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Enriching field crops with micronutrients through biofortification is a cutting-edge new approach to addressing the food insecurity

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Abstract
Globally, nutrition deficiency is of the major key factor, and around the world about more than 3 billion people were suffering from micronutrient deficiency. The yield barriers were fulfilled by the green revolution; however, the nutritional quality of the developed crops is affected. Biofortification is a feasible and cost-effective means of addressing micronutrients to these populations. This review summarizes various strategies of biofortification, methods, varieties released and the pros and negatives of the technique.

Keywords: Biofortification, green revolution, micronutrients, nutrition

1. Introduction
In day-to-day life**,** a total of 49 dietary nutrients are essential, which include nine amino acids (isoleucine, leucine, histidine, methionine, phenylalanine, lysine, threonine, tryptophan and valine), two fatty acids (linoleic acid and linolenic acid), seven macro-elements (N, Na, K, Ca, Mg, S, P and Cl), 16 micro-elements (Fe, Zn, Mn, Cu, I, B, F, Se, Mo, Ni, Cr, Si, V, As, Sn and Co) and 13 vitamins (A, D, E, K, C, B_1, B_2, B_3, Niacin, B_6, Folate, Biotin, B_{12} (Rehman *et al.*, 2019) [24]. The only source of sulphur to the human beings is through the dietary intake of cysteine and methionine-containing foods (Nimni *et al.*, 2007) [19]. Deficiencies of these minerals and vitamins in humans, affect a high proportion of the world's population, especially in developing countries (Stein *et al.*, 2010) [27]. Due to the inadequate consumption of a balanced diet, malnutrition results in poor health, a reduction in immunity to various diseases and a significant loss in annual Gross Domestic Product (GDP) (Yadava *et al.*, 2018) [35]. This malnutrition is commonly termed "hidden hunger". According to FAO, 2019, in the globe more than 820 million people are facing hidden hunger and undernourishment, particularly in developing countries. This hidden hunger can be alleviated by Biofortification.
Biofortification is a process by which the density of the essential dietary nutrients is increased through plant breeding techniques, modern agronomic practices and advanced transgenic techniques. Another alternative for increased density of nutrients is food fortification, however, it relays on external funding, expensive and there is no idea of long-term health benefits of these food supplements (Garg *et al.*, 2018) [9]. Therefore, biofortification that depends on different crop varieties is the long-term sustainable solution for rich dietary supply to the people. As per the reports of many global food hunger agencies, still, there are lots of countries are there where food hunger and malnutrition are still very big problems. The agriculture scientists and breeders have got great success in developing high-yielding varieties, but we have reached up to its plateau. Even continuous breeding efforts for developing higher-yielding varieties by speed breeding, molecular breeding, genetic engineering *etc*. have improved yield up to a certain level and countries became self-reliant. But on another hand still, we are in search of food that can satisfy the basic nutrition requirements of millions of hungry people all around the globe (Table 2). So, Biofortification may help in providing nutritional security besides providing food security.

2. Methods of biofortification
2.1 Agronomic biofortification
Agronomic fortification is an external application of nutrient-rich fertilizers to foliage or soil

to increase the micronutrient concentration in the edible parts of the crop and thus increase the intake of essential micronutrients by consumers.

Table 1: Various bio fortified nutrients, their functions and requirement on daily basis

