lead to various health issues including night blindness, impaired iron mobilization, keratomalacia (a condition affecting the cornea), and increased susceptibility to diseases (WHO, 2009). Provitamin A is present in normal maize usually in the range of 2-3 μg/g, which is 5 times less than the recommended daily allowance (RGA) required by humans (Pixley *et al.,* 2013). Natural favorable variations or polymorphisms have been observed in specific genes associated with the carotenoid synthesis pathway in maize. These genes include lycopene-ε-cyclase (lcyE) located on chromosome 8 and carotene hydroxylase (crtRB1) located on chromosome 10. These favorable variations have been extensively utilized to enhance the provitamin A content in maize. Notably, a preferred allele of crtRB1, specifically the 3'TE allele, has been shown to significantly increase the levels of β-carotene, sometimes up to tenfold, in maize inbreds. This allele plays a crucial role in boosting the provitamin A content of maize varieties. (Muthusamy *et al.,* 2014). The MABB approach has been utilized to enhance proA content in parental lines of promising hybrids by pyramiding both the alleles of genes (Muthusamy *et al.,* 2014; Zunjare *et al.,* 2018; Gupta *et al.,* 2013). Hybrids biofortified with proA include Pusa Vivek QPM9 Improved, Pusa Vivek Hybrid-27, Pusa HQPM-5 Improved and Pusa HQPM-7 Improved. Besides MABB, a transgenic approach attempted using crtB and crtI of bacteria Erwinia herbicola enhanced proA upto 10 μg/g.

Vitamin-E is another crucial nutrient essential for human growth and development. Its deficiency can lead to various health issues, including cancer, neurological disorders, and Alzheimer's disease, among others (Muzhingi *et al.,* 2017). In the gene cassette of vte4, specific insertion/deletion (InDel) markers have been associated with an increased content of α-tocopherol, a form of vitamin E (Das *et al.,* 2019a). Additionally, the identification of Indels and Single Nucleotide Polymorphisms (SNPs) (Das *et al.,* 2019b) provides valuable markers that can be effectively employed for enhancing α-tocopherol levels in maize using marker-assisted selection approaches. This offers a promising strategy for improving the vitamin E content in maize varieties.

4.3 High Fe and Zn Maize

Iron (Fe) and zinc (Zn) are essential micronutrients that play crucial roles in proper cell functioning and various enzymatic activities. However, the Fe content in maize kernels can be influenced by factors such as environmental conditions, genetic background, and positional effects. The molecular and biochemical mechanisms underlying the distribution of Fe and Zn in maize are not yet fully understood, despite the successful mapping of Quantitative Trait Loci (QTL) and identification of genomic regions associated with these traits (Qin *et al.,* 2012; Baxter *et al.,* 2013; Yuan *et al.,* 2019). Significant genetic variation for kernel Zn has been identified in maize germplasm collections (Hindu *et al.,* 2018; Prasanna *et al.,* 2020). Enhancing the levels of Fe and Zn in maize kernels through molecular breeding has proven to be a challenging task due to the involvement of multiple loci. However, reducing the content of phytic acid, an anti-nutritional factor, can increase the bioavailability of Zn and Fe (Prasanna *et al.,* 2020). Phytic acid binds to Zn and Fe due to opposite charges, thereby reducing their availability. An additional benefit of reducing phytic acid is a decrease in the environmental footprint associated with its undigested passage into the excreta of monogastric animals when consumed (Raboy *et al.,* 2000; Hossain *et al.,* 2022). Marker- assisted backcross breeding (MABB) has been employed to improve Quality Protein Maize (QPM) inbreds containing mutations in o2 and crtRB1 genes while also possessing low phytic acid traits (Bhatt *et al.,* 2018).

4.4 Quality Enhancement of Specialty Corn

Specialty corns are highly economical corn types that are specifically different than normal maize with specific reference to kernel characteristics. Sweet corn is preferred for higher sugars in kernels caused by to accumulation of phyto-glycogen instead of normal starch in endosperm. Recessive forms of alleles, su1 and sh2 have been used by plant breeders to modify and improve sweet corn (Mehta *et al.,* 2017). Biofortification of sweet corn through o2 and crtRB1 has been attempted where proA and lysine got enriched by 6 times and 2 times compared to parental normal sweet corn lines (Mehta *et al.,* 2020). In addition to this, genes Pr1 and C1 have been introgressed using the MABB approach (Jompuk *et al.,* 2020).

