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Biofortification of Crops: An Avenue to Alleviate Malnutrition

J. P. Aditya¹, Anuradha Bhartiya², R.S. Pal³ and L. Kant⁴

Modern day agriculture is a boon to strengthen the adequate food supply to masses in terms of meeting energy requirements. Whereas, it also caused major dependence on few staple crops with low dietary diversity particularly of micronutrients and resulted in high malnutrition or “hidden hunger” especially in Asian and African countries. Micronutrient deficiencies are inevitable among poor sections due to their non affordability to diversify their diets with adequate amounts of fruits, vegetables or animal-source foods that contain large amounts of micronutrients. Globally, over two-thirds of the population lack one or more essential mineral elements mainly Fe, Zn and Vitamin A which retards normal growth and development process. To combat micronutrient deficiencies there is need to explicitly reorient agriculture to promote human health together with improving production levels of staple crops, therefore, crop biofortification promises the long-term and better nutritional accessibility to rural unprivileged masses at doorstep in very cost-effective manner.

Humans require at least 49 nutrients to meet their metabolic needs and inadequate consumption of even one of these nutrient will result in sickness, poor health, impaired development. Food we all eating is coming from agricultural system and Agriculture is primary source of minerals and vitamins but food systems are failing to provide sufficient minerals and vitamins because we mostly focus on increasing production, productivity and profitability of farmers and agricultural industries. We never explicitly designed agriculture to promote human health and health comes from the pharmacy in forms of supplementation of mineral and vitamin pills and powders.

During green revolution, there was rapid increase of cereal production compare to human population, therefore, cereal prices fell, on the other hand prices of non staple foods like pulses, fruits, vegetables, eggs, meat and fish were high due to their

low production and poor masses continued eating more cereals so poverty is both cause and effect of hidden hunger.

Global micronutrient deficiency

Worldwide, micronutrient deficiency is high in African & Asian countries, Latin and Meso-America. Micronutrient deficiencies are also referred as hidden hunger because its symptoms cannot be seen directly such as lowers IQ, lower resistance to disease and fatigue. International and National media refer malnourishment as co-existence of “Grain mountains and hungry millions” because one side there are lot of food grain production whereas another side there are millions of people who are not getting sufficient food. The diets of over two-thirds of the world's population lack one or more essential mineral elements. Globally, the most common trace element deficiencies are iron

Table 1: Essential nutrients for sustaining human life

Sl. Essential Nutrients	Particulars
1. Water and energy	Water, carbohydrates
2. Protein (amino acids)	Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Theonine, Tryptophan, Valine
3. Lipid-fat(fatty acids)	Linoleic acid, Linolenic acid
4. Macro-elements	P, K, Na, Ca, Mg, S, Cl
5. Micro-elements	Fe, Zn, Cu, Mn, I, F, B, Se, Mo, Ni, Cr, V, Si, As, Sn, Co
6. Vitamins	A(Retinol), D(Calciferol), E(Tocopherol), K(Napthoquenone), C(Ascorbic acid), B1(Thiamine), B2(Riboflavin), B3(Pantathenic acid), B5(Niacin/Nicotinamide), B6(Pyridoxin), B7(Biotin), B9(Folic acid), B12(Cobalamin)

¹⁻³Scientist; ⁴Head, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand



(Fe) deficiency over 60% followed by zinc (Zn) deficiency (over 30%), iodine (I) deficiency (30%) and selenium (Se) deficiency (15%). The most widely prevalent vitamin deficiencies of public health significance are Vitamin A, followed by Vitamin B9 (Folate) and B12 (Cobaltamin). Micronutrients deficiency occur across the life cycle whether it is new borns (up to 2 months), children (0-12 years), Adolescents (13-18 years), Adults (19-59 years) or Seniors (60 years and above) but its effects are acute during the first 1,000 days (0-24 months) of a child's life from conception to the age of two years (2nd birthday of the child). WHO recognized three most common forms of malnutrition *viz.* Fe, Zn and Vitamin A worldwide hence briefly described below.

Vitamin A is essential for good vision and cell differentiation. Its deficiency results in blindness, impaired immune system function, abnormal fetal development, increased child and maternal mortality, growth retardation, damage to mucous membranes, reproductive disorders. In children it is associated with anemia, diarrhea and measles.

Zinc is involved in more body functions than any other mineral. It is essential to more than 200 enzyme systems, normal growth and development, the maintenance of body tissues, sexual function, vision and the immune system. Its deficiency cause decreased resistance to infectious diseases, stunting and impaired growth in children and increased infant and child mortality. Direct measures of the prevalence of zinc deficiency are scarce as the recommended methods for measuring zinc

deficiency is not widely used. Stunting is commonly used as a proxy to estimate risk of zinc deficiency in a population. Zinc deficiency in children is commonly associated with diarrhea, pneumonia, stunting and child mortality.