Nutrient	Function	Nutrient requirement on daily basis	Source food of nutrient	References
Protein	It provides essential amino acids for growth and tissue repair. Its deficiency leads to poor intellectual development, disorderly physical functioning and even mortality. Diet deficient in protein leads to kwashiorkor and marasmus disorders among humans	0.83g protein/kg	Pulses & Milk	Campbell *et al.*, 2008 [5]
Vitamin A	Vitamin A, also known as Retinol, is important for visual health, immune function, foetal growth and development.	700 µg per day for men and 600 µg per day for women	Green leafy vegetables,	Penniston *et al.*, 2003 [21]
Vitamin C	Vitamin C, also known as ascorbic acid, helps in protecting cells and keeping them healthy, maintaining healthy skin, blood vessels, bones and cartilage. It also aids in wound healing. Lack of vitamin C can lead to scurvy disease	90 mg/d for men and 75 mg/d for women	Citrus, lemon	Valdés F 2006 [30]
Iron	Iron is important in making red blood cells which carry oxygen around the body and its deficiency results in anaemia	Men over age 18 need 8.7mg/day and woman between 19-50 age need 14.8mg/day and women over age 50 need 8.7mg/day	White beans	Ghosh *et al.*, 2019 [29]
Zinc	Zinc helps in making new cells, enzymes, wound healing, processing carbohydrates, fat and protein. Severe deficiency results in dermatitis, retarded growth, diarrhoea, mental disturbance and delayed sexual maturation	Men of age 19-64 need 9.5mg/day and women require 7mg/day	Mushroom & lentils	Maret and Sandstead, 2006 [17]
Lysine	Lysine is the building block in protein synthesis. Deficiency of lysine leads to fatigue, dizziness, nausea, anaemia, delayed growth, loss of appetite and degeneration of reproductive tissue	30 mg/kg body weight/day for adults and 35 mg/kg body weight/day for of 3–10-year-old children	Maize	Millward *et al.*, 2012 [16]
Tryptophan	Tryptophan is an essential constituent of the diet. It plays an important role in protein synthesis and it is also precursor of a variety of biologically active compounds like serotonin, melatonin, tryptamine, quinolinic acid and kynurenic acid. Its deficiency leads to depression, anxiety and impatience. Weight loss and slow growth in children are the major symptoms of tryptophan deficiency	5-10mg/day	Maize (waxy corn)	Lindseth, *et al.*, 2015 [14]
Anthocyanin	These are pigments that give red, purple, and blue colours in plant parts. Anthocyanins act as antioxidants and help in removing harmful free radicals produced inside the body. Anthocyanin possesses antidiabetic, anticancer, anti-inflammatory, anti-microbial, anti-obesity effects, as well as plays a role in prevention of cardiovascular diseases	12.5mg/day	Vegetables	Mattioli, *et al.*, 2020 [18]
Oleic acid	It is a monounsaturated fatty acid present in oil. Monounsaturated fat in the diet is associated with decreased low-density lipoprotein (LDL) cholesterol and reduced risk of coronary heart disease	Oleic acid intake recommendations are based on the recommendations for intakes of total fat (around 30% of the total energy), maximum intakes of saturated fatty acids (10% or less), and minimum and maximum intakes of PUFA (6–10% of the energy). Therefore, dietary intake of oleic acid should be therefore in the range of 10–15%	Ground nut	Obici *et al.*, 2002 [20]
Linoleic Acid	It is a polyunsaturated fatty acid present in oil. It reduces total and LDL cholesterol, therefore good for cardiovascular functions	The daily requirement of the parent omega-3 fatty acid LA is 17-20 g for men and 12-13 g for women	Linseed	Liou YA *et al.*, 2007 [15]
Euric acid	It is a monounsaturated fatty acid found in rapeseed and mustard oil. High concentration of erucic acid in edible oils impairs myocardial conductance, causes lipidosis in children and increases blood cholesterol	<2.0 per cent of erucic acid in oil is desirable for health	Rape seed, mustard	Yadava *et al.*, 2018 [35]
Calcium	Calcium helps in building strong bones and teeth and regulates muscle contraction. It also helps in clotting the blood normally, and its deficiency causes rickets	Adults aged 19-64 need 700mg of calcium per day	Spinach, Cowpeas okra	Ross *et al.*, 2011 [25]

Table 2: Various crops and their bio fortified varieties released in 2021 on the occasion of the 75th anniversary of FAO

