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Review

Current Status and Potential of Biofortification to Enhance Crop Nutritional Quality: An Overview

Seema Sheoran [1,*], Sandeep Kumar [1,*], Vinita Ramtekey [2], Priyajoy Kar [3], Ram Swaroop Meena [4] and Chetan Kumar Jangir [5]

1 ICAR-Indian Agricultural Research Institute, Regional Station, Karnal 132001, India
2 ICAR-Indian Institute of Seed Science, Mau 275103, India; vinita14ramtekey@gmail.com
3 ICAR-Indian Institute of Maize Research, Ludhiana 141004, India; karpriyajoy@gmail.com
4 Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, India; meenars@bhu.ac.in
5 Division of Soil Science, ICAR-National Research Centre on Seed Spices, Ajmer 305006, India; chetanjangir710@gmail.com
* Correspondence: seema.sheoran@icar.gov.in (S.S.); sandeep.kumar5@icar.gov.in (S.K.)

Abstract: Around 2 billion people are suffering from chronic malnutrition or "hidden hunger", which is the result of many diseases and disorders, including cognitive degeneration, stunting growth, and mortality. Thus, biofortification of staple food crops enriched with micronutrients is a more sustainable option for providing nutritional supplements and managing malnutrition in a society. Since 2001, when the concept of biofortification came to light, different research activities have been carried out, like the development of target populations, breeding or genetic engineering, and the release of biofortified cultivars, in addition to conducting nutritional efficacy trials and delivery plan development. Although, being a cost-effective intervention, it still faces many challenges, like easy accessibility of biofortified cultivars, stakeholders' acceptance, and the availability of biofortified germplasm in the public domain, which varies from region to region. Hence, this review is focused on the recent potential, efforts made to crop biofortification, impacts analysis on human health, cost-effectiveness, and future perspectives to further strengthen biofortification programs. Through regular interventions of sustainable techniques and methodologies, biofortification holds huge potential to solve the malnutrition problem through regular interventions of nutrient-enriched staple food options for billions of people globally.

Keywords: biofortification; cost-effectiveness; COVID-19; health effects; malnutrition

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1. Introduction

The world population is anticipated to rise from 7.87 billion in 2021 to 8.6 and 9.8 billion by 2030 and 2050, respectively. The agricultural production and supply chain are the most vulnerable to current global crises like climate change and the COVID-19 pandemic. The pandemic threatens global human life and health, which will be further worsened by intensifying hunger and malnutrition from disrupting the food supply chain mainly in developing countries [1], and it is escalating the challenges for global food security [2]. Malnutrition has serious socio-economic consequences, especially in developing and underdeveloped countries where people follow unbalanced diets. Even after profuse scientific breakthroughs, a large section of the population still cannot access or afford an adequate quality diet, which causes malnutrition and undernutrition.

About 815 million people are undernourished due to an insufficient or low-quality diet or its poor absorption, of which about 780 million people belong to developing countries [3]. Children are most susceptible to malnutrition, as about 45% of children's deaths (<5 years) are due to malnutrition, while 151 million children (22.2%) are stunted and 51 million (7.5%) are underweight for their heights [4]. Despite consuming a carbohydrate-rich diet, the problem of hidden hunger persists, as we are unable to fulfil micronutrient requirements [5]. According to an estimate, about 2 billion people are suffering from micronutrient malnutrition or "hidden hunger" worldwide [6–8].

Micronutrient deficiency or "hidden hunger" for iron (Fe), zinc (Zn), vitamin-A, iodine (I), and calcium (Ca) is extensively widespread among all age groups. As per an estimate, nearly 60, 30, and 15% of the world's population is deficient in Zn, Fe, and I, respectively [9,10]. Micronutrients play a vital role in healthy body functions, but their deficiency leads to many adverse effects like poor growth and development and cognitive diminishment, in addition to the increased risk of disease and mortality. Most of the disorders caused due to micronutrient deficiency can be reversed with the proper diet, while some cause lifelong impairments, such as iodine deficiency in early pregnancy, which causes intellectual incapacity in children [11].

Considering the severity of its consequences, eradicating malnutrition is the only sustainable solution to achieving a healthy world [12,13]. In 2015, the global community discoursed the "Sustainable Development Goals" (SDGs) to alleviate malnutrition in all of its forms [14]. Among the 17 SDG goals, SDG2, "Zero Hunger," aims to transform the world into a hunger-free zone by facilitating food and nutritional security, and SDG3, "Good Health and Well-Being," aims to ensure healthy lives for people of all ages [15]. Hence, in addition to ensuring global food security, fortification of food crops is a potential approach to enhancing human immunity to fight the pandemic situation.

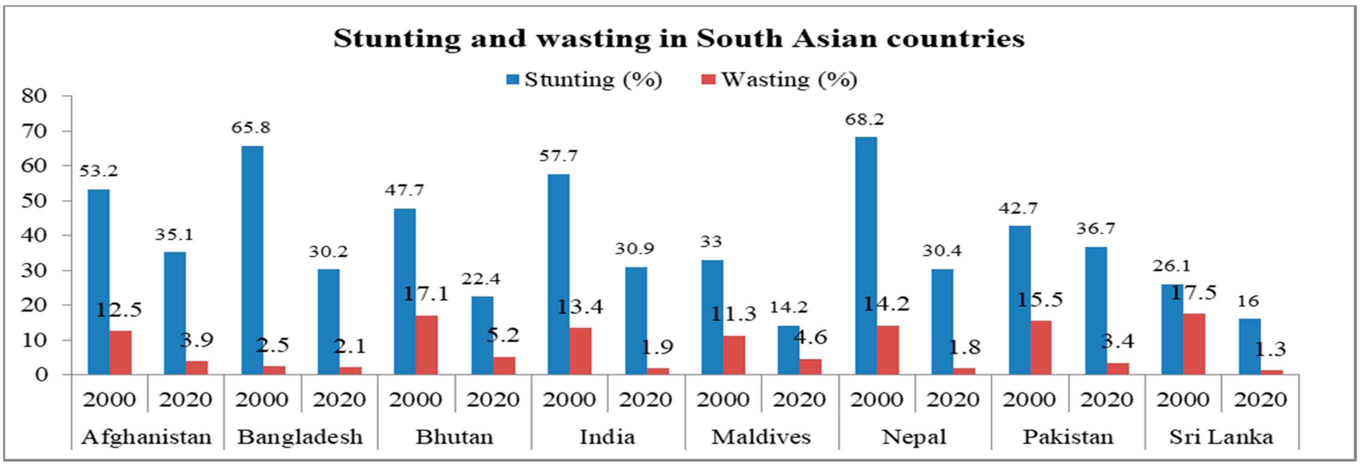
Fortification is the organized process of intentionally increasing an essential micronutrient (i.e., vitamins and minerals) in staple foods to enhance their nutritional quality and, in addition, provide a health benefit to the public with negligible risk. Food fortification can be performed either by directly taking supplements, commercial fortification, or diversifying or modifying the diet (i.e., biofortification) [16]. Biofortification is the process of enriching the nutritional status of staple food crops by mounting the nutrient content or bioavailability either through agronomic methods, conventional breeding, or biotechnological tools [17]. Commercial fortification and nutritional supplements are costly, and lack of access to the market and healthcare systems combined with no long-term health benefits data makes these options unattractive [18]. Genetic biofortification is a cost-effective approach with a one-time investment to fight hidden hunger, as unlike commercial fortification, there is no need to buy or add fortificants repeatedly to the food [19].

The idea of biofortification was initially originated around the green revolution period (1966–1985), and the first step to solving any micronutrient deficiency through biofortification was initiated in the early 1990s by economist Howarth Bouis [20]. The term "biofortification" was coined by Steve Beebe (the bean researcher) in 2001, and since then, a huge amount of funding has been invested in this direction by the World Bank, the Bill and Melinda Gates Foundation, the Consultative Group on International Agricultural Research (CGIAR), the US and UK governments, the European Union (EU), and the Asian Development Bank. In this direction, great initiatives have been undertaken by CGIAR institutes worldwide, such as the International Food Policy Research Institute (IFPRI) and the International Centre for Tropical Agriculture (CIAT) under a program called HarvestPlus [21] to develop biofortified varieties for major staple crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and cassava (*Manihot esculenta*) [22,23].