Iron is the most common micronutrient deficiency in the world and a leading cause of anemia. Because global data for iron deficiency does not exist, anemia is used as an indirect indicator. In childhood and adolescence it causes impairs mental development and learning capacity however, in adults it reduces the ability to do physical labour, reduced cognitive capability, increased maternal mortality, complications with childbirth and increased infant mortality.

Prevalence of anemia in preschool age children (under 3-4 years of age) and nutritional stunting cause by Zn deficiency in children (under 5 years of age) are high in most of the Asian and African countries. Zn deficiency is problem of both human and soil and it has been observed that wherever soils are deficient in Zn and Fe, human population of that regions are also deficient in Fe and Zn because crop grown on that soil does not contain sufficient amount of Fe and Zn in the grain to provide human as soil itself is lacking.

Indian scenario

Malnutrition is widespread, acute especially among children and women and more than 70% of Indian women and kids have serious nutritional deficiencies. In India more than one-fifth of the population lives in poverty and more than 15% of the

people are undernourished thus vulnerable to various health problems. The most commonly observed deficiencies in unbalanced diet are Fe, Zn, Ca, I, Mg, Se and vitamin A.

In India about

- 47% children suffering from protein energy malnutrition(PEM).
- 74% children under age of 3, 43% preschool children, 90% adolescent girls and 50% of women are severely suffering from Fe deficiency.
- 27% of total population is affected by Zn deficiency.
- Vitamin “A” deficiency: About 57% of pre-schoolers and their mothers have subclinical Vitamin A Deficiency (VAD).
- 42% children are underweight.
- 58% children are stunted by two years of age.

So looking to the seriousness of these deficiencies, Indian government and policy makers mainstream food and nutrition through our public distribution system. It is estimated that every \$1 invested in proven nutrition programme offers

benefits worth \$16.

For reducing malnutrition in the country, Government of India (GOI) framed National Nutrition Policy (NNP) in 1993 under Department of Women & Child Development, Ministry of Human Resource Development then and now Ministry of Women & Child Development. Since then several programmes have been implemented through distribution of food and supplements to the targeted groups in the country but due to higher cost of processed food supplements and low income level of the target groups, it is challenging to ensure continuous supply of needed nutrients to target groups.

There are different ways and means suggested for fighting micronutrient deficiency like dietary diversity (eating fruits, vegetables, egg, meat, fish); supplementation (Fe, Zn pills and protein powders); fortification or food fortification (food processing in factory viz., Fe and protein rice flour, Vitamin A rich milk) and now biofortification.

The term biofortification is made up of two

Table2: Antinutrients and Promoters of Fe & Zn Bioavailability

Antinutrients	Nutrient	Source
Phytate (Ip6), phytic acid or phytin	Fe, Zn, Ca and Mg	Cereal grains & whole legume seeds
Polyphenolics (tannins),	Fe, Zn, Ca and Mg	Tea, coffee, beans and sorghum
Fibres (e.g. cellulose, hemicellulose, lignin, cutin, suberin, etc.)	Fe and Zn	Whole cereal grain products (e.g. wheat, rice, maize, oat, barley, rye)
Oxalic acid	Fe and Zn	Spinach leaves, rhubarb
Haemagglutinins (e.g. lectins)	Fe and Zn	Most legumes and wheat
Goitrogens	Fe and Zn	Brassicas and Alliums
Heavy metals (e.g. Cd, Hg, Pb, etc.)	Fe and Zn	Contaminated leafy vegetables and roots
Oxalate	Ca	Certain fruit and vegetables
Promoters	Nutrient	Source
Ascorbic acid (Vitamin C), fumarate, malate, citrate and β-carotene	Fe and Zn	Fruits and vegetables
Cysteine-rich polypeptides	Fe, Zn and Cu	Plant and animal
Inulin	Fe, Zn, Ca and Mg	Cereal grains and Legume seeds
Nicotianamine	Fe	All higher plants
Haemoglobin	Fe	Animal meats
Certain amino acids (e.g. methionine, cysteine, histidine, and lysine)	Fe and/or Zn	Animal meats
Long-chain fatty acids (e.g. palmitate)	Zn	Human breast milk
Fats and lipids	Vitamin A	Animal fats, vegetable fats
Selenium	I	Sea foods, tropical nuts
Iron, zinc	Vitamin A	Animal meats

