Chapter 10
Crop Breeding and Biotechnological Advances Towards Nutrition and Environment Security

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10.1 Introduction

The concerns about nutrition security, human health and environment have gained substantial momentum, mainly due to rapid increases in global population and food insecurity. Historically, plant breeding remains key for improving crop yield, but their role on nutrition, environment and human health have not explored. Plant breeding can address the nutritional insecurity, human health problems and environmental degradation, by manipulation genetic makeup, creating new genotypes and adding different kinds of compounds including health-promoting bioactive compounds.

Nutrition is the major source of energy and building block for human life. Good quality of food i.e. nutrient rich food influences the quality of individual life, including the ability to maintain the body system for effective movement, work, and good physical appearance. Around 2.3 billion people in the world were moderately or severely food insecure in 2021 and 11.7% of the global population faced food insecurity at severe levels (FAO et al. 2022). The FAO et al. (2022) estimated that about 3.1 billion people are unable to afford a healthy diet that causes malnutrition. The number is 112 million more people compared to 2019. In addition, the Ukraine-Russia war will again increase this gap in food and nutrition security due to two of the biggest producers of agriculture and staple cereals globally.

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"Hidden hunger" (deficiency of micronutrients) is a global concern, where an estimated two billion people suffer from a chronic deficiency (or inadequate intake) of essential vitamins and minerals (micronutrients) in the daily diet (Muthayya et al. 2013). Worldwide, the most widespread micronutrient deficiencies are iron, zinc, iodine, and vitamins (vitamin A, folate (B9), and vitamin B12). Although distributed globally, over 98% of malnourished persons reside in developing countries, where particularly young children and women of reproductive age living in low-income countries are the most vulnerable to this "hidden hunger. Therefore, a good solution to this issue may be found effective if everyone in the society has access to safe and adequate food, which meets the energy requirements and ensure it functional adequacy of an organism. For improving the nutrition status, environment should also be in good condition.

Environment degradation is a major concern worldwide. Climate changes and the unwise applications of pesticides, and chemicals in agriculture have devastated the environment. Temperature is increasing each year (Fig. 10.1) and the world is experiencing adjustment difficulties. Unanticipated climate events include drought, extreme temperature, flood, storm, wildfire, etc. have hampered crop production significantly each year across the world (Razzaq et al. 2021). The extreme events of climate change have increased the loss of agricultural land and accelerated biotic and abiotic stresses (Raza et al. 2019). Thus the development of climate resilient varieties through crop improvement would be extremely beneficial to tackle such climate events as well as to improve the environment.

Pesticides are poisonous to almost all living beings and serious problem for each ecosystem. However, about, two million tons of pesticides are utilized annually worldwide and about 44% of farmers are poisoned by pesticides every year (Boedeker et al. 2020; Sharma et al. 2019). The best option to get rid of such a situation is resistance breeding through which crop varieties are developed with either

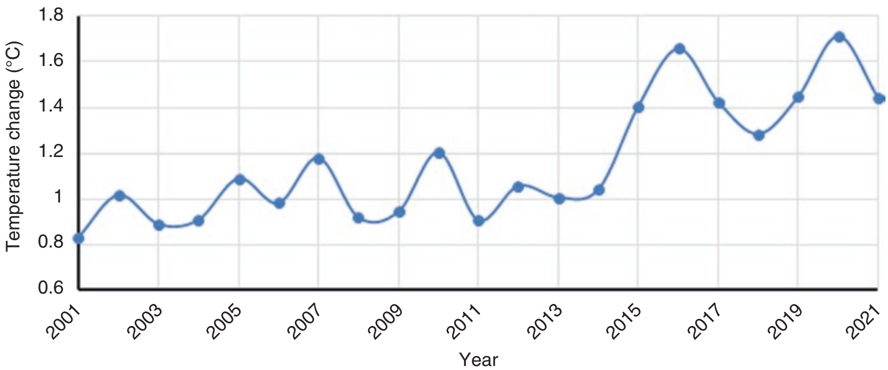

Fig. 10.1 The trend of annual changes in the world's temperature from 2001 to 2021. (Source: FAO (https://www.fao.org/faostat/en/#data/ET))

resistance or tolerance to any kind of stress. Therefore, crop improvement is the most important strategy for nutrition and environmental security along with the improved health system anywhere in the world.

10.2 Breeding for Nutrition Security and Human Health

Nutrition takes part in various catalytic and regulatory functions of the main metabolic process, including absorption, transportation, redux and biosynthesis of organic compounds, genetic information, etc. A deficiency in nutrition, including mineral and vitamins, disrupt these functions, thus leads to serious illness. Nutrition security builds on food security, emphasizing the co-existence of food insecurity and diet-related diseases and disparities (USDA 2022).

Malnutrition is a global health problem (Table 10.1). Therefore, the United Nations General Assembly session which was organized on 1 April 2016 also emphasized the importance of healthy diets and food systems for global human health. The period from 2016 through 2025 was announced as the UN Decade of Action on Nutrition. However, until now, the global agricultural research and development system has been designed only giving top priority to increasing grain yield and crop productivity, instead of focusing on increasing nutrition for the promotion of human health. Therefore, this system led to a gap in micronutrient deficiency in staple food crops, mainly rice, wheat, and maize; thereby increasing micronutrient malnutrition among consumers, particularly in developing countries. Currently, some nutrition-sensitive agriculture research and development activities are shifting from higher yield to nutrient-rich crops in sufficient quantity to fight against hidden hunger or micronutrient malnutrition, especially where their food source is cereals-based and micronutrient-poor (Yu and Tian 2017; Garg et al. 2018; Stangoulis and Knez 2022). Therefore, the purpose of this work was to examine the global scenario of malnutrition, major micronutrients and their key functions, and the main approaches associated with a long-term biofortification strategy to eliminate

Table 10.1 The global status of malnutrition and its impact on the economy

SN	Types of malnutrition	Affected people (%)	Age group
1	Hidden hunger	2 billion	All
2	Undernourished	820 million	All
3	Stunted	149 million (21.9%)	Children <5 yrs
4	Wasted	49.5 million (7.3%)	Children <5 yrs
5	Death	45%	Children <5 yrs
6	South Asian regions		
6.1	Stunted	31%	Children <5 yrs
6.2	Wasted	14.3%	Children <5 yrs
7	GDP losses in Asia & Africa	11%	
8	The cost associated with malnutrition	USD 3.5 trillion per year	

Source: GNR (2016, 2018, 2020), UNICEF et al. (2020)

malnutrition, concentrating on several kinds of cereal as the most popular staple food crops.

10.2.1 Conventional Plant Breeding

Conventional plant breeding (CPB) has been going on for hundreds of years and is still popular in developing new varieties with desirable traits in the offspring. Early generation farmers discovered that some crop plants could be artificially mated or cross-pollinated to increase yield. In the 20th century, the plant breeding system was further developed for the purposeful manipulation of quality traits in plants to create new varieties with a set of desired characteristics. The development of both the open-pollinated (OP) and hybrid varieties has dramatically increased the productivity and quality of various crop varieties for food, feed, fibers, and other industrial purposes.

