



Recent Progress in Cereals Biofortification to Alleviate Malnutrition in India: An Overview

11

Pankaj Kumar, Arun Kumar, Karuna Dhiman, and Dinesh Kumar Srivastava

Abstract

As the world's population increases, food insecurity and malnutrition due to essential micronutrient(s) deficiency are emerging as the two foremost challenges, and need urgent attention. Micronutrient deficiency among women, children, and adolescents is a big challenge in developing countries like India. People in such countries suffer not only from hunger but more from hidden hunger due to a lack of essential vitamins and minerals (micronutrients). Malnutrition is a major food-related primary health problem worldwide, including India, where the main staple food crops are cereals. Cereals contribute a significant part

Pankaj Kumar, Arun Kumar, Karuna Dhiman and Dinesh Kumar Srivastava contributed equally.

P. Kumar (✉)

Department of Biotechnology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

Biotechnology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

e-mail: pksharmabiotech@yspuniversity.ac.in

A. Kumar

Biotechnology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

e-mail: arunkumar@ihbt.res.in

K. Dhiman

Department of Biotechnology, College of Horticulture and Forestry (Affiliated to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan), Neri, Hamirpur, Himachal Pradesh, India

D. K. Srivastava

Department of Biotechnology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

to human nutrition and are a vital source of energy for human diets. Supplements, a balanced diet, fortifications, and biofortification are strategies to alleviate micronutrient malnutrition. Biofortification is a new nutritional revolution to deliver nutrient-rich food to every individual. Cereal crop biofortification is a promising way to serve a larger section of our society, including rural and poor populations. Cereal biofortification can provide a comparatively cost-effective, sustainable, and long-term means of delivering sufficient micronutrients to rural communities in developing nations. Conventional breeding, application of genomic tools, agronomical and transgenic approaches are some of the common strategies for crop biofortification. Therefore, this chapter provides insights from the cited literature on recent progress in cereals biofortification to alleviate malnutrition in India using different crop breeding and transgenic approaches.

Keywords

Biofortification · Genetic engineering · Cereals · Malnutrition · Micronutrients

11.1 Introduction

In developing countries like India, people are largely dependent on staple food crops such as rice, wheat, maize, millets, and sorghum. Today, our nation has attained self-sufficiency in food supply due to the green revolution that significantly increased the food grain production from 50.82 million tonnes in 1950–1951 to 284.83 million tonnes in the year 2017–2018 (Table 11.1). However, more than two billion people, especially women and pre-school age children, are micronutrient malnourished caused by a deficiency of micronutrients in the diet. More than half of the human population of developing countries of Asia and Africa are deficient in micronutrients such as Fe and Zn (White and Broadley 2009; Gomez-Galera et al. 2010). According to National Family Health Survey (NFHS), Ministry of Health and Family Welfare (MOHFW), Government of India, International Food Policy Research Institute

Table 11.1 Indian Statistical report on total cereals and total food grain production. (In Million Tonnes)

Year	2015–2016	2016–2017	2017–2018	2018–2019	2019–2020 ^a
Rice	104.41	109.70	112.76	116.48	118.43
Wheat	92.29	98.51	99.87	103.60	107.59
Maize	22.57	25.90	28.75	27.72	28.64
Total cereals production	235.22	251.98	259.60	263.14	273.50
Total food grain production	251.57	275.11	285.01	285.21	296.65

Sources: Agricultural Statistics Division, Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare

^aFourth advance estimates of production of food grains for 2020–2021

Table 11.2 Essential nutrients for well-being of human life^a. (modified from Bouis and Welch 2010; Garg et al. 2018)

Air, water, and energy	Amino acids/ proteins	Fats/ lipids	Essential macro elements	Essential trace elements	Vitamins
Oxygen	Histidine	Linoleic acid	Na	Fe	A (retinol)
Water	Isoleucine	Linolenic acid	K	Zn	D (calciferol)
Carbohydrates	Leucine		Ca	Cu	E (α-tocopherol)
	Lysine		Mg	Mn	K (phyllloquinone)
	Methionine		S	I	C (ascorbic acid)
	Phenylalanine		P	F	B1 (thiamin)
	Threonine		Cl	Se	B2 (riboflavin)
	Tryptophan			Mo	B3 (niacin)
	Valine			Co (in B12)	B5 (pantothenic acid)
				B	B6 (pyroxidine)
				B7 (biotin)	
				B9 (folic acid, folacin)	
				B12 (cobalamin)	

^aVarious additional valuable substances in foods are also known to contribute to better health

(IFPRI), and World Health Organization (WHO)/World Bank Group-Joint Child Malnutrition Estimates-2017, two billion people are malnourished, and 795 million are undernourished worldwide. Also, around 155 million children (<5 years) are stunted (low height-for-age), 52 million wasted (low weight-for-height), and 17 million severely wasted. Malnutrition contributes to a loss of 11% GDP in Asia and Africa. Indian scenario of malnutrition showed that 195 million people (15.2%) of the population is undernourished. Human beings require around forty known nutrients in their diet to live healthy and productive lives (Table 11.2). But unfortunately, major staple cereals contain insufficient amounts of essential nutrients such as vitamin A, iron (Fe), zinc (Zn), calcium (Ca), manganese (Mn), copper (Cu), iodine (I), or selenium (Se) to meet daily requirements (Neeraja et al. 2017). Supplementation, dietary diversification, fortification, and biofortification adapted to conditions in different countries, and regions are the comprehensive strategies to alleviate micronutrient malnutrition (Zimmerman and Hurrell 2007; Stein 2010). Key food crops with enhanced nutrients can be obtained by biofortification, which involves the genetic enhancement of micronutrients (Bouis et al. 2013). Unlike fortification (addition of exogenous nutrients as in iodized salt), biofortification methods increase the nutrients of crops at source through agricultural interventions,

viz. agronomy, breeding, and biotechnology. Also, growth and production in soils with depleted or unavailable minerals can be improved using these biofortified crops (Cakmak 2008; Borg et al. 2009). Staple food crops can be biofortified to enhance micronutrient concentrations in edible parts to address hidden hunger, with the potential to reach the neediest of the population (Haug et al. 2007; Bouis and Welch 2010; Lyons and Cakmak 2012). Thus biofortification, which links nutritious agricultural products with human health, can be more effective and sustainable than the other approaches used to combat mineral malnutrition (Lyons 2014). Along with national and international biofortification programs, the Indian Council of Agricultural Research, Government of India, has taken leads for biofortification of cereal crops by targeting the enhancement of nutrients in staple food crops.

11.2 Biofortification Approaches

Biofortification approaches focus on enhancing the nutritional contents of the crop through agricultural interventions, viz. agronomy, breeding, and biotechnology (Fig. 11.1). These approaches are discussed as follows:

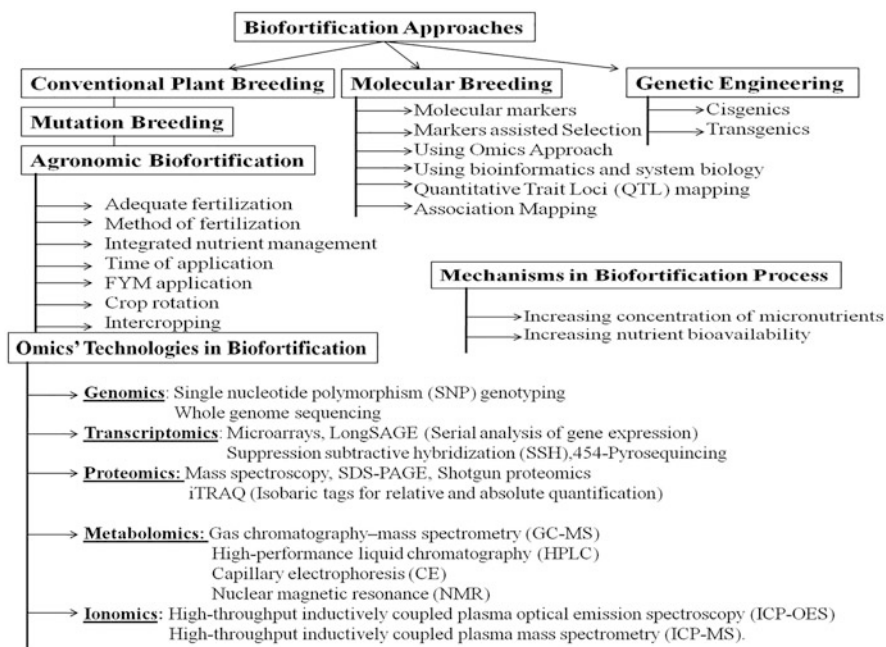


Fig. 11.1 Overview of biofortification approaches, mechanism, and omics technology as biofortification tools in cereals biofortification (modified from Carvalho and Vasconcelos 2013)

11.2.1 Biofortification Through Agronomic Approaches

The utmost demand for nutritional security of the developing world can be met by enhancing the dietary values of important staple food crops for certain essential micronutrients using agronomic biofortification. The physical application of nutrients to temporarily improve the nutritional and health status of crops through agronomical biofortification can improve human health by the consumption of such crops (Cakmak and Kutman 2017). Poor rural masses will never have money to buy mineral supplements. Even they cannot afford to improve the components of their diet by incorporating animal products; thus, agronomic biofortification will be a boon to such people. The agronomic biofortification approach generally relies on the application of mineral fertilizers, and either solubilization/or mobilization of nutrients from the soil to the edible parts of plants. Microbial cultures of plant growth-promoting soil microorganisms can also be used to enhance the nutrient mobility from soil to edible parts of plants and improve their nutritional status, in addition to fertilizer. Phyto-availability of these mineral elements can also be increased by using soil microorganisms like different species of *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter* (Smith and Read 2007). Crop productivity of crops grown in nitrogen-limited conditions can be increased using N₂-fixing bacteria (Hardarson and Broughton 2004). Organic acids, siderophores, and enzymes capable of degrading organic compounds and increasing mineral concentrations in edible produce are released by mycorrhizal fungi associated with many crops (Rengel et al. 1999; Cavagnaro 2008). In developing countries of Asia and Africa, cereals are the staple food crops, so agronomic biofortification is the easiest and fastest way for grain's micronutrient (Fe and Zn) enhancement. Agronomic addition of the appropriate nutrient as an inorganic compound to the fertilizer increases the mineral content of the plant as demonstrated successfully in crops like rice, wheat, and maize (Bouis and Welch 2010).