Waxy corn has culinary importance in many countries. Waxy corn is characterized with >95% amylopectin (Zhou *et al.,* 2016). Mutant allele wx1 inhibits the action of granule-bound starch synthase-1 and was reported to be promising in inducing waxy kernels. Waxy line biofortifications have been attempted by introgressing o16 and wx1 where lysine content increased by 16% (Yang *et al.,* 2013). Talukder *et al.* (2022) combine wx1 and o2 alleles in parental lines of hybrids using MABB. The improved hybrids contained 98% amylopectin with an increase in lysine and higher grain yield.

QPM popcorn conversions have been usually unsuccessful owing to poor recovery of popping traits. Ren *et al.* (2018) reported successful popcorn QPM conversion with o2 transfer while selecting for kernel zein profile-based.

V. BIOFORTIFICATION OF RICE

There were 33 million people in the year 2017, who were consuming biofortified crops in 2017 from Africa, Asia and Latin America (Heidkamp *et al.,* 2021). The Philippines became the first country to commercialize biofortified rice, the Golden Rice, in the year 2021. Vast genetic resources and technical proficiency with IRRI have facilitated the investigation of various avenues for biofortification in rice. Golden Rice is designed to complement existing strategies in addressing Vitamin A Deficiency (VAD). Food Standards Australia New Zealand and Health Canada conducted assessments and determined that Golden Rice is as safe as regular rice (https://www.irri.org/golden- rice). Hulling of paddy gives brown rice, and unpolished rice, also called brown rice, contains high amounts of minerals and nutrients such as iron, zinc, phosphorus, vitamin B1, and vitamin B2. The preference for white rice by the consumers' palate, rendered the condition unavailability of essential nutrients from white rice in a highly aggravated condition (Majumder *et al.,* 2019). Polishing of rice reduces protein content by more than 20% and brings down iron and zinc to 4.1 ppm and 18 ppm. The significant and foremost achievements in rice biofortification are the development of Golden Rice, and high iron and zinc rice. Plant breeders have long been interested in utilizing conventional plant breeding methods to achieve iron biofortification in rice varieties that exhibit both high iron content and high yield, in addition to disease resistance and preferred seed quality (Virmani and Ahmed, 2007). One significantly important variety is IR68144, with semi-dwarf and high-yielding properties. Another micronutrient, zinc, has been enhanced in rice to develop biofortified rice enriched with zinc. The breeding strategies employed to biofortify rice with iron and zinc are the same. The biofortified rice contains >22 ppm zinc in the polished form of rice. Some of the important varieties with enhanced zinc content are DRR Dhan 45, Zinco Rice MS, and CR Dhan 315 (Yadava *et al.*, 2022). Rice biofortification aims to tackle hidden hunger by not only addressing iron, zinc, and vitamin A deficiencies but also targeting other minerals and vitamins.

In addition to the biofortification of rice, simultaneous breeding for rice grain quality is a key aspect, as it ultimately decides the consumer acceptance and commercialization of developed rice varieties. Higher yield and superior quality are the two major goals of any rice breeding program. Increased yield is unquestionably proof of a significant development in contemporary rice science and technology, as shown by the linear growth of the worldwide rice yield at 47 kg per hectare per year from 1961 to 2021 in the FAOSTAT database (FAO, 2023). However, the incongruity between high yield and superior quality is a pervasive scientific issue, and rice quality has not received enough attention during the actual process of rice variety development. Thus, improving rice quality has become more crucial as living standards rise.

The four main components of rice grain quality are milling quality, appearance quality, eating and cooking quality, and nutritional quality (Gong *et al.*, 2023). Brown rice recovery and head rice recovery are two indicators of the milling quality, which refers to the rice's capacity to endure the processing process. In terms of appearance quality, grain length, grain width, the length/width ratio, chalkiness, and grain translucency are the main factors to consider. Three starch-related characteristics, namely amylose content, gel consistency, and gelatinization temperature, are linked to eating and cooking quality, which mostly reflects the qualities and flavor of cooked rice. Meanwhile, the quantity and quality of proteins, lipids, minerals, and other bioactive compounds that are good for human health in rice determines its nutritional quality (Custodio *et al.*, 2019; Li *et al.*, 2022).