Conventional plant breeding-based biofortification is a well-accepted method to improve the micronutrients, such as minerals (Fe, Se, Zn, Cu, Mg), vitamins (A, E, C) and essential amino acids (lysine, tryptophan) in crop varieties (Garg et al. 2018). In this system, parent lines containing high nutrients are crossed with recipient (target) lines over several generations to produce crop varieties with nutrients and agronomic traits (Garg et al. 2018). Using this technology, a good number of crops have been developed for fortification which harbor or be better able to uptake higher levels of micronutrients such as Fe, Se and Zn from the soil and then collect them in edible portions. For example, the world's first zinc enriched rice varieties (BRRIdhan 62, BRRIdhan 72 and BRRIdhan 64) developed by HarvestPlus were released in 2013 by Bangladesh Rice Research Institute (BRRI) that contains a maximum Zn content ranging from 20–22 ppm against the world average of 14–16 ppm in other varieties (Josh 2013; Garg et al. 2018). Similarly, crops like rice, maize, wheat, millets, lentil, groundnut, linseed, cauliflower, potato, sweet potato, etc. also can be increased their nutrient contents which are beneficial for improving health condition of human beings. It is the most sustainable and trustful approach to address the global micronutrients issue (Table 10.2). However, developing micronutrient enrich varieties is a time-consuming process, that can limit its effectiveness in micronutrient-deficient soil, limited due to the non-availability of enough genetic variation in the crossable gene pools or genetic exchange between closely related species (Garg et al. 2018; Kumar et al. 2019). In addition, it also lacks the modulation of target gene expression underlying the micronutrient accumulation. Therefore, modern plant breeding tools such as genetic engineering can be effectively and efficiently used to address these issues.

Table 10.2 Nutrition level improvement in different crops (OPVs+hybrids) through conventional breeding system

Crops	Nutrients	Baseline levels	Levels achieved	References
Rice	Protein	7–8%	>10%	Yadava et al. (2020)
	Iron	12 mg/kg	21 mg/kg	Gregorio et al. (2000)
	Zinc	12–16 ppm	>20 ppm	Yadava et al. (2020)
		14–16 ppm	>20 ppm	Josh (2013), Garg et al. (2018)
			40%	Josh (2013), Garg et al. (2018)
Maize	PVA	0.5–1.5 ppm	>5 ppm	Yadava et al. (2020)
	Lysine	1.5–2.0%	>2.5%	Yadava et al. (2020)
	Tryptophan	0.3–0.4%	>0.6%	Yadava et al. (2020)
Wheat	Protein	8–10%	>12%	Yadava et al. (2020)
	Iron	28–32 ppm	>38 ppm	Yadava et al. (2020)
	Zinc	30–32 ppm	>37 ppm	Yadava et al. (2020)
			30–40%	Thapa et al. (2022)
Pearl Millet	Iron	45–50 ppm	>70 ppm	Yadava et al. (2020)
	Zinc	30–35 ppm	>40 ppm	Yadava et al. (2020)
Finger Millet	Iron	25.0 ppm	>38 ppm	Yadava et al. (2020)
	Zinc	16.0 ppm	> 24 ppm	Yadava et al. (2020)
	Calcium	200 mg/100 g	>400 mg/100 g	Yadava et al. (2020)
Lentil	Iron	45–50 ppm	>62 ppm	Yadava et al. (2020)
	Zinc	35–40 ppm	>50 ppm	Yadava et al. (2020)
Groundnut	Oleic acid	45–52%	>70%	Yadava et al. (2020)
Linseed	Linoleic acid	20–25%	>58%	Yadava et al. (2020)
Cauliflower	PVA	Negligible	>8.0 ppm	Yadava et al. (2020)
Potato	Anthocyanin	Negligible	>0.60 ppm	Yadava et al. (2020)
Sweet Potato	PVA	2–3 mg/100 g	>13 mg/100 g	Yadava et al. (2020)
	Anthocyanin	Negligible	>80 mg/100 g	Yadava et al. (2020)

10.2.2 Genetic Engineering

Genetic engineering is a modern tool for generating transgenics and has also been deployed to transgenes directly into elite genotypes for increasing yield, tolerance to biotic and abiotic stresses, and effectively sustainably fighting against minerals, vitamins, and protein deficiency. It is the third and modest approach to biofortification for enhancing nutrient concentration in crops, where the success rate and acceptability of breeding is much higher (Garg et al. 2018, Table 10.3). This approach can serve as a fast and cost-effective strategy to alleviate different micronutrients deficiency. A superior genotype, once developed, can be used for many years without any additional recurring cost. However, it also has some limitations (Table 10.4).

The genetic engineering-based bio-fortification of crops emerges as a self-targeted and non-recurrent approach to address micronutrient malnutrition or hidden hunger, especially, where breeding is not possible due to lack of genetic

Table 10.3 Nutrition level improvement in different crops through genetic engineering system

Crops	Transformation systems	Targeted gene(s)	Targeted tissue	Total increase in nutrition levels	Author(s)
Iron					
Rice	*Agrobacterium*	Overexpression of soybean ferritin gene *Soyfer H-1*	Endosperm	38.1 μg/g DW	Goto et al. (1999)
Rice	*Bombardment*	Soybean *ferritin*	Leaves	99.00% ppm Dw	Drakakaki et al. (2000)
Rice	*Agrobacterium*	*Phaseolus ferritin*	Endosperm	22.07 μg/g/seed DW (2-fold higher than the control)	Lucca et al. (2001)
Rice	*Agrobacterium*	SoyferH2, soybean Ferritin gene	Endosperm	4.0 μg/g DW (4.4 times higher than the control)	Masuda et al. (2012)
Rice	*Agrobacterium*	Barley nicotianamine synthase gene *HvNAS1*	Endosperm	4.5-fold higher than the control	Masuda et al. (2009)
Rice	*Agrobacterium*	Soybean *ferritin*, *Aspergillus flavBous* phytase, *OsNAS1*	Endosperm	6-fold higher iron concentration-fold higher Fe than the control	Wirth et al. (2009)
Rice	*Agrobacterium*	*Overexpression of two OsNAS2*	Endosperm	19–81 μg/g DW (4-fold increased)	Johnson et al. (2011)
Wheat	*Bombardment*	Soybean *ferritin*	Leaves	47% ppm DW	Drakakaki et al. (2000)
Wheat	*Bombardment*	y1and *crtI*	Endosperm	4.96 μg/g DW (10.8-fold)	Cong et al. (2009)
Zinc					
Rice	*Agrobacterium*	Barley nicotianamine synthase gene *HvNAS1*	Endosperm	35 μg/g (>2.5 fold)	Masuda et al. (2009)
Rice	*Agrobacterium*	Overexpression of *two OsNAS2*	Endosperm	30–95 μg/g (2.5-fold increased	Johnson et al. (2005)
Vitamin A					
Rice	*Agrobacterium*	*Photoene synthase (psy)* from daffodil, *phytoene desaturase (CrtI)* gene from *Erwinia uredovora*	Endosperm	1.6 μg/g DW	Ye et al. (2000)
Rice	*Agrobacterium*	*Phytoene synthase (psy)* from maize, *Crtl* from the *Erwinia uredovora*	Endosperm	37 μg/g DW (23-fold than the original golden rice)	Paine et al. (2005)

(continued)

Table 10.3 (continued)

Crops	Transformation systems	Targeted gene(s)	Targeted tissue	Total increase in nutrition levels	Author(s)
Rice	*Agrobacterium*	*psy and lycopene β-cyclase (β-lcy)* both from daffodil (*Narcissus pseudonarcissus)*	Endosperm	1.6 μg/g DW	Beyer et al. (2002)
Rice	*Agrobacterium*	*crt1, psy1* and *pmi*	Endosperm	1.96–7.31 μg/g DW	GoC (2018)
Maize	*Bombardment*	Bacterial *crtB* and *crtI*	Endosperm	9.8 μg/g DW (>34 fold increased)	Aluru et al. (2008)
Maize	*Bombardment*	*Zmpsy1* and *PacrtI*	Endosperm	60 μg/g β-carotene (112-fold increased)	Naqvi et al. (2009)
Wheat	*Bombardment*	*psy* (maize) *and crtI (Erwinia uredovora)*	Endosperm	4.96 μg/g DW	Cong et al. (2009)
Ascorbate					
Maize	*Bombardment*	Dehydroascorbate reductase (*dhar*)	Endosperm	110 μg/g DW (6-fold Ascorbate)	Naqvi et al. (2009)
Folate					
Maize	*Bombardment*	*E. coli folE*	Endosperm	1.94 μg/g DW	Naqvi et al. (2009)
Vaccine					
Rice	*Agrobacterium*	*7Crp*	Endosperm	15% of total seed protein	Takaiwa (2007). Yang et al. (2007)

variability or when a particular micronutrient does not naturally exist in crops (Garg et al. 2018; Kumar et al. 2019). In this system of developing transgenic crops for micronutrients biofortification, two criteria are considered the most important such as (i) selection of widely adapted genotype of economically important crops, and (ii) accumulation of nutrients in the edible portion of the crop plant with diverse effect on plant physiology or development and economic yield, human health, ecology, and environment (Vanderschuren et al. 2013). This technique is a valid alternative where conventional plant breeding system fails to achieve significant genotypic improvement in nutritional levels in crops (e.g., provitamin A in rice), or when the conventional breeding cannot address the crop plants having propagated vegetatively (e.g., banana).

