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Biofortification: Golden way to save life from micronutrient deficiency- A review

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ABSTRACT

More than half of the world's population is affected by micronutrient malnutrition or deficiency and painstaking to be among the most serious global challenges to humankind. Modern plant breeding along with agronomic manipulation has been oriented toward achieving high agronomic yields than nutritional qualities. However, other efforts are expanding to alleviating the problems through industrial fortification or pharmaceutical supplementation which is very costly for the commoners. Micronutrient malnutrition or hidden hunger is very common among adolescent girls, women and preschool children caused mainly by low dietary intake of micronutrients, especially zinc and iron. Biofortification, the process of increasing the bioavailable concentrations of essential elements in edible portions of crop plants through agronomic intervention or genetic selection, may be the solution to malnutrition or deficiency mitigation. The Consultative Group on International Agricultural Research has been investigating the genetic potential to improve bioavailability of Fe and Zn in staple food crops such as rice, wheat, maize, common beans, and cassava.

Key words: Agronomic, Biofortification, Food crops**,** Genetic, Micronutrients.

Nearly half of all children's death below five years is due to malnutrition, translating into the loss of about 3 million young lives a year. It puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and contributes to delayed recovery. The interaction between malnutrition and infection can create a potentially lethal cycle of worsening illness and deteriorating nutritional status. Poor nutrition in the first 1,000 days of a child's life leads to stunted growth, which is associated with impaired cognitive ability and reduced school and work performance. Measures of child under nutrition are used to track development progress. West and Central Africa has progressed least since the year 2000, when compared to other regions. Meanwhile, the number of overweight children worldwide another aspect of malnutrition is rising. In general it is two types:

1. Malnutrition both under and over-nutrition leading to obesity- Dysfunctional food systems that cannot supply all the nutrients and health-promoting factors.
2. Malnutrition caused by deficiencies of vitamins and minerals - Hidden hunger, affected people do not show the physical symptoms usually associated with hunger and malnutrition.

The Biofortification Program will have focus on three micronutrients that are widely recognized by the World Health Organization (WHO) as limiting in conventional food: iron, zinc, and vitamin A. Full-time breeding programs are proposed for six staple foods for which feasibility studies have already been completed and which are consumed by the majority of the world's poor in Africa, Asia, and Latin America: rice, wheat, maize, cassava, sweet potatoes, and common beans. Pre-breeding feasibility studies are proposed for eleven additional staples: bananas, barley, cowpeas, groundnuts, lentils, millet, pigeon peas, plantains, potatoes, sorghum, and yams. As per the data released by World Health Organization on global occurrence of nutritional deficiencies, particularly micronutrient deficiencies Zn deficiency ranks 11th among the 20 most important factors in the world whereas Zn and Fe deficiency ranks 5th and 6th respectively, among the 10 most important factors in developing countries (Cakmak, 2008). The country suffering from Fe and vitamin A deficiency are India, Pakistan, China, Bangladesh, Central Africa, Iran and Turkey. However, Zn-deficient countries are India, Pakistan, China, Iran and Turkey. Singh and Behera (2011) has analyzed three lakh soil samples from across India and reported that 49 per cent soils were deficient in zinc, 12 per cent iron, 3 per cent copper, 5 per cent manganese, 33 per cent boron and 13 per cent in Molybdenum.

Forms of malnutrition

1. Stunted growth: Low height for age
2. Child wasting: Low weight for height
3. Child overweight: Height weight for height
4. Adult overweight: Carrying excess body fat with a body mass index ≥ 25

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5. Micronutrient deficiency: Iron, folic acid, vitamin A, zinc, iodine below healthy thresholds
6. Adult obesity: Carrying excess body fat with a body mass index ≥ 30
7. Non-communicable diseases: Diabetes, heart disease, and some cancers.

In 2016, 22.9 per cent, or just under one in four children under age 5 worldwide had stunted growth that said, overall trends are positive. Between 2000 and 2016, stunting prevalence globally declined from 32.7 per cent to 22.9 per cent, and the number of children affected fell from 198 million to 155 million (Fig. 1). In 2016, about one in two stunted children lived in South Asia and one in three in sub-Saharan Africa. Globally, stunting declined from one in three to just under one in four between 2000 and 2016.