11.2.2 Biofortification Through Conventional Breeding

The most accepted method of biofortification is conventional breeding. As compared to transgenic and agronomic-based strategies, this approach is sustainable and cost-effective. India is one of the mega centers of agro-biodiversity, but little effort has been made to evaluate the promising germplasm for enhanced nutrients in crops. With some identified donors for high nutrients, varieties are being developed through conventional breeding by crossing with popular varieties. Conventional breeding can be feasible if there is sufficient genotypic variation for the trait of interest. Levels of minerals and vitamins in crops can be improved by utilizing these variations in breeding programs. In conventional plant breeding, plants with desired nutrient and agronomic traits can be produced by crossing parent lines (with high nutrients) with the recipient lines with desirable agronomic traits over several generations. In case of limited genetic variation present in the gene pool crossing to distant relatives and moving the trait slowly into the commercial cultivars can be

done. Alternatively, mutagenesis of commercial varieties can be done to introduce new variations. HarvestPlus is the program that was launched by the Consultative Group on International Agricultural Research (CGIAR) along with the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI) to breed biofortified staple food crops. This program is investing heavily to boost three key nutrients—vitamin A, Fe, and Zn and is targeting the staple crops, wheat, rice, maize, cassava, pearl millet, beans, and sweet potato in Asia and Africa (Bouis and Welch 2010). It is directed to produce staple food crops with enhanced bioavailable essential minerals and vitamins that will have a measurable impact on improving the micronutrient status of target populations, primarily resource-poor people in the developing world. The Biocassava Plus program had been initiated to improve the nutrition status of the cassava crop. The breeding lines with adequate amounts of nutrients and promising yield thus developed are evaluated under the Indian Council of Agricultural Research (ICAR)-All India Coordinated Research Projects (AICRP) for varietal release. Recent approaches for biofortification include identification of genomic regions/candidate genes for high nutrients through tagging/identification of major genes or mapping of quantitative trait loci (QTL) followed by their introgression into popular varieties. Being a genetic solution, growing biofortified crops does not require any additional expenditure for farmers as this approach uses the intrinsic properties of crops. Since biofortified crops are developed through conventional breeding, regulatory constraints are not applicable for their release (Bouis and Welch 2010).

11.3 Transgenic Approach for Biofortification

Genetic engineering offers an alternative for increasing the concentration and bioavailability of micronutrients in the edible crop tissues when there is not sufficient variation among the genotypes for the desired character/trait within the species, or when the crop itself is not suitable for conventional plant breeding (due to incompatibility barrier) (Prasad et al. 2015). Thus, the transgenic approach can be an appropriate alternative in case of limited or no genetic variation in nutrient content among plant varieties. Plant transformation may be faster than conventional breeding to achieve the nutritional target, and this can be a valid alternative, where breeding approaches are not successful (Brinch Pedersen et al. 2006; Zhu et al. 2007). Genes from novel sources for desirable target traits can be introduced using this approach with unlimited access to the genes of interest, targeted expression in tissues of interest, rapid and direct application by introduction into popular varieties, and stacking of different genes. In the development of transgenic crops, the ability to identify and characterize gene function and then utilize these genes to engineer plant metabolism is essential (Christou and Twyman 2004).

Furthermore, metabolic engineering can be exploited to transplant alternative pathways using pathways from bacteria and other organisms (Newell-McGloughlin 2008). Genetic modifications can also be used to reduce the concentration of anti-nutrients which limit the bioavailability of nutrients in plants for redistribution of

micronutrients between tissues, enhancing the micronutrient concentration in the edible portions of commercial crops, increasing the efficiency of biochemical pathways in edible tissues, and/or even the reconstruction of selected pathways (Shewmaker et al. 1999; Agrawal et al. 2005; Yang et al. 2002). Unlike nutrition-based organizational and agronomic biofortification programs, the transgenic approach is an alternative and sustainable (White and Broadley 2005; Hefferon 2016). Transgenic lines were developed for β -carotene, high zinc, high protein, high iron, low phytate, and folic acid. Successful examples of transgenic products are high lysine maize, high unsaturated fatty acid soybean, high provitamin A and iron-rich cassava, and high provitamin A Golden rice (Garg et al. 2018).

11.3.1 Cereals Biofortification to Alleviate Malnutrition in India

The principal cereal food grain cultivated in India is rice, followed by wheat, maize, sorghum, and pearl millet. To date, a lot of research work which has been carried out in cereals crops with proof of concept using various biofortification approaches through national and international biofortification collaboration programs are discussed as follows:

11.3.1.1 Rice

Staple food crops such as rice (*Oryza sativa*) play a pivotal role in the Indian economy. Rice consumption is ~220 g per day, and thus, rice is a significant calorie supplement for two-thirds of the Indian population. Polished rice is a poor source of micronutrients (Eric and Eddie 2012). Various rice-growing countries, including India, have a primary consideration of emphasizing increasing the nutritional quality of rice. Biological and genetic enrichment of food products with vital nutrients, vitamins, and proteins aimed at rice biofortification program. Production of nutrient-packed rice grains in a sustainable way so that the product reaches the malnourished population in rural India can be possible with rice biofortification with vital nutrients so that the farmer can grow the variety indefinitely without any additional input. Conventional breeding can be used to enhance Zn and protein contents in polished rice, whereas transgenic technology appeared to be the only viable solution for increasing β -carotene and Fe.

Fe and Zn

Iron deficiency is one of the most prevalent micronutrient deficiencies affecting more than two billion people worldwide (World Health Organization 2016). Low dietary intake of Zn puts one-third of the world population at risk, including two billion people in Asia and four hundred million in Saharan Africa (Hotz and Brown 2004; Myers et al. 2015). Iron and zinc concentrations in brown rice are in the range of 6.3–24.4 $\mu\text{g/g}$ and 13.5–28.4 $\mu\text{g/g}$, respectively. Therefore, there was an approximately twofold difference in iron and zinc concentrations, suggesting a vast genetic potential to increase the concentration of these micronutrients in rice grains (Gregorio et al. 1999). Many promising donors were identified by screening

thousands of rice germplasm lines for Fe and Zn contents in brown and polished grain across the world. However, approximate loss of micronutrients Fe and Zn during the polishing is about 90% and 40%, respectively (Babu 2013; Pinson et al. 2015). A total of 126 accessions, including cultivated *indica* and *japonica* rice cultivars, germplasm accessions, and wild rice genotypes of brown rice, were analyzed for Fe and Zn concentration in brown rice by Anuradha et al. (2012) in which Fe concentration ranged from 6.2 ppm to 71.6 ppm, and Zn concentration ranged from 26.2 ppm to 67.3 ppm.

Similarly, Roy and Sharma (2014) analyzed rice landraces (84 cultivars), which were collected from various agro-ecological regions of West Bengal and adjoining areas for Fe and Zn. The concentration of Fe and Zn varied from 0.25 $\mu\text{g/g}$ to 34.8 $\mu\text{g/g}$ and 0.85 $\mu\text{g/g}$ to 195.3 $\mu\text{g/g}$, respectively. Identification of these micronutrient-rich genotypes opens up the possibilities for the linkage mapping of genomic regions or QTLs responsible for mineral uptake and translocation, which can be subsequently used as a donor for developing nutrient-enriched varieties. Genes associated with Zn metabolism and QTL for grain Zn concentration have been reported in rice (Swamy et al. 2016). ICAR-Indian Institute of Rice Research (IIRR), Hyderabad has released “DRR Dhan 45” using a donor from the HarvestPlus program. Chhattisgarh zinc rice-1, one of the high nutrient content varieties in polished rice, was released for the state of Chhattisgarh by Indira Gandhi Krishi Viswavidyalaya (IGKV), and “Mukul” (CR Dhan 311) was released for the state of Odisha by ICAR-National Rice Research Institute (NRRI). Expression of ferritin genes, nicotianamine synthase genes (NAS), or ferritin in conjunction with NAS genes to increase the Fe concentration of rice endosperm using transgenic technology could lead to a twofold and sixfold increase via single-gene and multi-gene approaches, respectively. Expression of Fe storage protein ferritin under the control of endosperm-specific promoters can increase the Fe storage capacity of rice grains. This approach can increase the concentration of Fe in the seeds of transformants by approximately twofold in polished seeds. Iron stored in ferritin is an important source for humans to avoid iron deficiency. Therefore, transgenic rice with 3–4 times as much Fe than wild-type rice was developed using different sources of the ferritin gene (Masuda et al. 2012; Paul et al. 2014).