Development of transgenic biofortified crop initially involves substantial amount of time, efforts, and investment during new variety research and development stage, but in a long run it is a cost-effective and sustainable approach (Table 10.4). This system has no taxonomic barriers and even synthetic genes can be constructed and

Table 10.4 Biofortification strategies for enhancing micronutrients in food

SN	Biofortification strategies	Tools of biofortification	Pros	Cons
1	**Food fortification**	Wheat and rice with Fe, Vit.12 & Folic acid Milk & edible oil with Vit. A & D Double fortified salt with iodine & Fe Other details in Table 10.5	1. Cost-effective 2. Lower the risk of multiple deficiencies 3. Does not require any behavior change 4. An overdose of nutrients is unlikely 5. Not altered its intrinsic characteristics of food	1. A small amount of food intake is less likely to benefit 2. Same recurrent cost year after year, therefore, ordinary people cannot afford to buy the staples without funding support 3. Feasible for developed countries only and quite limited to small farmers and rural poor in developing countries 4. No long-term solution 5. Only complementary but not a replacement
2	**Biofortification**			
2.1	Agronomic	Mineral fertilization: foliar and soil mineral fertilizing Other details in Table 10.6	1. Simple, inexpensive, rapid enhancement 2. Does not require any food intake behavioral change 3. Overdose of nutrition is unlikely 4. Intrinsic characteristics of food are not altered	1. Only works with minerals; very dependent on crop and cultivar; not possible to target edible organs 2. The rural poor cannot afford and access micro-nutrient fertilizers 3. Application of imbalance dose may affect the health and yield of plants thereby affecting the grain micronutrients.
2.2	Conventional plant breeding	Using genetic variability for the development of micronutrient-enhanced crop varieties Other details in Table 10.2	1. Uses intrinsic properties of crop 2. New variety development with desired traits for micronutrients 3. A well-accepted and sustainable method 4. Varieties able to uptake higher levels of micronutrients from the soil	1. Depends on existing gene pool; takes a long time; traits might need to be introgressed from wild relatives; possible intellectual property rights or regulatory constraints 2. Limits the effective uptake of micronutrients from the micronutrient deficit soil 3. Limited due to the non-availability of enough genetic variability in the gene pool 4. Genetic exchange is possible between closely related species 5. Needs special requirement to produce grain and seed

(continued)

Table 10.4 (continued)

SN	Biofortification strategies	Tools of biofortification	Pros	Cons
2.3	Genetic engineering/ modification technology	Molecular breeding: Marker-assisted breeding, Genetic engineering (Transgenic/ cisgenic, ZFNs, TALENs, CRISPR/cas9) of direct genes introduction or manipulation into target crops Other details in Table 10.6	1. Rapid; unconstrained by gene pool; targeted expression in edible organs; applicable directly to elite cultivars 2. Modern tools for generating new crop varieties crossing between related or unrelated species 3. Superior variety can be used for many years 4. Useful for bio-vaccine also	1. Regulatory landscape; political and socio-economic issues relevant to transgenic plants; possible intellectual property constraints 2. Environmental impact assessment is time and cost consuming 3. Farmers and consumers may not accept it easily 4. Requires high-tech human resources and sophisticated laboratory

used with its construct (promoter-gene-terminator). Several crops have been genetically modified to enhance micronutrients (Garg et al. 2018, Table 10.3). Among micronutrients, minerals, vitamins, protein, fatty acids, bio-vaccines have been targeted by using various genes to improve nutrients in food crops (Garg et al. 2018, Table 10.3). In addition, with the advances of powerful new gene-editing tools like transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 and increased availability of fully sequenced genomes in staple crops have created new rooms for this biofortification (Nemudryi et al. 2014; Kumari et al. 2021).

In addition, transgenic technology is also used to develop biopharmaceutical plants for a natural plant vaccine. For example, the transgenic rice (*7Crp*)-based edible vaccine is effective for the treatment of Japanese cedar pollinosis when transgenic rice grain containing the structurally disrupted CryJ1 and CryJ2 antigens (universal antigen) is orally administered. The clinical symptoms of pollinosis, sneezing frequency and infiltration of inflammatory cells such as eosinophils and neutrophils were also significantly reduced in the nasal tissue (Takaiwa 2007; Yang et al. 2007). The consumption of approximately 12 g of 7Crp rice (at approximately 50 mg per 20 mg of grain) per day should be sufficient. Japanese citizens eat approximately 100 g of rice per day as a staple food, and therefore 7Crp seed can be used to supplement the daily diet by mixing with normal rice (Takaiwa 2007). Biopharmaceutical plants may need to be grown in a contained environment, with either physical or geographic isolation (Takaiwa 2007).

Currently, some organizations such as the World Health Organization (WHO), the Consultative Group on International Agricultural Research (CGIAR), Food Agricultural Organization, Global Harvest Initiative (GHI), HarvestPlus and Global Alliance for Improved Nutrition (GAIN) have been involved in developing biofortified high yielding crop varieties using biotechnological tools to address the deficiency of iron, zinc, protein and vitamins (A, B9 (folate), B12 and other B vitamins) to narrow down the gap of "hidden hunger" or nutrition security in the world in general and developing countries in particular. Breeding approaches have also greater role on improving human health through developing specific nutrient rich as well as specialty varieties.

10.2.3 Biofortification Strategy

Biofortification is a short- to a long-term strategy to increase the number of essential micro- and macronutrients in the major food sources that insure their bioavailability in the human body system. There are mainly two strategies for micronutrient fortification such as food fortification and biofortification of edible crops, which are common and effective in addressing malnutrition and micronutrient deficiencies (Kumar et al. 2019, Table 10.5).

Food fortification Food fortification is defined as the practice of **adding vitamins and minerals to commonly consumed foods during processing such as synthetic capsules or tablets, and** value-added cereals **to enhance the nutritional value for human health benefits with minimal risk to health** (Olson et al. 2021, Table 10.5). It is a proven, safe, and cost-effective strategy to improve micronutrients in foods (Table 10.4). In addition, existing systems of supplementations and food fortification of staple food with minerals and vitamins can address the issue of adequate nutrition security (Kumar et al. 2019). It is a strategy to fill the nutrient gap which has the advantage of being able to deliver nutrients to large segments of the population without requiring radical changes in their food consumption patterns (Kumar et al. 2019). Food fortification includes mass, target, market-driven (or commercial), household, (or community), and microbial fortifications. Food fortification has a decades-long global history that includes butter, margarine and sugar with vitamin

Table10.5 Food fortification

Food item	Fortifying nutrient or agent
Cereals	Vitamins, minerals
Beverages	Vitamins, minerals
Infant formulas, cookies	Iron
Milk, margarine	Vitamin A, D
Oil	Iron, vitamin A
Salt	Iodine, iron
Soy milk, orange juice	Calcium
Sugar, monosodium, glutamate, tea	Vitamin A
Vegetable mixtures amino acids, proteins	Vitamins, minerals
Wheat whole flour (Aata)	Vitamin D, synthetic vitamin A, iron
Wheat flour (Maida), bread, rice	Folic acid, vitamin B1, 2, 12; niacin, iron

Source: Modified from Sirohi et al. (2018)

A, salt with iodine, vitamin with milk, and vitamin B in cereals in many countries. However, there are still some drawbacks to current food fortification practices in the world (Table 10.4).