Worldwide in 2016, 41 million, or 6 per cent, of children under age 5 were overweight. Eastern Europe and Central Asia had the highest overweight prevalence in 2016 with 12.8 per cent affected, followed by Middle East and North Africa at 10.7 per cent and North America at 7.8 per cent (Fig. 2) The lowest overweight prevalence in 2016 was seen in West and Central Africa, at 3.7 per cent, followed by Eastern and Southern Africa at 4.2 per cent. East Asia and the Pacific had the highest number of overweight children in 2016 with 8.6 million affected, followed by South Asia with an estimated 7.4 million overweight. Overall these two Asian regions account for nearly two out of every five overweight children in the world. Eastern Europe and Central Asia is the only region that has seen a statistically significant increase in number of overweight children between 2000 and 2016. The number of overweight under-fives has increased significantly between 2000 and 2016 in Eastern Europe and Central Asia.

Over the past decade, momentum around nutrition has been steadily building: In the year 2012, World Health Assembly adopted the 2025 Global Targets for Maternal,

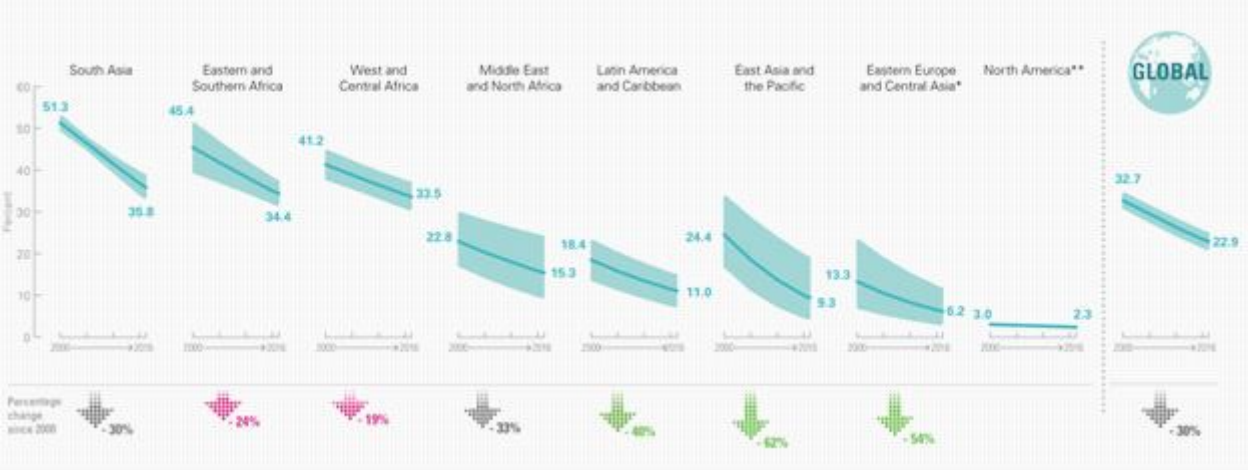


Fig 1: Graphical illustration of stunted child growth across world.
(Source: UNICEF/WHO/World Bank Joint Child Malnutrition Estimates, May 2017 edition)