Protein

Essential amino acids are crucial for normal growth and metabolism and cannot be synthesized de novo by humans, especially lysine (Lys) and methionine (Met) (Lee et al. 2003; Ufaz and Galili 2008; Cohen et al. 2014). Milled rice grains are a poor source of essential amino acids. Therefore, one of the main goals of breeders is to increase the lysine content in cereal grains, especially rice, to enhance the nutritional value of grains and prevent nutrient deficiency diseases such as kwashiorkor (Toride 2004). Lysine content has been enhanced in some cereals by a combination of conventional breeding and mutagenesis approaches. Moreover, it is challenging to improve this trait in most grains due to the limited availability of lysine-rich germplasm resources, particularly in the case of rice (Sun and Liu 2004). Lysine levels in crops can be increased with the development of molecular biological

techniques, and three strategies have been developed (Birla et al. 2015). The direct approach is over-expressing lysine-rich proteins in grains of rice (Wong et al. 2015), maize (Yu et al. 2004), and sorghum (*Sorghum bicolor* L.) (Zhao et al. 2003). Another approach is to modify seed storage proteins, e.g. silencing of 13 KDa prolamin encoding genes increased total lysine content by 56% and thus altered nutritional quality in rice (Kawakatsu et al. 2010). The last approach is to use metabolic engineering to regulate the key genes involved in lysine metabolism to increase lysine content in plants (Zhu and Galili 2003; Long et al. 2013). Several landraces and released varieties have been characterized for their protein and amino acid profiles in rice, and “Heera,” an old variety of rice, was found to have >10% protein. The mean crude protein content of the varieties, as estimated using the Kjeldahl method, was in the range of 6–8% (Juliano 1993). CR Dhan 310 with >10% protein in polished rice developed by NRI has also been nationally released. Genomic regions and genes associated with protein in rice have been deciphered (Rawat et al. 2013; Bao 2014; Garg et al. 2018).

Provitamin A

Vitamin A is a fat-soluble vitamin playing an essential role in vision, bone growth, reproduction, and in the maintenance of healthy skin, hair, and mucous membranes (FAO/WHO 2002). Among 118 countries primarily in Africa and South-East Asia, Vitamin A deficiency (VAD) is a global public health problem (Rostami et al. 2007). The vitamin A status of the poor can be addressed by an emerging strategy of biofortification of staple crops with provitamin A, and carotenoids (Tanumihardjo 2008; Tanumihardjo et al. 2008). For example, a bioengineered provitamin A enriched rice in India, Philippines, and Brazil is Golden Rice. In Asian countries, up to 73% of energy intake can be from rice. So vitamin A intake of vulnerable groups in developing countries can be increased by the enrichment of rice with vitamin A. Three genes for biosynthesis of β -carotene in grain were used to create golden rice, and the latest version is GR2R with >20 ppm of total carotenoids (Rawat et al. 2013). IARI, IIRR, and Tamil Nadu Agricultural University (TNAU) in India were the three research groups which were involved in the development of Indian versions of golden rice from the original prototype in collaboration with the International Rice Research Institute (IRRI) supported by the Department of Biotechnology (DBT), India.

11.3.1.2 Wheat

Wheat is the second important staple cereal after rice in India and the foremost target crop under the biofortification program and has significantly contributed in reducing hunger and malnutrition. Low genetic variation in cultivated wheat has been observed for Zn and Fe. However, wild relatives (*Triticum spelta*, *Aegilops tauschii*), emmer wheat, and different landraces are known to have wide variation for grain micronutrient (Zn and Fe) concentrations up to 190 ppm, and have been exploited for improvement of modern elite cultivars using biofortification approaches (Monasterio and Graham 2000; Cakmak et al. 2004; Ortiz-Monasterio et al. 2007; Garg et al. 2018).

Fe and Zn

Huge genetic variability for grain yield and micronutrient (Zn and Fe) has been observed in wheat germplasm. Wheat germplasm screening studies in hexaploid wheat genotypes revealed twofold variation, i.e. 25–55 ppm for micronutrient (Zn and Fe) contents, whereas in diploid wheat genotypes, it was fourfold (up to 100 ppm) (Chhuneja et al. 2006; Ortiz-Monasterio et al. 2007). The wheat varieties developed before the green revolution showed higher Zn and Fe contents than that of varieties developed after the green revolution, as reported by ICAR-Indian Institute of Wheat and Barley Research (IIWBR). The possible reason could be the selection for high-yielding varieties rather than the varieties with high nutritional quality (Neeraja et al. 2017). But because of today's need for nutritional security, Department of Biotechnology, Government of India, has started a wheat biofortification program for enhanced micronutrients using conventional and molecular breeding, and different biofortified varieties have been released in India. Indian Council of Agricultural Research, in collaboration with HarvestPlus wheat Biofortification program, has released different biofortified wheat varieties with 4–10 ppm higher Zn content. Biofortified wheat (high Zn content) varieties such as “BHU 1, BHU 3, BHU 5, BHU 6, BHU 7, and BHU 18” were released in India in 2014.

Along with high zinc content, BHU 1 and BHU 6 also had shown higher yield and disease resistance. Punjab Agricultural University, Ludhiana, India has also recently released high Zn wheat variety “PBW1Zn.” Indian Institute of Wheat and Barley Research, Karnal, India, has also developed and released high Zn and Fe content wheat variety “WB2” (Rawat et al. 2013; Garg et al. 2018). Using the transgenic breeding biofortification approach, the Fe content in wheat genotypes has been enhanced by the expression of the ferritin gene (*TaFer1-A*) from wheat (Borg et al. 2012) and soybean (Xiaoyan et al. 2012).

Low Phytate

To decrease the phytate or phytic acid content in wheat, sizeable genetic variability in synthetic wheat hexaploid genotypes up to sixfold for the phytase level has been reported. Therefore, the Indian Institute of Wheat and Barley Research (IIWBR) developed a microlevel test to transfer the high phytate level traits into the high-yielding backgrounds (Ram et al. 2010). Expression of the phytochrome gene (*phyA*) resulted in increased phytase activity, leading to enhanced iron bioavailability (Brinch-Pederson et al. 2000). Bhati et al. (2016) reported a decrease in phytic acid by silencing of wheat *ABCC13* transporter.

Protein

Wild tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) has been identified with high grain protein content and micronutrient using molecular breeding approaches. Distelfeld et al. (2007) identified quantitative trait loci (QTL) (*Gpc-B1*) in *dicoccoides* wheat for high grain protein content and transferred into cultivated bread and durum wheat. In India, under the AICRP-Wheat biofortification program, Punjab Agricultural University (PAU), IIWBR and IARI transferred the *Gpc-B1* QTL into different high-yielding wheat accessions and developed genotypes

are being tested. Using transgenic breeding approaches of biofortification, transfer of the *Amaranthus* albumin gene (*Amal* gene) into elite wheat cultivars resulted in increased wheat grain protein content, particularly essential amino acid content (lysine, methionine, cysteine, and tyrosine) (Tamas et al. 2009).

Provitamin A

Provitamin A is also an important targeted nutrient under wheat biofortification through breeding approaches. Indian Agricultural Research Institute (IARI), New Delhi has commercially released the high provitamin A durum wheat variety “HI 8627.” By expressing bacterial *psy* gene and carotene desaturase genes (*crtB*, *crtI*) using transgenic technology, wheat provitamin content was enhanced (Cong et al. 2009; Garg et al. 2018).

11.3.1.3 Maize

Maize is utilized as a human food and livestock feed, and thus it assumes worldwide significance. India is the second-most important maize growing country in Asia and is the world’s sixth-largest producer and the fifth largest consumer of maize (Prasanna 2014). Around 73% of farmland dedicated to maize production worldwide is located in the developing world. In India, 10% of the total production of maize is used for human food, while 60% is utilized for poultry- and animal-feed (Yadav et al. 2015). Important breakthroughs in maize biofortification are because of the vital role of maize in global diets and the rich genetic diversity of the crop. Thus biofortification of maize, including enhancement of protein quality coupled to the enrichment of micronutrients like provitamin A, Fe, and Zn in grain assumes great significance.

Fe and Zn

Fe and Zn contents are low in maize, a staple crop of Southern and Eastern Africa (CIMMYT 2000). Banziger and Long (2000) reported genetic variability for Fe and Zn in white grained tropical maize germplasm ranging from 16.4–22.9 µg/g and 14.7–24.0 µg/g, respectively. Accessions (1814) were also evaluated in 13 trials over 6 years, and a range in the grain of 9.6–63.2 mg-Fe/kg and 12.9–57.6 mg-Zn/kg was reported (Neeraja et al. 2017). Fe content was 15–159 ppm in mid-altitude and 14–134 ppm for low land inbred lines, and Zn content was 12–96 ppm for mid-altitude and 24–96 ppm for lowland inbred lines in maize germplasm, showing that sufficient genetic variation is available in maize germplasm (Pixley et al. 2011). In India, wide genetic variation for kernel Fe and Zn in a diverse set of normal and quality protein maize (QPM)-inbreds was reported by Chakraborti et al. (2009), Chakraborti et al. (2011), Prasanna et al. (2011), Agrawal et al. (2012), Guleria et al. (2013), Goswami et al. (2014), Mallikarjuna et al. (2014), and Pandey et al. (2015). The presence of ample variability for kernel Fe and Zn indicates the possibility of genetic enhancement of these micronutrients in maize. Accumulation of Fe and Zn in the maize kernel is governed by polygenes (Gorsline et al. 1964; Arnold and Bauman 1976). QTLs governing the accumulation of these micronutrients in

maize have also been reported (Lungaho et al. 2011; Simic et al. 2011; Qin et al. 2012; Baxter et al. 2013).