Bio-fortification Biofortification or biological fortification is an approach to enhance the micronutrient (minerals and vitamins) contents of agricultural produce with increased bioavailability to the human population that is developed and grown using modern agronomic practices, conventional plant breeding and biotechnology techniques (Garg et al. 2018). It also involves strategies that spin around targeting modulation of movement pathway (root uptake, transport, remobilization, storage, and enhanced bioavailability) of mineral nutrients, pulling nutrients from the soil, and pushing them to economic parts of plants in their bioavailable forms (Kumar et al. 2019). Garg et al. (2018), Hefferon (2019), Kumar et al. (2019) highlighted three systems of biofortification in plants, including agronomic, conventional plant breeding and genetic engineering (modern biotechnology). Biofortification of agricultural crop varieties offers a cost-effective, timely availability, sustainable and long-term solution approach to providing micronutrient-rich crop varieties to the needy and poor people, especially the people in developing countries (Garg et al. 2018). However, genetic engineering was found to be the most effective than agronomic and conventional breeding biofortification systems.

Agronomic biofortification The application of micronutrients or inorganic minerals such as iron (Fe), Selenium (Se) and zinc (Zn) fertilizers in the soil, foliar application, seed priming or coating of seed before planting, improves plant growth and development as well as biofortifying plants to improve nutrition for human consumption. High concentrations of zinc can be achieved in roots and left with soil fertilizer and even with foliar Zn-fertilizers (Wei et al. 2012). Although the use of the externally added micronutrients in the form of fertilizer can be effective, the relative efficiency of biofortification can vary from one plant to another and nutrients available in the soils. Sometimes, if the application of micronutrients is more than the recommended dose, it is deleterious to plants. However, agronomic inputs such as fertilizers may be harmful to the environment, and not be affordable or accessible to the rural poor, particularly in the developing world. Micronutrients such as Zn concentrations in fruits, seeds, and tubers are generally significantly lower.

Generally, plants have ability to absorb enough Fe and Zn from the soil to meet their physiological and metabolic requirements if the soil is rich in Fe and Zn. Nearly 50% of cereal growing areas in the developing world have been found deficient in Zn, which leads to the lower concentration of Zn in the grain of crops grown in such soils (Graham and Welch 1996; Cakmak, 2008). The application of Fe, Se and Zn fertilizers possess different positive responses to improve the availability of these minerals in plant growth and development and their accumulation in the grains to improve nutrition for human consumption (Table 10.3). The micronutrients such as Fe, Se and Zn availability to plant also depends on its rate and time of application; the genetic makeup of the crop; soil moisture; availability of other fertilizer concentrations in the soil, and the environment where the crop is grown.

Table 10.6 Agronomic biofortification system used for Iron (Fe), Selenium (Se) and Zinc (Zn) micronutrients food fortification

Crops	Micronutrients	Methods of application	Nutrient increment in grains/seeds over control (%)	References
Rice (*Oryza sativa* L.)	Fe, se and Zn	Foliar spray	Fe: 37.1 Se: 194.1 Zn: 36.7	Fang et al. (2008)
	Fe	Foliar spray	67.2	He et al. (2013)
	Zn	Foliar spray	22.47–24.04	Wei et al. (2012)
		Foliar spray	26.18	Ram et al. (2016)
		Seed priming	580.0	Johnson et al. (2005)
Maize (*Zea mays* L.)	Zn	Foliar	35.2–42.9	Wang et al. (2012)
		Soil	51	Zhang et al. (2013)
		Soil and foliar	Soil: 43.9 Foliar: 45.6	Rehman et al. (2018)
Wheat (*Triticum aestivum* L.)	Zn	Foliar	26.4–32.3	Wang et al. (2012)
		Foliar spray	47.14	Ram et al. (2016)
		Seed priming	900.0	Johnson et al. (2005)
Chickpea (*Cicer arietinum* L.)	Fe and Zn	Foliar spray	Fe: 34.54–35.28 Zn: 17.68–19.20	Pal et al. (2019)
	Zn	Seed priming	1067.0	Johnson et al. (2005)
Common beans (*Phaseolus vulgaris*)	Zn	Foliar spray	15.04	Ram et al. (2016)
Lentil (*Lens culinaris*)	Zn	Seed priming	1160.0	Johnson et al. (2005)

High concentrations of iron and zinc can be achieved in plant roots and leaves by application of Fe and Zn-fertilizers in soil and on foliar (Table 10.5). The seed priming was found to be more effective to increase the Zn content in rice, wheat, chickpea, and lentil (Johnson et al. 2005, Table 10.3). However, Harris et al. (2008) reported that seed priming of chickpea and wheat with Zn significantly increased grain Zn concentration by 29% and 12% respectively, which is far lower than report made by Johnson et al. (2005) (Table 10.6). Hence, the foliar application of Zn alone was also found to be effective to increase the grain Zn concentration in wheat by 84%, while soil Zn application showed an average increase of 12% over control (Zou et al. 2012). The response of these fertilizers is crop as well as the method of application-specific. For example, among rice, wheat, chickpea, and lentil the response of chickpea to seed priming with Zn was found to be the highest grain zinc content followed by lentil, wheat, and rice (Johnson et al. 2005), whereas, among rice, wheat, and maize, the response of wheat to foliar Zn application was the highest, followed by rice and maize (Table 10.6). Generally, Zn concentrations in fruits, seeds, and tubers are significantly lower using agronomic biofortification. Hence, micronutrient fertilizers may not be affordable or accessible to the rural poor in developing countries.

10.2.4 Other Nutritional and Human Health Issues

In addition to manipulation of major nutrients, there are many other potential of using crop breeding for better providing nutrition and health to human. Though very few studies have been observed, followings are potential areas of crop breeding to improve the nutrition security and human health.

- Plant based vaccine could be produced by developing varieties using biotechnological tools.
- Nutrient contents of any economical yield of any variety are major important for nutrition security and human health. Crop breeding can breed genotypes with higher protein content as well as very specific nutrient-dense varieties.
- Consumers prefer to have food with high energy provider, rather than amount. There is a need to considering research on developing varieties that produce grains and economical parts with high energy.
- There are many success cases of treatment of human diseases by eating very specific foods and herbs. Breeding on herbal plants are very limited. To improve the health of human, chemists and plant breeders need to work together so that specific human disease suppressor can be identified and incorporated in the varieties as well content plant development. In many areas, there are plant species which generate higher amount of oxygen. Varieties with such traits if developed, could be very important to human health.
- The requirement of types of food and their nutrient composition differs from children to adult to aged people. Varieties suitable to different age-people could be bred, so that people can easily get balance nutritional and easily digestible foods.
- To be healthy, consumers prefer food with low glycemic index and high rutine content food. Many people aware now a days to have lectin and gluten free food. Crop breeding has greatly contributed to develop varieties with these properties.