Thus far, our main focus has been to increase crop production and productivity, neglecting the aspect of the nutritional status of developed crop cultivars and also human health. This causes a rapid increment in micronutrient shortages in food crops, thereby augmenting the malnutrition problem among consumers. With the awareness of this fact, the agricultural system is shifting to develop high-quality, nutrient-dense food crops in addition to increasing quantity-wise production. This will help to alleviate "hidden hunger" or "micronutrient malnutrition", especially in developing countries [16]. According to the Copenhagen Consensus, reducing malnutrition can solve 5 out of 10 of the world's problems, and biofortification has been ranked the 5th main area to invest in to solve this problem [24]. Therefore, recently, micronutrient biofortification has increased exponentially, and this review focuses on the status and future potential of biofortification in crop plants to enhance nutritional values in the benefit of human health. We review the impacts of the COVID-19 pandemic on intensifying food and nutritional challenges and human health issues, and we then discuss the effectiveness of recent novel biofortification approaches like molecular and genetic engineering and agronomic biofortification, as well as their potential to alleviate hidden hunger. This article extends to the current efforts and achievements attempted in crop biofortification globally and their impact on the nutritional and human health status, in addition to the cost-effectiveness and monetizing benefits, compared with other interventions. There is an urgent need for policy support and implementation to achieve the SDG goals.

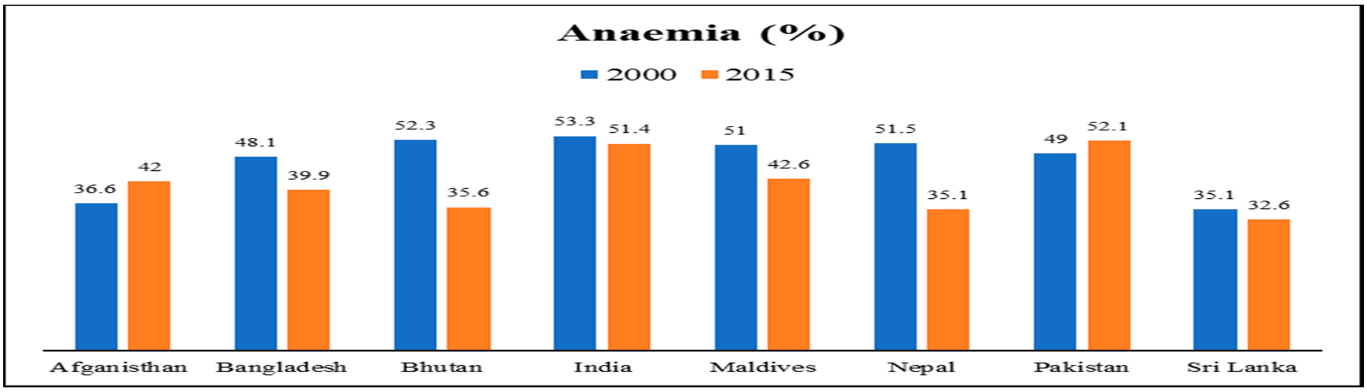
2. Health Issues and Nutritional Challenges Due to Malnutrition

The global population is expected to reach 9 billion by 2050, raising serious concerns for nutritional and qualitative feeding [25]. Micronutrient deficiency is associated with several physiological impacts, including stunted physical and intellectual growth in children, anemia and maternal mortality resulting in impaired cognitive functions, and several disorders like blindness and poor productivity [26]. In particular, vitamin A deficiency (VAD) has been regarded as a chronic public health issue in developing economies, which are more prone to economic instability, inadequate dietary intake, and faulty food distribution systems [27]. The nutritional crisis in South Asian countries is extremely alarming. Despite recent economic growth and poverty reduction policies, malnutrition remains widespread, and it is popularly known as the "South Asian Enigma" by policymakers [28]. The problem of malnutrition is so prevalent that 88% of the Asian and African countries face two or three forms of malnutrition simultaneously. An inadequate food supply, low household income, poor healthcare infrastructure, inappropriate childcare, and food insecurity have been recognized as the principal indicators of rising malnutrition prevalence in South Asia [29]. Amidst being among the fastest developing regions, South Asia represents a paradoxical situation, leading to the malnutrition front. South Asia is home to 33.3% and 15.3% of moderately or severely stunted and wasted children (<5 years), respectively, and 3.1% of the total of overweight children [28]. Observing the levels and trends of the World Health Organization (WHO) nutrition indicators (Figure 1), it can be noted that despite significant improvements in certain indicators, the countries in the South Asian region are still far from meeting the SDG targets. While child malnutrition is a major concern in the region, it is overshadowed by the region's most serious problem (i.e., approximately 40–50% of reproductive-age women are anemic).



(**a**)

Figure 1. *Cont.*



(**b**)

Figure 1. Trends in nutrition indicator level (stunting, wasting, and anemia) in eight South Asian countries from 2000 to 2020. (**a**) The stunting rate decreased in all countries such as Afghanistan (−34.0), Bangladesh (−54.1), Bhutan (−53.0), India (−46.4), Maldives (−57.0), Nepal (−55.4), Sri Lanka (−38.7), and Pakistan (−14.1). Wasting rates declined in 6 countries: Afghanistan (−68.8), Bhutan (−16.0), Maldives (−59.3), Nepal (−87.3), Pakistan (−78.1), Sri Lanka (−92.6), Bangladesh (−16.0), and India (−85.8). (**b**) Anemic conditions also show similar trends except in Afghanistan (5.4) and Pakistan (3.1) Data source: UNICEF, WHO, and World Bank [9].

The most vulnerable population groups are the young school kids, commonly with VAD. The South Asian countries represent the highest child malnutrition status, which stymies their economic development by upsetting a large section of the population [30,31]. Zn and Fe deficiencies have been the most common, owing to the fact that very few corrective measures have been implemented to address this nutritional issue [27,32]. Malnutrition affects approximately 293 million children under the age of 5 and 468 million reproducible women worldwide, and curing them could cost billions of dollars each year [27,33,34].

Impact of the COVID-19 Pandemic on Food and Nutrition

During the lockdown, meeting food and nutritional needs has been difficult for many of the poorer households due to increased food prices and livelihood losses. Given the interconnections, it is evident that food security, public health, and climate change must all be tackled together to maximize synergies and reduce trade-offs between food production and climate adaptation and mitigation [35–37]. The COVID-19 pandemic has disrupted the economy, food, and health system, which are projected to increase all forms of malnutrition. According to the IFPRI estimate, an extra 140 million people will be forced into extreme poverty in 2020 because of the pandemic, living on less than USD 1.90 per day [38]. From 1990 to 2020, the number of malnourished children has decreased from 253 to 144 million, but the COVID-19 pandemic has reverted this positive effect of the last three decades, as an additional 2.6 million children will be severely malnourished by 2022 (https://www.unitlife.org/impact-of-covid-19-on-malnutrition; assessed on 15 January 2022). The major causes of it are the loss of income, which amounted to USD 3.5 trillion (i.e., 5.5% of the global GDP) in the first three quarters of 2020, as per the International Labor Organization (ILO), disruption in the food chain supply, strained health systems, and access to other services during the lockdown, further jeopardizing maternal and child health and mortality [39]. As the economic and food system crises worsen, other kinds of malnutrition, such as micronutrient malnutrition, child stunting, and maternal nutrition, are predicted to rise [40]. As per the UNICEF report, in addition to the 47 million children affected by waste and 144 million affected by stunting in 2019 before the pandemic, an additional 6.7 million children are on the edge of becoming wasted during their first year as a worsened result of the pandemic, out of which 57.6% are from South Asia and 21.8% are from Sub-Saharan Africa [41]. In the early months of the pandemic, there was a 30% decline in coverage of services related to improving nutrition outcomes for women and children, with up to 75–100% under lockdown contexts [42]. The substantial influence of the COVID-19 pandemic on early life nutrition may have inter-generational implications for infant growth and development, as well as long-term effects on schooling, illness risk, and overall human capital building [43].

3. Biofortification Approaches

In general, the staple cereals, pulses, oilseeds, vegetables, and fruits are crops majorly focused upon for biofortification through these methods, targeting mainly Zn, Fe, magnesium (Mg), selenium (Se), I, folic acid, carotenoids, and vitamin A [44]. To achieve sustainable and substantial biofortification, different approaches like conventional plant breeding, molecular breeding, genetic engineering, and agronomic approaches provide a durable solution (Figure 2). These methods are for the long run, with a one-time investment to deploy target genes for essential micronutrients. In this way, molecular and genetic engineering are cost-effective, precise, and accurate approaches that enhance the nutritive value of staple crops [45,46].