Crop	Variety	Nutrient	Base level	Achieved level	Yield
Rice	CR Dhan310	Protein	7-8%	10.3%	45 q/ha
	DRR Dhan45	Zinc	12-16 ppm	22.6 ppm	50 q/ha
	DRR Dhan48	Zinc	12-16ppm	24 ppm	52 q/ha
	Zinco Rice MS	Zinc	12-16 ppm	27.4 ppm	58.0 q/ha
	CR Dhan311	Protein Zinc	78% 12-16 ppm	10.1% 20.1 ppm	46.2 q/ha
	CR Dhan315	Zinc	12-16 ppm	24.9 ppm	50 q/ha
Wheat	WB02	Iron Zinc	28-32 ppm 30-32 ppm	40 ppm 42 ppm	51.6 q/ha
	HPBW01	Iron Zinc	28-32 ppm 30-32 ppm	40 ppm 40.6 ppm	51.7 q/ha
	PusaTejas HI8759	Protein Iron Zinc	8-10% 28-32 ppm 30-32 ppm	12.0% 41.1 ppm 42.8 ppm	57 q/ha
	PusaUjala HI1605	Protein Iron	8-10% 28-32 ppm	13.0% 43 ppm	30 q/ha
	HD3171	Zinc	30-32 ppm	47.1 ppm	28 q/ha
	HI8777	Iron Zinc	28-32 ppm 30-32 ppm	48.7 ppm 43.6 ppm	18.5 q/ha
	MACS4028	Protein Iron Zinc	8-10% 28-32 ppm 30-32 ppm	14.7% 46.1 ppm 4.03 ppm	19.3 q/ha
	PBW752	Protein	8-10%	12.4%	49.7 q/ha
	PBW757	Zinc	30-32ppm	42.3 ppm	36.7 q/ha
	Karan Vandana DBW187	Iron	28-32 ppm	43.1 ppm	>48.8 q/ha
	DBW173	Protein Iron	8-10% 28-32 ppm	12.5% 40.7 ppm	47.2 q/ha
	UAS 375	Protein	8-10%	13.8%	21.4 q/ha
	DDW 47	Protein Iron	8-10% 28-32 ppm	12.7% 40.1 ppm	37.3 q/ha
	PBW 771	Zinc	30-32 ppm	41.4 ppm	50.3 q/ha
	HI 8802	Protein	8-10%	13%	29.1 q/ha
	HI 8805	Protein Iron	8-10% 28-32 ppm	12.8% 40.4 ppm	30.4 q/ha
	HD3249	Iron	28-32 ppm	42.5 ppm	48.8 q/ha
	MACS 4058	Protein Iron Zinc	8-10% 28-32 ppm 30-32 ppm	14.7% 39.5 ppm 37.8 ppm	29.6 q/ha
	HD3298	Protein Iron	8-10% 28-32 ppm	12.1% 43.1 ppm	43.7 q/ha
	HI1633	Protein, Iron Zinc	8-10% 28-32 ppm 30-32 ppm	12.4% 41.6 ppm 41.1 ppm	41.7 q/ha
	DBW 303	Protein	8-10%	12.1%	81.2 q/ha
	DDW48	Protein	8-10%	12.1%	47.4 q/ha
Maize	Vivek QPM9	Lysine Tryptophan	1.5-2% 0.3-0.4%	4.19% 0.83%	52q/ha
	PusaHM4 improved	Lysine Tryptophan	1.5-2% 0.3-0.4%	3.62% 0.91%	64.2q/ha
	Pusa HM8 improved	Lysine Tryptophan	1.5-2% 0.3-0.4%	4.18% 1.06%	62.6q/ha
	Pusa HM9 improved	Lysine Tryptophan	1.5-2% 0.3-0.4%	2.97% 0.68%	52q/ha
	Pusa Vivek QPM9 improved	Provitamin lysine Tryptophan	1.0-2ppm 1.5-2% 0.3-0.4%	8.15ppm 2.67% 0.74%	>55.9q/ha
	Pusa VH27 Improved	Provitamin-A	1-2ppm	5.49ppm	48.5q/ha
	Pusa HQPM5 improved	Provitamin lysine Tryptophan	1-2ppm 1.5-2% 0.3-0.4%	6.77ppm 4.25% 0.94%	>72.6q/ha
	Pusa HQPM7 improved	Provitamin, lysine& Tryptophan	1-2ppm 1.5-2% 0.3-0.4%	7.10ppm 4.19% 0.93%	74.5q/ha
	IQMH201	Lysine Tryptophan	1.5-2% 0.3-0.4%	3.03% 0.73%	84.8q/ha
	IQMH202	Lysine	1.5-2%	3.04%	72q/ha