10.3 Breeding for Environmental Security

The production of food has soared in recent decades as populations have boomed and global economies have improved. As a result of this growing demand, the food industry is now negatively impacting the planet like never before. Massive amounts of planet-warming greenhouse gas emissions are released every year from the production of food, water resources are being depleted and contaminated, and important ecosystems are being destroyed by deforestation for pastures and crops (Statistica 2021). Environmental biotechnology (EB) is one field of biotechnology that can play a positive and important role in detoxifying and eliminating pollutants and cleaning up the contaminated sites of ecosystems (Gu 2021). Environmental biotechnology involves the utilization of biotechnological processes that have applications in waste and wastewater treatment, bioremediation, bioleaching, biofuels, and biopolymers which contribute to controlling environmental pollution. It is also integrated with agricultural biotechnology for those applications and focuses on modifying microorganisms and other living organisms including plants for such purposes. Identification of useful microorganisms and their candidate genes can be utilized in agricultural biotechnology for developing improved biotech crops. Biotech crop farming has a lot of controversies and has faced resistance in the past. Some of governments have banned all GMO crops in their countries. The controversies mainly involve government regulations, biotechnology companies, and scientists. Some of the areas of concern include the health of the consumer, impact on the environment, impact on farmers, and government regulations. Some of the advocacy groups like the Center for Food Safety have called for a thorough examination of the risks associated with GM food before it is allowed for consumption even though scientists have persisted that GMO food poses no threat to life (WorldAtlas 2017). The benefits and challenges of genetically modified crops have attracted enormous public attention, primarily around four issues: food safety, toxicity and pest resistance, crop yield effects, and shifts in profits and control to major corporations. Although the merits of existing GM crops can be debated, GM technology may offer other useful potential benefits, particularly traits that help crops resist diseases that cannot be addressed by any other means (World Resources Institute 2014).

The world needs to close a 69% gap between the crops produced in 2006 and the crops that the world needs by 2050. Assuming the present course of diets, population growth, and rates of food loss and waste, crop yields will need to grow one-third more in the coming 44 years than they did in the last 44 years to avoid net expansion of harvested cropland (World Resources Institute 2014). Genetically modified (GM) crop technology has been used by many farmers around the world for more than 20 years and currently nearly 17 million farmers a year plant seeds containing this technology (Brookes and Barfoot 2020). The technology is also changing agriculture's carbon footprint, helping farmers adopt more sustainable practices such as reduced tillage, which has decreased the burning of fossil fuels and allowed more carbon to be retained in the soil (Qaim and Traxler 2005; Brookes and

Barfoot 2020). Plant breeding contributes to reducing greenhouse gas emissions: about 3.4 billion tons of direct CO_2 emissions were avoided in Europe because of plant breeding innovation over the last 15 years (Europeanseed 2016). GM crop technology has been widely used for more than 20 years in many countries and is mainly found in the four crops canola, maize, cotton and soybean. While increasing global yield by 22%, GM crops reduced pesticide (active ingredient) usage by 37% and environmental impact (insecticide and herbicide use) by 18% (Klümper and Qaim 2014). Recently, the adoption of GM crops technology for insect-resistant maize, cotton and soybean, and herbicide-tolerant soybean, maize and canola has reduced the use of pesticides by 775.4 million kg (8.3%) and also decreased the environmental impact associated with it by 18.5% (Brookes and Barfoot 2020). Therefore, the genetic engineering technique aims at developing crops that are resistant to diseases, pests, and extreme environmental conditions while reducing spoilage and improving the nutrients of the produce. The crops are sometimes referred to as Genetically Modified Crops or simply as GMCs or biotech crops. High yield and low cost of pesticides have increased farm profitability. Because of improved profit margins, farmers in most countries have adopted biotech crop farming (WorldAtlas 2017). In 2018, crops containing this type of technology accounted for 48% of the global plantings of these four crops. In 2019, the total acreage of genetically modified crops worldwide came to some 190.4 million hectares. Genetic modifications of crops are done for many reasons, for example, to attain desirable traits or to make crops resistant to pests or herbicides. Since 2003, the acreage of genetically modified crops has generally been increasing, and globally, soybeans and corn are the most commonly adopted biotech products (Statistica 2022).

Concerning the environment, cultivars can be developed that require less tilling, thereby bringing down soil erosion and nitrogen leakage. More drought-tolerant cultivars will decrease the need for irrigation which is a major cause of environmental problems. Plants with improved nitrogen efficiency will diminish the use of fertilizers, and pesticide-resistant crops the use of pesticides (Houehanou et al. 2014). It is widely accepted that increases in atmospheric levels of greenhouse gases such as carbon dioxide, methane and nitrous oxide are detrimental to the global environment. Therefore, if the adoption of crop biotechnology contributes to a reduction in the level of greenhouse gas emissions from agriculture, this represents a positive development for the world (Intergovernmental Panel on Climate 2006).

Currently, commercialized genetically modified (GM) crops have reduced the impacts of agriculture on biodiversity through enhanced adoption of conservation tillage practices, reduction of insecticide use and use of more environmentally benign herbicides and increasing yields to alleviate pressure to convert additional land into agricultural use (Carpenter 2011). Most of the genetically modified (GM) plants currently commercialized encompass a handful of crop species (soybean, corn, cotton and canola) with agronomic characteristics (traits) directed against some biotic stresses (pest resistance, herbicide tolerance, or both) and are created by multinational companies (Ricroch and Hénard-Damave 2016) for enhanced productivity, socio-economic benefit and environmental security. Some of the biotech crops developed through advanced breeding technologies for reducing the impact of agrochemical use and environmental pollution are discussed below.

10.3.1 Breeding Crops for Reduced Herbicide and Pesticide Use

The release of herbicide-tolerant glyphosate-tolerant Roundup Ready® from Monsanto and glufosinate-tolerant Liberty Link® from Bayer is known as the first commercialized transgenic crop in the USA in the 1990s (Heap 2014). Even though the adoption of glyphosate-tolerant crops has resulted in weed species developing resistance to the herbicide (ISAAA 2017), transgenic crops are accepted and cultivated in several areas in the world and contributed much more in terms of reducing chemical herbicide use and reducing the environmental pollution. Even though efforts are there in creating transgenic crops, only a few are being commercialized. Mostly commercial crops such as maize (*Zea mays*), soybean (*Glycine max*), and cotton (*Gossypium* sp.) are used in research due to the high cost of research work. In terms of wheat and rice, even though several transgenic traits are produced such as glyphosate-tolerant wheat (Hu et al. 2003) and Golden Rice (Ye et al. 2000), they seem to be absent from the commercial scale.

Tolerance to specific herbicides (notably glyphosate and glufosinate and tolerance to additional active ingredients like 2 4 D and dicamba) in maize, cotton, canola (spring oilseed rape), soybean, sugar beet and alfalfa has been achieved in the last decade (Brookes and Barfoot 2020). This GM Herbicide Tolerant (GM HT) technology allows for the 'over the top' spraying of GM HT crops with these specific broad-spectrum herbicides, that target both grass and broad-leaved weeds but does not harm the crop itself. Resistance to specific insect pests of maize, cotton, soybeans and brinjal using GM insect resistance (GM IR), or 'Bt' technology offers farmers resistance in the plants to major pests such as stem and stalk borers, earworms, cutworms and rootworm in maize, bollworm/budworm in cotton, caterpillars in soybeans and the fruit and shoot borer in brinjal. Instead of applying insecticide for pest control, a very specific and safe insecticide is delivered via the plant itself through the 'Bt' gene expression. In addition, the GM papaya and squash referred to above are resistant to important viruses (e.g., ringspot in papaya), the GM apples are non-browning and the GM potatoes have low asparagine (low acrylamide which is a potential carcinogen) and reduced bruising (Brookes and Barfoot 2020). Development of those genetically modified crops has significantly reduced the use of agrochemicals reducing environmental pollution as well as conserving biodiversity.