Low phytate

Phytic acid/phytate, which is an anti-nutritional component, plays a major role in reducing the bioavailability of Fe and Zn; thus, it is an important target for biofortification in maize. Phytate in the seed has a primary function to store phosphorus as an energy source and antioxidants essentially required for the germinating seeds, and 80% of the total phosphorus in the maize grain is present as phytic acid (Raboy et al. 2000). But the positively charged minerals like Fe and Zn get chelated by the negative charge of the phytic acid and make them unavailable in the animal gut (Raboy 2001). Undigested phytic acid, when released into the environment, causes environmental pollution because phytic acid in grains reduces the availability of phosphorus to poultry since monogastric animals cannot digest it (Cromwell and Coffey 1991). Low phytic acid mutants can be produced by the mutations in genes encoding myo-inositol-3-Pi synthase (*MIPS*) and inositol polyphosphate kinases. Myo-inositol-3-Pi synthase (*MIPS*) followed by inositol phosphate kinases convert glucose 6-phosphate to inositol-3-phosphate for phytic acid synthesis in plants. Chemical or radiation-induced mutagenesis is being used to develop low phytic acid (*lpa*) mutants. These *lpa* mutants include *lpa1* mutant of maize (Raboy et al. 2000), barley (Larson et al. 1998; Rasmussen and Hatzack 1998), and rice (Larson et al. 2000). In maize, *lpa1*, *lpa2*, and *lpa3* are three low phytic acid (*lpa*) mutants with 66%, 50%, and 50% reduction in phytic acid content. The seeds of *lpa* mutants have been found to be viable and normal. In India, scientists at Tamil Nadu Agricultural University (TNAU), Coimbatore, successfully introgressed the *lpa2-2* allele into elite inbreds and marker-assisted introgression of *lpa1* and *lpa2* mutants in early maturing inbreds, viz. “CM145” and “V334,” has been carried out at Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS) (Kumar et al. 2014; Gupta et al. 2015). Breeding for low phytate maize also offers several advantages both as food and feed.

Protein

In many developing countries of Latin America, Africa, and Asia, maize is the major staple food and often the only source of protein. Early work on maize biofortification was mainly focused on improving protein quality (Vasal 2000; Krivanek et al. 2007). There is a low level of essential amino acids such as lysine and tryptophan in traditional maize and thus possess poor endosperm protein, which is essential to humans and monogastric animals (FAO 1999). *Opaque 2* mutant maize with high lysine and tryptophan content was discovered in Connecticut, USA, while screening for maize lines with better amino acid (Mertz et al. 1964). Several modern maize varieties collectively referred to as quality protein maize (QPM), which have improved protein quality, and agronomic characteristics were produced by conventional breeding approach and are currently being actively disseminated, particularly in Sub-Saharan Africa (Krivanek et al. 2007). With the discovery of maize mutant *opaque2* having enhanced nutritional quality, the number of open-pollinated

varieties and hybrids in quality protein maize (QPM) genetic backgrounds have been released worldwide, and to develop locally adapted QPM germplasm, marker-assisted selection has been applied (Danson et al. 2006). In India, in comparison to more than a hundred non-QPM/normal maize hybrids, only a dozen QPM hybrids have been released (Yadav et al. 2015). In 1970, India released its first soft endosperm-based nutritious maize composites, then in 1997, first hard-endosperm QPM composite was released, and the first QPM hybrid was released in 2001. Since then, various ICAR institutes and SAUs in India are engaged in the development of the QPM version of elite commercial hybrids for different agro-ecologies of the country (Gupta et al. 2009). The first report of marker-aided selection (MAS) of *opaque2* was the commercial release of “Vivek QPM.” QPM provides a valuable model for the development, evaluation, targeting, and dissemination of biofortified crops, as most biofortification efforts are still in the early stages of research and development.

11.3.1.4 Pearl Millet

Pearl millet is one of the most important staple food crops grown in the arid and semi-arid tropical regions of Africa and Asia, and can be used to achieve food and nutritional security. The advantage of growing pearl millet is that it can tolerate high temperature, adaptation to soil salinity, and drought that increase the significance of this crop in varied adverse climatic conditions. Pearl millet also has inherent high nutritional values (dietary carbohydrates, energy, protein, and minerals (calcium, Fe, and Zn) and climate-resilient nature (drought and heat). To a great extent, pearl millet research progress is ongoing to assess the magnitude of genetic variability, optimizing efficient germplasm screening procedures, development and adoption with the objective of improvement in the breeding efficiency for pearl millet grain iron (Fe) and zinc (Zn) contents. Pearl millet is one of the key crops under the HarvestPlus biofortification challenge program, and most of the research work for its nutrition enhancement (high in Zn and Fe) is ongoing at ICRISAT in collaboration with AICRP at ICAR institutes/universities in India. The Indian government has also added pearl millet to the cereals of the public distribution system under the National Food Security Act/Mission (Govindaraj et al. 2018).

Pearl millet germplasm has been screened and explored for genetic diversification for high Fe and Zn contents at national and international biofortification programs. Large genetic variability (30–140 mg/kg Fe and 20–90 mg/kg Zn) for genetic improvement of grain Fe with Zn micronutrients content was reported in pearl millet populations, and parental lines were effectively utilized to develop high-yielding cultivars with high Fe and Zn contents in advanced breeding lines and hybrids (Rai et al. 2012). “Dhanashakti” is the first biofortified pearl millet cultivar rich in Fe that has been released in India and included in the Nutri-Farm Pilot Project of the Government of India for addressing Fe deficiency in India (Rai et al. 2014). Dhanashakti and Chakti (high Fe and Zn contents) are the open-pollinated varieties of pearl millet with hybrids (ICMH 1202, ICMH 1203, and ICMH 1301) which have shown high grain yield (>3.5 tons/ha) and high levels of Fe (70–75 mg/kg) and Zn (35–40 mg/kg) contents. Presently, India is growing more than 70,000 ha of

biofortified pearl millet hybrids/varieties. This increased adoption is due to significantly higher yields with enhanced nutrients, resistance to downy mildew disease, and tolerance to drought. Clinical research on biofortified “Dhanshakti” pearl millet cultivar has shown that 200 g of its daily consumption meets 100% of the recommended daily allowance (RDA) of Fe in adult men and children and 60% of the RDA in non-pregnant and nonlactating women in India (Neeraja et al. 2017). In India, the ICAR-AICRP-Pearl millet biofortification program has led to the development of various biofortified breeding lines with enhanced micronutrient (>80 ppm Fe and >50 ppm Zn) content, and currently being evaluated for their consistent performance. High-Fe (62–65 ppm) pearl millet cultivars “ICTP 8203” has been commercially grown in the Maharashtra state of India on more than 200,000 hectares. Biofortified pearl millet hybrid “MH 1928” with high Fe (>61 ppm) along with higher grain yield has been released at the national level, and various other hybrids with high Fe and Zn (>70 ppm) contents are in the pipeline and at testing stage (Govindaraj et al. 2018).

11.3.1.5 Sorghum

Sorghum is one of the top ten crops that feed this world and an important cereal crop in hot and dry agro-ecologies. After pearl millet, sorghum is the second cheapest source of energy and micronutrients and fifth most important cereal crop globally, and in the semi-arid tropics (Parthasarathy et al. 2006; Reddy and Reddy 2018). Sorghum has high photosynthetic efficiency as it is a C₄ species, and also it has inherent high biomass yield potential. Under the climate change scenario, sorghum has proved to be more relevant for food security because of the high levels of tolerance to drought and high temperature and adaptation to soils. Therefore, the biofortification of sorghum by increasing protein and mineral micronutrients (especially Fe and Zn) is of high priority as sorghum is among the cheapest sources of micronutrients. Therefore, to tackle India’s double burden of malnutrition, a biofortified variety of unpopular staple food has attracted the attention of scientists. In India, sorghum contributes around 50% of the total cereal intake (75 kg grain per head per year), especially by rural consumers in the major production regions. Public bred cultivars, and parental lines showed wide variability for grain Fe (12–68 ppm) and Zn (11–44 ppm) in the studies at ICAR-Indian Institute of Millets Research (IIMR) (Hariprasanna et al. 2014). The Indian national program on sorghum with comprehensive testing in co-ordinated trials has released over 31 hybrids and 25 varieties for commercial cultivation (Reddy and Reddy 2018).

Increasing mineral micronutrients (especially Fe and Zn) in the grain is of widespread interest, which can be achieved by biofortification of sorghum (Pfeiffer and McClafferty 2007; Zhao 2008; Kumar et al. 2009). Kumar et al. (2013) observed that rainy season-specific sorghum commercial hybrids possess better Fe and Zn contents than post-rainy sorghums. Agronomic attempts by external application of Fe and Zn fertilizers were also made for biofortification, but there was no significant increase in grain Fe or Zn (Mishra et al. 2015). Bioavailability of grain Fe and Zn was affected by high variability for anti-nutritional factors like polyphenols, phytate, and fibers (Hariprasanna et al. 2015). It was reported that the bioaccessibility of Fe

and Zn from sorghum was very low at 4.13% and 5.51%, respectively (Hemalatha et al. 2007). A total of 2267 core germplasm accessions were screened at ICRISAT, and promising donors were identified with Fe ranging from 20–70 ppm and Zn from 13–47 ppm under the HarvestPlus program. They developed improved breeding lines with high yield and high grain Fe and Zn by exploiting the large variability in core collections. In the Maharashtra state of India, ICRISAT-bred biofortified sorghum line ICSR 14001 with 50% higher Fe and Zn than base-level out-yielded all other entries in the state multi-location trials. All India Coordinated Sorghum Improvement Project (AICSIP) proved ICSR 14001 superior under on-farm testing, and it is under testing towards its commercialization. Parbhani Shakti is being touted as India's first biofortified variety of sorghum, a plant from which grain and other crops are grown. Sorghum hybrids with high Fe and Zn are being developed at national and international programs. Recently in Nigeria, a sorghum variety "12KNICSV-188" with three times higher Fe (129 ppm) content and high yield was released.