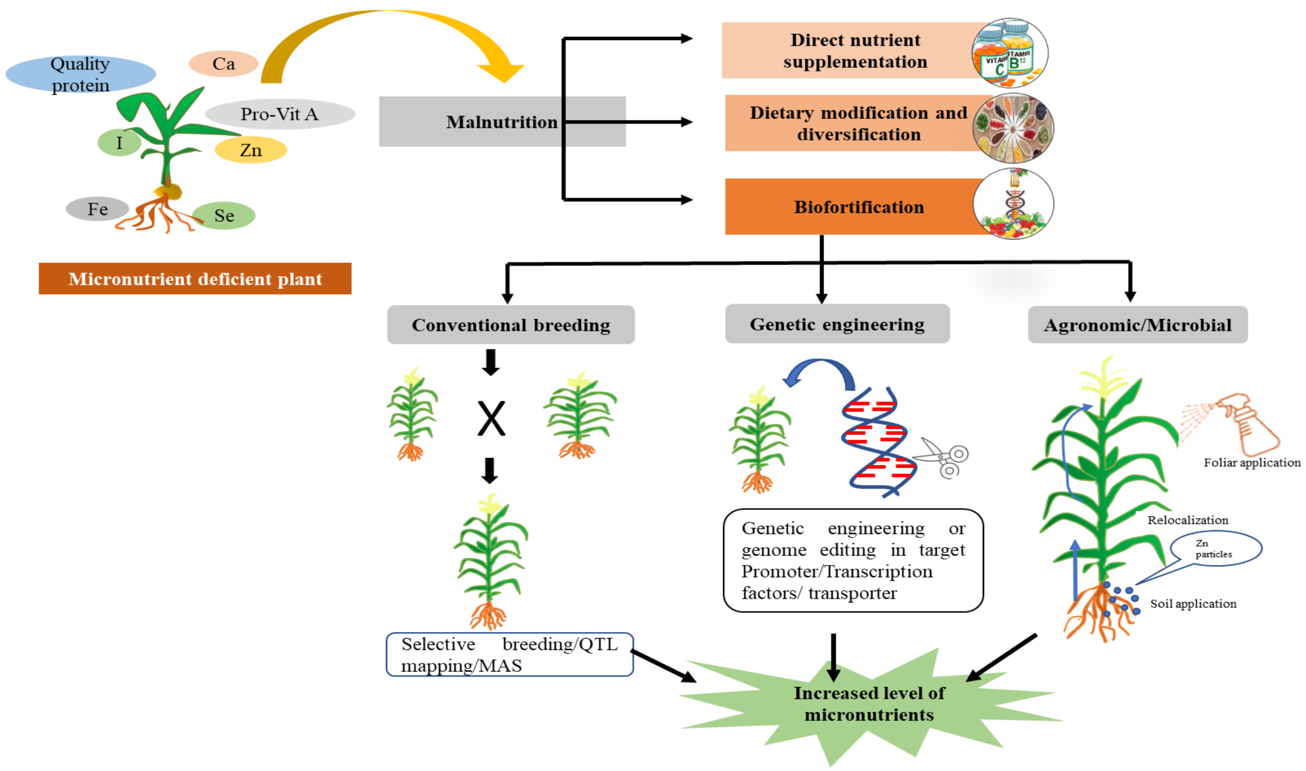


Figure 2. Malnutrition-alleviating approaches through direct (e.g., supplements or diet modification) and indirect interventions (e.g., biofortification).

3.1. Conventional Plant Breeding

Over the period, conventional plant breeding strategies have resulted in the development of several varieties of different staple crops with notable improvement in essential micronutrients [47,48] (Table 1). It is the most widely accepted and most trusted approach for biofortification. This process requires the existence of genetic variability in crops. Plant breeders can effectively utilize the germplasm belonging to primary, secondary, and tertiary gene pools to identify the essential genes required for the development of biofortified varieties [46]. Several investigations have been carried out to detect the genetic variability for micronutrient assessment [49–52], such as in the case of traditional and brown rice germplasm, where nutrients like Zn and Fe were found to be in higher quantities compared with white or polished rice [50,53]. One of the important examples of the conventional breeding method is the development of quality protein maize (QPM) that is widely accepted by farmers. However, using conventional methods, multiple gains can also be achieved, such as Fe- and Zn-enriched rice and wheat with a higher yield. Another biofortified crop—orange-fleshed sweet potatoes (OFSP)—was developed in Africa under the HarvestPlus program by enhancing both the nutrient and yield traits [54]. Nonetheless, breeders eventually depend upon minor genetic diversity present in the gene pool, which affects the cross-compatibility of plants. The mutation breeding approach can also be used to improve grain quality with irradiations and chemical treatments to induce greater genetic variability, but no practical results have been obtained yet. Moreover, the major limitation of the conventional breeding approach is that it is very time-consuming and totally dependent on genes or alleles already present in the gene pool of crops. This reduces the efficiency of conventional breeding methods. To overcome this issue, researchers prefer advanced molecular breeding approaches and genetic engineering, which bypass such barriers.

Table 1. Biofortified crop varieties developed through conventional or molecular breeding approaches.

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
Rice	Fe and Zn	BRRI dhan 62, BRRI dhan 72, BRRI dhan 64	18–25 mg kg^{-1} Zn	Conventional breeding	Bangladesh	CIAT, HarvestPlus
		Binadhan-20	20–31 mg/L Fe	MABB	Bangladesh	[55]
		IR68144-3B-2-2-3, Jalmagna	21 mg/kg Fe	Selection	India	[56]
	Zn	DRR Dhan 49, DRR Dhan 48, DRR Dhan 45	22.6–25.2 ppm	Backcross and pedigree selection	India	IIRR, India (https://www.icar-iirr.org/index.php/institute-research/institue-technologies-developed/33-iirr-technologies/107-technology-5; assessed on 26 November 2021)
		Zinco Rice MS	27.4 ppm	Pure line selection	India	IGKV, India
	Protein	CR Dhan 311 (Mukul), CR Dhan 315	10.2%	Backcross followed by pedigree selection	India	NRRI, India (https://icar-nrri.in/wp-content/uploads/2019/06/2.-leaflet_highprotein_final.pdf; assessed on 6 June 2021)
Wheat	Zn	BHU 1, BHU 3, BHU 5, BHU 6, BHU 17, BHU 18, Zinc Shakti (Chitra)	40–45 ppm	Conventional methods	India	CIAT, CIMMYT, Harvest Plus
		PBW1Zn	40.6 ppm Zn	Conventional	India	PAU, India
	Fe, Zn, and protein	Pusa Tejas (HI 8759) (durum), MACS 4028 (durum)	42.1 ppm Fe, 42.8 ppm Zn, 12% protein	Pure line selection	India	[57]
	Protein and Fe	Pusa Ujala (HI 1605)	43 ppm Fe, 35 ppm Zn, 13% protein	Pure line selection	India	IARI India
	Protein	PBW 752	12.5% protein	Conventional	India	PAU, India

Table 1. *Cont.*

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
	Zn	HD 3171, PBW 757	47.1 ppm Zn, 42.3 ppm Zn	Hybridization and selection	India	IARI; PAU, India
		BARI Gom 33	-	Conventional breeding	Bangladesh	[58]
		Zincol 2016, NR 419, 421	33.9 ppm Zn,	-do-	Pakistan	CIMMYT
		Zinc Gahun 1, Zinc Gahun 2, Borlaug 2020,	-	-do-	Nepal	CIMMYT
	Fe and Zn	WB2	40 ppm Fe, 42 ppm Zn	Pure line selection	India	IIWBR, India
		HPBW-01	40 ppm Fe, 40.6 ppm Zn	-do-	India	PAU, India
		HI 8777 (durum)	48.7 ppm Fe, 43.6 ppm Zn	Conventional breeding		IARI, India
	Carotene	HI 8627	6–9 ppm	-do-	India	IARI, India
	Anthocyanins	Black-grained wheat	17.71% protein	-do-	China	[59]
		NABIMG-9, NABIMG-10, NABIMG-11	-	Backcross	India	[60]
		Indigo		Conventional breeding	Austria	[59]
Maize	Lysine and tryptophan	Pusa HM4 Improved, Pusa HM8 Improved, Pusa HM9 Improved, IQMH 201 (LQMH 1), IQMH 202 (LQMH 2), IQMH 203 (LQMH 3)	3.62% lysine, 0.91% tryptophan (HM4)	MAS	India	CIMMYT; VPKAS, India; IARI, India
			4.18% lysine	-		
			1.06% tryptophan (HM8)			
		CML140, CML194, P70	-	Selection	China	CIMMYT
		BR-451, BR-473	-	Conventional	Brazil	CIMMYT
		QS-7705	-	Hybrid	South Africa	CIMMYT
		CML176, CML170	-	Selection	Mexico	CIMMYT
	Provitamin A, lysine and tryptophan	Pusa Vivek QPM9 Improved, Pusa HQPM 5 Improved, Pusa HQPM 7 Improved	8.15 ppm provitamin, 2.67% lysine, 0.74% tryptophan	MABB	India	IARI, India
	Provitamin A	Pusa VH 27 Improved	5.49 ppm	-do-	India	IARI, India
		CSIR-CRI Honampa (OPV)	6.2 µg/g	Conventional	Africa	CIMMYT
		Ife maizehyb-3, Ife maizehyb-4, Sammaz 38 (OPV), Sammaz 39 (OPV)	6.3–8.0 µg/g	-do-	Nigeria	CIIMYT