Important environmental benefits have also occurred in China and India from the adoption of genetically modified insect resistant (GM IR) cotton, with a reduction in insecticide active ingredient use of over 276 million kg (1996–2018). The adoption of GM insect resistant and herbicide tolerant technology has reduced pesticide spraying by 775.4 million kg (8.3%) and, as a result, decreased the environmental impact by 18.5% associated with herbicide and insecticide use on maize, soybean, canola and/or cotton crops (as measured by the indicator, the Environmental Impact Quotient (EIQ)). The technology has also facilitated important cuts in fuel use and tillage changes, resulting in a significant reduction in the release of greenhouse gas emissions from the GM cropping area. In 2018, this was equivalent to removing 15.27 million cars from the roads (Brookes and Barfoot 2020).

Biotech herbicide tolerance (HT) technology has facilitated changes in farming systems. Thus, biotech HT technology (especially in soybeans) has played an important role in enabling farmers to capitalise on the availability of a low-cost, broad-spectrum herbicide (glyphosate) and in turn, facilitated the move away from conventional to low/no-tillage production systems in both North and South America. This change in the production system has delivered important environmental benefits, notably reduced levels of GHG emissions (from reduced tractor fuel use and additional soil carbon sequestration). Concerning biotech HT crops, however, overreliance on the use of glyphosate by some farmers, in some regions, has contributed to the development of weed resistance. As a result, farmers are increasingly adopting a mix of reactive and proactive weed management strategies incorporating a mix of herbicides. Despite this, the overall environmental gains arising from the use of biotech crops have been, and continue to be, substantial (Brookes and Barfoot 2012).

Transgenic *Arabidopsis* and tobacco plants that express EbF synthase genes from peppermint and sweet wormwood have been demonstrated to repel aphids and attract their natural enemies, including ladybugs and parasitoid wasps. This technology would remove the practice of applying insecticides, which are undesirable both for causing environmental harm as well as inviting the possibility of insecticide resistance (Hefferon 2016). Recently, several commercial products have been available with multiple herbicide-tolerant traits. The use of single lepidopteran insect resistance genes derived from *Bacillus thuringiensis* in Bt cotton, Bt maize, Bt potato, etc. is the best examples known to save the agricultural industry from great losses. The combination of insect resistance and herbicide-resistant traits is considered a turning point in transgenic crops. The percentage of the planted area has been found to grow with this especially in USA and Brazil (Ma et al. 2017) contributing to decreased use of chemical herbicides and pesticides thus promoting organic agriculture.

Introduction of g-glutathione synthetase, a GST gene, into poplar plants leads to higher concentrations of glutathione, and the plants show tolerance toward two chloroacetanilide herbicides, acetochlor, and metolachlor (Gullner et al. 2001). Indian mustard (*Brassica juncea*) expressing this gene shows increased tolerance to atrazine, 1-chloro-2,4-dinitrobenzene (CDNB), metolachlor, and phenanthrene (Flocco et al. 2004). Maize GSTs are known to detoxify triazine and chloroacetanilide herbicides, and transgenic tobacco plants expressing maize GST I have been shown to remediate alachlor (Karavangeli et al. 2005). In rice, GSTs conjugate the herbicide prechirachlor with glutathione, a reaction induced by the safener fenclorim (Scarponi et al. 2003). Using RNAi, GST activity toward cinnamic acid, CDNB, and prechirachlor can be reduced by as much as 77% in transgenic calli (Deng et al.

2003). These studies demonstrate the important role of GSTs in protecting plants against general herbicide stress contributing for reducing the negative impact of herbicides on the environment.

In addition to the approaches involving P450 and GST genes, various transgenic plants that exhibit herbicide tolerance can be used for phytoremediation. Transgenic alfalfa, tobacco, and *Arabidopsis* plants expressing a bacterial atrazine chlorohydrolase (atzA) gene show enhanced metabolic activity against atrazine (Wang et al. 2005). These innovations in plant biotechnology have a great advantage in the elimination and detoxification of agrochemicals used in agriculture thus controlling environmental pollution.

10.3.2 Breeding Crops for Reduced Fuel Use and GHG Emission

The fuel savings associated with making fewer spray runs in GM IR crops of maize and cotton (relative to conventional crops) and the switch from Conventional Tillage (CT) to Reduced Tillage or No Tillage (RT/NT) farming systems facilitated by GM HT crops have resulted in permanent savings in carbon dioxide emissions. The widespread adoption and maintenance of RT/NT production systems in North and South America, facilitated by GM HT crops (especially in soybeans), has improved growers' ability to control competing weeds, reducing the need to rely on soil cultivation and seed-bed preparation as means to getting good levels of weed control. As a result, as well as tractor fuel use for tillage being reduced, soil quality has been enhanced and levels of soil erosion cut. In turn, more carbon remains in the soil and this leads to lower GHG emissions. In 2018, this amounted to a saving of 2456 million kg of carbon dioxide, arising from reduced fuel use of 920 million liters. The largest fuel use-related reductions in carbon dioxide emissions have come from the adoption of genetically modified herbicide technology (GM HT) technology in soybeans. These savings have been the greatest in South America. Over the period 1996–2018, the cumulative permanent reduction in fuel use has been about 34,172 million kg of carbon dioxide, arising from reduced fuel use of 12,799 million liters. In terms of car equivalents, this is equal to taking 22.65 million cars off the road for a year (Brookes and Barfoot 2020).

10.3.3 Breeding Crops for Reclamation of Soil and Water

Phytoremediation of herbicides has been well studied using conventional plants. Transgenic plants produced for metabolizing herbicides and long-persisting pollutants can be used for the phytoremediation of foreign chemicals in contaminated soil and water. The genes involved in the metabolism of chemical compounds can be isolated from various organisms, including bacteria, fungi, plants, and animals, and these genes are then introduced into candidate plants. Transgenic plants expressing mammalian P450s and the other enzymes showed tolerance and phytoremediation activity toward target herbicides. Transgenic plants can also enhance the absorption and detoxification of pollutants, thereby aiding the phytoremediation of contaminated environments (Kawahigashi 2009). As with P450s, several GSTs seem to be involved in herbicide metabolism in plants. The genetic engineering of plants to facilitate the reclamation of soils and waters contaminated with inorganic pollutants is a relatively new and evolving field, benefiting from the heterologous expression of genes that increase the capacity of plants to mobilize, stabilize and/or accumulate metals. The efficiency of phytoremediation relies on the mechanisms underlying metal accumulation and tolerance, such as metal uptake, translocation and detoxification. The transfer of genes involved in any of these processes into fast-growing, high biomass crops may improve their reclamation potential (Fasani et al. 2018). So far, positive results for either phytostabilization of heavy metals in soils, or their removal, were achieved with the yeast vacuolar transporter YCF1 gene (Shim et al. 2013), and metal ligands (Martínez et al. 2006) that are efficient in inducing both tolerance and accumulation by allowing metal chelation and compartmentalization. Regarding the specific instance of Hg decontamination, the combination of bacterial merA and merB genes has proved promising in a wide variety of species tested, including fast-growing trees such as poplar (Dai et al. 2009).

Similarly, the phosphorus absorbance of plants has been enhanced using genetic transformation (Hirsch and Sussman 1999). Engineering plants to enhance their ability to efficiently absorb soil nutrients can reduce the use of harmful agrochemicals, in turn reducing environmental pollution. Also increasing the tolerance of the crops to withstand high metal levels in the soil is also practiced in tobacco (*Nicotiana tabacum*) and papaya (*Carica papaya*) plants in making them tolerant to aluminum (De la Fuente-Martínez and Herrera-Estrella 1999).

Similarly, tobacco plants transformed with an extracellular fungal laccase from *Coriolus versicolar* (Sonoki et al. 2005) secrete laccase into the rhizosphere and remove the pollutants bisphenol A and PCP with high efficiency (20 mg per gram dry weight). For TNT, tobacco plants have been genetically engineered to express a bacterial (*Enterobacter cloacae*) NADPH-dependent nitroreductase, which enhances conversion to aminodinitrotoluene within the roots of the engineered plants. Plants expressing the bacterial gene tolerate and degrade TNT, at levels lethal to wild-type plants. Interestingly, the plants also naturally contained an enzyme that could remove the toxic aminodinitrotoluene. Similarly, a gene *XplA* has been transferred from *Rhodococcus rhodochrous* into the plant *Arabidopsis thaliana* with subsequent decontamination of RDX (Vaishnav and Demain 2009).