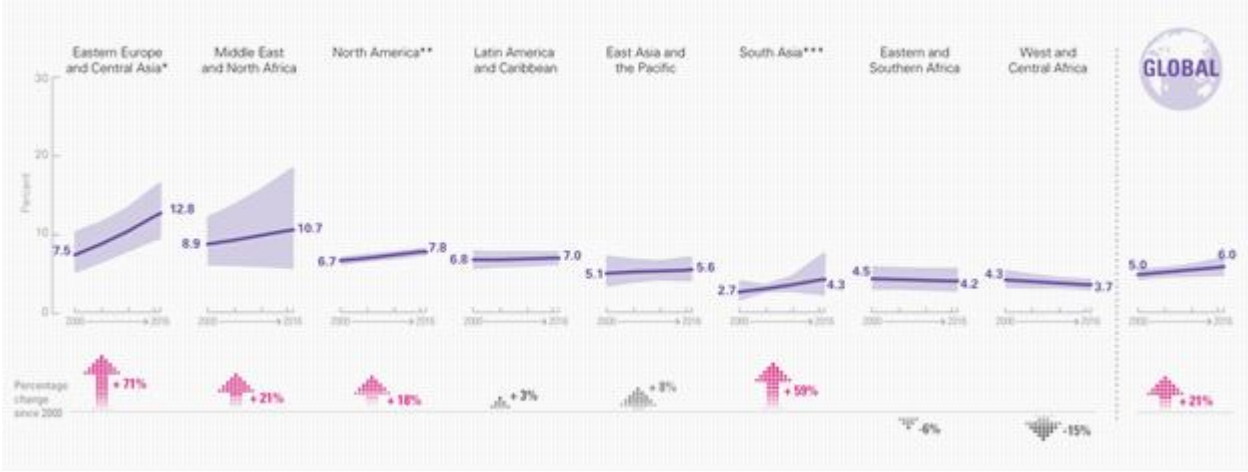


Fig 2: Graphical illustration of overweight child across world.
(Source: UNICEF/WHO/World Bank Joint Child Malnutrition Estimates, 2017 edition)

Infant and Young Child Nutrition. The following year, it went on to adopt targets for non-communicable diseases (NCDs), including those relevant to nutrition. Also in 2013, at the first Nutrition for Growth (N4G) Summit, donors committed US$23 billion to actions to improve nutrition. With the Second International Conference on Nutrition (ICN2) in 2014 and with the recent naming of 2016-2025 as the United Nations Decade of Action on Nutrition, more and more people have begun to recognize the importance of addressing malnutrition in all its forms. In 2015, the UN Sustainable Development Goals enshrined the objective of "ending all forms of malnutrition," challenging the world to think and act differently on malnutrition-to focus on all its faces and work to end it, for all people, by 2030.

Indian Scenario

1. Intake of micronutrients in daily diet is < 50% RDA in over 70% of Indian population
2. Alarmingly high deficit among children, adolescents, and pregnant and lactating women
3. About 57% of pre-schoolers and their mothers have subclinical VAD.
4. Iron deficiency *anaemia* (IDA) is the most serious; 62% of pre-school children are deficient in vitamin A, leading to an annual 3.3 lakh child deaths; and 58.7% of pregnant women, 63.2% lactating mothers and 69.5% of pre-school children are *anaemic*.
5. The prevalence of Zn deficiency has not been adequately investigated, partly due to lack of suitable biomarkers.
6. In 2004, DBT initiated the India Biofortification Programme for rice, wheat and maize biofortifed with Fe, Zn and provitamins. A.
7. In XI Plan, DBT funded biofortification of groundnut and pigeon pea for alleviating vitamin A and sorghum biofortification for high Fe and Zn content
8. High-iron pearl millet variety ICTP 8203 Fe developed by ICRISAT was released as 'Dhanshakti' in Maharashtra during April, 2013.

Biofortification

It is derived from Greek word *"bios"* means *"life"* and Latin word *"fortificare"* means *"making strong"*. It can be defined as the process of increasing the bioavailable concentrations of an element in edible portions of crop plants through agronomic intervention or genetic approaches. So, Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices; conventional plant breeding or modern biotechnology approaches (Meena *et al.*, 2017). The other way of process of breeding food crops that is rich in bioavailable micronutrients. These crops load high levels of minerals and vitamins in their seeds and roots, which are then harvested and consumed.

Biofortification differs in supplementation, addition of an element to the diet to make up for an insufficiency (capsules) and from fortification in such a way that aims to increase nutrient levels in crops during plant growth rather than through manual means during processing of the crops. It may therefore present a way to reach populations where supplementation and conventional fortification activities may be difficult to implement and/or limited.

Examples of biofortification projects include:

1. Iron-biofortification of rice, beans, sweet potato, cassava and legumes;
2. Zinc-biofortification of wheat, rice, beans, sweet potato and maize;
3. Provitamin A carotenoid-biofortification of sweet potato, maize and cassava; and
4. Amino acid and protein-biofortification of sorghum and cassava.