Table 1. *Cont.*

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
Pearl millet	Fe and Zn	HHB 299, AHB 1269Fe, ABV 04, Phule Mahashakti, RHB 233, RHB 234, Dhanashakti	73.0 ppm Fe, 41.0 ppm Zn (HHB 299), 91.0 ppm Fe, and 43.0 ppm Zn (AHB1269), 70 ppm Fe, and 63 ppm Zn (ABV 04)	Conventional	India	HAU, VNMKV, India with ICRISAT; MPKV, India
		Hybrid ICMH 1201 (Shakti-1201)		breeding		
	Fe	AHB 1200Fe	73.0 ppm, 83.0 ppm	-do-	India	VNMKV and HAU in collaboration with ICRISAT
		HHB 311				
		GB 8735 and ICTP 8203 (OPV)	53.60 mg, 55.07 mg	-do-	West Africa	[61]
Sorghum	Fe	ICSR 14001, ICSH 14002	45 ppm Fe and 32 ppm Zn	-do-	India	ICRISAT, HarvestPlus
		12KNICSV (Deko)-188 12KNICSV-22 (Zabuwa)	128.99 ppm Fe	-do-	Nigeria	ICRISAT, HarvestPlus
Finger millet (*Eleusine coracana*)	Fe	VR 929 (Vegavathi)	131.8 mg/kg Fe and 33.2 mg/kg Zn	Pedigree selection	India	ANGRAU, India
	Ca, Fe, Zn	CFMV1 (Indravati),	58.0 ppm Fe, 44.0 ppm Zn, 428 mg/100 g Ca,	-	India	ANGRAU, India; NAU, India
		CFMV 2	39.0 ppm Fe, 25.0 ppm Zn, 454 mg/100 g Ca	-		
Little millet (*Panicum sumatrense*)	Fe and Zn	CLMV1	59.0 ppm Fe, 35.0 ppm Zn	-	India	IIMR, India
Lentil (*Lens culinaris*)	Fe	Pusa Ageti Masoor	65.0 ppm Fe	Conventional	India	IARI, India
	Fe and Zn	IPL 220, L4704, Pusa Vaibhav	73.0 ppm Fe, 51.0 ppm Zn (IPL 220)	-do-	India	IARI India, ICARDA, HarvestPlus
		Idlib-2, Idlib-3	-		Syria	ICARDA, HarvestPlus
		Alemaya	-		Ethiopia	ICARDA, HarvestPlus
		Barimasur-6,	86 ppm Fe and 63 ppm Zn	-do-	Bangladesh	ICARDA, HarvestPlus
		Barimasur-4,	86 ppm Fe and 51 ppm Zn	-do-		
		Barimasur-7	81 ppm Fe and 61 ppm Zn	-do-		
Cowpea (*Vigna unguiculata*)	Fe	Pant Lobia-1,	82 ppm Fe and 40 ppm Zn (Pant Lobia-1), 100 ppm Fe, and 37 ppm Zn (Pant Lobia-2), 67 ppm Fe, and 38 ppm Zn (Pant Lobia-3), 51 ppm Fe, and 36 ppm Zn (Pant Lobia-4)	-do-	India	GBPAUT, HarvestPlus
		Pant Lobia-2,				
		Pant Lobia-3,				
		Pant Lobia-4,				
		Pant Lobia-7				

Table 1. *Cont.*

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
Groundnut (*Arachis hypogea*)	Oleic acid	Girnar 4, Girnar 5	78.4–78.5%	Marker-assisted breeding	India	DGR, India
Linseed (*Linum usitatissimum*)	Linoleic acid	TL 99	58.9% Linoleic acid	Mutagenesis	India	BARC, India
Mustard (*Brassica rapa*)	Erucic acid	Pusa Mustard 30,	1.20%,	Pedigree selection	India	IARI, India
		Pusa Mustard 32	1.32%			
	Erucic acid and Glucosinolates	Pusa Double Zero Mustard 31	0.76% Erucic acid and 29.41 ppm Glucosinolates	-do-	India	IARI, India
Soybean (*Glysine max*)	Kunitz Trypsin Inhibitor Free	NRC 127	-	Marker-assisted backcrossing	India	IISR, India
	Lipoxigenase-2 free	NRC 132	-	Modified marker-assisted backcrossing	India	IISR, India
	Oleic acid	NRC 147	42.00%	Pedigree selection	India	IISR, India
Potato (*Solanum tuberosum*)	Anthocyanin	Kufri Manik,	0.68 ppm,	-	India	CPRI, India
		Kufri Neelkanth	1.0 ppm	Hybridization and selection		
Sweet potato (*Ipomoea batatas*)	Provitamin A	Bhu Sona	14.0 mg/100 g	Pure line selection	India	CTCRI, India
		Kokota, Olympia, Zambezi	-	-	Zambia	CIP, HarvestPlus
		Vita, Naspot 13 O, Ejumula	-	Clonal selection	Uganda	CIP, HarvestPlus
		Beauregard, Resisto, W-119	-	Conventional	USA	[62]
Cauliflower (*Brassica oleracea* var. *botrytis*)	Provitamin A	Pusa Beta Kesari 1	8.0–10.0 ppm	Pure line selection	India	IARI, India
Tomato	Anthocyanin	Sun Black	7.1 mg/100 FW	Conventional breeding	Italy	[62]
		Black Galaxy	-	-do-	Israel	[63]
Greater yam (*Dioscorea alata*)	Anthocyanin, protein, Zn	Sree Neelima	50 mg/100 g anthocyanin, 15.4% protein, and 49.8 ppm Zn	Selection	India	CTCRI, India
	Anthocyanin, Fe, Ca	Da 340	141.4 mg/100 g anthocyanin, 136.2 ppm Fe, and 1890 ppm Ca	-	India	CTCRI, India
Cassava	Vitamin A	NR07/0220-UMUCASS44, TMS01/1368-UMUCASS36	-	-	Nigeria	IITA, HarvestPlus
		Kindisa (TMS 2001/1661); I011661		-	DRC	IITA, HarvestPlus

Table 1. *Cont.*

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
Pomegranate (*Punica granatum*)	Fe, Zn, vitamin C	Solapur Lal	5.6–6.1 mg/100 g Fe, 0.64–0.69 mg/ 100 g Zn, and 19.4–19.8 mg/ 100 g Vit C	Conventional breeding	India	NRCP, India

CIAT: International Center for Tropical Agriculture; IIRR: Indian Institute of Rice Research; NRRI: National Rice Research Institute; IGKV: Indira Gandhi Krishi Vishwavidyalaya; CIMMYT: International Maize and Wheat Improvement Center; IARI: Indian Agriculture Research Institute; PAU: Punjab Agricultural University; IIWBR: Indian Institute of Wheat and Barley Research; VPKAS: Vivekananda Parvatiya Krishi Anusandhan Sansthan; HAU: Haryana Agricultural University; VNMKV: Vasantrao Naik Marathwada Krishi Vidyapeeth; MPKV: Mahatma Phule Krishi Vishwavidyalaya; ICRISAT: International Crops Research Institute for the Semi-Arid Tropics; NAU: Navasari Agricultural University; IIMR: Indian Institute of Maize Research; ICARDA; International Center for Agricultural Research in the Dry Areas; GBPUAT: Govind Ballabh Pant University of Agriculture and Technology; DGR: Directorate of Groundnut Research; BARC: Bhabha Atomic Research Center; IISR: Indian Institute of Soybean Research; CPRI: Central Potato Research Institute; CIP: International Potato Center; NRCP: National Research Center on Pomegranate; IITA: International Institute of Tropical Agriculture; DRC: Democratic Republic of the Congo; MAS: Marker-assisted selection; MABB: Marker-assisted backcross breeding.