Rice grain quality is a complex trait impacted by both genetic and environmental influences (Kumar *et al.*, 2018). The starch content, which affects cooking and sensory evaluation, as well as grain shape and chalkiness in terms of appearance, are key traits that affect rice quality (Jayhoon *et al.*, 2023). The physical properties of the rice grain, such as the pericarp, seed coat, aleurone layer, starchy endosperm, and embryo, are strongly associated with the quality of the rice grain (Lu and Luh, 1991).

VI. CONCLUSION

Breeding-based biofortification appears to be a promising strategy to combat malnourishment and associated risks. The approach is significantly efficient and sustainable considering the biofortification through soil inputs and transgenic approaches. The nutrient-enriched maize would have a successful impact on poultry and other direct industrial uses. Multi-nutrient enriched ‘second generation’ biofortified maize inbreds could be tested for more hybrid combinations. This can potentially increase the genetic base of present biofortified maize hybrids besides selection for new alleles for biotic and abiotic stress found in new parental lines. The present efforts can be further strengthened by institutional collaborations and public-private partnerships.

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Questions

1. Why is there a need for crop biofortification?
2. What are the methods to biofortify crops?
3. How does introgression of opaque2 pose challenges to breeders? What strategies can be employed to improve kernel endosperm characteristics in opaque2-based breeding programs?
4. What quality characteristics should be simultaneously considered while attempting the biofortification of rice?
5. What are the achievements in specialty corn biofortification in India?

Self-Assessment

1. Which of the following crop varieties were developed through transgenic technology?
   a. HQPM1
   b. Shaktiman 1
   c. Golden Rice
   d. HD 3086

2. Malnutrition that is primarily caused due to micronutrient deficiencies in staple diet
   a. Hidden Hunger
   b. Wasting
   c. Anemia
   d. Stunting

3. An elected fellow of National Academy of Agricultural Sciences was awarded the World Food Prize in 2000.
   a. Dr. S K Vasal
   b. Dr. Rattan Lal
   c. Prof. Yuan Longping
   d. Dr. Normal Borlaug

4. From which backcross generation onwards does >90% of average recurrent parent genome recovery is observed without applying selection?
   a. BC2
   b. BC3
   c. BC4
   d. BC6

5. Among the following set of markers, which would be best suitable for foreground selection of gene of interest in an introgression based breeding program?
   a. Linked SSR marker
   b. Functional SSR Marker
   c. Linked RAPD Marker
   d. SNP in LD with putative candidate gene

6. Which are the following breeding methods are employed to derive an essentially derived variety?
   a. Marker assisted backcross breeding
   b. Intercross with sibbing
   c. SDN1 based genome editing and selection
   d. Both (a) and (c)

7. First country starts commercial produciton of golden rice and second country falters as of August 2023.
   a. Phillipines, Bangladesh
   b. Bangladesh, USA
   c. Phillipines, England
   d. Indonesia, Japan

8. Which of the following maize types are not commercialised in India?
   a. Biofortified Dent corn
   b. None of the above
   c. Biofortified Flint Corn
   d. Biofortified Popcorn

9. The first QPM maize hybrid derived by using marker-assisted selection was?
   b. Vivek QPM 9
   c. Pusa HM 8 Improved
   d. Pusa Vivek QPM 9 improved
   e. APTQH5

10. The genes targeted for provitamin A biofortification in maize were?
    a. o2, crtRB1
    b. crtRB1, lcyE
    c. o16, fatB
    d. o2, su2

11. Psy gene was which sourch was found to be most efficient in developing 2nd generation golden rice?
    a. Narcissus pseudonarcissus
    b. Daffodil
    c. E. coli
    d. Zea mays

12. The following are not encompassed under the prospect of biofortified crops
    a. QPM
    b. Low phytic acid
    c. Golden rice
    d. High iron and zinc rice

13. The pleiotropic effect of o2 are not observed in high lysine mutant
    a. o16
    b. dzr1
    c. crtRB1
    d. lpa1-1

14. Which are not the essential amino acids
    a. Lysine
    b. Proline
    c. Tryptophan
    d. Methionine