10.3.4 Breeding Crops for Oil Spills and Explosives Pollution Control

The use of inherent aquatic plants along with recent omics tools have been used to improve the aquatic plant's phytoremediation ability to a great extent for controlling aquatic pollution (Agarwal and Rani 2022). These plants have an extensive root system that can filter and immobilize sediments, contaminants, fertilizer and pesticide run-off thereby reducing water pollution. Free-floating aquatic plants of the Lemnaceae family, mostly *L. minor*, can transform and eliminate azo-dyes like Acid blue dye (AB92) and other textile dyes by converting them into their by-products(Ansari et al. 2020; Khataee et al. 2012). American waterweed (*Elodea sp.*) is also known to bioaccumulate and phyto transform the DDT (dichlorodiphenyltrichloroethane) into DDD (Ekperusi et al. 2020). A study in duckweed indicated that *L. paucicostata* bioaccumulated less than 1% and significantly biodegraded 97.74% of hydrocarbons in wetlands and it is reasonable to infer that *L. paucicostata* is an effective aquatic macrophyte for the removal of petroleum hydrocarbons in moderately polluted waters (Ekperusi et al. 2020). Azolla and *H. verticillata* are reported to have the ability to remediate the toxic fly ash from the water body (Pandey 2012; Srivastava et al. 2010). Duckweed is popularly known for transforming the higher carbon chain hydrocarbons into lower carbon chain hydrocarbons (C30–C40), which are eventually conjugated and sequestered by the plants (Ekperusi et al. 2020; Agarwal and Rani 2022). Studies found that *E. crassipes* (water hyacinth) can be an effective aquatic plant for phytodegradation of bisphenol A (2,2-bis (4-hydroxyphenyl) propane) and ethion from pesticide-contaminated water (De Laet et al. 2019; Dhir 2013). Aquatic macrophytes that can survive in contaminated environments and can detoxify the water bodies include species of *Lemna, Eichornnia, Pteris, Wolfia, Spirodela, Hydrilla, Pistia, Typha* and *Crysopogon,* etc. (Agarwal and Rani 2022). Genomic approaches have identified genes that play an important role in the tolerance mechanisms and phytoremediation ability of the plant toward various contaminants (Agarwal and Rani 2022).

Studies of proteomic changes in the hyperaccumulator plant *Phytolacca americana* upon exposure to Cd stress revealed that 11 genes that were found responsible for photosynthesis and glutathione metabolism pathway were downregulated. Similarly, a proteomic study in Al stressed *Glycine max* revealed 21 proteins were attributable to the antioxidant defense system and were upregulated while 14 newly stored and 5 other proteins were downregulated (Agarwal and Rani 2022). These newly formed proteins were found to be concomitant with signal transduction, biosynthesis of cysteine synthase enzyme and sulfur metabolism in plant cells, which was confirmed with western blot. A somatic hybrid developed using *T. caerulescens* and *B. napus* and *T. caerulescens* and *B. juncea* showed enhanced biomass production and more Zn accumulation and Pb phytoextraction respectively (Rascio and Navari-Izzo 2011). A transgenic *Petunia* hybrid plant with the *CAXcd* (an Arabidopsis CAX1 mutant) gene for enhanced Cd tolerance and accumulation was also reported (Wu et al. 2011). The transgenic plants were able to accumulate up to 2.5 times more Cd than the controls. Similarly, transgenic *B. juncea* can better remediate selenium from contaminated soil as well as hydroponically, by the expression of *APS* (ATP sulfurylase) and *SMT* genes. Moreover, it is critically important to understand the functions and regulations of genes involved in metal uptake, hyperaccumulation, translocation via the xylem, detoxification and sequestration mechanisms to strategically manage the contaminated water systems without harming the wild species of the plants (Agarwal and Rani 2022). For the enhanced phytoremediation of organic or refractory pollutants, transgenic tobacco was used to express several genes comprising Nfs1 (encodes for nitroreductase), onr gene (organic nitrate reductase) that encodes for pentaerythritol tetranitrate reductase enzyme, (PETN) for the enhanced removal of GNT and TNT (Abhilash et al. 2009). It was also reported that the use of CYP450 2E1 (Cytochrome P450 Monooxygenase enzyme) gene in *N. tobaccum* can be used for the enhanced degradation of TCE, ethylene dibromide, anthracene and chlorpyriphos (Dixit et al. 2008). Up-regulation of ECS and GS in transgenic *B. juncea* can be used to amplify tolerance of plants towards atrazine, 1-chloro-2, 4-dinitrobenzene, phenanthrene, metolachlor (Flocco et al. 2004). XplA and XplB genes isolated from a soil bacterium, *Rhodococcus rhodochorus* (genus Nocardiaceae) were used to increase detoxification of RDX in *A. thaliana* plants as reported by Jackson et al. (2007). *Solanum tuberosum* was modified using CYP1A1, CYP2B6 and CYP2C19 to improve the resistance of transgenic plants toward sulfonylurea and other herbicides (Inui and Ohkawa 2005).

CRISPR-mediated strategy for enhanced phytoremediation focuses on scrutinizing and expressing the target genes to upsurge the synthesis of metal ligands, metal transporters, increased phytohormones and root exudates (Rai et al. 2021; Basharat et al. 2018). A plasma membrane protein (NtCBP4) was introduced in *N. tobaccum* plants for enhanced accumulation of lead in the plant, but simultaneously it caused sensitivity towards Pb in the plant system. Similar results were found when the MerC gene was transferred and expressed in two model plants, *Arabidopsis* and tobacco plants, causing sensitivity in these plants to mercury (Hg). Additionally, similar reports against the organic pollutants are also reported that such sensitivity against RDX and TNT was observed in the transgenic plants being developed for enhanced phytoremediation of these organic contaminants (Jaiswal et al. 2019). Yang et al. (2019) have reported the use of the CRISPR/ Cas9 system to produce the OsNRAMP5 knockout plants for meliorating the tolerance of *O. sativa* exposed to Cd stress.

Evaluation of the biodiversity and life forms of plant species in the impacted sites showed that phytoremediation with *C. esculentus*, alone or in a mix-culture with *C. laxus* and *L. peploides*, reduces the TPH (total petroleum hydrocarbons) to such an extent that the native plant community was progressively reestablished by replacing the cultivated species resulting in the ecological recovery of the affected soil. From the phytoremediation treatments, a mix-culture of *C. laxus*, *C. esculentus*, and *L. peploides* in soil removed 20.3% were polyaromatic hydrocarbons (PAH) and 34.2% were asphaltenes (ASF) and was able to remove up 93% of the TPH, while in unvegetated soil, the TPH removal was 12.6%. These results demonstrate that native Cyperus species from weathered oil spill-affected sites, specifically *C. esculentus* and *C. laxus*, alone or in a mix-culture, have potential for phytoremediation of soils from tropical wetlands contaminated with weathered oil hydrocarbons (Palma-Cruz et al. 2016). A study revealed that arsenic could be removed through volatilization from the contaminated soil by bacteria that have the *arsM* gene expressed ad it is possible to use microorganisms expressing *arsM* as an inexpensive, efficient strategy for arsenic bioremediation from contaminated water and soil (Liu et al. 2011). In addition, rice plant is efficient in arsenic (As) accumulation due to enhanced soil As release under flooded condition and its effective As uptake. Therefore, rice plant can be used to remove bioavailable As from paddy soil. The removal of rice roots resulted in ~19% lower the diffusive gradients in thin films (DGT)-As in post-harvest soil compared to without removing the roots (He et al. 2020). Therefore, the use of such information in plant breeding with the aid of genetic engineering and omics technology can be used as a successful tool for developing commercial crops with pollution-controlling properties considering the health and environmental benefit.