Criteria for biofortification

1. Crop productivity must be maintained/enhanced to guarantee farmer acceptance (high yielding)
2. Micronutrient enrichment levels must have significant impact on human health (effective)
3. Enriched levels must be relatively stable (stability)
4. Bioavailability in enriched lines must be tested in humans to ensure that they improve the micronutrient status of people preparing and consuming them (efficacious)
5. Consumer acceptance has to be tested (taste and cooking quality).

Approaches of Biofortification

A. Agronomic approaches: Agronomic biofortification is the easiest and cheaper way to enhance micronutrient concentrations in food crops and reduce dietary deficiencies (Fig. 3). Today, increasing micronutrient focus on staple food crops, especially in cereal grains, represents an important humanitarian challenge and a high-priority research area. Soil and foliar application of micronutrient fertilizer such as zinc and iron can be used to varying effectiveness. This biofortification, especially in the case of foliar application, is highly effective for iron and selenium, while also for iodine and cobalt. However, soil application is recommended for zinc element. It is also reported that if micronutrients applied through EDTA, it become available to plants and ultimately increases concentration in seeds. As an effective strategy for reducing micronutrient deficiency, zinc and iron provides one of the best and quickest avenues for it, particularly within cereal crops. Knowledge of the different forms of Zn fertilizer and timing of foliar Zn application is crucial for enhancing grain Zn. The most effective method for increasing grain Zn is the soil þ foliar application method, which may result in an about 3-fold increase in grain Zn concentration (Cakmak *et al.*, 2010). Shivay *et al.* (2008) reported that Zn application to soil as Zn enriched urea (ZEU) not only increased yield but also Zn concentration in rice and wheat grains (Table 1). Barua and Saikia (2018) had reported that application of $ZnSO_4$ @ 25 kg ha^{-1} as basal + foliar spray @ 0.5 % at three

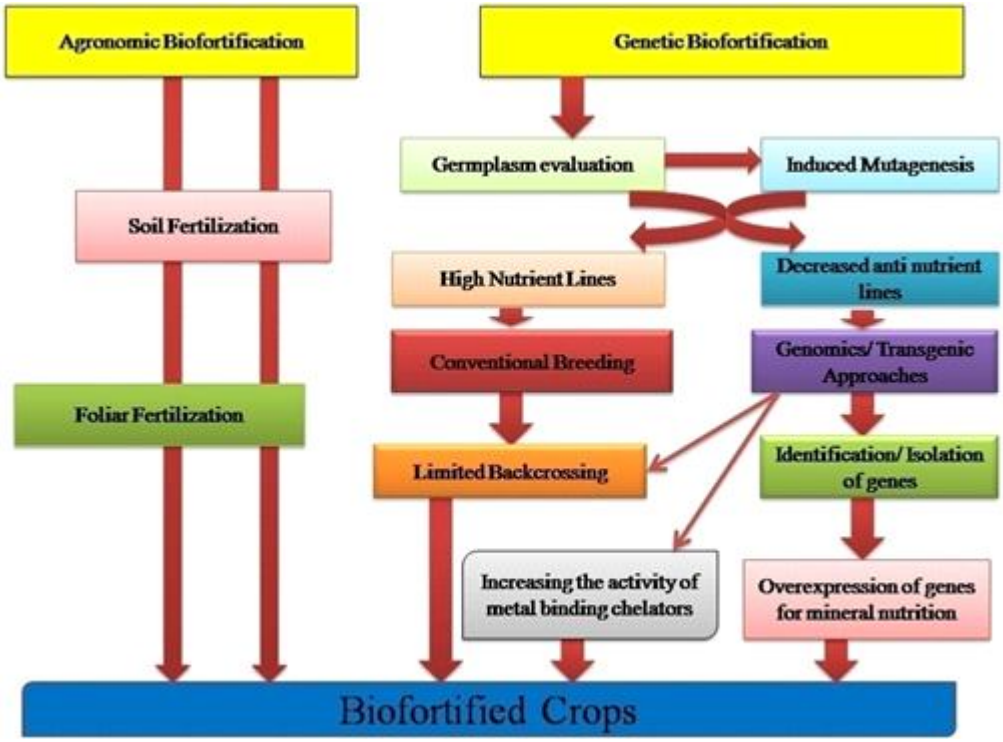


Fig 3: Different methods for Biofortification (Source: Sharma *et al.,* 2017).

stages reveal significantly highest Zn content in grain (35.09 mg/kg) and brown rice (28.31mg kg^{-1}). According to Datta *et al.* (2012) Fe as foliar spray recorded highest accumulation of Fe in grains of various cultivars of rice (Table 2). Similarly, Uma Shankar Ram *et al.* (2013) also reported that application of Zn-EDTA @ 1.0 kg ha^{-1} as basal and Fe-EDTA @ 0.5 kg ha-1 as foliar increased Zn (176.1 ppm) and Fe content (80.14 ppm) in rice grain (Table 3).

