GENETIC ENHANCEMENT OF MICRONUTRIENT CONTENT IN CEREALS

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SUMMARY

Cereals provide more than half of global human caloric intake. Though cereals are nutritious, their nutritional density, especially of micronutrients, is far below recommended level in the products we consume. Due to this, over three billion individuals are malnourished globally. About 45 micronutrients (required in μg to mg quantity) including some mineral elements, vitamins, essential amino acids and fatty acids are vital to human body. In addition, antinutrients in food staples, reduced accumulation by plants and low bioavailability of minerals contribute further to micronutrient deficiencies. Vitamin-A, iron and iodine deficiency disorders are the most common. Hence, there is a need to evolve viable strategies of micronutrient enrichment in dietary staples. Of the three major strategies *viz*., dietary diversification, supplementation and food fortification, agronomic and/or genetic fortification is the cost effective and sustainable way to address the problem of malnutrition. Considering the severity of malnutrition, the HarvestPlus initiated genetic biofortification program of staple food crops focusing on most limiting nutrients *viz*., iron, zinc, and vitamin A. Most of the HarvestPlus biofortified varieties are bred through conventional breeding. However, MAS and transgenics

are also used to biofortify crops in the research programmes other than the HarvestPlus. More than 150 biofortified varieties of 10 crops have been released in 30 countries. More than 20 million people are consuming biofortified crops such as vitamin-A maize, iron pearl millet, zinc rice, zinc wheat, etc. Continuous efforts are needed still to biofortify staples and to encourage adoption further by farmers and consumers. With our present technological competence, multibiofortified staples can be developed by gene stacking. Much work on biofortification is still needed through collaborative and multi-sectoral approaches along with the strong policy support. In future, in addition to deployment of conventionally bred biofortified varieties, transgenic biofortified varieties may be approved and deployed.

Key findings: Importance of micronutrients in human nutrition and the advances made through genetic biofortification of major cereals, *viz*., maize, rice, wheat, sorghum and pearl millet, to combat the problem of malnutrition, are presented in this review.

INTRODUCTION

The food security along with nutritional security plays a very important role in sustaining human health. *Let food be thy medicine*, the quote by Hippocrates more than 2000 years ago, was the title of the 2016 Borlaug Dialogue (WFPO, 2016). The definition of food security by Rome Declaration of the World Food Summit-1996, stressed the availability of 'nutritious food' not just 'sufficient food' to sustain a healthy and active life. Even after achieving staple food security in many countries after the Green Revolution, nutritional security with respect to micronutrients has not been achieved fully as their content in the staples is far less than the daily requirement for the body. Hence, the enhancement of nutritional content of crops through biofortification is now an important thrust area of the second

or the 'Evergreen Revolution'. Biofortification, defined as the enrichment of staple food crops with desired micronutrients (Zhao and Shewry, 2011), is apparently a sustainable way to alleviate micronutrient deficiency.

Biofortification of crops can be achieved either by adding the appropriate mineral or inorganic compound during fertilizer application, by traditional plant breeding, or through the biotechnological tools. Though biofortification through mineral fertilizer application is the simplest method, the success of this approach depends upon the extent of mineral mobility and the potential of mineral accumulation among plant species, leading to highly variable results. This also requires regular micronutrient application to the soil and hence, increases both cost and labour. The people worldwide,

especially the poor, rely on staple crops such as rice, wheat and maize for their food and nutritional needs. However, the predominant cultivars of cereal crops have limited content of micronutrients including iron and zinc in grains (Cakmak *et al.*, 2000; Graham *et al.*, 2001; Bouis, 2003; Rawat *et al.*, 2009a). Hence, the genetic enhancement of micronutrient content of crops through conventional plant breeding or through genetic engineering would provide the solution to the problem of hidden hunger and can reach populations in the rural and remote areas of the developing world. This review attempted to document and discuss the status of micronutrient *vis a vis* their quality in cereal staples and the efforts for genetic enhancement of micronutrient content of cereal crops *viz*., maize, rice, wheat, sorghum and pearl millet.

MICRONUTRIENTS

There are about 300 chemical substances recognized as nutrients from the food sources. Out of these, 45 are classified as essential nutrients. These essential nutrients, required in small quantities ranging from few micrograms to milligrams, are known as "micronutrients" which include some mineral elements, vitamins, amino acids, and fatty acids those enable the living organisms to utilise the macronutrients (Beers, 2003). Although carbohydrates, proteins and fats can be interchanged as the sources of energy, no substitute to any of the micronutrients is available in human body, thus the deficiency of these nutrients causes major imparities. Among the micronutrients, about 13 vitamins, 9 amino acids, 2 fatty acids and 20 mineral elements (mineral micronutrients), which

cannot be synthesised by the human body, should essentially come from food (World Bank, 2008). No single food source provides all the nutrients we need. Hence, several diverse foods are needed to provide appropriate quantities of both macro- and micronutrients to constitute a '*balanced diet*'. Of the micronutrients, mineral nutrients are accumulated in certain plant parts mainly by transport from the soil solution across the plasma membrane. Therefore, the mode of mineral transport and accumulation plays important role in the composition of these micronutrients in dietary staples.

Mineral micronutrient transport, accumulation and availability

Higher plants have evolved two strategies to acquire iron (Fe) from the soil *viz*, (i) reduction based (strategy I) and (ii) chelation based (strategy II) (Romheld and Marschner, 1986). Reduction based strategy is mostly seen in all the higher plants except graminaceous plants. Fe starvation activates H^+-ATPase in plants and extrudes protons into the rhizosphere, which lowers the pH of the soil solution. The decrease in every unit of pH, increases the solubility of Fe^{3+} by 1000 fold (Olsen *et al.*, 1981). The reduction of freely available Fe^{3+} to Fe^{2+} is mediated by NADPH-dependent ferric oxide reductases (*FROs*). FROs have been cloned and characterized in model and crop plants (*Arabidopsis*: Robinson *et al.*, 1999; Pea: Waters *et al.*, 2002). The import of available Fe^{2+} to root epidermal cells is carried out by plasma membrane located ZIP family Iron Regulated Transporters (IRTs). Several IRTs have been identified in crops species including the model

plant *Arabidopsis viz.*, *OsIRT1* and *OsIRT2* in rice (Ishimaru *et al.*, 2006), *ZmIRT1* in maize (Li *et al.*, 2013), *HvIRT1* in barley (Pedas *et al.*, 2008), *LeIRT1* and *LeIRT2* in tomato (Eckhardt *et al.*, 2001) and *Arabidopsis* (Eide *et al.*, 1996). Strategy II or chelation based uptake of iron is seen in graminaceous plants on iron limitation, where roots of grasses release low molecular weight Fe^{3+} specific ligands made up of non-proteomic amino acids called phytosiderophores (PS) (Kobayashi and Nishizawa, 2012). The S-adenosylmethionine decarboxylase (SAM-DC) mediates the conversion of precursor methionine to S-adenosylmethionine (SAM). Nicotianamine synthase (NAS) mediate the synthesis of nicotianamine (NA) from SAM (Mizuno *et al.*, 2003; Hind and Guerinot, 2012). Later NA is converted to unstable 3'-oxo form by NA aminotransferase (NAAT) and subsequently, the reduction of 3'-oxo by deoxymugineic-acid synthase results in 2'- deoxy mugineic acid (DMA). The secreted PS in rhizosphere forms complexes of PS-Fe^{3+} and transport into root epidermis *via* members of Oligo-Peptide Transporter (OPT) *i.e.,* yellow stripe-like (YSL) proteins (Curie *et al.*, 2001; Yen *et al.*, 2001). As compared to iron, zinc uptake from soil is simple. Zinc predominately enters the roots as a divalent cation through ZIP transporters (Marschner, 1995) from soil. In addition, roots of strategy-II plants release PS under zinc deficiency to increase the uptake of zinc (Zhang *et al.*, 1991). Further, researchers demonstrated zinc uptake both in free (Zn^{2+}) and complex form (PS-Zn^{2+}) in maize *ys1* mutant (von Wiren *et al.*, 1996).

After uptake, iron and zinc have to be distributed to other plant tissues and storage organs *via* xylem and phloem mediated transport. Iron enters the xylem as citrate-iron complex. Citrate plays a prominent role in chelating and trafficking of iron for its transport into the apoplastic place of xylem (Curie *et al.*, 2009; Conte and walker, 2011) *via* Ferric Reductase Defective 3 (FRD3) and ferroportin proteins (Durrett *et al.*, 2007; Roschzttardtz *et al.*, 2011). Phenolic Efflux Zero 1 (PEZ1) loads the phenolic compounds, protocatechuic acid and caffeic acid into xylem to solubilise the precipitated apoplastic iron (Ishimaru *et al.*, 2011). The members of *p*-type ATPases, *AtHMA2* and *AtHMA4* are the active zinc-pumps involved in loading of zinc to root xylem (Verret *et al.*, 2004; Sinclair *et al.*, 2007). Slightly acidic pH of xylem sap leads to translocation of zinc as a free cation, although citric acid, reported to be involved in trafficking of zinc, is the prominent component in xylem sap (Lu *et al.*, 2013). The distribution of minerals to developing organs and seeds depends to a great extent on vascular tissues of the phloem. Phloem sap possesses alkaline pH in which metals are sparingly soluble and reactive metals such as iron can undergo valence changes, and leads to production of highly reactive oxygen species *via* fenton reactions. Therefore, mineral complexations with ligands such as NA, DMA can provide better solubility and protection to the living cells and facilitate transport to the nutrient sinks. The movement of iron and zinc in complex with NA was observed in various crops (Curie *et al.*, 2001; Waters *et al.*, 2006). In addition to phloem and xylem, grains acquire iron and zinc through

senescence assisted remobilization from leaves. Delayed senescence through down-regulation of an NAC transcription factor, NAM-B1, resulted in lower grain protein, iron and zinc concentration owing to reduced nutrient remobilization (Uauy *et al.*, 2006; Waters *et al.*, 2009). The understanding on mineral dynamics in plants would help to device strategy for enhancing mineral accumulation and availability in staple crops.

Micronutrient malnutrition

Micronutrients are one of the essential players in the metabolic activities of the human body (Bendik *et al.*, 2014) those have to be met from staple foods. However, less than recommended level of micronutrients in staples has resulted in large scale malnutrition. The problem of food and nutrition insecurity relates not only to the total food supply, but also to a decline in crop diversity and changing food patterns away from traditional diets. There are different strategies to combat malnutrition, those include guidelines for balanced diet, dietary diversification, use of specific nutrient supplements, etc. and public health measures to control disease (Chinma and Igyor, 2007). No single strategy is universally assured of success. Reasonably, a fusion of intervention strategies is needed for sustainable development in human nutrition and prevention of nutrition-related chronic diseases (Emire *et al.*, 2013). The effect of nutritional status on principal lifecycle events, morbidity and mortality, reproductive performance, cognitive development, work, etc. is appropriately documented in malnourished populations (Bouis and Welch, 2010). The micronutrients related chronic diseases and their prevalence are presented in Table 1.

Though Rome Declaration envisaged reducing the number of malnourished people to half by 2015, one-third of the global population still suffers from micronutrient malnutrition (IFPRI, 2016). Hence, development of micronutrient dense cultivars of staple cereals and their consumption would help meet the recommended dietary allowance (RDA). The breeding targets for biofortified crops were designed to meet the specific dietary needs and consumption patterns of people with emphasis on women and children under HarvestPlus project by a committee of breeders, nutritionists and policy makers (Bouis and Saltzman, 2017).

IMPORTANCE OF CEREALS AS A SOURCE OF MICRONUTRIENTS

Cereals are the edible seeds or grains of the grass family, Gramineae (Bender and Bender, 1999). They have been part of the human diet since centuries. They have become a popular part of the human diet due to cultivability in a variety of areas, soils, climatic conditions and higher productivity compared with other crops. The cereals and cereal products are the main sources of energy, carbohydrates, proteins and fibre to humans. The estimate of per capita cereal consumption in developing countries is 166 kg per annum, while it is 133 kg in developed countries (FAO, 2003). Cereals provide a range of macro- and micronutrients and a range of phytochemicals which may provide some of the health benefits (Goldberg, 2003). However, the density of micronutrients is far below RDA. This coupled with presence of

Table 1. Micronutrients related chronic diseases and their worldwide prevalence.