3.2. Molecular Breeding

The general procedure for the development of a biofortified variety is the identification and transfer of desirable genes from a donor to a recipient parental line that is agronomically superior via molecular breeding tools. The advancements in molecular breeding programs strengthen and speed up the development of biofortified varieties introgressed with essential minerals that help fight against malnutrition [64]. The molecular dissection of germplasm lines helps in the detection of genes or quantitative trait loci (QTL) associated with micronutrients like β-carotenoids, Fe, Zn, essential amino acids in rice, wheat, maize, and pearl millet (*Pennisetum glaucum*) [10,65–68]. The introgression of these genes or QTL results in the development of cultivars with enhanced nutrient contents [69,70]. With the development of genomics resources, the application of marker-assisted breeding tools has rapidly boomed for biofortification-related gene mapping and their introgression into elite cultivars. Molecular breeding has been mainly applied to staple crops like cereals, pulses, millets, fruits, and vegetables for the development of biofortified varieties [45,60,71] (Table 1). This helps to reduce the generation numbers and allows the screening of a large number of plants at the seedling stage only. This approach can also be used to identify recessive traits in plants that cannot be located by conventional breeding techniques. Furthermore, the identification and validation of genes or QTL vis-a-vis understanding the molecular basis of the accumulation of minerals in grain will facilitate breeding for a high micronutrient concentration with the assurance of their bioavailability in crops through marker-assisted selection (MAS) [46].

3.3. Genetic Engineering

Genetic engineering is the preferred option when there is limited or no genetic diversity related to the essential nutrients in crops [17]. This approach is utilized to transfer and overexpress the desired heritable traits from any unrelated plant species or organisms like bacteria to the staple crops, which may or may not be related to taxonomic and evolutionary aspects. Genetic engineering enables the direct introduction of targeted genes into elite cultivars to boost essential nutrients through two distinct processes: first by modifying the pathway of nutrient uptake and utilization and second by increasing nutrient bioavailability or decreasing anti-nutritional factors [63]. Several key factors are required for successful genetic engineering of a targeted gene, such as reliable tissue culture and regeneration methods, the development of gene constructs with suitable promoters, efficient transformation methods, and multiplication and characterization of transformed plants for introduced traits by conventional breeding methods [72]. There are several approaches, such as overexpression, gene stacking, RNA interference (RNAi), and clustered regularly interspaced short palindromic repeats (CRISPR) or CRISPR-associated protein-9 nuclease (Cas9)-mediated genome editing, for regulating the gene of interest's expression. Novel target-specific genome editing methods, viz. zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9, have shown brilliant results in several crops' biofortification [73], such as rice [74], wheat [75], and tomatoes (*Solanum lycopersicum*) [76] (Table 2). They possess the immense potential to develop biofortified varieties within less time and cost [77,78]. Recent advancements in biotechnological approaches have enabled the development of a large number of commercial crop varieties through genetic engineering with increased essential micronutrients, minerals, fatty acids, and amino acids [45], such as Fe-dense rice [79], wheat [80], and sorghum (*Sorghum bicolor*) [81], which have helped improve the human health status through enhanced nutrition [17] (Table 2). In the case of some micronutrients like Fe and Zn, their absorption is vulnerable due to anti-nutritional factors like phytic acid. Hence, the genetic modification of their pathway has helped by increasing Fe absorption or decreasing anti-nutrient factors [82,83]. Genetic engineering also allows the development of multi-nutrient-enriched varieties through the inserting of a single DNA cassette, in addition to improving the post-harvest stability of vitamins, along with favorable agronomic traits and biotic or abiotic stress resilience. Recently, multiple micronutrient contents (i.e., Zn, Fe, and β-carotene) have been simultaneously increased in rice through introgressing a single DNA fragment [84]. Similarly, Zhao et al. [85] targeted lysine, vitamin A, Fe, and Zn bioavailability in sorghum through genetic transformation. Hence, this approach facilitates new perspectives for developing multi-nutrient-dense crop cultivars in a single step. In this direction, metabolic engineering applications will provide a leap forward by designing strategies to jointly target different micronutrients, taking into account their stability. In this process, it is also required to consider the undesirable consequences of micronutrients' impacts on other traits, for which a number of policy interventions are proposed for their regulation.

Table 2. Genetic engineering approaches for the development of biofortified varieties.

Targeted Crop	Targeted Nutrients	Gene	Donor Organism or Technique	References
Rice	Fe	*AtIRT1*, *AtNAS1*, *PvFER*	Arabidopsis, common bean	[86,87]
	Fe	*Soyfer H-1*	Soybean	[88]
		Phaseolus ferritin	Common bean	[89]
	Fe, Zn, β-carotene	*AtNAS1*, *PvFERRITIN*, *CRTI*, *ZmPSY*	Arabidopsis, common bean, maize	[84]
	Vitamin A	*Phytoene synthase (PSY)*, *phytoene desaturase (CrtI)*	Daffodil, *Erwinia uredovora, maize*	[90,91]
	Zn	*HvNAS1*	Barley (*Hordeum vulgare*)	[92]
		Ferritin, phytase, *OsNAS1*	Soybean, *Aspergillus flavus*, rice	[93]
	Methionine and cysteine	*Sulfur-rich protein*, *S2SA*	Sesame (*Sesamum indicum*)	[94]
	Lysine	*lysC*, *dapA*	Bacteria	[10]
Wheat	Vitamin A	*psy1*, *crtI*, *CrtB+ Crtl*	Maize, bacteria	[95,96]
	Fe	*Ferritin*	Soybean	[97]
		TaFer1 and *TaFer2*	Wheat	[98]
	Low-phytate	*phyA*	*Aspergillus niger*	[99]
	Low-phytate	*phyA*	*Aspergillus japonicus*	[83]
	Amylose	*SBEIIa*	Wheat	[100]
	Anthocyanin	*Dhn12*, *Itr1*, and *Ltp1*	Barley	[101]
Maize	Carotenoid	*crtI*	Bacteria	[102]
	Vitamin E	*HGGT*	Barley	[103]
	Vitamin A or multivitamin	*crtB* and *crtI*, *psy1*	Bacteria	[104]
	Fe	*lpa1-1*, *ferritin*	Maize and soybean	[105]
	Low-phytate	*phyA2*	*Aspergillus niger*	[106]
		MRP ATP-binding cassette	Maize	[107]
	Lysin and total protein	*sb401*	*Solanum berthaultii*	[108]
	Lipid, protein (lysin) and starch	*AtGIF1*, *OstGIF1*, *ZmGIF1*	Arabidopsis, rice, maize	[108]

Table 2. *Cont.*

Targeted Crop	Targeted Nutrients	Gene	Donor Organism or Technique	References
Sorghum	Carotenoids	-	-	[109]
	Lysin, vitamin A, Fe and Zn	*PSY1, CRTI, At-DXS HGGT*	Maize, *Pantoea ananatis*, Arabidopsis, barley	[85]
	Asparagine content	ENGase	CRISPR/Cas9	[110]
Soybean	Amino acid	*MB-16*	Soybean	[111]
	β-carotenoid	*PSY*	*Pantoea ananatis*	[112]
		PAC	*Capsicum* and *Pantoea ananatis*	[113]
	Vitamin E	*At-VTE3*	Arabidopsis	[114]
	Sulfur	*Zein*	Maize	[115]
Common bean (*Phaseolus vulgaris*)	Methionine and cysteine	*uidA* and *be2s2*	-	[116]
Potato	Beta carotene	*Or*	Cauliflower	[117]
	Vitamin C	*GalUR*	Strawberry	[118]
	Methionine and anthocyanin	*CgS*, *PAL*	Arabidopsis	[119]
	Methionine	*StMGL1*	*Solanum tuberosum*	[120]
	Phenolic acids and anthocyanins	*CHS*, *CHI*, *DFR*	Barley and *Petunia hybrida*	[121]
Cassava	Fe	*Vascular iron transporter VIT1*, *iron transporter IRT1*, *ferritin*(*FER1*)	Arabidopsis	[122]
	Beta carotene Provitamin A	*PSY*, *CrtI*, *nptII*, *crtB* and *DXS*	*Pantoea ananatis*	[123,124]
Linseed	Flavonoid	*CHS*, *CHI*, *DFR*	*Petunia hybrida*	[125]
	Carotenoid	*crtB*	*Pantoea ananatis*	[126]
Canola (*Brassica napus*)	Carotenoid	*crtB*, *crtE*, *crtZ*, *crtY*, *crtI*, *crtW*, and *idi*	*Pantoea ananatis* and *Brevundimonas* sp.	[127]
	Lysine	*AK* and *DHDPS*	*Corynebacterium* and *Escherichia coli*	[128]
	Fatty acids	*Ch FatB2*	*Cuphea hookeriana*	[129]
Tomato	*β*-carotene	*β-Lcy*	Arabidopsis	[130]
		β-cyclase	*Erwinia herbicola*, *Narcissus pseudonarcissus*	[131]
	Astaxanthin	-	*Chlamydomonas reinhardtii* and *Haematococcus pluvialis*	[132]
	Xanthophyll	*b-Lcy*, *b-Chy*	Arabidopsis and pepper	[133]
	Iodine	*HMT*, *S3H*, and *SAMT*	*Solanum lycopersicum* L.	[134]
Camelina sativa	Low polyunsaturated fatty acids	*FAD2*	Targeted mutagenesis by CRISPR/Cas9	[135]
Tobacco	Protein	*XylT*, *FucT*	CRISPR/Cas9	[136,137]