B. Genetics approaches

Conventional breeding: It refers to crop breeding for varieties with higher micronutrient content. Exploiting the genetic variation in crop plants for micronutrient density is one of the most powerful tools in biofortification of crops, which can reach the poor in rural areas, with low recurrent costs and long-term sustainability. Breeding for specific nutritional qualities require nutrient density traits in high yield cultivars and strategies based on these genetic findings to determine the best selection technique (Fig. 3). Micronutrient-enrichment traits are available within the genomes of some major staple food crops, which can be used for substantial increases in the levels of iron, zinc and pro-vitamin A carotenoids as well as other nutrients and health promoting factors without negatively impacting crop yield.

The highest micronutrient densities, which are approximately double that of popular modern cultivars and indicating the existing genetic potential, can be successfully combined with high yield. Major success stories in this field are QPM maize (Quality Protein Maize) and high carotene sweet potato. The polymorphism in crtRB1gene is associated with β-carotene levels in maize kernels. The randomly selected 10 seeds of selected maize lines in BC_2F_1 population cobs when analyzed for β-carotene content through HPLC, showed confirmation of improvement in β-carotene content of 2.37 µg/g (Pitambara and Nagarajan, 2016). The conventional breeding is the tools to improve not only micronutrients, but also pro-vitamins-A. Increasing the concentration of provitamin A in the edible portions of staple crops may have greater promise to reduce the incidence of vitamin A deficiency (Naveen *et al.*, 2016). It is well known facts that vitamin A is not obtainable from plants; however, its precursors, the pro-vitamin A (ß-carotene) are found in high amount in maize particularly yellow maize, sorghum, pasta and wheat. However, it is also still higher in cassava and sweet potato, but in other staple food crops such as wheat, rice and groundnut, is present at low concentration. Similarly, Rai *et al.* (2012) reported that open pollinated varieties of pearlmillet like ICTP 8203 has good amount of Fe density (52 mg kg^{-1}), whereas ICMV 221 has contained 61 mg Zn kg^{-1} (Table 4). Sometimes, conventional method has become very complex to achieve aim oriented breeding. Therefore, breeding has its own limitations in some crops due to a narrow range of the germplasm, lack of micronutrients traits in wild species, and hybridization barriers. It is not possible in vegetatively propagated crop plants. The different biofortified varieties in various crops have been release recently by ICAR (Table 5).

Table 1: Effect of zinc-enriched urea on grain yield and Zn content in direct rice and residual wheat.

Treatments	Zn added (kg ha^{-1})	Grain yield (t ha^{-1})		Zn content in grain (mg kg^{-1})	
Prilled urea	-	4.50	3.34	26.6	45.4
0.5% ZEU	1.3	4.82	3.46	27.9	47.0
1.0% ZEU	2.6	5.02	3.58	32.7	48.5
2.0% ZEU	5.2	5.20	3.75	39.0	51.4
3.0% ZEU	7.8	5.40	3.93	42.1	56.6
CD (*P*=0.05)	-	0.20	0.31	3.4	2.4

Source: Shivay *et al.* (2008)

Table 2: Effect of methods of Fe application and cultivars on the Fe content (mg kg^{-1}) in grain of aerobically grown rice.