Micronutrient	Deficiency Prevalence	Major Deficiency Disorders
Iodine	2 billion at risk	Goiter, hypothyroidism, iodine deficiency disorder, increased risk of stillbirth, birth defects, infant mortality cognitive impairment.
Iron	2 billion	Iron deficiency anaemia, reduced learning and work capacity, increased maternal and infant mortality, low birth weight.
Zinc	High in developing countries	Poor pregnancy outcome, impaired growth (stunting), genetic disorders, decreased resistance to infectious diseases.
Vitamin A	254 million preschool children	Night blindness, xerophthalmia, increased risk of mortality in children and pregnant women.
Folate (Vitamin B6)	Insufficient data	Megalobastic anaemia, neural tube and other birth defects, heart disease, stroke, impaired cognitive function, depression.
Cobolamine (Vitamin B12)	Insufficient data	Megaloblastic anaemia (associated with Helicobacter pylori induced gastric atrophy).
Thiamine (Vitamin B1)	Common in developing countries	Beriberi (cardiac and neurologic), Wernicke and Korsakov syndromes Alcoholic confusion and paralysis).
Riboflavin (Vitamin B2)	Common in developing countries and in famines, displaced persons.	Non-specific – fatigue, eye changes, dermatitis, brain dysfunction, impaired iron absorption.
Niacin (Vitamin B3)	Common in developing countries	Pellagra (dermatitis, diarrhea, dementia, death).
Vitamin B6	Common in developing countries and in famines, displaced persons.	Dermatitis, neurological disorders, convulsions, anaemia, elevated plasma homocysteine.
Vitamin C	Common in famines, displaced persons	Scurvy (fatigue, haemorrhages, low resistance to infection, anaemia)
Vitamin D	Widespread in all age groups, low exposure to ultra violet rays of sun	Rickets, osteomalacia, osteoporosis, colorectal cancer.
Calcium	Estimated to be widespread	Decreased bone mineralization, rickets, osteoporosis.
Selenium	Common in Asia, Scandinavia, Siberia.	Cardiomyopathy, increased cancer and cardiovascular risk.
Fluoride	Widespread	Increased dental decay, affects bone health.

Source: Adapted from Allen *et al*. (2006).

many antinutritional factors make human population consuming cereal rich diets prone to micronutrient deficiency disorders. The food staples like wheat, rice, maize, pearl millet and sorghum are rich in starch but having very low contents of mineral elements. The importance of each of the five cereals has been briefly discussed below.

Maize

Maize is a staple food of people in the sub-Saharan Africa, Africa and some parts of America and of livestock worldwide. Globally, maize is cultivated on an area of 177 mha, with a production of 967 mt and productivity of 4,920 kg ha^{-1} (USDA, 2017). Maize supplies more than five percent dietary energy to humans; provides nutrients for animals and serves as a basic raw material for

industries. In many developing countries, maize is a major staple food and the consumption per capita is very high (Danilo, 2003). In Sub-Saharan Africa, maize is consumed by 50 percent of the population and is the preferred food for one-third of all malnourished children and 900 million poor people worldwide. By 2050, global maize consumption is expected to increase from 32 to 52 kg per person per year. Maize is also dubbed as a "Poor Man's Nutricereal" as it contains about 10% protein, 4% oil, 70% carbohydrate, 2.3% crude fibre, 10.4% albuminoides and 1.4% ash and also has more vitamin A, nicotinic acid, riboflavin and vitamin E (Nuss and Tanumihardjo, 2010) compared to other cereals, albeit less than the RDA.

Rice

Rice is the staple food for more than half of the world population and provides about 20% of the world's dietary energy supply and 15% of per capita protein. It is cultivated in more than 100 countries, with an area of about 158 mha, producing more than 700 mt annually, of which Asia contributes 90%. It is the main dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. Though raw rice contains higher quantities of minerals, vitamins, and fibre, all constituents, except carbohydrates, are reduced by milling (NRRI Annual Reports, 1949-50 and 1950-51). Thus, cooked rice after milling is composed of 68% water, 28% carbohydrates, 3% protein, negligible fat, less than 10% of the Daily Value (DV) of the micronutrients, moderate amounts of B vitamins, iron, and manganese (10–17% DV) and provides about 130 calories of energy. Although rice protein is high in nutritional quality compared with other major cereals, protein content is modest. Rice eaters and growers constitute bulk of the world's population living in poverty.

Wheat

Wheat is the main staple cereal of temperate regions covering North America, Europe, Australia, New Zealand, the Southern Cone countries (Argentina, Chile, Brazil, Paraguay, Uruguay) and the Greater Middle East. It is a widely grown cereal crop contributing towards 28% of the edible dry matter and also contributing towards two third of the daily calorie needs in the developing countries. It is occupying about 20% of the world's total cultivated land and a staple food crop for one third of the world's population. Globally, wheat is grown on 220 mha with a production of 749 mt (FAO, 2016). It is staple food of South Asia and its consumption by an individual is around 100–150 g/day (Joshi *et al.*, 2010). This is a major source of energy as it provides 327 calories per 100 grams and also a rich source of multiple minerals, vitamins and protein. Additionally, wheat contains 13% water, 71% carbohydrates and also has 13% protein. Each 100 g of wheat contain 3.2 mg of iron and 2.6 mg of zinc that can fulfill only 17% and 20%, respectively, of the daily requirement of adults. Wheat is the leading source of vegetal protein in human diet. The protein content in wheat is relatively high compared with other major cereals but, relatively low in protein quality for supplying essential amino acids.

Sorghum

Sorghum is the fifth most important cereal crop grown in more than 100 countries in Africa, Asia, Oceania and the Americas. It is cultivated on about 44.77 mha producing 63.93 mt of grains with an average productivity of 1428 kg ha^{-1} (FAO, 2016). It is one of the widely consumed (by about 500 million poor and most food-insecure people) cereals in the sub-tropical and semi-arid regions of Western and Central Africa, Eastern and Southern Africa and India (Kresovich *et al.*, 2005). In countries like China, Mexico, etc. grains are important as animal and bird feed. In large parts of the developing world, stover represents up to 50% of the total value of the crop, particularly in drought years (ICRISAT & FAO, 1996). Sorghum has a nutritional profile better than the fine cereals like rice and almost on par with wheat (Hemalatha *et al.*, 2007). A vast majority of the population in Africa and central India depend on sorghum for their dietary energy and micronutrientrequirement (Rao *et al.*, 2006). Sorghum accounts for about 35% of the total intake of calories, protein, iron and zinc in the dominant consumption regions in low income group populations (Rao *et al.*, 2006). Sorghum is a rich source of various phytochemicals, which are secondary plant metabolites or integral cellular components, such as phenolic compounds, anthocyanins, phytosterols and policosanols (Awika and Rooney, 2004). These phytochemicals have potential to impact human health significantly through high antioxidant activity against different free radicals *in vitro.* The bioavailability of iron (4.13 ± 0.33%) and zinc (5.51 ± 0.32%) in sorghum is poor compared to rice, maize, or wheat and pulses due to tannin, phytate, fibre, etc. (Hemalatha *et al.*, 2007).

Pearl Millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal grown in semi-arid regions. It is a staple food of more than 90 million people living in pearl millet growing regions and is widely cultivated as a source of fodder (Rao *et al.*, 2006). Pearl millet is grown over 33 mha in the world, mostly in arid and low fertility regions, where it is often the only viable cereal. It has a good nutritional profile among cereals and supports the nutritional requirement of both human and livestock. Pearl millet is staple food source and is also used as a feed and fodder crop in Asian, African and Latin American countries. Pearl millet can meet 35% of total intake of calories, protein, iron and zinc in the areas where it is grown as staple crop. Pearl millet flour is used in fortification of foodstuff to improve nutritional quality of food products (Sonkar and Singh, 2015). Products prepared using pearl millet have been shown to have lower glycemic index than similar products produced from wheat thus increasing the food value of pearl millet for those prone to Type-2 diabetes (T2D). Consumption of pearl milletalso obstruct cancer and for relieving of celiac and several other non-communicable diseases (Nambiar *et al.*, 2011). These nutritional and nutraceutical properties make this crop highly valued for daily consumption (Satyavathi *et al.*, 2015). A comparison of major and micro nutrients in five cereals is presented in Table 2 and Figure 1.

Table 2. Comparison of nutrient composition in 100g edible portions of various cereals.

Food gain	Carb.(g)	Protein (g)	Fat (g)	Energy (KCal)	Crude fibre (g)	Ca (mg)	P (mg)	Carotene (μg)
Wheat (whole)	71.2	11.8	1.5	346	1.2	41	306	64
Rice (raw, milled)	78.2	6.8	0.5	345	0.2	10	160	0
Maize	66.2	11.1	3.6	342	2.7	10	348	90
Sorghum	72.6	10.4	1.9	349	1.6	25	222	47
Pearl millet	67.5	11.6	5.0	361	1.2	42	296	132
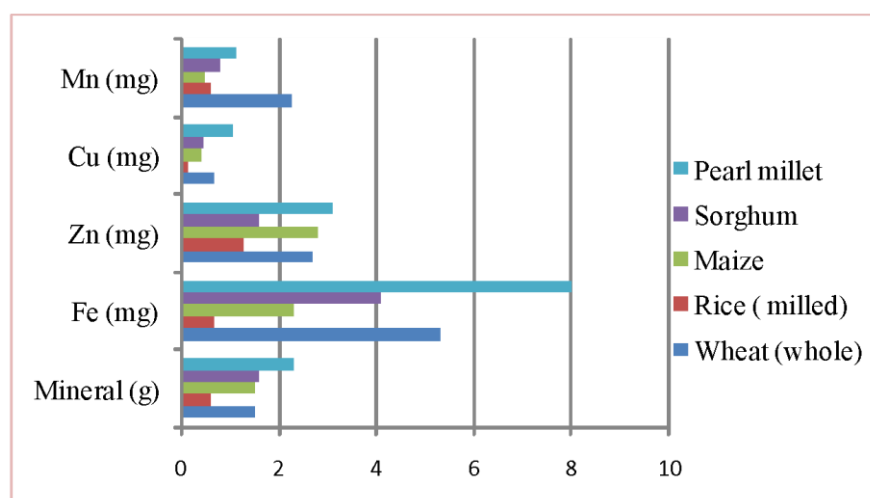
Figure 1. Comparison of micronutrient composition in five cereals.(Source: Gopalan *et al*., 2007)

TARGETS AND STRATEGIES FOR MICRONUTRIENT ENRICHMENT OF STAPLES

Although the most severe cases of micronutrient deficiencies are found in developing countries, people in developed countries also suffer from various forms of malnutrition. Women and children in poverty are most at risk for deficiencies of all these micronutrients (Tulchinsky, 2010).

Deficiency of iron and zinc are ranked ninth and eleventh, respectively in the list of global diseases (Phuke *et al*., 2017). Iron and zinc deficiency together are affecting nearly half of the global poor (Hotz and Brown, 2004). The prevalence of iron and zinc deficiency is more severe and predominant in countries having cereal rich diets lacking diversity in food components (Borril *et al*., 2014). Iron deficiency anaemia and zinc deficiency are responsible for many severe health problems, resulting in impaired physical growth, mental development and learning capacity including increased risk of infections and DNA damage (Bouis, 2003; Hotz and Brown, 2004; Gibson, 2006; Prasad, 2007). The zinc deficiency is more serious among children of below 5 years and is causing death of approximately 433,000 children every

year. The situation is even more adverse in developing countries (Shazad *et al*., 2014). Vitamin A deficiency affects about one third of children living in low and middle income settings, mainly in sub-Saharan Africa and South Asia. Vitamin A deficiency weakens the immune system and increases a child's risk of infections and deaths due to measles, diarrhea, etc.

Mineral malnutrition can successfully be addressed by three major strategies; (i) by dietary diversification; by increasing the intake of diverse foods rich in essential minerals such as fish, animal products, vegetables, etc. in diets, (ii) mineral supplementation/medical supplementation in the form of tablets or syrup and (iii) food fortification by adding specific micronutrients and/or increasing the bioavailability of mineral elements in staple crops. However, these strategies have not been very successful due to political, socioeconomic, infrastructure-related, and technical constraints prevalent in most developing countries. For these reasons, the biofortification of crops through the application of mineral fertilizers (agronomic biofortification), combined with genetic enhancement of micronutrient content through crop improvement procedures to increase the inherent capacity of varieties to accumulate specific micronutrients has been advocated (White and Broadley, 2009).

Considering the severity of the health effects caused by micronutrient deficiencies, the HarvestPlus initiative of the CGIAR consortium (www.harvestplus.org) in collaboration with national and international partners has initiated biofortification program of staple food crops. The program is focusing on three micronutrients widely recognized by the World Health Organization (WHO) as limiting: iron, zinc, and vitamin A. In the first phase, three cereals, maize, rice and wheat and in the second phase pearl millet and sorghum along with other crops are targeted. Full-time crop improvement programs for staple food crops, *viz*., rice, wheat, maize sorghum, pearl millet, cassava, sweet potatoes, common beans, etc. are in operation (Welch and Graham, 2004; Cakmak, 2008; Peleg *et al*., 2009). Presently, major focus is on iron, zinc and vitamin A in these crops, although the research efforts on other micronutrients and crops have already been initiated.