Table3: Breeding targets for different nutrient in different crop

Crop	Nutrient	Baseline (ppm)	Target level (ppm)	Target increase (ppm)
Rice	Zn	16	28	12
Wheat	Zn	25	37	12
Common Bean	Fe	50	94	44
Pearlmillet	Fe	47	77	30
Cowpea	Fe	30	63	33
Lentil	Fe	40	70	30
Maize	Vitamin A	0	15	15
Sweet potato	Vitamin A	2	32	30
Cassava	Vitamin A	0	15	15
Banana/Plantain	Vitamin A	10-18	17-106	58
Potato	Fe & Zn	19 & 14	48 & 33	29 & 19
Sorghum	Fe & Zn	30 & 20	60 & 32	30 & 12

Source: Harvest Plus

words greek word 'bios' means 'life' and latin word 'fortificare' means 'make strong'. It means 'make life strong'. There is no universally accepted and officially declared definition of biofortification. People define it in their own ways like “It is a process of increasing the density of vitamins and minerals in a crop through plant breeding, transgenic techniques or agronomic practices”. Or “It is a process to increase the bioavailability and the concentration of nutrients in crops through both conventional plant breeding and recombinant DNA technology (genetic engineering)”. Or “It is the process of breeding food crops that are rich in micronutrients, such as vitamin A, zinc, and iron” and many more definitions are there. The issue of definition was raised in 2012 by harvest plus and different draft definition of biofortification was proposed before 'the Codex Committee on Food Labelling' (CCFL) which further referred it to another committee 'the Codex Committee on Nutrition and Foods of Special Dietary Use' (CCNFSDU), which then requested establishing a consensual definition of biofortification and yet it has to be finalized.

Measuring vitamin a content in crops

Beta-carotene is the most potent and widespread form of provitamin A. HarvestPlus has recommended HPLC as a reference method and the spectrophotometer reading for Total Carotenoid Content (TCC) but only limited number of samples per day can be analysed using these techniques.

Therefore, it is desirable that first to go for pre-selection of sample from a large number of sample using devices/equipments like 1. Near-Infrared Spectroscopy (NIRS), 2. Hunter color quantification with a chromameter and 3. iCheck™ Carotene - introduced by BioAnalyt then analyse limited no. of samples with HPLC and spectrophotometer.

Measuring trace micronutrient levels in crops

Various techniques have been employed for accurate and quick determination of metal particularly iron (Fe) and zinc (Zn), in plant material. These techniques include 1. ICP-OES (inductively coupled plasma optical emission spectroscopy); 2. AAS (atomic absorption spectroscopy); 3. Colorimetric Approaches / Colorimetric staining; 4. XRF (X-ray Fluorescence Spectroscopy). Among these techniques, ICP-OES analysis is considered the “gold standard” due to the high accuracy and low limits of detection for micronutrient analysis in plant material. However, this is time consuming and expensive both so XRF is alternate ideal means for screening large numbers of samples. Whole grain samples of rice, wheat, and pearl millet and flour samples for larger grains such as beans and maize can be analysed. Although XRF is less accurate than ICP analysis but it analyse sample quickly between 30 seconds to two minutes depending on the crop and able to analyze 100–200 samples per day. Therefore using XRF analysis, identify samples having highest levels of iron and zinc, and these samples can further

analyze (such as ICP-OES) for more accurate micronutrient determination.

Bioavailability

It measures the amount of nutrients digested, absorbed, utilized and is not available. It differs from bioaccessibility which refers to the amount available for absorption after digestion. Bioavailability of micronutrients is complex phenomenon and depends upon several factors. Various models have been suggested to determine the bioavailability of micronutrients in plant foods to humans such as cultured human intestinal cells (i.e. Caco-2 cell model), animal models (e.g. rats, pigs, and poultry) and small-scale human clinical. Bioavailability of micronutrient depends on crop type, presence of fat for provitamin A, antinutrients and promoters.

Brief history and origin of biofortification

Kernel of an Idea of biofortification was conceived by Howarth Bouis, an economist at IFPRI, Washington, D.C. in 1990s. He thought what if we could produce a new variety of seed that was higher in micronutrients say Fe and Zn. He discussed his idea with Dr. Robin and Dr. Ross who were plant nutritionist. They accepted and agreed this idea and initiated work on screening of germplasm to identify high-nutrient breeding parents, and feasibility of a conventional breeding approach to develop high-yielding and high-nutrient varieties and a project called “CGIAR Micronutrients Project” started in this direction. The term “biofortification” was coined by Steve Beebe, a bean researcher at CIAT in early 2001. By April, 2002 the “Biofortification Challenge Program (BCP)” was started and over time, a Phase I (2003-2008) for the BCP had been defined as a “discovery” stage where target population identified, set nutrient targets, validate nutrient targets, discover and screen crop genes. BCP was renamed as “Harvest Plus” by mid-2003. Phase 2 (2009-2013) of harvest plus was called 'Development' phase to improve and evaluate crops, test nutritional efficacy of crops, study farmer adoption and consumer acceptance. Phase 3 (2014-2018) of harvest plus called 'delivery' phase