3.4. Agronomic Biofortification

For hundreds of years, mineral fertilizers have been applied to plants or soil to facilitate the increased nutrients of crop plants. Based on a similar principle, the agronomic biofortification approach has been used to enrich cereal grains with minerals [138]. It is commonly believed that the application of mineral nutrients from external sources advances their concentration in developing grains as well as improving soluble and mobilizable mineral elements in the soil. In developing nations, particularly in Africa and Asia, agronomic biofortification is the fastest and easiest method to supply food grains with Zn, Fe, or additional essential micronutrients for the human body [139]. Agronomic biofortification, like supplements and fortification, is probably best used in a specific situation or in conjunction with other strategies. It is used as a foliar application when minerals cannot be easily translocated to edible tissues [46]. This approach has been adopted worldwide due to its straightforwardness and timeliness. Pre-harvest agricultural practices that increase the nutritional value of food are supported by following such approaches [140].

This method incorporates the use of organic manures, synthetic fertilizers, and biofertilizers, as well as seed priming via soil or foliar application [141]. According to Zou et al. [142], the foliar Zn application in wheat biofortifies the wheat grains with Zn without reducing yields. Similarly, Zhang et al. [143] also investigated the impact of varying Zn fertilizer placement in maize roots, leading to Zn accumulation in maize plants and an enhanced grain Zn content of up to 51%. Adding phenylalanine to spinach increased the folate content twofold, reflecting 76.5% of the recommended daily allowance for adults [144]. Through the agronomic biofortification process, the Si (silicon) content of pods increased almost threefold without affecting the yield and appearance of the product [145]. Similarly, Barrameda-Medina et al. [146] suggested that supplementation with Zn at 80–100 μM is ideal for healthy plant growth, as well as for enhancing the concentration of Zn in the edible part of cauliflower. Zou et al. [147] observed the increased concentrations of multiple nutrients such as iodine (I), Zn, Fe, and Se simultaneously after their foliar application as a cocktail solution on wheat grain. It demonstrated that agronomic biofortification is also an effective strategy to biofortify any crop with multiple nutrients simultaneously without any yield trade-off. Similarly, Prom-U-Thai et al. [148] also studied the effects of micronutrient cocktails composed of Zn, I, Fe, and Se on rice through a foliar application in five countries: India, China, Brazil, Pakistan, and Thailand. The results showed that irrespective of the rice varieties and variable soil conditions in different countries, there was a significant increment in the Zn, I, and Se concentrations. Sahin [149] attempted the combined biofortification of lettuce with I, Se, and Zn, evaluating their effects on essential and non-essential elements, and found a significant increase in the Se and Zn concentrations in the leaves. Thus, the adoption of this approach would significantly boost daily micronutrient intake and help in combating micronutrient malnutrition. The application of these inorganic fertilizers has a few disadvantages: it increases the cost of food, limiting its availability to poor populations, and causes environmental degradation [150]. Hence, being eco-friendly and cost-effective, the application of organic fertilizers is another of the most sustainable approaches to biofortifying crops. Ramzani et al. [151] conducted an experiment to test the Fe biofortification of wheat using biochar (BC), poultry manure (PM), and normal and sulfur-treated low-pH calcareous soil. The results showed that with Fe-applied BC, the concentrations of Fe and ferritin in the grains increased by 1.4 and 1.2 times, respectively, while the polyphenol and phytate concentrations were reduced by 44% and 35%, respectively, over the controls. In contrast to organic and inorganic fertilizers, biofertilizers contain microbial inoculants, which provide plants with growth- and productivity-enhancing microorganisms [152]. Ramesh et al. [153] concluded that the *Bacillus aryabhattai* strains MDSR14 and MDSR7 considerably enhanced Zn mobilization and its content in wheat and soybean, and hence they could be utilized as suitable bioinoculants for biofertilization and biofortification.

Seed priming is another method of biofortification, in which the seeds are soaked in nutrient-rich solutions before planting. Crops are typically seed-primed to improve germination, seedling establishment, and robust root systems and yield [154]. It is a low-cost and simple method for increasing nutrient availability for farmers [155]. According to Praharaj et al. [156], the Zn concentration in wheat grains improved significantly after the seeds were primed with different concentrations of a zinc sulfate heptahydrate solution.

4. Current Efforts, Achievements, and Future Possibilities in the Biofortification of Food Crops

In the dawn of the 21st century, biofortification is an attractive tactic to achieve nutritional security, thereby reducing hidden hunger [157]. At present, HarvestPlus, the Biocassava project, and the National Agricultural Research Organization (NARO) are the major projects initiated for nutritional security via the development of biofortified varieties. Through the partnership of several programs and projects such as HarvestPlus, Reaching

Agents of Change (RAC), Sweet Potato Action for Security and Health in Africa (SASHA), and Building Nutritious Food Baskets (BNFB), biofortified crops have been developed, distributed, and promoted across the nation [158]. Recently, in light of biofortification, the Indian Council of Agricultural Research (ICAR) started a consortia research platform on "Biofortification in Selected Crops for Nutritional Security", where the main attention was given to cereals and millets for nutrient enhancement [159]. Currently, several research programs are carried out for the identification of genetic loci or genomic regions associated with traits related to biofortification, followed by introgression of identified genes or QTLs to accelerate the breeding program [150,160–163]. Several QTLs and single-nucleotide polymorphism (SNPs) have been identified in different crops controlling essential micronutrients such as Fe, Zn, low phytate, vitamins, and amino acids [164–166]. Research has been conducted toward dissection of an anti-nutritional factor whose presence led to reduced bioavailability of essential nutrients in crops. For example, the absorption of Fe and Zn is affected by phytic acid [167]. However, in crops where there is no availability of genetic variation, the transgenic approach makes a significant contribution. Most of the crops have been targeted through genetic engineering, but the practical utility is minimal in farmers' fields and the human diet. For example, golden rice enriched with vitamin-A was developed a long way back in 2005, and after a lengthy and tiring procedure, it received approval in 2018 [168]. Among all the micronutrients, major biofortification research is carried out on Fe, Zn, β-carotene, and essential amino acid increments of crops, which is economical and practical for 90% of the world's population [45,169–171]. Since 2001, when the concept of biofortification was practically utilized, the target populations were first identified from 2003 to 2008. The biofortified crops were first bred and released after conducting nutritional efficacy trials and delivery plan development between 2009 and 2013. Since then, more than 140 biofortified varieties for 10 major staple crops have been released in about 30 countries and are under consideration for production in another 60 countries [172]. In many countries, like Brazil, India, and China, several biofortified varieties are released and used each year. In 2020 alone, India released 17 biofortified varieties for 8 crops (rice, wheat, maize, finger millet, little millet, mustard, groundnut (*Arachis hypogaea*), and yam) on the occasion of the 75th anniversary of the Food and Agriculture Organization (FAO), containing more than 1.5–3.0 times extra nutrition than the conventional varieties [45,170,171].