APPROACHES FOR GENETIC ENHANCEMENT OF MICRONUTRIENT CONTENT

The selection of approach for genetic enhancement of micronutrient content needs data on the micronutrient deficiency and requirement of targeted populations, genetic variability, the genes and metabolic pathways those regulate nutrient biosynthesis. Accordingly, the HarvestPlus has devised a strategy for breeding for micronutrient rich crop as depicted in Figure 2.
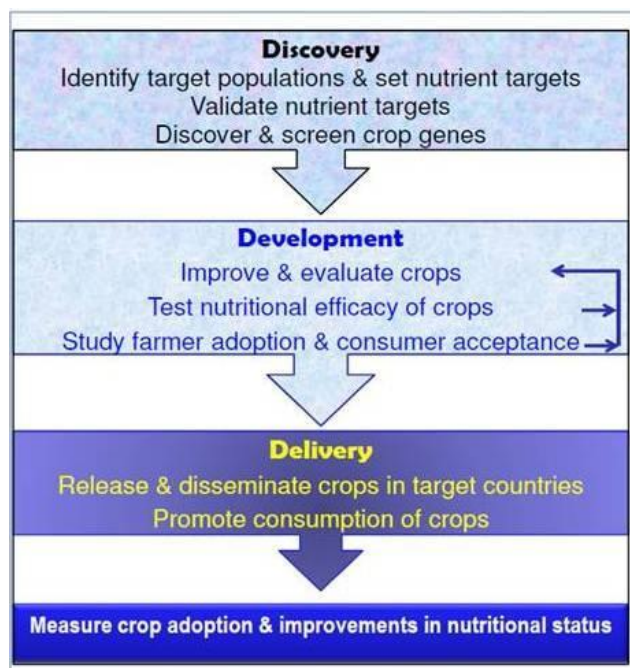
Figure 2. HarvestPlus strategy of genetic enhancement of micronutrients in staples to alleviate hidden hunger

Though, at the stage of development, both conventional and genetic engineering approaches can be utilized, use of conventional breeding is limited mainly because of low genetic variability for micronutrients among the germplasm. However, both conventional and modern breeding approaches succeeded in delivering the iron and zinc biofortified products. The exploitation of natural genetic variation for grain iron and zinc is one of the major strategies to improve the micronutrients in staple intakes.

Wide range of genetic variation for grain micronutrient concentration was reported among major cerelas *viz.*, wheat (Morgounov *et al.*, 2007), rice (Liang *et al.*, 2007), maize (Mallikarjuna *et al.*, 2014, 2015; Mallikarjuna, 2015), sorghum (Nguni *et al.*, 2011) and pearl millet (Velu *et al.*, 2007) (Table 3). It is possible to use the genetic variation in breeding programs to increase micronutrients concentration through appropriate breeding strategies. Various crop studies have highlighted strong and positive correlation among grain iron and zinc and non-significant to significant association between grain micronutrients' concentration and grain yield (Table 3). These evidences suggest the scope for simultaneous improvement of iron and zinc concentration without compromising the grain yield in spite of dilution effect in crops like maize.

Advances in genomics and molecular biology provided various breeding tools and strategies at the plant breeder's disposal to develop micronutrient rich food crops. Several genomic regions and quantitative trait loci (QTL) were identified for grain iron and zinc concentrations in major cereals and millets (Table 4).

Table 3. Genetic variability and correlation for grain micronutrient concentration among major cereals and millets.

No.	Germplasm	Range (mg kg^{-1})			Correlation		Reference
						Grain yield	
		Fe	Zn	Fe vs Zn	vs Fe	vs Zn	
Rice							
1	Accessions	2.00-17.49	9.80-32.44	-	-	-	Gangashetty *et al.* (2013)
2	Accessions	-	18.90-36.90	-	-	0.35^{NS},	Bekele *et al.* (2013)
3	RILs (Madhukar×Swarna)	17.30-53.70	22.50-27.20	0.32*	-	-	Anuradha *et al.* (2012)
4	Accessions	6.30-24.40	13.50-58.40	-	-	-	Gregorio *et al.* (2000)
Wheat							
1	Spelt wheat Lines	27.00-86.00	39.00-115.0	0.78***	−0.49*	−0.62*	Gomez-Becerra *et al.* (2010)
2	Cultivars	25.00-56.00	20.00-39.00	0.79***	−0.41*	−0.64***	Morgounov *et al.*(2007)
3	Accessions	20.00-88.00	15.00-43.00	0.53***	−0.51	−0.67	Oury *et al.* (2006)
Maize							
1	Inbred lines	23.94-42.41	11.83-21.44	0.44**	−0.06^{NS}	−0.16^{NS}	Mallikarjuna *et al.* (2015)
2	RILs (B73 × Mo17)	-	-	0.56*	−0.23*	−0.17*	Baxter *et al.* (2013)
3	Inbred lines and Accessions	20.38-54.29	7.01-29.88	NS	-	-	Agrawal *et al.* (2012)
Sorghum							
1	RILs (296B × PVK801)	10.8.0-76.40	10.20-58.70	0.79**	−0.34**	−0.34**	Phuke *et al.* (2017)
2	Accessions	26.00-60.00	21.00-57.00	0.75**	−0.36*	−0.46*	Ashok Kumar *et al.* (2012a)
3	Varieties	27.40-81.80	20.30-55.30	0.46**	−0.20	−0.32**	Nguni *et al.* (2011)
Pearl millet							
1	RILs (ICMB 841-P3 × 863B-P2)	28.40-124.00	28.70-119.80	0.59**-0.91**	-	-	Kumar *et al.* (2016)
2	Parents and Hybrids	28.80-82.10	25.80-55.50	0.88**	−0.11^{NSa}, −0.29^{**b}	−0.18^{NS}, −0.26^{**b}	Kanatti *et al.* (2014a)
3	Hybrids	36.00-76.00	22.00-44.00	0.69**	−0.55**	−0.32^{NS}	Kanatti *et al.* (2014b)

^{NS},*, **, ***Non-significant, Significant at *P*< 0.05, *P*< 0.01 and *P*< 0.001, respectively; ^{a}parental lines, ^{b}hybrids

Table 4. QTLs identified for grain iron and zinc in important cereals.

No. Parents		Mapping population	No. of QTLs detected*		R^2 (%)		Reference
			Fe	Zn	Fe	Zn	
Rice							
1	Madhukar×Swarna	RILs	7	6	69-71	29-35	Anuradha *et al.* (2012)
2	Bala × Azucena	RILs	3	1	3.2-4.4	3.7	Norton *et al.* (2010)
3	*O. indica*, cv.Teqing × *O. rufipogon*	ILs	2	3	5.0-7-0	5.0-19.0	Garcia Oliveira *et al.* (2009)
Wheat							
1	Saricanak98 × MM5/4	RILs	3	4	9.1-11.7	10.0-16.9	Velu *et al.* (2017)
2	Berkut × Krichauff	DHs	1	2	22.2	23.1-35.9	Tiwari *et al.* (2016)
3	*Triticum boeoticum* (pau5088) × *T. monococcum* (pau14087)	RILs	3	2	7.0-12.6	9.0-18.8	Tiwari *et al.* (2009)
Maize							
1	B73 × Mo17	IBM-RI	2	3	9–11	5-10	Baxter *et al.* (2013)
2	Mu6 × SDM and Mu 17 × SDM	F$_{2:3}$	4	7	10-21	6-21	Qin *et al.* (2012)
3	B84 ×Os6-2	F$_4$	3	1	21.1	4.2	Simic *et al.* (2012)
Sorghum							
1	296B × PVK801	RILs	9	12	2.8-6.6	0.3-5.7	Kotla *et al.* (2016)
Pearl millet							
1	ICMB 841-P3 × 863B-P2	RILs	3	3	0.6-20.5	7.0-34.1	Kumar *et al.* (2016)

*Across environment

These identified linked markers and genes could assist greatly to adopt molecular markers-based breeding approaches to develop grain iron and zinc rich cultivars. The homeostasis of iron and zinc involves several transporters (Mallikarjuna *et al.*, 2016). Overexpression of transporter genes allowed the breeder/biotechnologist to adopt single gene based approach to handle the complex traits like grain mineral concentrations. Targeted expression of native iron and zinc transporters and transgenes lead to several successful experimental transgenic events for higher grain iron and zinc concentration. In rice, expression of soybean *ferritin* gene under the control of the glutelin promoter resulted in enhanced grain iron and zinc concentration in brown and polished rice (Vasconcelos *et al.*, 2003). Overexpression of native NAS gene family proved sufficiency of single gene for effective iron and zinc biofortification of rice endosperm (Johnson *et al.*, 2011). Field evaluation of transgenic events developed through overexpression of rice nicotianamine synthase and soybean *ferritin* genes showed grain accumulation of 15 µg g^{-1} iron and 45.7 µg g^{-1} zinc which is above the HarvestPlus target (Trijatmiko *et al.*, 2016).

As pointed out earlier, there has been only a limited success in enhancement through conventional breeding except for iron and zinc genetic biofortification. Moreover, conventional breeding is not useful for those target micronutrients which are not at all produced by the crop. Thus, the current approach involves an array of genetic engineering (GE) protocols to develop micronutrient enriched staple crops. The current developments with respect to use and potential of different genetic enhancement approaches for micronutrients have been summarized below in Table 5.

In addition to the above methods, some other variants of gene editing protocols *viz.,* Engineered Meganucleases (EMNs), Zinc Finger Nucleases (ZFNs), Oligonucleotide Mediated Mutagenesis (OMM), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9), base editing- CRISPR 2- CRISPR-Cas-13, homologous recombination, non-homologous end joining, site-directed nucleases, double-stranded break and homology directed repair protocols also have potential to be used in the genetic enhancement of micronutrient content in staple foods. The elaborate research efforts on enhancement of micronutrients content involving more number of and multiple micronutrients have gone into maize and rice compared with other cereals. Accordingly, the efforts on genetic enhancement are reviewed hereunder starting with the maize crop.

GENETIC ENHANCEMENT OF MICRONUTRIENTS CONTENT IN MAIZE

Maize is inferior to other cereals in nutritional value. Its protein is of poor quality as it has low lysine and tryptophan contents. Hence, protein quality improvement has been the major focus in maize and the efforts on improvement of provitamin-A, iron and zinc contents are in progress.

Essential minerals: iron and zinc

Wide variation for kernel iron and zinc concentration was reported in different germplasm categories of maize ranging from temperate, tropical, flint, dent and QPM (Gupta *et al*., 2015; Mallikarjuna, 2015). Strong and positive correlation between grain iron and zinc, while non-significant to significant association between grain micronutrients concentration and grain yield have been reported (Table 3). These evidences suggest the scope for simultaneous improvement of iron and zinc concentration without compromising the grain yield inspite of dilution effect in crops like maize. Chakraborti *et al*. (2009a) reported significant positive correlation between grain zinc and grain yield in maize. Simultaneous improvement of micronutrients without yield penalty by exploiting the genetic correlation among micronutrients and grain yield is major issue to be considered.

Very few QTL studies were reported for grain iron and zinc concentration in maize (Baxter *et al*., 2013; Lungaho *et al*., 2011; Qin *et al*., 2012; Simic *et al*., 2012). However, several genomic regions and QTLs were identified for grain iron and zinc concentrations in major cereals and millets (Table 4). The QTL analysis

Table 5. The approaches for genetic enhancement of micronutrient composition in crops.