11.3.2 The Recent Breakthroughs in Cereal Biofortification in India

For achieving nutritional security, biofortification, along with dietary supplementation and diversification, is a sustainable approach. India has executed an "enormous scale-up" of two national projects, i.e. Integrated Child Development Services and National Health Mission, to address nutrition. Still, these do not seem to accomplish sufficient inclusion (Menon et al. 2017). In India, National Nutrition Strategy (NNS), which envisages a Kuposhan Mukta Bharat, was launched on 5th September 2017, by National Institution for Transforming India (NITI Aayog). The main strategies of this program are to provide nutritious food, income and livelihood, health service, and drinking water and sanitation, which will possibly contribute to national food nutrition security. HarvestPlus is the Consultative Group on International Agricultural Research (CGIAR) Biofortification Challenge program. The main objective of this program is to tackle the global hidden hunger/malnutrition issues by a collaborative approach, including different national and international organizations/institutions/agencies (Bouis and Saltzman 2017). This biofortification program worked as a collaborative effort that involved multi-CGIAR teams including various plant scientists, plant geneticists/breeders, nutritionists, food scientists, economists, and social specialists. The goal of this group was to address micronutrient malnutrition by producing staple food crops with improved levels of bioavailable essential nutrients (minerals and vitamins) to enhance the nutrient status of target populations, primarily resource-poor people in the developing countries, such as India. Three essential issues were recognized to make biofortification fruitful: (I) development of biofortified varieties/hybrids must be having high yields and that are advantageous to the agriculturist/farmers, (II) the developed varieties should have a bioavailability of nutrients and effective in reducing hidden hunger problems due to micronutrient deficiencies, and (III) the biofortified crop should be as per farmer's and consumer's

DISCOVERY

- Identify target populations to enhance its nutritional status
- Set nutrient target levels
- Screening and applied biotechnology (for germplasm screening and gene discovery)

**DEVELOPMENT**

- Crop improvement research (Breed biofortified crops)
- Genotype by environment studies
- Testing performance of new crop varieties
- Nutrient retention and bioavailability studies
- Nutritional efficacy in humans

**DELIVERY**

- Official release of biofortified crops
- Develop strategies to disseminate the seed
- Product marketing, consumption and consumer acceptance of biofortified crops

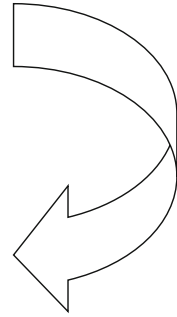
**IMPROVED NUTRITIONAL STATUS OF TARGET POPULATIONS**

Fig. 11.2 HarvestPlus pathway for biofortification (modified from Bouis et al. 2011)

acceptance in target regions where people are severely suffered from micronutrient malnutrition {(Fig. 11.2) (Hotz and McClafferty 2007; Bouis and Saltzman 2017)}.

In India, initially, most of the scientific research efforts were made in agriculture to achieve food grain' self-sufficiency. But today, to resolve the major issues of the country's nutritional security, staple food crop biofortification to overcome the hidden hunger problem has become of utmost importance (Neeraja et al. 2017). By understanding the importance of crop biofortification, the Indian Council of Agriculture Research (ICAR), Govt. of India has endorsed the Consortia Research Platform (CRP) to enhance the nutritional status of the country's major staple food crops such as rice, wheat, maize, sorghum, pearl millet, and small millet by collaboration among different ICAR institutes, state agricultural universities (SAU), traditional universities, and Indian Council of Medical Research (ICMR). Consortia Research Platform (CRP) has focused on developing cereals biofortified with enhanced β -carotene, quality protein, Fe, and Zn using different biofortification approaches (Neeraja et al. 2017; Yadava et al. 2017). All human beings on this

earth have a right to nutritious food, so biofortification is a cost-effective and sustainable approach to address the hidden hunger/malnutrition problem. So far, the Indian Council of Agricultural Research (ICAR) has significantly improved the nutritional quality in high-yielding varieties of the country's staple food crops and released a large number of biofortified crop varieties as a sustainable way to alleviate malnutrition which could integrate into the food chain and ensure nutritional security (Table 11.3). For the rice biofortification program, ICAR consortia research platform, HarvestPlus, Department of Biotechnology (DBT), Department of Science and Technology (DST), and other supporting agencies, along with the Indian Institute of Rice Research (IIRR), Hyderabad, National Rice Research Institute (NRRRI), Cuttack, Odisha, Indian Agricultural Research Institute (IARI), New Delhi, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, University of Agricultural Sciences (UAS), Bengaluru, and Tamil Nadu Agricultural University (TNAU), Coimbatore have screened and identified rice germplasm with high Zn, Fe, and protein contents. Different rice breeding lines with improved nutritional content are being evaluated under the All India Coordinated Research Project (AICRP) on Rice.

Similarly, for wheat biofortification programs, the Indian Institute of Wheat and Barley Research (IIWBR), IARI, Punjab agricultural university (PAU), Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), UAS, Dharwad have screened and identified wheat germplasm with higher Fe and Zn contents and are being tested at multi-locations. Different biofortified wheat lines high in Zn content developed through the HarvestPlus biofortification program and ICAR are under field trials in India and Pakistan. Globally, particularly in India, various biofortified QPM hybrids with improved lysine and tryptophan contents have been released and commercially cultivated by farmers. Various experimental QPM hybrids are currently under different stages of the All India Coordinated Research Project (AICRP) on Maize. Recently, the Indian Agricultural Research Institute (IARI), New Delhi has developed a provitamin A-rich maize hybrid by introgression of *crTRB1* into parental lines of elite hybrids under AICRP-Maize. By combined introgression of *opaque2*, *crTRB1*, and *lcyE* genes, IARI led to the development of multi-nutrient maize (Neeraja et al. 2017). Biofortified pearl millet variety "Dhanashakti" rich in iron (71 ppm) largely adopted by farmers in India has been released by the All India Coordinated Research Project (AICRP) on Pearl millet along with the private seed sector.

However, the critical concern for the developed biofortified food crops is the bioavailability of enhanced nutrients like Fe and Zn in human beings which are primarily affected by anti-nutritional factors like phytate/phytic acid or phytin (Raboy 2001). Bioavailability means a measurable amount of the nutrient quantity ingested that experiences intestinal absorption and utilization. Therefore, the bioavailability of nutrients from biofortified food crops is often the key to success (Neeraja et al. 2017). Recently, biofortified rice pure line varieties, i.e. CR Dhan 310 (protein-rich variety) and DRR Dhan 45 (Zn rich variety); biofortified wheat pure line varieties, i.e., WB 02 (Zn and Fe rich variety), and HPBW 01 (Fe and Zn rich variety); biofortified maize hybrid, i.e., Pusa Vivek QPM9 Improved (provitamin A, lysine and tryptophan-rich hybrid) and Improved Pusa (HM4,

Table 11.3 Cereals biofortified varieties developed by the Indian Council of Agriculture Research (ICAR), India as a sustainable way to alleviate malnutrition

Biofortified varieties/ hybrid	Improved nutrient content	Grain yield	Maturity	Adaptation	Developed by
<i>Rice</i>					
CR Dhan 310 (protein-rich pure line variety)	10.3% protein in polished grain as compared to 7.0–8.0% in popular varieties	45.0 q/ha	125 days	Odisha, Madhya Pradesh, and Uttar Pradesh	ICAR-National Rice Research Institute, Cuttack, Odisha
DRR Dhan 45 (Zn rich pure line variety)	High in Zn content (22.6 ppm) in polished grains in comparison to 12.0–16.0 ppm in popular varieties	50.0 q/ha	125–130 days	Karnataka, Tamil Nadu, Andhra Pradesh, and Telangana	ICAR-Indian Institute of Rice Research, Hyderabad
<i>Wheat</i>					
Wheat WB 02 (zinc and Fe rich pure line variety)	Rich in Zn (42.0 ppm) and Fe (40.0 ppm) in comparison to 32.0 ppm Zn and 28.0–32.0 ppm Fe in popular varieties	51.6 q/ha	142 days	Punjab, Haryana, Delhi, Rajasthan (excluding Kota and Udaipur division), Western UP (except Jhansi division), Jammu and Kathua district of J & K, Paonia Valley and Una district of HP and Tarai region of Uttarakhand	ICAR-Indian Institute of Wheat and Barley Research, Kamal, Haryana
HPBW 01 (Fe and Zn rich pure line variety)	Contains high Fe (40.0 ppm) and Zn (40.6 ppm) in comparison to 28.0–32.0 ppm Fe and 32.0 ppm Zn in popular varieties	51.7 q/ha	141 days	Punjab, Haryana, Delhi, Rajasthan (excluding Kota and Udaipur division), Western UP (except Jhansi division), Jammu and Kathua district of J & K, Paonia	Punjab Agricultural University, Ludhiana under ICAR-All India Coordinated Research Project on Wheat and Barley

				Valley and Una district of HP and Tarai region of Uttarakhand	
<i>Maize</i>					
Maize Pusa Vivek QPM9 Improved (provitamin A, lysine and tryptophan-rich hybrid)	Country's first provitamin A rich maize • High provitamin A (8.15 ppm), lysine (2.67%) and tryptophan (0.74%) as compared to 1.0–2.0 ppm provitamin A, 1.5–2.0% lysine and 0.3–0.4% tryptophan content in popular hybrids	55.9 q/ha [Northern Hills Zone (NHZ)] and 59.2 q/ha [Peninsular Zone (PZ)]	93 days (NHZ) and 83 days (PZ)	Kharif season in J&K, Himachal Pradesh, Uttarakhand (Hill region), North Eastern states, Maharashtra, Karnataka, AP, Telangana, and Tamil Nadu	ICAR-Indian Agricultural Research Institute, New Delhi
Pusa HM4 Improved (lysine and tryptophan-rich hybrid)	Contains 0.91% tryptophan and 3.62% lysine which is significantly higher than popular hybrids (0.3–0.4% tryptophan and 1.5–2.0% lysine)	64.2 q/ha	87 days	Kharif season in Punjab, Haryana, Delhi, Uttarakhand (Plain), Uttar Pradesh (Western region)]	ICAR-Indian Agricultural Research Institute, New Delhi
Pusa HM8 Improved (lysine and tryptophan-rich hybrid)	Rich in tryptophan (1.06%) and lysine (4.18%) as compared to 0.3–0.4% tryptophan and 1.5–2.0% lysine in popular hybrids	62.6 q/ha	95 days	Kharif season in Maharashtra, Karnataka, Andhra Pradesh, Telangana, Tamil Nadu	ICAR-Indian Agricultural Research Institute, New Delhi
Pusa HM9 Improved (lysine and	Contains 0.68% tryptophan and 2.97% lysine compared to	52.0 q/ha	89 days	Kharif season in Bihar, Jharkhand, Odisha, Uttar Pradesh (Eastern	ICAR-Indian Agricultural Research Institute, New Delhi