Applied Fe	Cultivars				
	Sugandha-3	IR-64	IR-55419	IR-55423	Mean
Control	10.5	9.51	11.7	10.8	10.6
Soil (15.0 kg $FeSO_4$ ha^{-1})	16.6	9.53	10.1	10.9	11.8
Four foliar sprays (3% $FeSO_4$)	11.7	11.9	11.9	13.3	12.5
Two foliar sprays (3% $FeSO_4$)	10.2	12.1	11.4	11.2	11.2
Mean	12.3	10.7	11.3	11.6	

CD (*P*=0.05) Applied Fe-1.08, Cultivar-1.08, Applied Fe × Cultivar-2.16 Foliar sprays at 30, 45, 60 and 75 days after sowing (DAS)

Source: Datta *et al.* (2012)

Table 3: Effect of Zn, Fe and FYM on content of rice grain.

Treatments	Zn (ppm)	Fe (ppm)
Varieties		
NDR-359	155.555	53.308
HUBR-2-1	171.955	62.567
CD (*P* < 0.05)	4.987	1.743
Fertilizers		
100% RFD through inorganics	157.025	52.153
75% RFD through inorganics + 25% N through FYM	170.490	63.722
CD (*P* < 0.05)	4.987	1.743
Micro-nutrient (Zn and Fe)		
Control	148.368	39.087
Zn-EDTA @ 1.0 kg ha^{-1} (S)	176.078	44.348
Zn-EDTA @ 0.5 kg ha^{-1} (F)	169.843	58.088
Fe-EDTA @ 1.0 kg ha^{-1} (S)	158.513	48.018
Fe-EDTA @ 0.5 kg ha^{-1} (F)	161.806	80.144
Zn-EDTA @ 1.0 kg ha^{-1} (S) + Fe-EDTA@ 1.0 kg ha^{-1} (S)	168.227	58.389
Zn-EDTA @ 0.5 kg ha^{-1} (F) + Fe-EDTA @ 0.5 kg ha^{-1} (F)	166.279	64.807
Zn-EDTA @ 1.0 kg ha^{-1} (S) + Fe-EDTA @ 0.5 kg ha^{-1} (F)	169.618	66.977
Fe-EDTA @ 1.0 kg ha^{-1} (S) + Zn- EDTA @ 0.5 kg ha^{-1} (F)	156.092	61.579
CD (*P* < 0.05)	3.611	1.352

Source: Uma Shankar Ram *et al.* (2013)

Transgenic/Biotechnological approach: In contrast to plant breeding, the techniques of genetic engineering allow the transfer of heritable traits between completely unrelated species. In recent years, genetic engineering techniques have been used to introduce new traits into commercially important plants thereby producing combinations of features which could not been achieved by traditional breeding (Bhatnagar *et al.*, 2011).

Iron: Introduced the *Naat-A* (nicotianamine synthase) gene with a 35S promoter in rice was shown to result into some iron-efficient rice strains in calcareous soil (Takahashi, 2003). Nicotianamine synthase stimulates the production of siderophores in the roots that improves the uptake of iron in the plants. To increase iron content in rice seeds, the ferritin gene from *Phaseolus vulgaris* was expressed in rice endosperm under the control of the glutelin promoter that resulted in over two-fold increase in the iron content (Lucca *et al.*, 2001).

β-carotene: Ye *et al.* (2000), who introduced three genes, a daffodil phytoene synthase (psy1) gene under the control of seed specific Gt1 promoter, an phytoene desaturase gene (crtI) from *Erwinia uredovera* (codes for enzymes mediates four desaturation steps, for which two plants enzymes are required) driven by CaMV 35S promoter, and daffodil

Table 4: Mean performance of released open-pollinated varieties (OPVs) for grain iron (Fe) and zinc (Zn) density in pearl millet.

OPV	Breeding organization	Fe density (mg kg^{-1})	Zn density (mg kg^{-1})
MBC 2	AICPMIP, Mandor	43	38
CZP 980	CAZRI, Jodhpur	42	37
HC 10	CCSHAU, Hisar	49	45
HC 20	CCSHAU, Hisar	50	45
Pusa Composite 334	IARI, New Delhi	49	44
Pusa Composite 383	IARI, New Delhi	46	44
Pusa Composite 443	IARI, New Delhi	46	40
Raj 171	ICRISAT, Patancheru	49	45
ICMS 7703	ICRISAT, Patancheru	50	44
ICTP 8203	ICRISAT, Patancheru	67	52
ICMV 155	ICRISAT, Patancheru	48	43
ICMV 221	ICRISAT, Patancheru	61	45
JBV 2	JNKVV, Gwalior	47	44
AIMP 92901	NARP, Aurangabad	56	46
Co (Cu) 9	TNAU, Coimbatore	52	46
LSD (P<0.05)		3.9	2.7

Source: Rai *et al.* (2012)

Table 5: Different biofortified varieties under various crops.