Approach/protocol	Succesful examples
Conventional Plant Breeding Mutations hybridizations Selective breeding	Enhancement of iron, zinc and carbohydrate components in wheat, maize and barley mutants. (lysine-rich sorghum - Eswara Reddy and Jacobs,2002), lysine rich maize (Vasal, 2001)
Marker Technology Marker assisted Selection (MAS)	a) High lysine in maize: First successful demonstration of marker-assisted selection; Several QPM hybrids in maize (Gupta *et al.*, 2009, 2013) b) High provitamin-A in maize (Muthusamy *et al.*, 2014)
Genetic Engineering (GE) Recombinant DNA approach (rDNA)	a) High lysine: sorghum (Zhao *et al.*, 2003), rice (Sindhu *et al.*, 1997, Katsube *et al.*, 1999, Stoger *et al.*, 2001, Christou and Twyman, 2004), wheat (Stoger *et al.*, 2001; Christou and Twyman, 2004) b) High methionine: rice (Hagan *et al.*, 2003), maize (Lai and Messing, 2002)
Gene stacking approach	a) The three vitamin corn with five stacked genes (Naqvi *et al.*, 2009) b) Multi-biofortified rice with enhanced pro-vitamin A, zinc, iron and folate concentrations (De Steur *et al.*, 2012)
Gene silencing (GS) approach	a) Changing of the relative proportions of starch components amylose and amylopectin in wheat and potato (Lafiandra *et al.*, 2008); enhanced B-glucan in wheat b) Modifying levels of proteins and amino acids (Uauy *et al.*, 2006), Gil-Humanes *et al.*, 2010 in wheat; O'Quinn *et al.*, 2000); Yang *et al*. (2002) in maize c) Modifying levels of fatty acids (Liu *et al.*, 2002a, 2002b; Young *et al.*, 2004, ILSI, 2008) in maize. d) Reducing levels of allergens and antinutritional compounds reduced phytic acid: (Brinch-Pedersen *et al.*, 2000 in wheat; Lucca *et al.*, 2002 in Rice, Han, 2009 in maize). Gil-Humanes *et al*. (2010) used RNAi to shut down celiac disease related wheat gliadin T-cell epitopes. Reduced zein in maize- (Segal *et al.*, 2003) e) Reduced levels of steroidal glycoalkaloids in potato (McCue *et al.*, 2003) f) Decaffeinated coffee (Ogita *et al.*, 2003) g) Reducing levels of polyphenol oxidases (USFDA, 2015a,b in apple and potato) h) Reducing the levels of asparagine and reducing sugars (USFDA, 2015b in potato)
The'-omics' protocols	a) Identifying the quantitative and qualitative status of nutritionally significant compounds in a food crop b) The isolation and extraction genes
Metabolic engineering	Desired levels of qualitative and quantitative enhancement of compounds of significance in human nutrition. (Dharmapuri *et al.*, 2002- xanthophylls content in tomato; Diretto *et al.*, 2007a -carotenoid in potato; Fujisawa *et al.*, 2008-carotenoid in flax seed; Shintani and DellaPenna, 1998-vitamin E in plants, Storozhenko *et al.*, 2007-folate in rice)
Direct multiple gene transfer approach	a) Expression of complex recombinant macromolecules into the plant genome in rice (Nicholson *et al.*, 2005). b) Engineered minichromosomes segregating independently of the host chromosomes in maize (Carlson *et al.*, 2007)
Synthetic proteins and nucleotides approach	Synthetic storage protein in sweet potato (Egnin and Prakash, 1997; Prakash and Jaynes, 2000)
Gene Editing Protocols Transcription Activator-Like Effector Nucleases (TALENs) approach	a) Several mutations in barley (Wendt *et al.*, 2013) b) Improved quality of soybean oil (Huan *et al.*, 2014)

identified the possible reasons behind the correlation between grain iron and zinc concentration either by co-localization or pleiotropic effect of QTLs which control iron and zinc homeostasis (Clemens, 2001; Qin *et al.*, 2012). Significant large effect QTLs need to be fine mapped and cloned to reveal the mechanism of grain iron and zinc accumulation and for better utility of QTLs in breeding programs.

Targeted expression of native iron and zinc transporters and transgenes lead to several successful experimental transgenic events for higher grain iron and zinc concentration. Few attempts were done to develop maize transgenics for higher grain iron and zinc accumulation. The homeostasis of iron and zinc involves several transporters (Mallikarjuna *et al.*, 2016). Overexpression of *NAS* and *NAAT* resulted in increased phytosiderophore synthesis (Zheng *et al.*, 2010). Simultaneous expression of soybean *ferritin* and *Aspergillus niger* phytase in maize resulted in decrease of phytate up to 95%, while increase in iron content by 20-70% (Drakakaki *et al.,* 2005). The expression of soybean *ferritin* transgene in maize endosperm altered expression of native iron homeostasis genes in the maize plant and accumulated significantly higher concentrations of calcium, and magnesium in addition to iron (Kanobe *et al.*, 2013). The above approaches can be coupled with expression of phytase which breaks down the antinutritional factor and facilitate the increased iron bioavailability during digestion.

Kernel provitamin-A

Vitamin A deficiency (VAD) is a global health problem and affects millions of people worldwide (Bouis and Saltzman, 2017). Carotenoids play vital role in human metabolism and among various carotenoid fractions, α-carotene, β-carotene and β-cryptoxanthin serve as the precursors for vitamin-A biosynthesis, while lutein and zeaxanthin act as scavengers for free radicals (Olson, 1999). Although maize possesses adequate genetic variation for kernel carotenoids compared to rice and wheat, traditional maize cultivars are low (1-2 µg g^{-1}) in provitamin-A (proA) (Buckner *et al.*, 1990). HarvestPlus has set a target of 15 µg g^{-1} of provitamin-A to alleviate VAD in humans (Bouis *et al.*, 2011).

Though several studies reported the genetic variation for total carotenoids in maize, reports on variation for different fractions of carotenoids in maize germplasm are limited (Gupta *et al.*, 2015). This is due to the fact that the phenotyping of individual fraction of carotenoids in segregating populations using high performance liquid chromatography involves high cost, posing a major limitation in breeding for proA rich maize (Vignesh *et al.*, 2013). Available reports also suggest large genetic variation for non-proA (lutein and zeaxanthin) carotenoids. However, the concentration of proA (α-carotene, β-carotene and β-cryptoxanthin) carotenoids reported is very low (<2 µg g^{-1}) to meet the target level (Chander *et al.*, 2008; Choudhary *et al.*, 2015; Menkir *et al.*, 2008; Muthusamy *et al.*, 2015a, b; Suwarno *et al.*, 2014; Tanumihardjo, 2011; Vignesh *et al.*, 2012) to exploit in the genetic improvement of proA in maize

(Pfeiffer and McClafferty, 2007). The proA carotenoids are predominantly governed by additive gene action, less influenced by genotype × environment interactions and are highly heritable traits (Chander *et al.*, 2008; Egesel *et al.*, 2003; Menkir and Maziya-Dixon, 2004; Menkir *et al.*, 2008; Muthusamy *et al.*, 2015a & 2016; Senete *et al.*, 2011).

Carotenoid biosynthesis pathway is well characterised in maize (Vallabhaneni *et al.*, 2009). Among the genes involved, *Yellow1* (*Y1)* gene or *psy1* (*phytoene synthase1*) plays a pivotal role by condensing two geranyl-geranyl pyroposphate molecules into one molecule of phytoene (Buckner *et al.*, 1990). White maize possesses *y1* allele in homozygous state and virtually devoid of any carotenoids. Plants with dominant *Y1* gene produce carotenoids, which imparts colour to the kernel in maize. The first branching point of the pathway is the cyclization of lycopene, where *lycopene epsilon cyclase* (*lcyE*) in association with other genes converts more lycopene to the β, ε branch, which produces more α-carotene and lutein, whereas the naturally existing mutant alleles of *lcyE* divert more lycopene to the β, β branch and produces β-carotene, β-cryptoxanthin, and zeaxanthin (Harjes *et al.*, 2008). Although, the favourable *lcyE* allele increases the proportion of β-carotene in the pathway, β-*carotene hydroxylase* (*crtRB1*) hydroxylates large amounts of β-carotene to produce β-cryptoxanthin (which has proA activity only half that of β-carotene) and zeaxanthin (which has no proA activity at all) (Yan *et al.*, 2010). Hence, these two key genes play vital role in enrichment of proA in maize with the *crtRB1* having more

profound effect than the *lcyE* gene (Zunjare *et al.*, 2017a). Maize genotypes with high proA and favourable alleles of key genes have been developed and are being distributed to different breeding centres worldwide under HarvestPlus programme (www.harvestplus.org). Breeder friendly PCR-based co-dominant markers have also been developed for these two key genes *viz.*, *lcyE* and *crtRB1* for rapid enhancement of β-carotene in the yellow maize (Babu *et al.*, 2013; Harjes *et al.*, 2008; Yan *et al.*, 2010; Zunjare *et al.*, 2017b). However, the frequency of these alleles in the germplasm is very low (Muthusamy *et al.*, 2015c) and nucleotide polymorphisms within these genes has been identified those cause differential accumulation of kernel β-carotene (Vignesh *et al.*, 2013; Zunjare *et al.*, 2018a).

MAS has been successfully employed to develop maize genotypes with high proA. Muthusamy *et al.* (2014) introgressed the favourable allele of *crtRB1* from HarvestPlus inbreds into seven elite parental inbreds of four high yielding commercial maize hybrids in India *viz.,* Vivek QPM-9, Vivek Hybrid-27, HM-4 and HM-8. The improved hybrids possessed enhanced kernel β-carotene as high as 21.7 µg g^{-1}, compared to 2.6 µg g^{-1} in the original hybrid (Muthusamy *et al.*, 2014; Choudhary *et al.*, 2014). Of them, β-carotene rich version of Vivek QPM 9 has been released as "Pusa Vivek QPM 9 Improved" by central variety release committee (CVRC) for commercial cultivation in India (Yadava *et al.*, 2017). This hybrid also possesses higher lysine and tryptophan, and is country's first multi-nutrient maize hybrid developed through targeted

breeding approaches. Further, Liu *et al*. (2015) also introgressed the *crtRB1* favourable allele into CML161 and CML171 through MAS and showed improvement of proA from 1.6 µg g^{-1} and 1.80 µg g^{-1} to 5.25 µg g^{-1} and 8.14 µg g^{-1} in CML161 and CML 171, respectively. Zunjare *et al*. (2018b) pyramided *lcyE* and *crtRB1* favourable alleles into four elite QPM inbreds *viz*., HKI161, HKI163, HKI193-1 and HKI193-2, the parental inbreds of four popular QPM hybrids *viz*., HQPM-1, HQPM-4, HQPM-5 and HQPM-7. The reconstituted hybrids showed 9.25 to 12.88 µg g^{-1} of proA, an average of 4.5-fold increase compared to original hybrids (2.14 to 2.48 µg g^{-1}) (Zunjare *et al*., 2018b). These hybrids are under different stages of testing under All India Coordinated Research Project on Maize. Around 11 proA rich hybrids/open pollinated varieties (OPVs) in Malawi, Zambia and Zimbabwe; while about 15 proA rich OPVs in Nigeria, Ghana and DR Congo have been developed and released (www.harvestplus.org).

Efforts were also made to enrich carotenoids in maize through genetic engineering or transgenic technology (Aluru *et al*., 2008; Zhu *et al*., 2008; Naqvi *et al*., 2009). Overexpression of *crtB* and *crtI* genes from bacteria (*Erwinia herbicola*) increased β-carotene content up to 10 µg g^{-1} in Hi-II maize line (Aluru *et al*., 2008). Zhu *et al*. (2008) and Naqvi *et al*. (2009) have also developed transgenic maize genotypes (with ~60 µg g^{-1} β-carotene) using combination of five genes (*psy1*, *crtI*, *lycb*, *bch* and *crtW*). Albeit the enhancement achieved, its successful adoption faces several socio-economic and political challenges.

The carotenoids including provitamin-A are highly sensitive to environmental factors like heat and light (De-Moura *et al*., 2015). Genotypes with higher retention of provitamin-A during storage has been identified (De-Moura *et al*., 2015; Taleon *et al*., 2017); and research efforts are also underway to identify and characterize gene(s) responsible for higher retention of pro vitamin-A during storage (Suwarno *et al*., 2015). With the series of provitamin-A hybrids and varieties being developed, efforts are also underway to study the bioavailability (Dubey *et al*., 2018), consumer preference and acceptability of proA maize hybrids (Muzhingi *et al*., 2011; Pillay *et al*., 2011).

Protein quality

Cereal proteins including maize proteins have poor nutritional value for humans due to reduced essential amino acids like lysine and tryptophan (Vasal, 2001). Lysine is the most important limiting amino acid in the maize endosperm protein, followed by tryptophan. Maize protein possesses lysine content of 2.7%, which is well below the recommendation by FAO for human nutrition. Although the germ protein has adequate lysine (5.4%), the abundance of endosperm proteins -zeins (average lysine content ~1.9%) reduces the level of lysine. This is due to 60-70% of endosperm protein consists of zeins, which contain few or no lysine residues (Coleman and Larkins, 1999). Similarly, the lack of tryptophan residues in zein proteins leads to low tryptophan content as well.

Lesser lysine and tryptophan result in poor protein quality and low biological value of the maize protein (Gupta *et al*., 2013). Monogastric animals like human and poultry birds cannot synthesize these amino acids

in their body and therefore, they need to be supplemented through diet. Discovery of recessive *opaque2* (*o2*) mutant was a major breakthrough, as it resulted in enhancing the lysine and tryptophan by nearly two folds compared to wild type (Mertz *et al.*, 1964). The *O2* gene product acts as a leucine zipper family of transcriptional factor that is required for the expression of zein genes. In contrast, the mutant protein possesses less affinity of binding to the promoter regions, thereby causing reduction in synthesis of zein protein by 50-70% (Bjarnason and Vasal, 1992). Thus, reduction inlysine deficient zein proteins followed by the enhanced synthesis of lysine-rich non-zein proteins causes enhancement of protein quality (Geetha *et al.*, 1991; Habben *et al.*, 1993). Recessive *o2* also significantly reduces transcription of lysine keto-reductase (LKR), the enzyme that degrades lysine in maize endosperm, thereby enhancing the concentration of lysine (Kemper *et al.*, 1999). Further, *o2* is involved in regulation of various metabolic pathways and causes enhanced synthesis of various lysine-rich proteins and enzymes (Jia *et al.*, 2013). Maize genotype with *o2* possesses 80% biological value as compared to 45% in normal maize. Further, based on nitrogen balance index protein quality of *o2* maize is 90% to that of milk. Decreased leucine/ isoleucine ratio in *o2* is helpful in liberating more tryptophan for niacin biosynthesis, and thus, helps to combat pellagra (Vasal *et al.*, 1980).