		Tryptophan	0.3-0.4%	0.66%	
	IQMH203	Lysine Tryptophan	1.5-2% 0.3-0.4%	3.48% 0.77%	63q/ha
Pearl Millet	HHB299	Iron Zinc	45-50ppm 30-35ppm	73ppm 41ppm	32.7q/ha
	AHB1200Fe	Iron	45-50ppm	730ppm	32q/ha
	AHB1269Fe	Iron Zinc	45-50ppm 30-35ppm	91ppm 43ppm	31.7q/ha
	ABV04	Iron Zinc	45-50ppm 30-35ppm	70ppm 63ppm	28.6q/ha
	Phule Mahashakti	Iron Zinc	45-50ppm 30-35ppm	87ppm 41ppm	29.3q/ha
	RHB233	Iron Zinc	45-50ppm 30-35ppm	83ppm 46ppm	31.6q/ha
	RHB234	Iron Zinc	45-50ppm 30-35ppm	84ppm 46ppm	31.7q/ha
	HHB311	Iron	45-50ppm	83ppm	31.7q/ha
Finger Millet	VR929 Vegavathi	Iron	25ppm	131.8ppm	36.1q/ha
	CFMV1 Indravati	Calcium Iron Zinc	200mg/100g 25ppm 16ppm	428mg/100g 58ppm 44ppm	31.1q/ha
	CFMV2	Calcium Iron Zinc	200mg/100g 25ppm 16ppm	454mg/100g 39ppm 25ppm	29.5q/ha
Little Millet	CLMV1	Iron Zinc	25ppm 20ppm	59ppm 35ppm	15.8q/ha
Lentil	PusaAgeti Masoor	Iron	45-50ppm	65ppm	13.0q/ha
	IPL220	Iron Zinc	45-50ppm 35-40ppm	73ppm 51.0ppm	13.8q/ha
Groundnut	Girnar 4	Oleic acid	45-52%	78.5%	32.2q/ha
	Girnar 5	Oleic acid	45-52%	78.4%	31.2q/ha
Linseed	TL 99	Linoleic acid	Linoleic acid>20%	Linoleic acid-58.9%	12.7q/ha
Mustard	Pusa Mustard 30	Erucic acid	Erucic acid>40%	Erucic acid-1.20%	18.2q/ha
	Pusa Double Zero Mustard31	Erucic acid Glucosinolates	Erucic acid>40% Glucosinolates-120ppm	Erucic acid-0.76% Glucosinolates-29.41ppm	23q/ha
	Pusa Mustard 32	Erucic acid	Erucic acid>40%	Erucic acid-1.32%	27.1q/ha
Soybean	NRC127	Kunitz Trypsin inhibitor	Kunitz Trypsin inhibitor 30-45mg/g	Free from Kunitz Trypsin inhibitor	18q/ha
	NRC132	Lipoxygenase-2	-	Free from lipoxygenase 2	>16.5q/ha
	NRC 147	Oleic acid	22-25%	42%	>14q/ha
Cauliflower	Pusa Beta Kesari1	Provitamin-A	Negligible	0.8-1.0ppm	40 -50t/ha
Potato	KufriManik	Anthocyanin	Negligible	0.68ppm	23t/ha
	Kurfi Neelkanth	Anthocyanin	Negligible	1.0ppm	36-38t/ha
Sweet potato	Bhu Sona	Provitamin -A	2-3mg/100g	14.0mg/100g	>19.8t/ha
	Bhu Krishna	Anthocyanin	Negligible	90.0mg/100g	>18t/ha
Greater Yam	SreeNeelima	Anthocyanin Protein zinc	negligible Crude 2.7% 22-32ppm	50mg/100g 15.4% 49.8ppm	35t/ha
	Da 340	Anthocyanin Iron Calcium	negligible 70-120ppm 800-1200ppm	141.4mg/100g 136.2ppm 1890ppm	80t/ha
Pomegranate	Solapur Lal	Iron Zinc Vitamin C	2.7-3.2mg/100g 0.50-0.54mg/100g 14.2-14.6mg/100g	5.6-6.1mg/100g 0.64-0.69mg/100g 19.4-19.8mg/100g	23-27t/ha