(continued)

Table 11.3 (continued)

Biofortified varieties/ hybrid	Improved nutrient content	Grain yield	Maturity	Adaptation	Developed by
tryptophan-rich hybrid)	0.3–0.4% tryptophan and 1.5–2.0% lysine in popular hybrids			region), and West Bengal	
<i>Pearl Millet</i>					
HHB 299 (Fe and Zn-rich hybrid)	High Fe (73.0 ppm) and zinc (41.0 ppm) as compared to 45.0–50.0 ppm Fe and 30.0–35.0 ppm Zn in popular varieties/ hybrids	32.7 q/ha	81 days	Kharrif season in Haryana, Rajasthan, Gujarat, Punjab, Delhi, Maharashtra, and Tamil Nadu	CCS-Haryana Agricultural University, Hisar in collaboration with ICRISAT, Patancheru under ICAR-All India Coordinated Research Project on Pearl millet
AHB 1200 (Fe rich hybrid)	Rich in Fe (73.0 ppm) in comparison to 45.0–50.0 ppm in popular varieties/ hybrids	32.0 q/ha	78 days	Kharrif season in Haryana, Rajasthan, Gujarat, Punjab, Delhi, Maharashtra, and Tamil Nadu	Vasandrao Naik Marathwada Krishi Vidyapeeth, Parbhani in collaboration with ICRISAT, Patancheru under ICAR-All India Coordinated Research Project on Pearl millet
<i>Sorghum</i>					
Improved variety (higher Fe and Zn) ICSR 14001, released as “Parbhani Shakti”	Fe concentration of 45 ppm and Zn 32 ppm compared to varieties that are currently being cultivated in India (Approx 30 ppm Fe and 20 ppm Zn). Higher	Yield levels are higher (>5.0 tons/ha) in post-rainy and summer seasons with irrigation	Tolerate higher temperatures (41 °C) at flowering and seed setting, but the flowering may be delayed (80 days)	Maharashtra and different sorghum growing regions of India It was released as a rainy season variety (<i>Kharrif</i>), but it can be	ICRISAT under the HarvestPlus sorghum biofortification project under All India Coordinated Sorghum Improvement Project and released for

	<p>protein (11.9%) and low phytate content (4.14 mg/100 g) compared to 10% protein and 7.0 mg/100 g phytate content in most sorghum cultivars</p>			<p>grown in post-rainy (<i>Rabi</i>) and summer seasons</p>	<p>cultivation by Vasantha Naik Marathwada Krishi Vidyaapeeth (VNMKV), Maharashtra</p>
--	---	--	--	---	--

HM8, HM9) (lysine and tryptophan-rich hybrid); biofortified Pearl millet hybrid, i.e., HHB 299 (Fe and Zn rich hybrid) and AHB 1200 (Fe rich hybrid) were released for commercial cultivation (Yadava et al. 2017; Neeraja et al. 2017).

In July 2018, India's first biofortified sorghum (jowar) variety ICSR 14001 was released as "Parbhani Shakti," which is rich in Fe and Zn than traditionally grown sorghum varieties. It was developed under HarvestPlus biofortification programs by International Crops Research Institute for the Semi-Arid Tropics (ICRIAT), Patancheru, Hyderabad, and All India Coordinated Sorghum Improvement Project and released for large-scale seed production, dissemination, and commercial cultivation by Vasant Naik Marathwada Krishi Vidyapeeth (VNMKV), Maharashtra. Biofortified sorghum "Parbhani Shakti" having Fe concentration 45 ppm and Zn 32 ppm as compared to other varieties that are at present cultivated in India (Approx 30 ppm Fe and 20 ppm Zn). Improved biofortified varieties have also shown higher protein (11.9%) and low phytate content (4.14 mg/100 g) compared to 10% protein and 7.0 mg/100 g phytate content in most sorghum cultivars. It also tolerates higher temperatures (41 °C) at flowering and seed set, but the flowering may be delayed (80 days). Yields are higher (>5.0 tons/ha) in post-rainy and summer seasons with irrigation. It was released as a rainy season variety (Kharif), but it can also be grown in Rabi and summer seasons in different sorghum growing regions. Recently, in 2020, different nutritionally enriched cereals biofortified varieties were released, viz. rice (CR Dhan 315, high zinc); wheat (HI 1633, rich in protein, iron and zinc; HD 3298, protein and iron-rich; DBW 303, protein-rich and DDW 48, protein-rich); maize (Ladhowal Quality Protein Maize Hybrid 1, 2, and 3, lysine and tryptophan-rich); finger millet (CFMV1 and 2, rich in calcium, iron, and zinc) and little millet (CLMV1, iron-zinc rich). ICAR has introduced the Nutrition-Sensitive Agricultural Resources and Innovations (NARI) program to promote family farming that linked agriculture to nutrition, nutrition-smart villages to boost nutritional stability, and KVKs are designing and promoting unique nutrition garden models to ensure access to locally accessible, balanced, and diversified diets with adequate macro and micronutrients. To alleviate malnutrition and make India Kuposhan Mukta by naturally enriched food ingredients, the production of biofortified crop varieties will be upgraded and connected to government programs of mid-day meal, Anganwadi, etc. This would also contribute to increased farmers' incomes and open up new means of developing entrepreneurship.

11.4 Conclusion

India is one of the richest agro-biodiversity countries, and scientific research is continuously evaluating the promising germplasm for enhanced nutrients in staple food crops to alleviate malnutrition. Biofortification is a promising, cost-effective, and sustainable agricultural strategy for improving the nutritional status of malnourished populations. With proper planning, collaboration, execution, and implementation by different organizations (National and Internationals) like ICAR-AICRP, DBT, ICMR, and HarvestPlus, biofortification of cereal crops for product

development, testing, and validation will help in achieving the nation's nutritional security. It will have a significant impact on the lives and health of a large number of poor individuals/malnourished populations of the country.

References

- Agrawal PK, Jaiswal SK, Prasanna BM, Hossain F, Saha S, Guleria SK, Gupta HS (2012) Genetic variability and stability for kernel iron and zinc concentration in maize (*Zea mays* L.) genotypes. *Indian J Genet* 72:421–428
- Agrawal PK, Kohli A, Twyman RM, Christou P (2005) Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. *Mol Breed* 16:247–260
- Anuradha K, Agarwal S, Batchu AK, Babu AP, Swamy BM, Longvah T, Sarla N (2012) Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. *J Phytology* 4(1):19–25
- Arnold JM, Bauman LF (1976) Inheritance of and interrelationships among maize kernel traits and elemental contents. *Crop Sci* 16:439–440
- Babu RV (2013) Importance and advantages of rice biofortification with iron and zinc. *J SAT Agric Res* 11:1–6
- Banziger M, Long J (2000) The potential for increasing the iron and zinc density of maize through plant breeding. *Food Nutr Bull* 21:397–400
- Bao J (2014) In: Yan W (ed) Genes and QTLs for rice grain quality improvement, Rice - Germplasm, Genetics and Improvement. InTech, London. <https://doi.org/10.5772/56621>
- Baxter IR, Gustin JL, Settles AM, Hoekenga OA (2013) Ionomic characterization of maize kernels in the intermated B73 × Mo17 population. *Crop Sci* 53:208–220
- Bhati KK, Alok A, Kumar A, Kaur J, Tiwari S, Pandey AK (2016) Silencing of ABC13 transporter in wheat reveals its involvement in grain development, phytic acid accumulation and lateral root formation. *J Exp Bot* 67(14):4379–4389
- Birla DS, Malik K, Sainger M, Chaudhary D, Jaiwal R, Jaiwal PK (2015) Progress and challenges in improving the nutritional quality of rice (*Oryza sativa* L.). *Crit Rev Food Sci Nutr* 57(11):2455–2481
- Borg S, Brinch-Pedersen H, Tauris B, Holm PB (2009) Iron transport, deposition and bioavailability in the wheat and barley grain. *Plant Soil* 325:15–24
- Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S et al (2012) Wheat ferritins: improving the iron content of the wheat grain. *J Cereal Sci* 56:204–213
- Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011 Mar) Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr Bull* 32(1 Suppl):S31–S40
- Bouis H, Low J, McEwan M, Tanumihardjo S (2013) Biofortification: evidence and lessons learned linking agriculture and nutrition. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), Washington, D.C.
- Bouis HE, Saltzman A (2017) Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Sec* 12:49–58
- Bouis HE, Welch RM (2010) Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the Global South. *Crop Sci* 50:20–32
- Brinch Pedersen H, Hatzack F, Stoger E (2006) Heat Stable Phytases in Transgenic Wheat (*Triticum aestivum* L.): Deposition Pattern, Thermostability, and Phytate Hydrolysis. *J Agric Food Chem* 54:4624–4632
- Brinch-Pederson H, Olesen A, Rasmussen SK, Holm PB (2000) Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. *Mol Breed* 6:195–206

- Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification. *Plant Soil* 302:1–17
- Cakmak I, Kutman UB (2017) Agronomic biofortification of cereals with zinc: a review. *Eur J Soil Sci* 69:172–180
- Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A et al (2004) *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci Plant Nutr* 50:1047–1054
- Carvalho SM, Vasconcelos MW (2013) Producing more with less: strategies and novel technologies for plant-based food biofortification. *Food Res Int* 54:961–971
- Cavagnaro TR (2008) The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. *Plant Soil* 304:315–325
- Chakraborti M, Prasanna BM, Hossain F, Mazumdar S, Singh AM, Guleria SK, Gupta HS (2011) Identification of kernel iron- and zinc-rich maize inbreds and analysis of genetic diversity using microsatellite markers. *J Plant Biochem Biotechnol* 20:224–233
- Chakraborti M, Prasanna BM, Hossain F, Singh AM, Guleria SK (2009) Genetic evaluation of kernel Fe and Zn concentrations and yield performance of selected maize (*Zea mays* L.) genotypes. *Range Manag Agrofor* 30:109–114
- Chhuneja P, Dhaliwal HS, Baines NS, Singh K (2006) *Aegilops kotschy* and *Aegilops tauschii* as sources of higher levels of grain iron and zinc. *Plant Breed* 125:529–531
- Christou P, Twyman RM (2004) The potential of genetically enhanced plants to address food insecurity. *Nutr Res Rev* 17:23–42
- CIMMYT (2000) In: Pingali PL (ed) World maize facts and trends: meeting world maize needs: technological opportunities and priorities. CIMMYT, Mexico, D.F.
- Cohen H, Israeli H, Matityahu I, Amir R (2014) Seed-specific expression of a feedback-insensitive form of cystathionine- γ -synthase in *Arabidopsis* stimulates metabolic and transcriptomic responses associated with desiccation stress. *Plant Physiol* 166:1575–1592
- Cong L, Wang C, Chen L, Liu H, Yang G, He G (2009) Expression of phytoene synthase1 and carotene desaturase crtI genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). *J Agric Food Chem* 57(18):8652–8660
- Cromwell GL, Coffey RD (1991) Phosphorus: a key essential nutrient, yet a possible major pollutant. Its central role in animal nutrition. In: Lyons TP (ed) *Biotechnology in the Feed Industry*. Alltech Tech Publishers, Nicholasville, pp 133–145
- Danson J, Mbogori M, Kimani M, Lagat M, Kuria A, Diallo A (2006) Marker-assisted introgression of *opaque2* gene into herbicide tolerant elite maize inbred lines. *Afr J Biotechnol* 5:2417–2422
- Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H (2007) Multiple QTL-effects of wheat *Gpc-B1* locus on grain protein and micronutrient concentrations. *Physiol Plant* 129:635–643
- Eric JW, Eddie CC (2012) International rice baseline with deterministic and stochastic projections, 2012–2021. *World Rice Outlook*, pp. 1–81
- FAO (1999) In: Food and Agriculture Organization (FAO) of the United Nations (ed) *Maize in human nutrition*. FAO Food and Nutrition Series No. 25, Rome
- FAO/WHO (2002) Human vitamin and mineral requirements. Available via <http://www.fao.org/DOCREP/004/Y2809E/y2809e00.htm#Contents>
- Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front Nutr* 5:12
- Gomez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T et al (2010) Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Res* 19:165–180
- Gorsline GW, Thomas WI, Baker DE (1964) Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al, and Fe concentrations by corn (*Zea mays* L.) leaves and grain. *Crop Sci* 4:207–210
- Goswami R, Kumar J, Hossain F, Thirunavukkarasu N, Manjaiah KM, Agrawal PK, Reddy SS, Guleria SK, Gupta HS (2014) Analyses of the genetic variability for kernel-iron and kernel-zinc

- in maize genotypes. In: Prasanna et al (eds) 12th Asian Maize Conference and Expert Consultation on Maize for Food, Feed and Nutritional Security, 30 October - 01 November, Book of Abstracts, Bangkok, Thailand, p 116
- Govindaraj M, Kanatti A, Rai KN (2018) Breeding biofortified pearl millet cultivars unlock millet markets for nutrition. In: Proceedings of 3rd International Millet Symposium (3rd International Symposium on Broomcorn Millet), August 8–12, 2018. Marriot Inn, Fort Collins, CO
- Gregorio GB, Senadhira D, Htut T, Graham RD (1999) Improving iron and zinc value of rice for human nutrition. *Agric Dev* 23(9):68–87
- Guleria SK, Chahota RK, Kumar P, Kumar A, Prasanna BM, Hossain F, Agrawal PK, Gupta HS (2013) Analysis of genetic variability and genotype \times year interactions on kernel zinc concentration in selected Indian and exotic maize (*Zea mays* L.) genotypes. *Indian J Agric Sci* 83:836–841
- Gupta HS, Hossain F, Nepolean T, Vignesh M, Mallikarjuna MG (2015) Understanding genetic and molecular bases of Fe and Zn accumulation towards development of micronutrient enriched maize. In: Rakshit et al (eds) *Nutrient Use Efficiency: From Basic to advances*, 1st edn. Springer, New Delhi, pp 255–282
- Gupta HS et al (2009) Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker-assisted breeding. *Curr Sci* 96:230–237
- Hardarson G, Broughton WJ (2004) Maximising the use of biological nitrogen fixation in agriculture. *Ann Bot* 93(4):477
- Hariprasanna K, Agte V, Elangovan M, Gite S, Kishore A (2015) Anti-nutritional factors and antioxidant capacity in selected genotypes of sorghum [*Sorghum bicolor* (L.) Moench]. *Int J Agric Sci* 7:620–625
- Hariprasanna K, Agte V, Elangovan M, Patil JV (2014) Genetic variability for grain iron and zinc content in cultivars, breeding lines and selected germplasm accessions of sorghum [*Sorghum bicolor* (L.) Moench]. *Indian J Genet* 74:42–49
- Haug A, Graham R, Christophersen O, Lyons G (2007) How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microb Ecol Health Dis* 19:209–228
- Hefferon KL (2016) Can biofortified crops help attain food security? *Curr Mol Biol Rep* 2 (4):180–185
- Hemalatha S, Platel K, Srinivasan K (2007) Zinc and Iron content and their bioaccessibility in cereals and pulses consumed in India. *Food Chem* 102:1328–1313
- Hotz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull* 25(1):194–195
- Hotz C, McClafferty B (2007) From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food Nutr Bull* 28(2):271–279
- Juliano B (1993) *Rice in Human Nutrition*. FAO Food Nutr., Ser. No. 26. International Rice Research Institute: Manila, Philippines
- Kawakatsu T, Hirose S, Yasuda H, Takaiwa F (2010) Reducing rice seed storage protein accumulation leads to changes in nutrient quality and storage organelle formation. *Plant Physiol* 154:1842–1854
- Krivaneck AF, De Groote H, Gunaratna NS, Diallo AO, Friesen D (2007) Breeding and Disseminating Quality Protein Maize (QPM) for Africa. *Afr J Biotechnol* 6(4):312–324
- Kumar A, Anuradha K, Ramaiah B (2013) Increasing grain Fe and Zn concentration in sorghum: progress and way forward. *J SAT Agric Res* 11(12):1–5
- Kumar A, Reddy BVS, Ramaiah B, Sanjana Reddy P, Sahrawat KL, Upadhyaya HD (2009) Genetic variability and plant character association of grain Fe and Zn in selected core collections of sorghum germplasm and breeding lines. *J SAT Agric Res* 7:1–4
- Kumar S et al (2014) Marker-assisted introgression of *lpa-2* locus responsible for low-phytic acid trait into an elite tropical maize inbred. *Plant Breed* 133:566–578
- Larson SR, Rutger JN, Young KA, Raboy V (2000) Isolation and genetic mapping of a non-lethal rice low phytic acid 1 mutation. *Crop Sci* 40:1397–1405