Due to its nutritional superiority, breeding efforts worldwide led to the rapid introgression of *o2* in diverse genetic backgrounds. However, the *o2* germplasm showed negative pleiotropic effects *viz.,* soft texture of the grain, lower seed density, brittleness and susceptibility to storage insects, kernel breakage during mechanical processing, thereby reducing the value of the grains (Wessel-Beaver and Lambert, 1982). Subsequently, breeders at CIMMYT, Mexico under the leadership of Dr. Surinder K. Vasal found partially modified kernels in some back cross derived *o2* genotypes. Following this, elaborate efforts were made to accumulate the frequency of endosperm modifier loci those eventually led to the development of nutritionally-rich hard endosperm *o2* genotype popularly phrased as 'Quality Protein Maize' or QPM (Vasal *et al.*, 1980). The QPM germplasm is comparable to normal maize in relation to hardness of the endosperm (Babu *et al.*, 2015; Hossain *et al.*, 2008a &b;Pandey *et al.*, 2016), but possesses nearly double the concentration of lysine and tryptophan. Studies also revealed that the simultaneous enhancement of tryptophan and lysine are possible due to their highly significant correlation in maize (Vivek *et al.*, 2008).

The conversion breeding programme of CIMMYT led to the development of several widely adapted QPM populations/pools/inbreds, which are widely used in the development of QPM cultivars in several Asian and Latin American countries (Vasal, 2001; Gupta *et al.*, 2009). Intensive efforts were made in different breeding centres in India, resulting in the release of a nearly a dozen QPM hybrids (Gupta *et al.*, 2015). *Opaque2* being recessive in nature, progeny testing becomes essential in backcross breeding programme. It eventually takes longer time. With the availability of *o2*-based SSRs, heterozygotes

could be selected in the backcross generation without progeny testing, and further marker-based background selection can recover the recurrent parent genome in just two backcrosses in place of 6-7 generations required in conventional backcrossing programme (Hossain *et al*., 2017). Several QPM lines or hybrids have now been developed through marker-assisted selection (MAS) worldwide (Gupta *et al*., 2013).

The first successful demonstration of MAS technology in maize was the development of Vivek QPM-9; where Vivek Hybrid-9 was converted in QPM by introgression of *o2* allele into the elite parental inbreds of the original hybrid (Gupta *et al*., 2009, 2013). Vivek QPM-9 possesses 30% higher lysine and 40% more tryptophan while retaining the same level of productivity of the original hybrid. Further, five commercial hybrids, *viz*., HM-4, HM-8, HM-9, HM-10, and HM-11 have been biofortified for lysine and tryptophan using MAS for *o2* gene (Hossain *et al*., 2017). These improved hybrids possess enhanced levels of lysine and tryptophan with similar yield potential compared to their original hybrids, and three hybrids *viz*., 'Pusa HM-4 Improved', 'Pusa HM-8 Improved' and 'Pusa HM-9 Improved' has now been released for commercial cultivation through CVRC (Yadava *et al*., 2017). Similar efforts to introgress *o2* allele into normal inbreds through MAS have been reported in India (Babu *et al*., 2005), Uganda (Manna *et al*., 2005), Kenya (Danson *et al*., 2006), Philippines (Magulama and Sales, 2009) and Thailand (Jompuk *et al*., 2011).

Further, recently a recessive *opaque16* (*o16*) mutant with higher lysine and tryptophan in maize has been reported (Yang *et al*., 2005; Zhang *et al*., 2013, Sarika *et al*., 2017). Besides, *o16* mutant does not cause softness in the kernel (Sarika *et al*., 2018). The *o16o16* possesses nearly two-fold more lysine (0.247%) and tryptophan (0.072%) than the wild type (0.125% lysine and 0.035% tryptophan (Sarika *et al*., 2018). Further, mutant combination of *o2* and *o16* possesses 40-80% higher lysine and tryptophan as compared with *o2* alone (Yang *et al*., 2005). Efforts were made in India to improve the popular QPM hybrids *viz*., HQPM-1, HQPM-4, HQPM-5 and HQPM-7 by pyramiding *o2* and *o16* (Sarika *et al*., 2017). In China, improved maize genotypes in combination with *o2* and *o16* have been developed through MAS (Yang *et al*., 2013; Zhang *et al*., 2013).

Kernel vitamin-E

Vitamin-E predominantly determined by the activity of tocopherols is made of α-, β-, γ- and δ-isoforms (DellaPenna and Pogson, 2006). Tocopherols in human body scavenge various reactive oxygen species (ROS), free radicals and quenches singlet oxygen (Bramley *et al*., 2000). It is also involved in membrane stability by protecting polyunsaturated fatty acids (PUFA) from lipid peroxidation. Vitamin-E prevents several diseases in humans such as Alzheimer's disease, cardiovascular disease, neurological disorders, cancer, cataracts, age-related macular degeneration and inflammatory disease (Traber and Sies, 1996). Human liver preferentially absorbs α-tocopherol due to efficient hepatic α-tocopherol transfer protein, and in turn α-tocopherol is present 10-times in higher concentration than γ-tocopherol in the bloodstream.

Besides, α-tocopherol possesses the highest vitamin-E and antioxidant activity in mammalian tissues, and six times more effective than γ-tocopherol (Feng *et al.*, 2015; Diepenbrock *et al.*, 2017). The RDA of vitamin-E in both adults and teenagers is 15 mg/day (Institute of Medicine 2000). It is estimated that over 20% of the people from both developed and developing world has suboptimal plasma α-tocopherol (Li *et al.*, 2012). In developing countries, vitamin-E deficiency is more common in premature infants and elderly people. Since tocopherols are produced only in plant systems, it is supplemented through diet in humans (Rocheford *et al.*, 2002).

Wide range of variation in tocopherols has been reported in maize kernel (Feng *et al.*, 2015; Muzhingi *et al.*, 2017). Presence of significant genetic variation for α-, γ-, δ- and total tocopherols in maize has been reported in RIL population (Chander *et al.*, 2008), sweet corn inbreds (Feng *et al.*, 2015) and $F_{2:3}$ progenies (Wong *et al.*, 2004); whereas wide variability only for α-, γ- and total tocopherols was observed in maize hybrids (Egesel *et al.*, 2003) and $F_{2:3}$ mapping population (Shutu *et al.*, 2012). Grams *et al.* (1970) reported much lesser proportion (20%) of α-tocopherol and higher γ-tocopherol (80%). Lower proportion of α-tocopherol and higher proportion of γ-tocopherol was also reported by Feng *et al.* (2015) (α-tocopherol: 15%; γ-tocopherol: 80%) and Egesel *et al.* (2003) (α-tocopherol: 21%; γ-tocopherol: 79%).

Several loci have been identified for higher vitamin-E activity in maize kernel (Wong *et al.*, 2003; Chander *et al.*, 2008; Shutu *et al.*, 2012; Diepenbrock *et al.*, 2017). However,

ZmVTE4 (*γ-tocopherol methyl transferase*) has been identified as the major genetic locus that can efficiently converts γ-tocopherol into α-tocopherol. The favourable haplotype had 3.2-fold more α-tocopherols content than the unfavourable haplotype of *ZmVTE4* (Li *et al.*, 2012). Presence of 118bp and 7bp InDels in the promoter region of the *ZmVTE4* lead to creation of different alleles of the gene. The 118bp InDel affects the transcripts level, while 7bp InDel regulates the secondary structure of the transcript and affects the translational efficiency of the *ZmVTE4*. Deletion for both 118bp and 7bp creates the most favourable allele (0/0) of *ZmVTE4*, followed by (0/118) and (0/7). The unfavourable allele (118/7) having insertion at both places is predominant in the natural populations, and inefficiently converts γ- into α-tocopherol, thereby accumulating low α-tocopherol, compared to other three versions of the favourable allele. Feng *et al.* (2015), introgressed the favourable allele of *ZmVTE4* into sweet corn inbreds using MAS and reported enhancement of α-tocopherol. At IARI, New Delhi, introgression of *ZmVTE4* into the parental lines of provitamin-A rich QPM hybrids has led to the significant enhancement of vitamin-E. These genotypes also possess higher lysine, tryptophan and provitamin-A.

GENETIC ENHANCEMENT OF MICRONUTRIENTS CONTENT IN RICE

In the last two decades, rice researchers have given major attention for improving the nutritional quality of rice grains with major emphasis on increasing grain protein, iron and zinc concentrations.

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Essential minerals: iron and zinc

A large number of studies were conducted to estimate the iron and zinc content in cultivated and wild rice. Among wild *Oryza* species; *O. nivara*, *O. rufipogon*, *O. barthii*, and *O. longistaminata* and African cultivated rice *O. glaberrima* have higher level of zinc. Indian Institute of Rice Research, Hyderabad, developed high grain zinc containing rice variety DRR Dhan 45 through conventional breeding with overall mean zinc content of 22.6 mg kg^{-1} in polished rice.

Several QTLs have been reported for higher concentration of iron and zinc in rice grains. A wide array of germplasm like Indica rice, Japonica rice, land races, aus genotypes and wild species have been used to map the QTLs and assigned them to chromosomes from different kinds of mapping populations like F_2, recombinant inbred lines (RILs), doubled haploids (DH), backcross inbred lines (BILs), etc. (Gross *et al*., 2003; Stangoulis *et al*., 2007; James *et al*., 2007; Lu *et al*., 2008; Garcia-Oliveira *et al*., 2009; Sperotto *et al*., 2010; Norton *et al*., 2010; Zhang *et al*., 2014; Anuradha *et al*., 2012). Some of these researchers were also able to identify the candidate genes for iron or zinc or both (Table 6).

Besides the attempts to utilize the available genetic variability, significant progress has been achieved in development of transgenic lines for increasing the quantity of mineral micronutrients as well as increasing their bioavailability. In order to improve the iron content in grain, *ferritin* gene has been transferred from soybean into rice (Goto *et al*.,

1999). Efforts have been made to reduce the phytic acid content, a major anti-nutritional factor in rice grain. It reduces the bioavailability of iron in the body by chelating it at low pH in the stomach (Gupta *et al*., 2015). Table 6 compiles the genes used for transformation to improve quality of rice grain for mineral micronutrients.

Vitamins

Unlike mineral micronutrients like iron and zinc, biofortification of some vitamins in rice is not possible throughconventional breeding or marker assisted backcross breeding. Amongvitamins, few accessions of pigmented rice were found to be rich in Vitamin-B and E (Kushwaha, 2016).

Among the B group of vitamins also, folate (Vit-B$_9$) is present in negligible amount in rice germplasm which is very essential for human health. Researchers have relied upon the transgenic approaches for increasing the contents of unavailable vitamins in rice grains.

Provitamin-A

The biggest success in this regard was achieved with the development of β-carotene rich golden rice which is the result of collaborative effort of Ingo Potrykus from ETH, Zurich and Peter Beyer from University of Freiburg. Some steps of the pathway for synthesis of β-carotene are turned off in the grains of rice whereas, the mechanism is fully functional in the leaves. By adding only two genes, coding for phytoene synthase and phytoene desaturase, it is possible to

Table 6. Candidate genes identified and the genes used for transformation for kernel iron and zinc content in rice.

Grain mineral	Candidate genes reported	Genes used for transformation*
Iron (Fe)	*OsYSL1*, *OsMTP1*, *OsFER1*, *OsFER2* (Anuradha et al., 2012; Gross et al., 2003)	*Osnas2, Afphytase,* and *Osnas1, Osnas3, OsYSL2, Ferritin genes: SoyferH1, PyFerritin, rgMT, Gm ferritin*
Zinc (Zn)	*OsARD2, OsIRT1, OsNAS1, OsNAS2* (Anuradha et al., 2012)	*Osnas2, Gm ferritin, Afphytase* and *Osnas1*
Fe and Zn	*OsNAS3, OsNRAMP1, Heavy metal ion transport*, *APRT* (Johnson et al., 2011); Anuradha et al., 2012)	*Nicotianamine synthase* (*NAS*) *genes* [*OsNAS1, OsNAS2,* and *OsNAS3, OsNAS3-D1, HvNAS1, AtNAS1* and *HvNAS1*], *HvNAAT, HvNAAT-A, HvNAAT −B, Osfer2, SoyFerH1, SoyFerH2, Pvferritin, OsIRO2, OsYSL2, OsYSL15, HvNAS1, Afphytase, MxIRT1*
Zn, Cu, and Ni	*OsNAS3*	*OsNAS3*

*Source: Adopted and updated from Mahender *et al*. (2016).

make the pathway fully functional in the grains also. By transformation of genes from daffodil and a soilbacterium *Pantoea* (previously known as *Erwinia*) into rice and expressing them in the grain (Ye *et al*., 2000), it was possible to synthesize β-carotene in the grains. Later, the concentration of β-carotene was further improved by transforming the *phytoene synthase* gene from maize (Paine *et al*., 2005). The genes for golden rice traits have transferred in popular Indian rice varieties through marker assisted backcross breeding strategy (Chikappa *et al*., 2011).