5. Impact of Biofortified Crop Cultivars in the Alleviation of Human Malnutrition

Biofortified crops are nutritionally dense in comparison with non-biofortified crops, with assumptions of similar micronutrient bioavailability [173] and retention after cooking, processing, and storage, so the consumption of biofortified staple crops improves the total micronutrient intake. Currently, over 20 million people have included biofortified food crops in their diets across the world [174]. The deployment and consumption of biofortified varieties has demonstrated positive effects on human health and wellbeing. However, the assessment of biofortified crops' impact on humans is tedious, as it is difficult to measure their effects in controlled conditions, but there are several studies attempting to study their effect on human health. The consumption of Fe-enriched biofortified crops like rice has increased the Fe stores in potentially pregnant women in the Philippines [175]. Fe-biofortified pearl millet has enhanced the Fe level in Indian school children, overcoming Fe deficiency [176], and Fe-biofortified beans have improved the Fe stores in Rwanda women [177]. It was assessed that the interpretation of the pro-vitamin A effect was more difficult, as the pro-vitamin A carotenoids are first engrossed by the body and then converted into vitamin A's active form as per the body's need. A few case studies were conducted to analyze the effect of the consumption of pro-vitamin A biofortified sweet potatoes, which overcame the vitamin A deficiency in Mozambique [178,179], South Africa [180], and Uganda [179], while in Bangladesh, a base experiment showed an increased pro-vitamin A concentration but no increment in vitamin A's status [181]. Consumption of pro-vitamin A-enriched yellow cassava increased the vitamin A status and pro-vitamin A concentrations in Kenyan school children [182]. The study related to the consumption of biofortified orange maize produced ambiguous results because of the difference in sensitivity and accuracy of the laboratory tests used. Hence, two independent studies carried out in Zambia showed that the pro-vitamin A concentrations increased in both, but vitamin A did not increase in either of the studies, possibly due to the very low level of VAD initially and the use of a more sensitive test to assess the vitamin A concentration [183]. Another study to see the effect of pro-vitamin A-rich maize feeding in children in Zambia significantly improved serum xanthophylls and retinol [184,185]. The positive effects of QPM have been demonstrated globally, as consumption of a QPM maize diet decreased the sick days among children in comparison with consumption of normal maize. The QPM consumption increased weight and height gain by 12% and 9% in young children, respectively, when compared with a control group fed only regular maize [186], and 100 g of QPM was sufficient to meet the lysine requirement in children [187]. Hence, all the studies provide sufficient evidence that the consumption of biofortified crops shows improvements in the human health status, and thus the development and promotion of biofortified crop cultivars would be helpful to achieve the SDGs by eradicating malnutrition [188]. Furthermore, with each biofortified crop variety developed, there is a need to conduct more of such trials in different population groups to accumulate evidence of its positive impact on the micronutrient status increments in humans.

6. Cost-Effectiveness and Monetizing Benefits of Biofortification

Through biofortification, the majority of the world's population is dependent on a single staple food and cannot afford a diverse diet to fulfill all nutrient requirements. Hence, through biofortification, basic food crops like wheat, rice, maize, and beans are enriched with deficient micronutrients [5]. It mainly targets the rural population, where food production and consumption will stay in the community, on the farm, or locally, and unlike commercial fortified food, there is no need to purchase the product repeatedly. Thus, developing and disseminating biofortified varieties requires only a one-time investment.

The evidence of biofortified crops' impact on public health has shown their benefits and positive effects on different populations of human groups, studied by conducting trials under controlled settings. In addition to the health benefits, it is also equally important to consider how biofortification is economically efficient, as it is a long-term process that includes huge research and developmental activities. The assessment of the cost-effectiveness of biofortification is a tedious task, as it varies with the type of biofortification attempted in different crops and countries (Figure 3). The Disability-Adjusted Life Years (DALYs) framework is mainly used to determine the cost-effectiveness by considering both mortality and morbidity results in a single analysis. The benefits are quantified using the number of DALYs saved and the costs per DALY saved to provide a constant way of ranking different interventions. However, the DALYs framework used to assess the cost-effectiveness is very data-intensive, and the calculations are based on many assumptions, which generate a certain level of uncertainty in the assessment.

Several studies have shown that biofortification leads to a decrease in the burden of micronutrient deficiency [189]. Furthermore, it has to decipher how much the biofortification process costs to achieve these reductions in burden. For crop biofortification, there are initial costs for basic breeding and research activities to develop the micronutrient-enriched biofortified lines, followed by marginal costs for testing, adaptive breeding, maintenance breeding, dissemination, and extension activities. According to the World Bank Report for 1993, any public health interventions costing less than USD 150 per DALY saved are highly cost-effective [190].

Ex ante evaluations of biofortified crops such as pro-vitamin A-enriched cassava, maize, and sweet potato from Nigeria, Brazil, Ethiopia, Kenya, and Uganda, as well as Fe- and Zn-enriched beans, rice, and wheat from Honduras, Nicaragua, Brazil, Bangladesh, India, the Philippines, and Pakistan, revealed that the majority of the costs per DALY saved for biofortification fell into the highly cost-effective category [191].

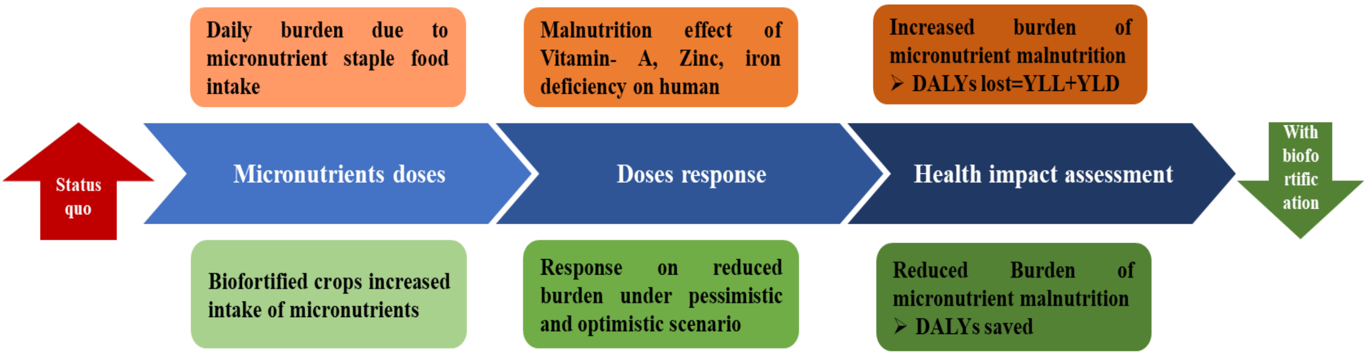


Figure 3. Impact assessment of biofortified crops on human health and cost-effectiveness. Abbreviations: DALYs = Disability-Adjusted Life Years; YLL = years of life lost; YLD = years lived with disability.

Similarly, many ex ante-based analyses have been undertaken to assess the cost-effectiveness of rice biofortification for folate [192], vitamin A [193,194], Fe [195], or Zn deficiency [196]. Under pessimistic assumptions, rice biofortification could reduce the particular micronutrient deficiency burden by 9% (vitamin A) to 19% (Fe) at a cost of less than USD 20 per DALY saved [197], while under optimistic assumptions, the impact and cost-effectiveness could be much higher [198]. Stein et al. [199] assessed the costs of biofortification of rice and wheat and concluded that to save one healthy life year, it costs just USD 0.36 for Fe biofortification in India, clearly making it a very cost-effective intervention. The dissemination of multi-biofortified rice with folate, pro-vitamin A, Zn, and Fe would save one DALY for USD 2.30 under optimistic assumptions and USD 9.60 under pessimistic assumptions, demonstrating that multi-biofortified rice is highly cost-effective [189]. Biofortification of orange-fleshed sweet potatoes has demonstrated its cost-effectiveness through its per DALY saving of USD 20 in Uganda [174]. According to estimates, one US dollar invested in biofortification could yield USD 17 in benefits. The cost-effectiveness of any nutrition program intervention, however, varies depending on the crop, micronutrient, and delivery country [174]. Thus, all these promising results encourage biofortification programs to be extended on a larger scale to reduce any form of malnutrition. In comparison with the other strategies, the impact of biofortification on human health and its cost-effectiveness strengthens its potential role in fighting micronutrient malnutrition. Still, the success of biofortification largely depends on whether the biofortified germplasm is in the public domain. In addition, it must be easily accessible and accepted by consumers and farmers from developing countries in their regular diets, which in turn vary region-wise.