- Larson SR, Young KA, Cook A, Blake TK, Raboy V (1998) Linkage mapping: two mutations that reduce phytic acid content of barley grain. *Theor Appl Genet* 97:141–146
- Lee TT, Wang MM, Hou RC, Chen LJ, Su RC, Wang CS, Tzen JT (2003) Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. *Biosci Biotechnol Biochem* 67:1699–1705
- Long XH, Liu QQ, Chan ML, Wang Q, Sun SSM (2013) Metabolic engineering and profiling of rice with increased lysine. *Plant Biotechnol J* 11:490–501
- Lungaho MG, Mwaniki AM, Szalma SJ, Hart J, Rutzke MA, Kochian LV, Glahn R, Hoekenga OA (2011) Genetic and physiological analysis of iron biofortification in maize kernels. *PLoS One* 6: e20429
- Lyons G (2014) Vitamin A. Why weren't we told this long ago. *World Nutr* 5:1125–1126
- Lyons G, Cakmak I (2012) Agronomic biofortification of food crops with micronutrients In: Bruulsema TW, Heffer P, Welch RM, Cakmak I Moran K (eds) *Fertilizing Crops to Improve Human Health: A Scientific Review*. International Plant Nutrition Institute, Norcross, GA, pp 97–122
- Mallikarjuna MG, Nepolean T, Hossain F, Manjaiah KM, Singh AM, Gupta HS (2014) Genetic variability and correlation of kernel micronutrient among exotic quality protein maize inbreds and their utility in breeding programme. *Indian J Genet* 74:166–173
- Masuda H et al (2012) Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Sci Rep* 2:1–7
- Menon P, Nguyen PH, Mani S, Kohli N, Avula R, Tran LM (2017) Trends in Nutrition Outcomes, Determinants, and Interventions in India (2006–2016). POSHAN Report No. 10. International Food Policy Research Institute, New Delhi
- Mertz ET, Bates LS, Nelson OE (1964) Mutant genes that change protein composition and increase lysine content of maize endosperm. *Science* 145:279–280
- Mishra JS, Hariprasanna K, Rao SS, Patil JV (2015) Biofortification of post-rainy sorghum (*Sorghum bicolor*) with zinc and iron through fertilization strategy. *Indian J Agric Sci* 85:721–724
- Monasterio I, Graham RD (2000) Breeding for trace minerals in wheat. *Food Nutr Bull* 21 (4):392–396
- Myers SS, Wessells KR, Kloog I, Zanoletti A, Schwartz J (2015) Effect of increased concentrations of atmospheric carbon dioxide on the global threat of zinc deficiency: a modelling study. *Lancet Glob Health* 3(10):639–645
- Neeraja CN, Ravindra BN, Ram S, Hossain F, Hariprasanna K, Rajpurohit BS, Longvah PT, Prasad KS, Sandhu JS, Datta SK (2017) Biofortification in cereals: Progress and prospects. *Curr Sci* 113(6):1050–1057
- Newell-McGloughlin M (2008) Nutritionally improved agricultural crops. *Plant Physiol* 147:939–953
- Ortiz-Monasterio I, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J Cereal Sci* 46:293–307
- Pandey N, Hossain F, Kumar K, Vishwakarma AK, Muthusamy V, Manjaiah KM, Agrawal PK, Guleria SK, Reddy SS, Nepolean T, Gupta HS (2015) Microsatellite marker-based genetic diversity among quality protein maize (QPM) inbreds differing for kernel iron and zinc. *Mol Plant Breed* 6(3):1–10
- Parthasarathy RP, BIRTHAL BS, Reddy BVS, Rai KN, Ramesh S (2006) Diagnostics of sorghum and pearl millet grains-based nutrition in India. *Int Sorghum Millets News* 147:93–96
- Paul S, Ali N, Datta SK, Datta K (2014) Development of an iron-enriched high-yielding *Indica* rice cultivar by introgression of a high-iron trait from transgenic iron-biofortified rice. *Plant Foods Hum Nutr* 69:203–208
- Pfeiffer WH, McClafferty B (2007) HarvestPlus: Breeding crops for better nutrition. *Crop Sci* 47: S88–S105

- Pinson SRM et al (2015) Worldwide genetic diversity for mineral element concentrations in rice grain. *Crop Sci* 55:294–311
- Pixley KV, Palacios N, Glahn RP (2011) The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value. *Field Crops Res* 123:153–160
- Prasad BVG, Mohanta S, Rahaman S, Bareilly P (2015) Bio-fortification in horticulture crops. *J Agric Eng Food Technol* 2350-0263:95–99
- Prasanna BM (2014) Maize research-for-development scenario: challenges and opportunities for Asia. In: Prasanna et al (eds) 12th Asian Maize Conference and Expert Consultation on Maize for Food, Feed and Nutritional Security, 30 October - 01 November. Book of Extended Summaries, Bangkok, Thailand, pp 2–11
- Prasanna BM, Mazumdar S, Chakraborti M, Hossain F, Manjaiah KM, Agrawal PK, Guleria SK, Gupta HS (2011) Genetic variability and genotype \times year interactions for kernel iron and zinc concentration in maize (*Zea mays* L.). *Indian J Agric Sci* 81:704–711
- Qin H, Cai Y, Liu Z, Wang G, Wang J, Guo Y, Wang H (2012) Identification of QTL for zinc and iron concentration in maize kernel and cob. *Euphytica* 187:345–358
- Raboy V (2001) Seeds for a better future: Low phytate grains to help overcome malnutrition and reduce pollution. *Trends Plant Sci* 6:458–462
- Raboy VP, Gerbasi KA, Stoneberg YS, Pickett SG, Bauman AT, Murthy PPN et al (2000) Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. *Plant Physiol* 124:355–368
- Rai KN, Govindaraj M, Rao AS (2012) Genetic enhancement of grain iron and zinc content in pearl millet. *Qual Assur Saf Crop* 4:119–125
- Rai KN, Gupta SK, Sharma R, Govindaraj M, Rao AS, Shivade H, Bonamigo LA (2014) Pearl millet breeding lines developed at ICRISAT: a reservoir of variability and useful source of non-target traits. *SAT eJournal* 2:1–13
- Ram S, Verma A, Sharma S (2010) Large variability exists in phytase levels among Indian wheat varieties and synthetic hexaploids. *J Cereal Sci* 52:486–490
- Rasmussen SK, Hatzack F (1998) Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. *Hereditas* 129:107–112
- Rawat N, Kumari N, Tiwari VK, Dhaliwal H (2013) Biofortification of cereals to overcome hidden hunger. *Plant Breed* 132:437–445
- Reddy PS, Reddy BVS (2018) History of Sorghum Improvement. In: *Breeding Sorghum for Diverse End Uses*. Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, Duxford, pp 61–75
- Rengel Z, Batten GD, Crowley DE (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Res* 60:27–40
- Rostami N, Farsar AR, Shiva N (2007) Prevalence of sub-clinical vitamin A deficiency in 2-5-year-old children in Tehran. *WHO EMRO Eastern Med. Health J* 13(2):1–6
- Roy SC, Sharma BD (2014) Assessment of genetic diversity in rice (*Oryza sativa* L.) germplasm based on agro-morphology traits and zinc-iron content for crop improvement. *Physiol Mol Biol Plants* 20(2):209–224
- Shewmaker CK, Sheehu JA, Daley M, Colburn S, Ke DY (1999) Seed-specific overexpression of phytoene synthase: increase in carotenoids and metabolic effects. *Plant J* 20:41–412
- Simic D, Mladenovic DS, Zdunic Z, Jambrovic A, Ledencan T, Brkic J, Brkic A, Brkic I (2011) Quantitative trait loci for biofortification traits in maize grain. *J Hered* 103:47–54
- Smith SE, Read DJ (2007) *Mycorrhizal Symbiosis*, 3rd edn. Elsevier, London
- Stein AJ (2010) Global impacts of human malnutrition. *Plant Soil* 335:133–154
- Sun SSM, Liu QQ (2004) Transgenic approaches to improve the nutritional quality of plant proteins. In *Vitro Cell Dev Biol Plants* 40:155–162
- Swamy BPM, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P, Reinke R, Slamet-Loedin IH (2016) Advances in breeding for high grain zinc in rice. *Rice* 9 (1):49

- Tamas C, Kisgyorgy BN, Rakszegi M, Wilkinson MD, Yang MS, Lang L et al (2009) Transgenic approach to improve wheat (*Triticum aestivum* L.) nutritional quality. *Plant Cell Rep* 28 (7):1085–1094
- Tanumihardjo SA (2008) Food-based approaches for ensuring adequate vitamin A nutrition. *Comp Rev Food Sci Food Saf* 7:373–381
- Tanumihardjo SA, Bouis H, Hotz C, Meenakshi JV, McClafferty B (2008) Biofortification of staple crops: an emerging strategy to combat hidden hunger. *Comp Rev Food Sci Food Saf* 7:329–334
- Toride Y (2004) Protein sources for the animal feed industry. In: *FAO Expert Consultation and Workshop, April-MAY, Bangkok, Thailand*. FAO, Rome, pp p161–p165
- Ufaz S, Galili G (2008) Improving the content of essential amino acids in crop plants: goals and opportunities. *Plant Physiol* 147:954–961
- Vasal SK (2000) The quality protein maize story. *Food Nutr Bull* 21:445–450
- White J, Broadley MR (2005) Biofortifying crops with essential mineral elements. *Trends Plant Sci* 10:586–593
- White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol* 182:49–84
- Wong HW, Liu Q, Sun SSM (2015) Biofortification of rice with lysine using endogenous histones. *Plant Mol Biol* 87:235–248
- World Health Organization (2016) World health report, 2016. Available via <http://www.who.int/whr/2016/>
- Xiaoyan S, Yan Z, Shubin W (2012) Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. *J Agric Biotechnol* 20:766–773
- Yadav OP et al (2015) Genetic improvement of maize in India: retrospect and prospects. *Agric Res* 4(4):325–338
- Yadava DK, Choudhury PR, Hossain F, Kumar D (2017) Biofortified Varieties: Sustainable Way to Alleviate Malnutrition. Indian Council of Agricultural Research, New Delhi
- Yang SH, Moran DL, Jia HW, Bicar EH, Lee M, Scott MP (2002) Expression of a synthetic porcine alpha-lactalbumin gene in the kernels of transgenic maize. *Transgenic Res* 11:11–20
- Yu J, Peng P, Zhang X, Zhao Q, Zhy D, Sun X, Liu J, Ao G (2004) Seed-specific expression of a lysine rich protein *sb401* gene significantly increases both lysine and total protein content in maize seeds. *Mol Breed* 14:1–7
- Zhao Z (2008) The Africa biofortified sorghum project—Applying biotechnology to develop nutritionally improved sorghum for Africa. Pages 273–277 in *Biotechnology and sustainable agriculture 2006 and beyond*. In: Xu Z, Li J, Xue J, Yang W (eds) *Proceedings of the 11th IAPTC&B Congress, Aug 31–18, 2006, Beijing, China*. Springer, Amsterdam
- Zhao Z, Glassman K, Sewalt V, Wang N, Miller M, Chang S, Thompson T, Catron S, Wu E, Bidney D, Kedebe Y, Jung R (2003) Nutritionally improved transgenic sorghum. In: Vasil IK (ed) *Plant Biotechnology 2002 and Beyond*. Springer, Dordrecht, pp 413–416
- Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic Strategies for the Nutritional Enhancement of Plants. *Trends Plant Sci* 12:548–555
- Zhu X, Galili G (2003) Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also transregulates the metabolism of other amino acids in Arabidopsis seeds. *Plant Cell* 15:845–853
- Zimmerman MB, Hurrel RF (2007) Nutritional iron deficiency. *Lancet* 370:511–519