Folate and ascorbic acid

Other vitamins targeted for rice biofortification are folate and ascorbic acid. Folate is a water soluble vitamin (B_9) which can be synthesized only in plants and microbes. The folate content in rice grains is very low. Its enhancement to the extent of 100 times was achieved through overexpression of two genes of the *pterin* and *para*-aminobenzoate branches in folate biosynthetic pathway from *Arabidopsis thaliana* (Storozhenko *et al*., 2007). However, the stability of folate in rice grains is low, which was enhanced through complexing folate to folate-binding proteins and folylpolyglutamate synthetase (FPGS) mediated folate polyglutamylation by metabolic engineering (Blancquaert *et al*., 2015). The efforts to increase ascorbate (Vitamin-C) content have not met with much success (De Steur *et al*., 2015). These suggest that increasing vitamin content in the grains involves manipulation of complex metabolic pathways for which genetic transformation is the only possible approach.

Protein content and quality

The second major component of rice endosperm is protein (vary from 5–12% of grain weight), which is the lowest among cereals. However, rice protein has got highest digestibility among cereal grains. Rice protein is also more balanced among cereal proteins due to higher content of

lysine and tryptophan, the limiting essential amino acids in other cereals. Hence increasing the grain protein content in rice will in turn enhance the content of essential amino acids those are likely to benefit a large section of malnourished population.

Increasing the grain protein content (GPC) in rice was an important breeding objective in rice since several decades, unlike vitamins and minerals, which became the breeding target in the last two decades. However, the genotypes identified with higher GPC were mostly low yielding, which led the scientists to think that yield and grain protein content are negatively correlated. With availability of methodologies like near infra red spectroscopy, it became possible to phenotype the seeds for GPC without grinding them. Mohanty *et al*. (2011) reported about presence of 16.41 and 15.27% crude protein content on dry weight basis in two rice landraces ARC 10063 and ARC 10075, respectively. Through backcross breeding, the high GPC trait of these two landraces were transferred to high yielding background of two popular Indian rice varieties, Naveen and Swarna. Two derivatives of Naveen have been released for cultivation as high protein rice in India *viz*., CR Dhan 310 (10.3% GPC) and CR Dhan 311 (GPC of 10.1% and zinc content of 20 mg kg^{-1}), with grain yield similar to their high yielding recurrent parent. From the backcross population, a consistent QTL (qGPC1.1) over the season in chromosome 1 has also been identified (DARE/ICAR Annual Report 2015-16 & 2016-17 and NRRI Annual Report 2014-15, Mahender *et al*., 2016). In rice, many QTLs along with associated markers have been identified covering all 12 chromosomes for GPC in brown rice as well as milled rice among which chromosomes 1, 2 and 7 were found to harbor most of the QTLs (Tan *et al*., 2001; Aluko *et al*., 2004; Weng *et al*., 2008; Zhang *et al*., 2008; Yu *et al*., 2009; Zhong *et al*., 2011; Yun *et al*., 2014). One QTL, *qPC1* present on long arm of chromosome 1, controls GPC through its regulation of synthesis and accumulation of glutelins, prolamins, globulins, albumins and starch. It encodes a putative amino acid transporter (*OsAAP6*) and controls GPC without affecting growth and grain yield. This suggested that GPC and nutritional quality could be improved without reduction in grain yield (Peng *et al*., 2014, Yang *et al*., 2015).

In order to increase the rice protein quality, Wong *et al*. (2015) overexpressed endogenous lysine-rich histone proteins (*RLRH1* and *RLRH2*) in the seeds of rice to increase the lysine content. The lysine content of transgenic seeds increased nearly 35 percent without disturbing the balance of other amino acids. Jiang *et al*. (2016) also successfully increased the total crude protein as well as amino acid content along with specific increase of lysine and threonine.

Antioxidants

Antioxidants are another important class of nutri-compounds that protect the cells from harmful effects of free radicals and reactive oxygen species. Among antioxidants, anthocyanins are considered as the most important ones. Fortunately in many rice genotypes, these compounds are present naturally. Several pigmented rice genotypes have been identified and characterized in India, including the Chakhao rice of Manipur, Kalbahat rice of Maharashtra, Njavara of Kerala,

etc. Njavara is well known for its medicinal significance in Ayurvedic medicines. However, the identified pigmented genotypes are low yielding and susceptible to lodging. Sanghamitra *et al*. (2017 a, b) studied different accession of pigmented rice and found that low amylose content in important purple rice genotypes, Chakhao, Mamihunger and Manipuri Black may lead to their lower acceptability by consumers. Grain yield along with the grain amylose content in these genotypes needs improvement through breeding. A significantly higher antioxidant property in the grains of these genotypes has been recognized. Higher heritability of the trait suggested greater scope for genetic improvement of anthocyanin and antioxidant contents in pigmented rice. Breeding interventions to improve their plant type and yield shall be highly beneficial in their popularization.

Anthocyanin pigmentation in rice grain has been found to be a qualitative trait due to presence-absence type of phenotypes, although the quantity of anthocyanin varies widely among genotypes as well as environments. Anthocyanin is under dominant gene control and two complementarily acting genes, *Rc* and *Rd* control the pericarp pigmentation. The *Rd* locus codes for dihydro flavonol reductase (DFR) enzyme and the *Rc* gene codes for Basic Helix-Loop-Helix (HLH) Protein. The *Rc* locus has been cloned and its three allelic variants have been well characterized. Its null allele (*rc*) with 14bp deletion creates frame shift mutation and a premature stop codon leading to white pericarp phenotype (Brooks *et al*., 2008) found in most rice varieties. As the pigmentation is present only in

pericarp, the quality advantage of this kind of rice will be lost if the grains are polished while processing. Zhu *et al*. (2017) have addressed this problem through development of 'purple endosperm rice' rich in anthocyanin content through genetic engineering approach.

GENETIC ENHANCEMENT OF MICRONUTRIENTS CONTENT IN WHEAT

The efforts on genetic enhancement in micronutrient content of wheat have been focused mainly on iron and zinc along with preliminary work on other micronutrients.

Essential minerals: iron and zinc

Wide variation for iron and zinc has been reported by several authors in different studies (Graham *et al*., 1999 (iron content: 28.8 to 56.5mg and zinc: 25.2 to 53.3 mg g^{-1} wheat flour); Morgounov *et al*., 2007; Rawat *et al.*, 2009a), but the availability of micronutrients in modern day varieties is partial. Wild relatives of wheat like *Triticum boeoticum*, *Triticum monococcum*, *Triticum dicoccoides*, *Aegilops tauschii* and *Aegilops speltoides* have been evaluated and found to be promising donors for iron and zinc (Cakmak *et al.*, 2000; Chhuneja *et al*., 2006; Rawat *et al*., 2009b). Cakmak *et al*. (2000) identified *T. dicoccoides* having both high concentration and range of variability for iron and zinc. High yielding and micronutrient rich wheat genotypes can be developed by genetic manipulations, since seeds could reach a larger number of people without necessarily changing consumer's behaviour (Ortiz-

Monasterio *et al*., 2007; Cakmak, 2008).

There were large scale efforts to screen genotypes for zinc and iron content under breeding programmes at CIMMYT, the National Agricultural Research System (NARS), and agricultural universities in India and other countries. There have been efforts to transfer zinc and iron richness traits into locally adapted agronomically competitive germplasm, considering consumer preferred end-use quality. High iron (85 mg kg^{-1}), zinc (125 mg kg^{-1}), and grain protein (250 g kg^{-1}) concentration traits with same genetic background were the focus of breeding strategies (Welch and Graham, 2002; Zvi *et al*., 2008). The genotypes accumulating higher levels of zinc and iron were chosen carefully for further breeding studies (Ortiz-Monasterio *et al*., 2007; Velu *et al*., 2014). Some of the studies have even shown presence of three to four-fold higher iron and zinc content in grains of wild relatives compared to the cultivated hexaploid wheats (Chhuneja *et al*., 2006; Rawat *et al*., 2009b).

Recently, improved nitrogen (N) management both through zinc available in soil as well as by foliar application has enabled zinc concentrations in grain crops such as wheat to be enhanced. Radio labeled ^{65}Zn has been shown to be taken up by the roots, translocated to shoots and to accumulate in the wheat grain (Grillet, 2014; Cakmak, 2009). Erenoglu *et al*. (2011) demonstrated that increasing the nitrogen supply in the soil can stimulate the root-to-shoot translocation of zinc and enhance its accumulation in wheat grain, possibly *via* increasing the abundance of transporter proteins in the presence of nitrogen (Cakmak,

2010). Nitrogen availability therefore, represents a key component for the zinc biofortification of wheat and thus can improve the nutritional status for many who reside in developing countries. The wheat endosperm was also reported to lack transporters those are essential for the translocation of iron into the endosperm region (Borg *et al.*, 2009, Mazzolini, 1985). Little or no studies were conducted on the translocation of phenolic acids into the wheat endosperm, and there is less information regarding the translocation or transporters involved in the translocation of phenolic compound and vitamins into the endosperm region. Waters and Sankaran (2011) summarized the genes implicated in the uptake of iron mainly in dicots, *Oryza*, maize and barley. No gene(s) involved in iron uptake have been reported for wheat. Thus, there is still a need to characterize and identify genes involved in the uptake of iron from soil to the seeds in wheat. Furthermore, Morrissey and Guerinot (2009) provided a comprehensive overview of genes and pathways involved mainly in iron uptake from roots to other plant compartments. Waters *et al*. (2009) conducted a more comprehensive investigation on the role of the *NAM-B1* gene, which affects iron and zinc in wheat.

There have been several efforts to identify genomic regions (QTL) controlling accumulation of higher zinc in the wheat grains. Cloning of a high grain protein (*Gpc-B1*) locus by Uauy *et al*. (2006) using wild wheat *T. turgidum* ssp. *dicoccoides,* which induced early senescence and increased sequestration of iron and zinc from leaves to grains, was a much needed and timely illustration

that manipulation of a single gene could enhance iron and zinc content of wheat grain. Several other studies were reported to have mapped QTL regions (Table 7) on chromosomes 2A and 7A (Tiwari *et al.*, 2009; Peleg *et al.*, 2009; Shi *et al.*, 2008; Genc *et al.*, 2009).

Gpc-B1 locus from *Triticum dicoccoides* was mapped and found to enhance zinc and iron concentrations and encoded a NAC transcription factor that was found responsible to accelerate senescence. Senescence, the programmed degradation of cell constituents, makes nutrients available for remobilization from leaves to developing seeds (Distelfeld *et al.*, 2007). During senescence, proteases are rapidly activated to degrade leaf proteins into amino acids (Guitman *et al.*, 1991).

The gene *Gpc-B1* controls grain protein, iron, and zinc concentrations in wheat (Uauy *et al.*, 2006). Later *Gpc-B1* was positionally cloned and reported to enhance iron, zinc, Mn, and protein concentrations by 18, 12, 29, and 38%, respectively (Distelfeld *et al.*, 2007). The marker Xuhw89 is tightly linked to the *Gpc-B1* locus (0.1 cM) and can effectively be used for MAS in early segregating generations to select for improved iron and zinc levels (Distelfeld *et al.*, 2006)

The increased grain iron content in wheat has been demonstrated by endosperm-specific expression of wheat or soybean *ferritin* genes (Borg *et al.*, 2012). Wheat transgenic plants expressing *Aspergillus japonicus* phytase gene (*phyA*) in wheat endosperm increased bioavailability of iron and zinc by 14.5% and 4%, respectively (Abid *et al.*, 2016). In addition, the doubled content of iron in both wheat and barley crops have been reported by overexpressing the

vacuolar iron transporter genes with endosperm-specific promoter (Connorton *et al.*, 2017).

Protein quantity

Sonmezoglu *et al.*, (2017) reported application of MAS for selection of *Gpc-B1* gene in the BC_4F_1 plants. The positive plants selected were having increased protein content along with yellow rust resistance as Yr-36 gene conferring resistance was closely associated with *Gpc-B1* gene. Tabbita *et al.*, (2017) reviewed all the reports of usage of *Gpc-B1* gene through MAS and concluded that *Gpc-B1* gene has the potential to increase nutritional and end use quality in a wide range of modern cultivars and environments. Zhao *et al.* (2015) identified a novel NAC1-type transcription factor, TaNAC-S, in wheat, with gene expression located primarily in the leaf/sheath tissues. Overexpression of TaNAC-S in transgenic wheat plants resulted in delayed leaf senescence, which led not only to increased GPCbut also to increased grain yields. Thisresult further verified the improved nitrogen remobilization from vegetative organs to growing grains in transgenic lines.