7. Policy Support to Promote Biofortified Cultivars

Strengthening the seed supply chain to manufacture and stream high-quality seeds is a key step toward the widespread adoption of biofortified crop varieties. Subsidized seed and other inputs would further contribute to the quick spread of nutritionally enhanced cultivars among farmers [200]. Farmers will be encouraged to grow more biofortified crops if they are assured of a remunerative price through a minimum support price or a premium price for biofortified grains on the market [159]. India's "National Nutrition Strategy" was recently unveiled by the National Institution for Transforming India (NITI) Aayog to reduce malnutrition in the country through food-based solutions [159]. Incorporating these biofortified cereals into government-sponsored programs such as the National Food Security Mission (NFSM) and Rashtriya Krishi Vikas Yojana (RKVY), as well as nutrition intervention programs such as the Integrated Child Development Service Scheme, the mid-day meal, nutrition education, and training through community food and nutrition extension units would help to provide the deprived population with much-needed awareness about balanced diets. In addition, initiatives like the National Nutrition Mission, Nutri-Sensitive Agricultural Resources and Innovations (NARI) program, and Nutri-Smart

Villages to enhance nutritional security have been started to encourage diversified diets at the community level. In South Asian countries, the program (i.e., the South Asia Food and Nutrition Security Initiative (SAFANSI)) was designed to address the South Asian malnutrition enigma through strengthening innovative actions to improve food and nutritional security. HarvestPlus, in partnership with various private companies, has scaled up biofortification in low- and middle-income South Asian countries. In Sub-Saharan Africa, government policies such as the National Multi-Sectoral Nutrition Action Plan (NMNAP), Tanzania Food and Nutrition Centre (TFNC), and Food and Nutrition Security Policy have been implemented, including biofortified crops as an important component in agriculture.

The NMNAP specifically aims to promote the cultivation and consumption of biofortified high-protein maize and cassava as well as vitamin A-enriched orange-fleshed sweet potato and bananas by focusing on the multiplication and distribution of seeds, seedlings, and cuttings of nutrient-dense crop varieties among farmers. Moreover, under the National Food Fortification Alliance (NFFA), the National Biofortification Task Force was formed to advocate for biofortification projects to alleviate malnutrition in Tanzania, while in Nigeria, projects like Working to Improve Nutrition in Northern Nigeria (WINNN) and the Rainbow Project played a significant role in including biofortified crops in national plans and policies [201].

Africa has implemented various policies and strategies such as the Pan African Nutrition Initiative, Africa Ten-Year Strategy for Vitamin and Mineral Deficiencies, Africa Regional Nutrition Strategy, Framework for African Food Security, Regional Economic Communities Nutrition Strategies–Southern African Development Community, West African Health Organization, and New Partnership for Africa's Development (NEPAD) Food and Nutrition Security strategy to address the micronutrient deficiency and hidden hunger issues as a whole [158]. In 2012, the "Feed the Future" program was launched to introduce biofortified sweet potato cultivars, in association with the U.S. Agency for International Development (USAID), HarvestPlus, and the Ugandan government [202]. Incorporating biofortified crops into these government-sponsored programs would especially benefit lactating children, elderly people, and pregnant women, aside from increasing their dissemination to the larger mass. Significant government policies supporting the use of several innovative initiatives would further improve the uptake and acceptance of biofortified crops. There is a need for partnerships between the private and public sectors to support the development of proven biofortified technologies. It is necessary to promote biofortified crops through seed markets and to incentivize them with premium prices to encourage farmers and seed companies to invest in biofortified crop production and development.

8. Constraints and Challenges of Crop Biofortification

To develop biofortified crop cultivars, the agronomic approach using micronutrient-fortified fertilizers is the simplest method, but it is highly variable due to the changing behavior of mineral transportation and accretion among different crop plants, in addition to variable soil compositions at different geographical locations. In addition, it is a cost- and labor-intensive approach, as it needs continuous inputs of micronutrients for the plant and soil regularly [12,203]. Furthermore, many times the micronutrients were accumulated in the non-edible portions of plants like leaves instead of the seeds or fruit, so this methodology is effective in certain specific plant species and minerals. In addition, the biggest drawback of this method is the adverse effects on the environment due to the over-application of fertilizers, which leads to their accumulation in soil and water reserves [204].

To biofortify crops, conventional breeding programs are the most successful and sustainable solution in the long run, but these are very time-consuming and require large genetic variability in the plant gene pool for improvement of micronutrient traits. Hence, for many traits like oil quality improvement or the Se increment, conventional breeding is not a successful approach due to limited variability, lower heritability, and linkage drag for these traits. For micronutrient traits, several genes are involved in controlling the mineral elements that are variable in different genetic and environmental backgrounds, so their estimation and introgression is a tedious task. Molecular breeding approaches seem to be an appropriate choice for more reliable and speedy selection, but the extensive literature review showed that only in a few major staple crops like rice, wheat, and maize were some varieties developed through marker-assisted breeding, as shown in Table 1. To overcome the limitations of conventional breeding methods, the transgenic option seems most viable for expanding the diversified genetic reservoir, but it has major limitations related to regulatory processes and mass acceptance [205]. Different countries have adopted various regulatory processes, which are both costly and time-consuming. Additionally, current politicians and environmentalists are not supportive of this method. In comparison with conventional breeding, transgenic development requires much higher efforts in research with a lower success rate of variety release, such as in the case of golden rice, as after 8 years of intensive research and publication in 2000, it received approval from governmental authorities in 2018.

There is also a need to optimize the post-harvest processing of biofortified crop cultivars to reduce the loss of large quantities of minerals during the milling and processing of the product [206]. The presence of certain anti-nutrients in crops like phytate, fibers, oxalate, tannins, and hemagglutinins reduces the bioavailability of certain micronutrients in the human body [207]. Hence, in addition to enriching the cultivars with micronutrient concentrations in the edible portions of crops, the amount of micronutrients absorbed by the consumers after cooking and processing should also be estimated [175]. The biofortified crop varieties should be agronomically equivalent or superior to the traditional varieties (i.e., they should be higher in yield and tolerance to biotic and abiotic stresses to compete with the already existing varieties so that farmers can accept or adopt them [47]).

The introduction of some nutrients like pro-vitamin A carotenoids will impart color to foods, so consumer acceptance will be affected when buying and eating such products as orange-fleshed maize, cassava, or sweet potato. Therefore, to motivating them to buy these products will be a challenging task. In addition, some sensory tastes will also be changed with increased pro-vitamin A concentrations. Hence, creating awareness and providing proper information about the health benefits associated with these biofortified crops will only change consumers' mindsets. Most biofortified crop cultivars are mostly assumed to be genetically modified crops, and most efforts are also using genetic modification or transgenics to biofortify the staple food crops. Hence, a functional regulatory framework to assess their benefits and risks to a larger extent is very much needed to build consumers' confidence in biofortified crops developed through genetic modifications, in addition to their education and awareness.

9. Conclusions and Future Perspectives

To alleviate malnutrition, biofortification is the most sustainable and cost-effective methodology to enrich the nutritional status of crops, which will improve the health of malnourished people across the world. Biofortification approaches through plant breeding, transgenic, and mineral fertilizer applications have great potential for addressing micronutrient malnutrition. However, this is a very challenging endeavor, so to achieve this, collaboration among different subject specialists like agronomists, plant breeders, biotechnologists, genetic engineers, and nutritionists is indispensable. Despite conventional breeding, transgenics are given more weight to biofortify crops, which subsequently face hurdles in regulatory processes and consumer acceptance. It was found that only 2.4% of transgenic biofortified rice varieties have been released, which shows that these crops still face a rigid regulatory obstacle. The developed varieties must be included in the seed chain to strengthen the formal and informal farming systems to produce and supply biofortified varieties. This would lead to a reduction in the hunger index and nutrition security achievement for a large group of people. Multi-biofortification also appears to be an efficient approach for introducing multiple micronutrients simultaneously into a cultivar, rather than the traditional way of introducing several biofortified crops or varieties with a single micronutrient to eradicate all forms of malnutrition. By contrast, multi-biofortified varieties could potentially achieve higher combined coverage and cost reductions through substantial savings in research and regulatory costs. Many countries are implementing biofortification of crops as a technique to eliminate micronutrient deficiencies and consequently enhance human health. Thus, to strengthen the biofortification program, future research should be focused on (1) integrating agronomic and genetic strategies to promote mineral transport to phloem-fed tissues and (2) identifying the mechanisms affecting mineral homeostasis in plant cells to increase micronutrient concentrations in edible crops. There is a need to establish communication and marketing strategies that consider ethical values when it comes to the production and use of biofortified staples. The same tactics may not be helpful in all nations to make it acceptable and persuade people to pay for micronutrient-enriched food. As a result, the target countries should be guided to use strategies that are beneficial to their people. Therefore, intensive efforts need to be made by the public sector to prepare the policy and guidelines for the promotion of acceptance of biofortified varieties by consumers. Among all the challenges, biofortification still holds huge potential for facilitating healthy food options to billions of people across the world, solving malnutrition problems through regular interventions of sustainable techniques or methodologies for hunger and a malnutrition-free world.

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