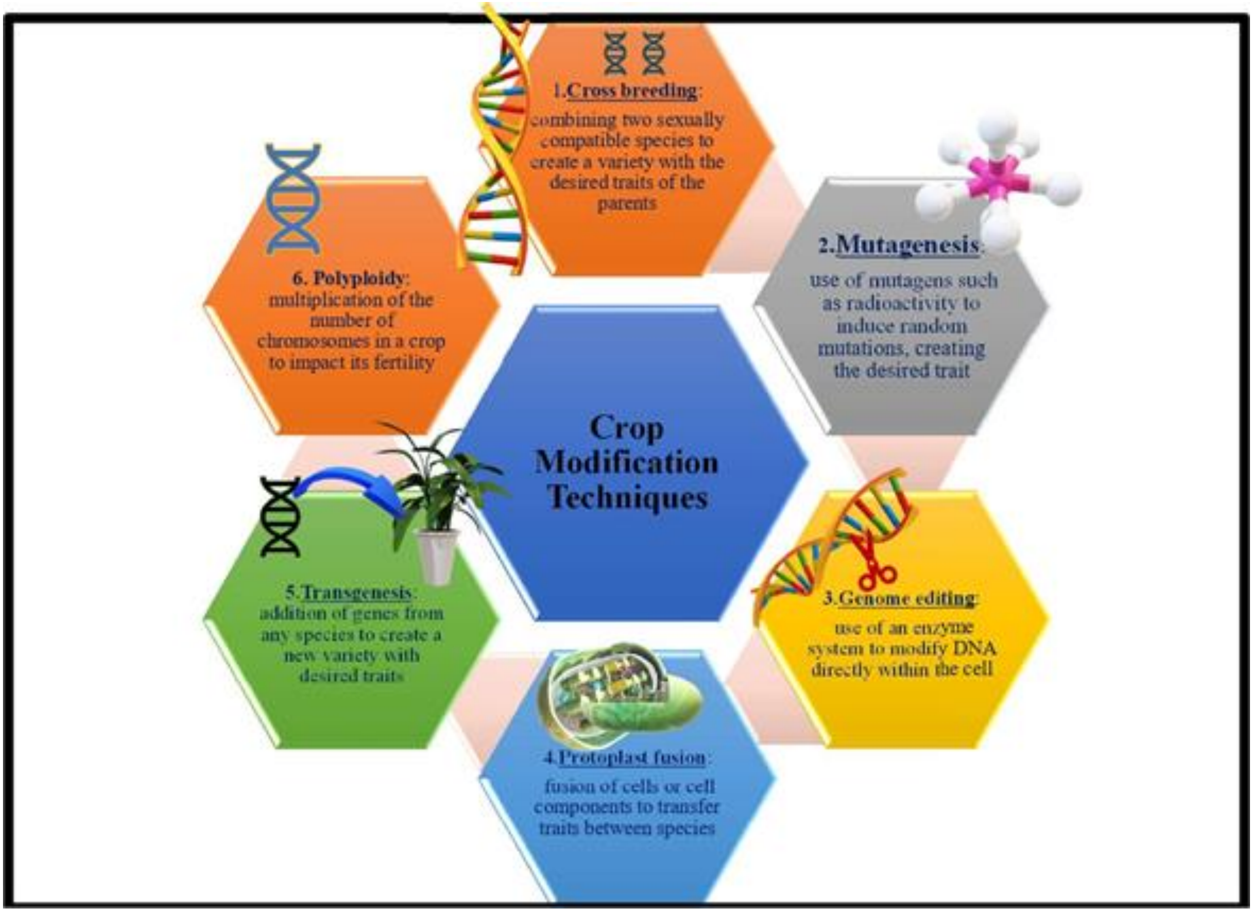

Fig 1: Crop modification techniques

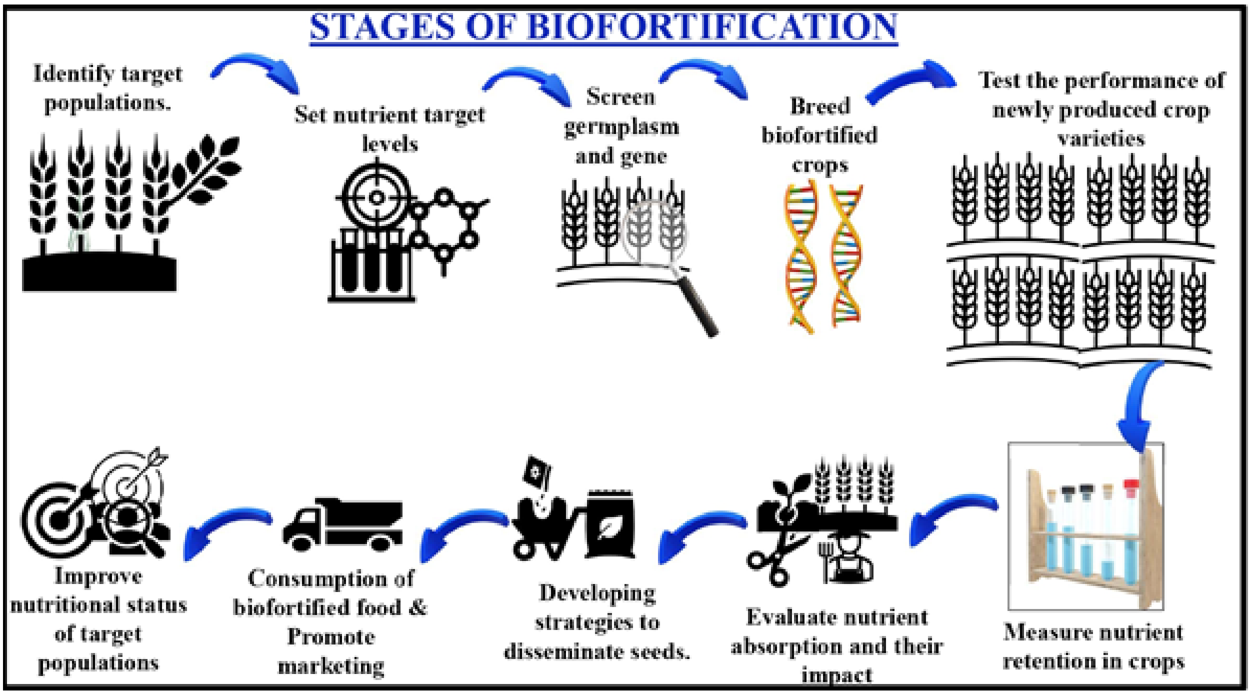

Fig 2: An overview of stages of biofortification

2.2 Conventional plant breeding

Plant breeding has been practised by farmers for hundreds of years. Conventional plant breeding refers to the crossing of plants in research fields to yield progeny with characteristics of both parents. In the case of biofortification, one of the crops in the initial cross has high levels of target micronutrients. An example of a conventionally plant-bred bio fortified crop is rice. Rice varieties that contain high levels of iron and zinc were crossed with high-yielding rice varieties to produce progeny with both high yield and increased levels of micronutrients.

2.3 Genetic modifications
Molecular techniques are used to transfer a specific trait from a donor to a recipient organism, like daffodil which synthesizes beta-carotene taken as a donor, and rice is taken as a recipient so that rice will get the ability to synthesize beta-carotene which is not present before in rice.

3. Crop modification techniques
Various crop modification methods are being employed for bio-fortification, which include conventional breeding, mutagenesis, genome editing, protoplast fusion, polyploidy and transgenics (Decourcelle *et al.*, 2015) [6] (Fig. 1). Conventional breeding depends on the availability of genetic variation for various nutrients/ vitamins in the germplasm that is sexually compatible (Strobbe, Van Der Straeten 2017) [28] with the target line.

4. Stages of biofortification
Figure 3 outlines the key activities involved in developing a bio fortified variety. The first and foremost activity involves screening the population for the target nutrient. Once identified, it is employed in genetic studies and developing molecular markers to facilitate breeding. Genotype x environment interaction is then determined at different locations. The most promising varieties are selected for MLTs over multiple seasons by national research partners and then are submitted for release.

5. Major Advantages and limitations of biofortification
- Biofortification can be introduced quickly and can produce nutritional benefits for the population in a short period.
- It improves the nutritional status of a large population covering both poor and rich equally.
- Bio fortified foods are having the same colour and taste as staple foods which are normally consumed by people and hence are consumer-friendly.