Crops	Biofortified varieties	Remarks
Rice	CR Dhan 310	10.3% protein in polished grain as compared to 7.0-8.0% in popular varieties
	DRR Dhan 45	High in zinc content (22.6 ppm) in polished grains in comparison to 12.0-16.0 ppm in popular varieties
Wheat	WB 02	Rich in zinc (42.0 ppm) and iron (40.0 ppm) in comparison to 32.0 ppm zinc and 28.0-32.0 ppm iron in popular varieties
	HPBW 01	Contains high iron (40.0 ppm) and zinc (40.6 ppm) in comparison to 28.0-32.0 ppm iron and 32.0 ppm zinc in popular varieties
Maize	Pusa Vivek QPM9	**Country's first provitamin-A rich maize,**
	Improved	High provitamin-A (8.15 ppm), lysine (2.67%) and tryptophan(0.74%) as compared to 1.0-2.0 ppm provitamin-A, 1.5-2.0% lysine and 0.3-0.4% tryptophan content in popular hybrids
	Pusa HM4	Contains 0.91% tryptophan and 3.62% lysine which is significantly higher than popular
	Improved	(0.3-0.4% tryptophan and 1.5-2.0% lysine)
	Pusa HM8	Rich in tryptophan (1.06%) and lysine (4.18%) as compared to 0.3-0.4% tryptophan and
	Improved	1.5-2.0% lysine in popular hybrids
	Pusa HM9	Contains 0.68% tryptophan and 2.97% lysine compared to 0.3-0.4% tryptophan and 1.5-
	Improved	2.0% lysine in popular hybrids
Pearl millet	HHB 299	High iron (73.0 ppm) and zinc (41.0 ppm) as compared to 45.0-50.0 ppm iron and 30.0-35.0 ppm zinc in popular varieties/hybrids
	AHB 1200	Rich in iron (73.0 ppm) in comparison to 45.0-50.0 ppm in popular varieties/hybrids
Lentil	Pusa Ageti Masoor	Contains 65.0 ppm iron as compared to 55.0 ppm iron in popular varieties
Sorghum	ICSR-14001(Line developed by ICRISAT)	50% higher iron and zinc than base level out yielded all other entries in the state multi-location trials in Maharashtra state
Mustard	Pusa Mustard 30	Contains low erucic acid (<2.0%) in oil as compared to >40% erucic acid in popular varieties
	Pusa Double Zero	**Country's first Canola Quality Indian mustard variety,** Low erucic acid (<2.0%) in oil and Mustard 31glucosinolates (<30.0 ppm) in seed meal as compared to >40.0% erucic acid and >120.0 ppm glucosinolates in popular varieties
Cauliflower	Pusa Beta Kesari 1	**Country's first biofortified cauliflower,** Contains high β-carotene (8.0-10.0 ppm) in comparison to negligible β-carotene content in popular varieties
Potato	Bhu Sona	High β-carotene (14.0 mg 100^{-1} g) content as compared to 2.0-3.0 mg 100^{-1}g β- carotene in popular varieties
Sweet potato	Bhu Krishna	High anthocyanin (90.0 mg 100^{-1}g) content in comparison to popular varieties which have negligible anthocyanin content
Pomegranate	Solapur Lal	High iron (5.6-6.1 mg 100^{-1}g), zinc (0.64-0.69 mg 100^{-1}g) and vitamin C (19.4 -19.8 mg 100^{-1}g) in fresh arils in comparison to 2.7-3.2 mg 100^{-1}g, 0.50-0.54 mg 100^{-1}g and 14.2-14.6 mg 100^{-1}g, respectively in popular variety Ganesh