Antinutritional factors

Wheat has been altered using biotechnology for a number of health benefits. For example, levels of celiac-disease causing gliadins have been lowered from wheat using RNAi-based technologies, and the level of free lysine, an essential amino acid, is generally scarce in wheat, has been increased. Genetically altered wheathas been tested for dough making quality and taste with

Table 7. QTLs identified for high grain micronutrients in wheat and rice.

Micronutrient	Wheat chromosome	Rice chromosome
Zinc (Zn)	4A, 4D, 2D and 3A (Shi et al., 2008) 7A (Tiwari et al., 2009) 3D, 4B, 6B, & 7A (Genc et al., 2009)	1 L and 2 L (Stangoulis et al., 2007)
Iron (Fe)	2A and 7A (Tiwari et al., 2009) 4 (Peleg et al., 2008)	2S, 8 L, and 12 L (Stangoulis et al., 2007)
Iron and zinc	7 (Peleg et al., 2008)	1, 3, 5, 7 and 12 (Anuradha et al.,2012)
Iron zinc and protein content	6B (Distelfeld et al., 2007)	-
Iron, zinc, copper, and manganese	5 (Ozkan et al., 2006)	-
Iron and zinc	7A (Peleg et al., 2009)	-

encouraging results. Biofortified wheat provides more options for the proportion of the population who are gluten sensitive or intolerant, and can also provide higher levels of micronutrients, such as iron and zinc, to the people using wheat as a staple (Gil-Humanes, 2014).

The `HarvestPlus' programme is developing and deploying new wheat varieties with higher grain zinc concentrations in South Asia (Singh and Velu, 2017). The HarvestPlus target is to enhance the grain zinc concentration in locally-adapted wheat varieties by 8±12 mg kg^{-1}, from a baseline of ~25 mg kg^{-1}, without compromising grain yield or other grain quality attributes. Through collaboration with Banaras Hindu University (BHU), Uttar Pradesh (UP), India, and several private Indian seed companies including Sai Seeds, Nirmal Seeds, and Astha Beej, three varieties with increased grain zinc concentration have been released in Eastern Uttar Pradesh, India: Akshai (BHU-3), Abhay (Zinc Shakthi), and BHU-6 (Velu *et al*., 2015).

A high zinc wheat variety `Zincol-2016' has been recommended for release and multiplication in Pakistan (PARC; http://www.harvestplus.org/ node/1647). In addition, biofortified wheat varieties *viz.*, 'WB02' and 'HPBW-01' have been released in India (Singh and Velu, 2017). The high bioavailability (% absorption) of zinc from wheat in human diets translates to significant nutritional impact. Nutrition trials with high zinc wheat have shown (i) an increase in zinc intake and (ii) a reduction in child morbidity. Few studies have reported the relative contributions of genotype (G), environment (E) and GxE interactions on wheat grain zinc concentration under widely differing field conditions. It is likely that optimal grain zinc concentrations will ultimately be achieved using a combination of crop improvement and agronomy (Velu *et al*., 2015).

GENETIC ENHANCEMENT OF MICRONUTRIENTS CONTENT IN PEARL MILLET

International Crops Research Institute for Semi Arid Tropic (ICRISAT) and National Agricultural Research Systems (NARS) partners started work on biofortification of pearl millet

almost 15 years back. Application of genomic resources for improvement of micronutrients in pearl millet is still in infancy. Therefore, more research efforts have to be done in this direction to ensure formulation ofprecise and speedy crop improvement program in near future.

Essential minerals: iron and zinc

In the beginning of this program, genetic variability for iron and zinc was surveyed among germplasm. Large variability for iron (40 to 180 mg kg^{-1}) and zinc (10 to 66 mg kg^{-1}) was observed in 27 lines of pearl millet (Jambunathan and Subramanian, 1988). Inheritance pattern of iron and zinc has also been established. The screening of pearlmillet germplasms in many studies indicated presence of sufficient variability for grain iron and zinc. However, number of seed parents (females- male sterile lines) having high iron and zinc is still very low. Hence more emphasis should be given on genetic diversification of females (seed parents) coupled with resistance to biotic and abiotic stresses and elevated level of micronutrients.

In pearl millet, only conventional approach was exploited for developing biofortified hybrids and varieties and genetic stocks in pearlmillet till now due to its cost effectiveness and limited availability of genomic resources. *Iniadi* germplasm from West Africa has been extensively utilized by different institutions for developing high iron and zinc lines. Being quantitative in nature, grain micronutrient concentration is highly influenced by the environmental conditions which make breeders' job difficult (Feil *et al*., 2005) as the presence of GxE interaction may come

in the way of effective selection of the genotypes (Gurmu *et al*., 2009).Therefore, multi-location testing should be done to identify the suitable variety (Moghaddam and Pourdad, 2009). Several high iron and zinc genetic stocks were identified by IARI, New Delhi and some of them like PPMI 904 were also registered in NBPGR, New Delhi (India) for high iron (91 mg kg^{-1}) and zinc (78 mg kg^{-1}). These genetic stocks were developed by conventional approaches and multi-location testing. By exploitation of genetic variability, open pollinated varieties like Dhanashakti and hybrids like Shakti 1201, HHB 299 and AHB 1200 have already been released and notified in India.

Recently, QTLs for high iron and zinc were mapped on linkage group 3 using 304 (96 SSRs; 208 DArT) markers explaining 19% phenotypic variation for iron QTL and 36% phenotypic variation for zinc QTL (Sushil *et al*., 2016). In open pollinated seeds, QTLs of iron explaining 16% phenotypic variance were mapped on linkage group 3 and 5, while two QTLs of zinc explaining 42% phenotypic variance were mapped on linkage group 3 and 7. Association between iron QTLs and zinc QTLs was strong and positive which indicates genes responsible for high iron and zinc may be linked. Anuradha *et al*. (2017) for the first time tried to mine the favorable alleles related to high iron and zinc content using association mapping panel of 130 diverse pearl millet lines and reported that markers Xpsmp 2261 (13.34% R^2-value), Xipes 0180 (R^2-value of 11.40%) and Xipes 0096 (R^2-value of 11.38%) were associated with iron and zinc content. Inbred line PPMI 1102 had highest favorable alleles followed by PPMFeZMP 199 and

PPMI 708 for both the micronutrients. Using *in-silico* analysis, Mahendrakar *et al*. (2018) reported 29 putative candidate genes responsible for higher iron and zinc content in pearl millet. It was further validated by real time PCR. Thus, with this preliminary information available on application of MAS and genomic selection, further validation of the markers associated with higher iron and zinc content is required to enrich pearl millet with micronutrients.

Efforts of ICRISAT, Indian Council of Agricultural Reasearch (ICAR) institutes and State Agricultural Universities (SAUs) led to development and identification of of iron and zinc enriched lines, varieties and hybrids. ICRISAT program on development of parental lines is being supported by HarvestPlus (Bouis and Saltzman*,* 2017). Iron enriched version of mega pearl millet variety ICTP 8203 developed by ICRISAT was officially released for Maharashtra state of India in 2012. Due to its wider adaptation and high iron and zinc content, variety ICTP 8203 was released and notified under the name of 'Dhanashakti' in 2013 for all pearl millet growing regions India (Rai *et al*., 2014a and 2014b). This variety was also included in the Nutri-farm Pilot Project started by Govt. of India to address the iron deficiency problem. ICRISAT has also developed high iron pearl millet hybrid Shakti 1201 (ICMH 1201) having 30% higher grain yield than Dhanashakti. Shakti 1201 is recommended for Northern and Peninsular India and is marketed by Shakti Vardhak Seeds as Truthfully Labelled Seeds under the brand name Shakti 1201 since 2014 (Govindaraj *et al*., 2016). In 2017, two iron enriched hybrids namely HHB 299 developed by CCSHAU, Hisar (India) and AHB 1200 developed by Vasantrao Naik Marathwada Krishi Vidyapeeth, Maharashtra (India), in collaboration with ICRISAT, have been released and notified for Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu.

GENETIC ENHANCEMENT OF MICRONUTRIENTS CONTENT IN SORGHUM

Sorghum had only limited information base and research related to genetic enhancement of micronutrients content till early 2000s. However, under the aegis of HarvestPlus, sorghum biofortification research gained momentum in the last few years.

Essential minerals: iron and zinc

Preliminary studies at ICRISAT indicated limited variability for grain iron and zinc contents (Reddy *et al*., 2005) in sorghum hybrid parents, advanced breeding lines and germplasm accessions. The grain iron content in the 84 lines ranged between 20 mg kg^{-1} (ICSR 93031) to 37 mg kg^{-1} (ICSB 472 and 296 B) with an average of 28 mg kg^{-1}, while grain zinc content ranged between 13 mg kg^{-1} (JJ 1041) to 31 mg kg^{-1} (IS 1199) with an average of 19 mg kg^{-1}. Large genetic variability for grain iron and zinc concentrations has been reported in sorghum recently (Ashok Kumar *et al*., 2009, 2010, 2012a; Reddy *et al*., 2010). Compared to post-rainy adapted sorghums predominantly grown for food use in India, the rainy season adapted commercial hybrids possessed better micronutrients content (up to 44 mg kg^{-1} iron and 33 mg kg^{-1} zinc). The hybrids GK 4035 and Mahabeej 703 showed higher

mean iron concentrations over two years indicating their stability for this trait. Characterization of popular Indian cultivars, hybrid parents, breeding lines and some selected germplasm accessions collected from the major sorghum growing states indicated sufficient variability for grain iron (12-83 mg kg^{-1}) and zinc (6-51 mg kg^{-1}) contents as well as high heritability (Hariprasanna *et al.*, 2014a). Based on the grain iron and zinc concentrations observed, the base level estimated is 30 mg kg^{-1} for iron and 20 mg kg^{-1} for zinc. Considering the extent of genetic variability observed among landraces, the level of sorghum consumption, nutrient retention in grain storage, milling and food preparation, and nutrient bioavailability, the target level for genetic improvement has been fixed at 60 mg kg^{-1} for grain iron and 32 mg kg^{-1} for zinc by HarvestPlus.

Large variability for both iron and zinc concentration among the core collection (2246) accessions of sorghum germplasm conserved at ICRISAT was also observed. The variability observed in core collection along with hybrid parents (>500 B lines and 100 R lines), breeding lines and commercial sorghum cultivars (67) has been put on public domain in the form of a database (Ashok Kumar *et al.*, 2012b). A number of germplasm accessions with relatively high iron (>60 mg kg^{-1}) were identified for use in the breeding programmes (Ashok Kumar *et al.*, 2013a). These donors are being used as sources for improving iron and zinc in sorghum.

Significant and positive association between the grain iron and zinc concentrations have been reported in most of the sorghum material studied (Ashok Kumar *et al.*, 2009, 2010, 2012a, 2013b; Reddy *et al.*, 2010; Hariprasanna *et al.*, 2014a; Phuke *et al.*, 2017). Among the Indian cultivars, parental lines, breeding lines and germplasm accessions, grain iron and zinc contents were found to be significantly and positively correlated (0.2–0.5, *P*<0.05) (Hariprasanna *et al.*, 2014a). Close association between iron and zinc indicate that genetic control of these two minerals are linked, or physiological mechanisms for uptake or accumulation in the grains are interconnected, and hence there is a possibility of simultaneous genetic improvement for both the micronutrients. Encouragingly grain iron and zinc content is not correlated with agronomic traits such as days to 50% flowering and plant height and grain traits such as size and hardness. Hence, combining agronomic superiority and high iron and zinc contents in cultivars will be practicable.

Grain iron and zinc in sorghum are quantitatively inherited. Both additive and non-additive gene effects were found to be important in the inheritance of grain iron and zinc, but dominant gene action was more predominant in case of iron (Ashok Kumar *et al.*, 2013c; Hariprasanna *et al.*, 2014b). In case of grain zinc, additive gene action was more important with a σ² gca/σ² sca ratio of 0.94. Highly significant correlation between mean grain zinc content of the parents and GCA was observed. The results suggest the scope for heterosis breeding to improve grain iron, while for improving grain zinc progeny selection in pedigree breeding will be effective. To develop heterotic hybrids for high iron levels in sorghum, at least one of the parents should possess high iron, and for zinc, both parents should possess high

mean values for grain zinc (Ashok Kumar *et al*., 2013c). It is possible to predict the hybrid performance based on parental performance as there is good correspondence between mid-parent values and hybrid performance for iron and zinc (Hariprasanna *et al*., 2014b).

Assessment of the stability of grain iron and zinc contents across growing regions is a pre-requisite. Weak genotype × soil fertility interaction has been reported for grain micronutrients. However, significant genotype × location (G × E) interactions were observed for both grain iron and zinc (Hariprasanna *et al*., 2012; Phuke *et al*., 2017). Substantial G × E for grain iron and zinc indicates differential nutrient accumulation by the genotypes over locations and hence, multi-location as well as multi-season testing is needed to assess stability of grain micronutrient content and to take care of G × E interaction while breeding for micronutrient rich sorghum lines.