- It is feasible to bio-fortify foods with several micronutrients simultaneously to treat multiple micronutrient deficiencies that often coexist in a population having a poor diet.
- Bio fortified foods often fail to reach the poorest segments of the general population due to their low purchase power and an underdeveloped distribution channel.
- The initial investment is high, and it may take years to develop a new variety.

6. Conclusion
In this review, we discussed current understanding of biofortification strategies and applications in diverse field crops for food security. Globally, soils deficient in Zn are more common than those poor in other micronutrients. Deficiencies in micronutrients can harm human and livestock health, which has been established. Micronutrient deficit in developing and undeveloped countries is mostly due to the great reliance of the human population on cereal-based diets.
Biofortification by fertilization appears to be sustainable and cost-effective in addressing vitamin insufficiency in humans. It is possible that management strategies such as organic or inorganic inputs can either serve as a direct nutrient source or contribute to the improvement of soil micronutrient bioavailability by altering its characteristics. Due to the overuse of fertilizers, a supplementary strategy is essential. However, there has been less research on transgenic biofortification in fodder crops, despite its versatility in improving micronutrient content. Due to the importance of microorganisms in enhancing nutrient availability and uptake, further research is needed to identify the best microbial culture for use in green technology. The use of nano-fertilizers to improve plant nutrition is a new area of study that necessitates in-depth investigations of the toxicity of the materials. That's why we contend that the knowledge presented here will help with long-term dietary needs by reducing micronutrient deficiency. An international definition of bio fortified products is the greatest barrier to the spread of biofortification, which is evident from the review of works included in this review.
If all of this is taken into consideration, it would be possible to begin the process of creating a globally recognized specific regulatory framework covering all stages of the supply chain that could lead to clear development and research objectives as well as increased credibility and acceptance of bio fortified products by the end consumers (Adeyeye *et al.*, 2019) [1].

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