Source: Yadava *et al.*, 2017

lycopene β-cyclase (lcy) driven by CaMV 35S promoter into T309 japonica rice line. This resulted in the accumulation of carotenoids (surprisingly, mostly β-carotene) in the endosperm. Interestingly, they were also able to produce β-carotene only by introducing the phytoene synthase and phytoene desaturase activities in the absence of heterologous β-cyclase. Rosati *et al.* (2000) were able to enhance the conversion of β-carotene from lycopene, which is normaly present in high amounts in tomato fruit, by transforming with β-lcy gene from Arabidopsis driven by fruit-specific promoter. In maize, the generation of transgenic maize with enhanced pro-vitamin A content in their kernels due to the overexpression of the bacterial genes crtB (phytoene synthase) and crtI (phytoene desaturase) under the control of a 'super g-zein promoter' for endosperm-specific expression was reported by Aluru *et al*. (2008). Data showed an increase of total carotenoids of up to 34-folds with a preferential accumulation of β-carotene in the maize endosperm. The introduction of the crtB (phytoene synthase) gene from *E. uredovora* in *Brassica napus* in mustard under the control of seed-specific napin promoter was shown to result in a 50-folds increase in the total carotenoid level (Shewmaker *et al.*, 1999). Diretto *et al.* (2006) silenced the lycopene β-cyclase (β-LCY) by Agrobacterium-mediated transformation of an antisense fragment of this gene under the control of the patatin promoter. The transgenic tubers thus produced showed a significant increase in β- carotenoid levels where the β-carotene showed a maximum increase of about 14-folds while the total carotenoids increased up to 2.5-folds.

Harvest Plus program

The HarvestPlus program is an international initiative that aims to address human micronutrient malnutrition through improving the micronutrient concentration of staple foods (Singh *et al.*, 2017). The program has targeted grain Zn levels of brown and polished rice, respectively, of 30 and 28 mg kg^{-1}. Achieving these targets will require strategic use of Zn fertilizers, as many rice fields have low available Zn levels. So, a combination of genetic and agronomic strategies is required to raise grain Zn concentration. The genetic approaches can be advanced through germplasm screening of old landraces, traditional varieties and wild species to create novel genetic tools to increase Zn concentration in rice grain (Nakandalage *et al.*, 2016).

Germplasm screening is the initial step for a breeding program to raise grain Zn concentration and to achieve breeding objectives and should be a wide genetic variation. In addition, substantial genetic variation of Zn concentration in brown rice (13.5–58.4 mg kg^{-1}) has been reported for a large collection of rice germplasm at the International Rice Research Institute (IRRI), with an average of 25.4 mg Zn kg^{-1} (Boonchuay *et al.*, 2013). The world's first Zn enriched rice variety was released in 2013 by the Bangladesh Rice Research Institute (BRRI Dhan 62), which is claimed to contain 20–22 mg Zn kg^{-1} for brown rice. Nonetheless, this is short of the target of 30 mg Zn kg^{-1} set by the HarvestPlus program (Shahzad *et al.*, 2014). It is suggest that Zn biofortification can be improved by drawing on under-utilized genetic materials, and by better understanding of Zn homeostasis of the rice plant.

CONCLUSION

No single type of intervention can by itself solve the problem of micronutrient deficiency based malnutrition. A comprehensive strategy involving multiple types of intrusions adapted to conditions in specific countries and regions is required. The final, permanent solution to micronutrient malnutrition in developing countries is a substantial improvement in dietary quality-higher consumption of pulses, fruits, vegetables, fish, and animal products, which the poor already desire but cannot afford even now a days. Meanwhile, breeding staple foods that are dense in minerals and vitamins provides a low-cost, sustainable strategy for reducing levels of this malnutrition. Biofortification complements existing strategies and has its own unique "niche," as conditioned by the comparative advantages outlined above and technical characteristics, most importantly the level of the "dose" that it can be expected to deliver. However, pro-vitamin A, Zinc and Fe concentration in food grains is influenced by plant-related factors (genetic), environmental factors (climatic) and crop management strategies (agronomic). Greater understanding of how these factors interact to influence grain Zn and Fe accumulation is vital for enriching Zn and Fe concentration in food grain. Improved Zn and Fe uptake along with efficient remobilization are identified as key holdup for biofortification. These blockages should be addressed by exploiting the wide genetic diversity of germplasm.

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