10.3.5 Breeding Plants for Other Environmental Issues

Agricultural research and production systems are not nature +ve in many areas. Due to which (in addition to other factors), environmental condition is deteriorating day by day. In breeding program, breeders paly more roles on determining the genotypes for next generation but in nature, environmental factors play crucial role to select the genotypes that suit best in next generation. Evolutionary population of any varieties are those populations, where environmental factors decide on advancing the progenies in next generation. Such population could be of greater role on tackling the different negative aspects of environment. Plant breeding has a very wide scope on dealing many following environmental conditions.

- Virus free plant from meristem tissue culture is an effective method for virus treatment, i.e. without using any chemicals and others compounds.
- Tissue culture can help to rescue the germplasm, which are at risk and not able to produce progeny. Even the number of plants within a short period can be made ready to transplant in environmentally degraded areas. This helps to restore the conditions in a faster way.
- Climate changes are the top most concern across the world and crop breeding is an option to develop the climate resilient crop populations. Faster growing varieties can be developed so that many drought areas can be made greenery.
- To speed up the restoration of degraded habitat, plant breeders can develop suitable broad genetic base varieties that suit to such areas.
- Production systems along with road side and city areas are affected by dust and other air and water pollutant. For such areas, dust and pollutant tolerant plant could be developed.
- The amount and types of waste are also increasing and they are damaging the production areas as well. Depending on the types of waste in the soil, adjustable varieties can be developed.
- With the advances on manipulating genetic makeup, nitrogen fixation plant varieties could be very effective means to increase the soil fertility. This helps to minimize the fertilizers required per unit area. Such nitrogen fixing system will also be useful to keep the air with balanced components.

- Ecological services are now decreasing in many areas. This is root cause of bad environments and for which suitable varieties which enhance the ecological services can be bred and grown in needy areas.
- To help purify the water and air purification, suitable varieties of different plant species can be developed as per the necessity of particular site.
- Soil erosion is another major problem on damaging the environment. Plant varieties with strong root system could be developed that helps to control soil erosion.
- Organic matter in the soil is very low in many production systems. To speed up the organic matter in the soil, high biomass producing plant varieties could be developed.

10.4 Breeding Constraints and Limitations

Along the technological advances, the nutritional aspects are being considered only on few crops e.g. rice, wheat, maize, lentil, etc. Under the environmental aspect, focus is only on drought, and insect pests. Health related crop breeding is almost none and there should be some initiation for breeding crops suitable for agro-hospital and agro-medical college. Static populations in the field along the rapid genetic erosion are creating havoc to getting the suitable genetic materials for better designing the genotypes for diverse human population and consumer demands. The advanced technologies, infrastructures and experts are not available in all countries for many different crop species especially on breeding for nutrition and environmental security. Conserving genetic diversity statically in Genebank and marketing single genotypes worldwide cannot favor evolution which is a must in the context of climate change, nutritional demand and health issues. Different varieties may be required to breed nutritionally suitable for different age people and can be produced at different seasons across the diversity agro-eco zones. This means large number of varieties of many different crops should be developed. Breeding strategies should also focus on improving underutilized crop species including local landraces in addition to major staple food crops for ensuring nutrition, health and environmental security. However, genetics of many underutilized crop species and landraces are not known.

The development of different nutrient-rich varieties is very costly and time-consuming. Nutrition profiling and metabolic assisted breeding demands both high skill and advance equipment. Crop breeding is far behind to support the new subject i.e. Medical Agriculture by developing varieties of agricultural medicine which are needed to run the agricultural hospital. The big challenge is breeding works with the concept of treatment and prevention of human diseases through healthy food, medicinal herb, agricultural exercise, balanced agroecosystem, waste management, nutrient-rich genotypes, and plant-based vaccines, etc.

Some of the areas of concern regarding the development and use of GM crops include the health of the consumer, impact on the environment, impact on farmers, and government regulations. However, a thorough examination of the risks associated with GM crops and their products before it is allowed for cultivation and consumption, is necessary. To get more environmentally friendly genetically modified crops for environmental security, it is important to use novel breeding approaches like omics technologies and genome editing tools to minimize the risk of transgenes being incorporated into new plants and for this, manpower is very limited across the world. All breeding researches are mostly carried out on chemical production system but for health and environment, varieties suitable for nature +ve production system have not been considered. To increase the soil organic matter, high biomass producing varieties are also very important and this aspect was given due attention in the past. To minimize the environmental shock to newly developed varieties, seed production and maintenance system of such varieties should be developed in such a way that, farmers can maintain seeds themselves without deteriorating genetic performance.

10.5 Conclusions

The major problems worldwide are food and nutrition insecurity, and environmental hazards including climate changes, pesticides, waste, etc. Worldwide, over 98% population is malnourished, mostly from developing countries such as South Asian and African continents. The environment is deteriorating day by day and human health is becoming challenging. Plant breeding plays a significant role for nutrition, human health and environmental security. Food fortification and biofortification (agronomic, conventional breeding and genetic engineering) approaches of edible crops are considered effective and have outstanding potential for ameliorating the problem of micronutrient malnutrition. Although food fortification is cost-effective and does not require any behavioral change, minimum risk, and no alteration of intrinsic characteristics of food; ordinary people cannot afford it without any financial support and not feasible for small farmers and rural poor in developing countries. Similarly, agronomic biofortification improves the grain nutrients such as iron, selenium, and zinc concentration for improving human health benefits. However, it is very crop and cultivar dependent, not possible to target edible organs, rural people cannot afford and access micro-nutrients and if its application dose is imbalanced, it may affect negatively to plant health and yield. With the advancement of conventional plant breeding systems, new crop varieties can be developed with desired micronutrient traits without disturbing their intrinsic properties, however, it takes a long time and depends on the existing gene pool. Therefore, advanced genetic engineering and molecular technologies such as transgenic/cis-genic, and genome editing are superior promising and sustainable tools for direct introducing or manipulating desired target genes to develop biofortified crop varieties. It is rapid, unconstrained by gene pool, targeted expression in edible organs, and apply directly to elite cultivars. The benefits and challenges of genetically modified crops have attracted enormous public concerns i.e., food safety, toxicity, pest resistance, crop yield effects and biosafety. Therefore, some regulatory, socio-economic, intellectual property rights, environmental and human health issues should be addressed properly which requires policy initiatives and government support for further research and development, and dissemination of biofortification technologies and practices globally.

Although GM technology is debated, it may offer other useful potential benefits by developing biotic and abiotic stress-resistant crops which ultimately reduce environmental pollution through reduced use of herbicides, pesticides, reduced greenhouse gas emissions, and other toxic pollutants of soil and water. Several novel biotechnological approaches have been developed to improve crops for enhanced yield, and biotic and abiotic stress resistance, however, the biosafety of those newly developed crops and their role in biodiversity conservation and environmental safety should be confirmed before cultivation, consumption and marketing globally. Crop breeding can also play an important role to improve the urban environment by developing suitable varieties for urban agriculture and dust-absorbing varieties to plant along the side of the road. Therefore, the adoption of new tools, technologies and approaches can help crop breeders to achieve a greener world, not only for increasing yield of any crops for farmers, but also for local communities who need nutritious crops/foods, healthy diet and a healthy environment. As there is necessity of consideration of nutrition, human health and environment aspects in plant breeding across the world, the number of breeders with the capacity of using different advanced tools, e.g. biotechnological, nutrient profiling, etc. should be increased in each country so that diverse types of varieties of many different plant/ crop species could be developed.

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