Use of modern approaches like deployment of molecular markers in sorghum biofortification is still in infancy. Little information is available on genomic regions/QTLs and candidate genes governing sorghum grain iron and zinc contents. Using 91 metal homeostasis candidate genes, homology (*in silico*) search was performed on sorghum genome and a total of 77 genes having homology with sorghum genome were identified (Anuradha *et al*., 2013). Genes associated with grain iron and zinc concentrations from maize and wheat showed 100% homology on the sorghum genome. Gene identical percentage (similarity of sequences) ranged from 71.9 (IDS3) to 95.9 (HMA) with average of 86.4%. Candidate genes (homologs) identified

in this study can be used for the development of functional markers for improving grain iron and zinc concentrations in sorghum (Anuradha *et al*., 2013).

A RIL population (342 individuals) derived from cross 296B × PVK801 was phenotyped at multi-locations and genotyped with SSRs and DArTs (Anuradha *et al*., 2016). Highly significant G × E interactions were observed for both micronutrients; grain iron showed greater variation than zinc. Sorghum genetic map was constructed with 2088 markers covering 1355.52 cM with an average marker interval of 0.6 cM. A large number (167) of small main-effect QTLs controlling iron and zinc concentration were identified. A total of 18 QTLs were stable across environments. Three QTLs for iron and 15 for zinc explained 3.94 to 5.09% and 3.17 to 9.42% of phenotypic variance, respectively. QTLs were analyzed *in silico* to identify underlying candidate genes, and 62 candidate genes involved in iron/zinc metabolism were identified within QTL interval; 23 were found in QTL with highest phenotypic effect (9.42%). Currently, genome-wide association studies (GWAS) as well as candidate gene based mapping of genomic regions for grain iron/zinc are being undertaken.

Development of sorghum varieties endowed with more micronutrients and grain yield is underway at different national and international organizations. Under HarvestPlus, ICRISAT has developed some elite sorghum hybrids with high grain iron and zinc in addition to higher yield. Five hybrids (45-49 mg kg^{-1} iron, 32-40 mg kg^{-1} zinc) and a R line/variety ICSR 14001 (42 mg kg^{-1} iron, 35 mg kg^{-1} zinc) meeting the

current breeding targets for grain zinc were tested under, multilocation trials. ICSR 14001 was later released as Parbani Shakti, the first biofortified sorghum variety in India, for cultivation in Maharashtra, Recently, a new sorghum variety, 12KNICSV-188 (Improved Deko, released as SAMSORG 45), with three times higher iron content (129 mg kg⁻¹ compared with 40 mg kg⁻¹ of other varieties) and high yield (2.4-2.8 t ha⁻¹, compared with<1 t ha⁻¹ yield of varieties) has been released in Nigeria. In India, a private company hybrid, 3204 with bold shiny white grains possesses 46 mg kg⁻¹ iron and 29 mg kg⁻¹ zinc. Another variety, Phule Rohini developed at Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri is found to have very high iron (90-100 mg kg⁻¹) and zinc (30-51 mg kg⁻¹) contents.

Provitamin-A and antinutritional factors

The grains of non-yellow endosperm lines had only traces of β-carotene content while, some yellow endosperm germplasm had β-carotene content ranging from 0.56 (IS 24724) to 1.132 mg kg⁻¹ (1S 26886). The analyses of 20 yellow endosperm sorghum germplasm lines by Kapoor and Naik (1970) also had revealed similar range of β-carotene contents (0.2 to 1.4 ppm). As sorghum grains contain more fibre and phytochemicals, attempts were made to characterize the grains for the levels of nutritional/anti-nutritional factors. The bioavailability of the grain iron and zinc is often adversely affected by phenols, phytate and fibre. Phytic acid complexes with micronutrients in other foods during intestinal digestion leading to reduced

bioavailability. Significant variation was observed among the tested genotypes for all the anti-nutritional factors (Hariprasanna *et al.*, 2013). The total polyphenols ranged from 44 to 1272 mg gallic acid eq. per 100 g, phytate content ranged from 720 to 3909 mg per 100 g while the anti-oxidant activity measured as Trolox equivalent anti-oxidant capacity (TEAC) ranged from 33 to 2238 mg trolox eq. per 100 g. Variability was also observed for fibre (5.2-20.9%), cyanogens (14.2-173.6 mg kg⁻¹) and trypsin inhibitor (1.6-12.8 TIU) (Hariprasanna *et al.*, 2015, 2016). Results were confirmed with high repeatability and high correlation (r = 0.6-0.9, *P*< 0.01) for polyphenols, phytates, cyanogens and TEAC using selected genotypes.

Efforts have been made to develop a transgenic sorghum with increased levels of lysine, Vitamin A, iron and zinc under the Africa Biofortified Sorghum (ABS) Project, started in 2005 (http://biosorghum.org). The project developed the world's first golden sorghum (with yellow/golden endosperm) as well as the world's first sorghum transformation system. The transgenic sorghum had elevated levels of provitamin-A (5.7-21µg g⁻¹ β-carotene), reduced phytate (35-65%), and an improved protein quality (tryptophan 10-20%, lysine 30-120%, threonine 30-40%). Bioavailability studies have shown increased zinc absorption of 30-40% and increased iron absorption of 20-30% when phytate levels were reduced by ≥30% and ≥80%, respectively. A total of 6 confined field trials were completed in Kenya and 8 in Nigeria from 2011 to 2016, and gene flow studies have shown no impact on wild sorghum or any negative impact in the environment. Fitness study in F₂generations of transgenic × wild species of sorghum also has ruled out

any significant changes on either the vegetative or reproductive parameters of the cross derivatives and enhancing the weedy features (Magomere *et al*., 2016). Currently, efforts are on for increasing the levels and stabilizing Vitamin-A within the plant, reducing phytate content through alternative approaches and in turn increasing mineral bioavailability.

Protein quality

Even in the era of biofortification, minor cereals and millets are not given due emphasis in nutritional enhancement efforts. An induced high lysine mutant sorghum, P721Q found to contain seed protein with better digestibility (Benmoussa *et al*., 2015). Eswara Reddy and Jacobs (2002) identified five lysine rich sorghum cultivars which contained 1.5 to 2.0 times more lysine than the others and even better than high lysine mutant P7210. Transgenic sorghum having high lysine content had been developed by Zhao *et al*. (2003). However, this was not carried forward till its commercialization.

CONCLUSIONS AND THE WAY FORWARD

The enormous progress made over the past decade on genetic enhancement of micronutrient content in staples has proven the potential of plant breeding and modern technology for genetic biofortification beyond doubt. The concept and the impact of these on human health have been explicitly shown in variety of crops and micronutrients, especially by HarvestPlus and its partners. The enrichment of nutrients in the grains is a sustainable way of supplying the deficient nutrients to masses (Singh *et*

al., 2016). Micronutrient enriched varieties neither add to costs of cultivation and use nor pose any risk of overdosing of nutrients. The importance of biofortified staples has been acknowledged by WHO/FAO also (Garcia-Casal *et al*., 2016). This was further exemplified by awarding the World Food Prize, 2016 to the pioneers of provitamin-A enriched orange-fleshed sweet potato (OFSP) for deploying this staple crop to nearly two million African households (De Steur *et al*., 2017). As of now, more than 150 biofortified varieties of 10 crops have been released in 30 countries. Candidate biofortified varieties across 12 crops are being evaluated for release in an additional 25 countries. More than 20 million people in developing countries are now growing and consuming biofortified crops (Bouis and Saltzman, 2017) such as vitamin-A cassava, vitamin-A maize, vitamin-A orange sweet potato, iron beans, iron pearl millet, zinc rice, and zinc wheat and improving their health.

In the future, deployment of conventionally bred biofortified varieties would increase. However, the approval, release and deployment of transgenic biofortified crop varieties such as provitamin-A rich 'Golden Rice', multi-biofortified 'BioCassava' (Sayre *et al*., 2011), etc., are still awaited. This situation severely discourages taking forward and popularizing the prototypes now ready. Unless the hitherto developed products get into commercial production by regulatory clearance, new micronutrient enriched varieties are unlikely to be developed. In addition, questions have also been raised about potential benefits, which also need to be addressed, in terms of actually meeting the RDA by

consuming micronutrient enriched crops.

Now that the breeding for enhanced micronutrient content in cereals has gained momentum, continuous efforts are needed to develop micronutrient rich, high yielding, stress tolerant crop varieties having consumer acceptability to encourage adoption further. When defining breeding strategies for micronutrient enrichment, one should consider the desired enhancement, food intake and retention, bioavailability, anti-nutritional factors, and consumer's acceptability (Ortiz-Monasterio *et al.*, 2007). Utilization of already enriched germplasm could give good remuneration for current breeding approaches. Success of quality breeding depends on the productivity of fortified variety/hybrid. Conversion of genetic architecture of proven inbred lines keeping all the genetic information intact except gene under target yields more précise result rather than following the strategy with genotypes of unknown potentiality. For example, conversion of tropical maize lines to QPM inbred lines enabled the rapid development of QPM hybrids (Babu *et al.*, 2005). Introgression of β-carotene hydroxylase in tropical based inbred lines showed 12.6 fold increases in kernel β-carotene (Muthusamy *et al.*, 2014). These would be the sources of donors for future improvement of nutritional quality in maize.

The genetic biofortification programmes must focus on traits those benefit the maximum number of people, widely used staple crops and fast and precise technologies facilitating stable crop performance (Kameswara Rao and Annadana, 2017). With our present technological competence as detailed by Newell-McGloughlin (2010), Dubock (2013), Patil and Kameswara Rao (2015), it would be possible to modify the nutritional content of food by targeting multiple traits (gene stacking) to improve human and animal nutrition. For instance, five genes stacked three vitamin corn (Naqvi *et al.*, 2009) and multi-biofortified rice in China (De Stuer *et al.*, 2012).

Besides, there is need for thorough testing of the stability of the transgenic events and their utility in trait transfer into high yielding back grounds through breeding interventions to avoid wastage of time disappointments, and financial resources. For instance, there were problems after transferring the golden rice trait to Swarna, a popular variety, from transgenic event GR2-R1 as donor. Although the provitamin-A level was upto 20 ppm, the plants had abnormal phenotypes with reduced grain yield due to disruption of the native *OsAux1* gene during transformation (Haritha *et al.*, 2017). Also, transformation by phytase gene was also not much successful for iron biofortification in rice due to loss of activity of the enzyme while boiling the rice grain (Chugh and Dhaliwal, 2013).

Developing new micronutrient-rich plant genotypes is a long-drawn process, and its effectiveness can be limited by the low amount of readily available pools of micronutrients in soil solution (Cakmak, 2008). Now, the efforts are also needed to enhance bioavailability of micronutrients through breeding and/or genetic engineering. The transgenic rice seeds overexpressing nicotianamine synthase (OsNAS3) have been shown to contain increased nicotianamine and elevated amounts of iron, zinc, and Cu along with higher

bioavailability of iron (Usuda *et al.*, 2008; Lee *et al.*, 2009).

To achieve the goal of providing nutritious food worldwide by reaching one billion malnourished people by 2030, much work is needed through collaborative, integrated and multi-sectoral approaches involving plant breeders, molecular biologists, nutritionists and even social scientists (Bouis and Saltzman, 2017). Additionally, biofortification must move beyond HarvestPlus. Considering the prevalence of chronic health issues due to malnutrition (Table 1), there is a need to include more and more micronutrients and expand the biofortification project beyond iron, zinc and Provitamin-A. Besides, research efforts should also focus on devising strategies to maintain the nutritional quality of micronutrient enriched grains during storage, transportation, etc. National governments, policymakers and multilateral institutions must ensure inclusion of biofortification on the nutrition agenda; higher priority to popularizing biofortified varieties to improve human health. Both public and private sector breeding programmes must include micronutrient enrichment as an integral objective. Cereals are the only staples for one-third of world's population especially in developing and underdeveloped nations of Sub-Saharan Africa and South-East Asia (Sofi *et al.*, 2009). For these areas with cereals as main source of energy (Venkaiah *et al.*, 2002), vitamins, minerals and essential amino acids (Poletti *et al.*, 2004), cereals should be the major breeding targets for enhanced nutrient content.

All the players including food processing units along the value chain must include biofortified varieties in their products. After nutritionally fortified edible parts like fruits, seeds, etc. are harvested; the nutrients in the inedible parts should be extracted and added to processed foods. They can also be administered as encapsulated supplements. Widely used food products such as those of tomato, corn, soybean, etc. should be made from biofortified varieties of these crops (Kameswara Rao and Annadana, 2017). There should be community-friendly nutrition education to increase awareness and motivation, active people's participation, nutrition oriented horticulture programmes, orientation of functionaries, and establishment of integrated micronutrient surveillance.

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