concentrate on delivery and scaling up of biofortified crops. In 2016 the 'world food prize' also known as 'nobel prize for food and agriculture' was given for the work of biofortification to Laureates Dr. Maria Andrade, Dr. Robert Mwangi, Dr. Jan Low and Dr. Howarth Bouis. During first phase six staple crops viz., rice, wheat, maize, cassava, sweet potato, beans were included that are consumed by majority of the world poor in Africa, Asia, and Latin America. In second phase, ten more crops were included Potato, Barley, Cowpeas, Groundnuts, Lentils, Millet, Bananas/Plantains, Sorghum, Pigeon Peas, Yams.

Why biofortification?

Because fortification and supplementation are short term public health interventions, required infrastructure, sophisticated processing technology, product control, purchasing power, access to market, health care system for their success.

Advantages of biofortification

1. It target poor households grow and eat-staple foods,
2. It is one-time investment to develop seeds that fortify themselves afterwards it may be shared,
3. Recurrent costs low and making it highly cost-effective,
4. Sustainable in the longer term, varieties will continue to be grown and consumed year after year,
5. Reach the poor in rural areas with poor access to markets or health care systems,
6. Produces higher yields in an environment friendly way.

Methods/Approaches of biofortification

1. Agronomic biofortification

- Application of fertilizer to the soil or leaves.
- It provides temporary micronutrient increase.
- Useful for micronutrients that can be directly absorbed by the plant, such as zinc.
- Less efficient and effective for micronutrients that are synthesized in the plant and cannot be absorbed directly.
- Successful examples are Se fertilization of crops

Table4: Biofortified varieties released in India in different crops

Sl.	Crop	Variety/Hybrid	Trait
1.	Paddy	CR Dhan 310	Protein 10.3% in polished grain as compare to 7-8% in popular varieties
		DRR Dhan 45	High Zn content (22.6 ppm) in polished grain in comparison to 12-16 ppm in popular varieties
		GNR 4	High Iron (91 ppm), dietary fibres (2.87%) and beta- carotene (0.53 ppm)
		DRR Dhan 48	High Zn content (22 ppm)
		DRR Dhan 49	High Zn content (25.2 ppm)
2.	Wheat	WB 02	High Zn content (42 ppm) & Fe content (40 ppm) in comparison to 32ppm Zn and 28-32 ppm fe in popular varieties.
		HPBW 01	High Fe content (40 ppm) & Zn content (40.6 ppm) in comparison to 28-32 ppm Fe and 30 ppm Zn in popular varieties.
3.	Maize	Pusa Vivek QPM 9	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
		Improved Pusa HM4	Contains 0.91% tryptophan and 3.62% lysine which is significantly higher than popular hybrids (0.3- 0.4% tryptophan and 1.5-2.0% lysine)
		Improved Pusa HM8	Rich in tryptophan (1.06%) and lysine (4.18%) as compared to 0.3-0.4% tryptophan and 1.5-2.0% lysine in popular hybrids
		Improved Pusa HM9	Contains 0.68% tryptophan and 2.97% lysine compared to 0.3-0.4% tryptophan and 1.5-2.0% lysine in popular hybrids
		Improved Vivek QPM 9	Posses 0.83% tryptophan, 4.19% lysine 37 ppm Fe, 29 ppm Zn as compared to 0.59% tryptophan, 3.25% lysine 30 ppm Fe, 25 ppm Zn in check Vivek Maize Hybrid 9
4.	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
5.	Mustard	Pusa Mustard 30	Contains low erucic acid (<2.0%) in oil as compared to >40% erucic acid in popular varieties
		Pusa Double Zero Mustard 31	Low erucic acid (<2.0%) in oil and glucosinolates (<30.0 ppm) in seed meal as compared to >40.0% erucic acid and >120.0 ppm glucosinolates in popular varieties
6.	Lentil	Pusa Ageti Masoor	Contains 65.0 ppm iron as compared to 55.0 ppm iron in popular varieties
7.	Cauliflower	Pusa Beta Kesari 1	Contains high β -carotene (8.0-10.0 ppm) in comparison to negligible β -carotene content in popular varieties
8.	Potato	Bhu Sona	High β -carotene (14.0 mg/100 g) content as compared to 2.0-3.0 mg/100 g β - carotene in popular varieties
9.	Sweet Potato	Bhu Krishna	High anthocyanin (90.0 mg/100g) content in comparison to popular varieties which have negligible anthocyanin content
10.	Pomegranate	Solapur Lal	High iron (5.6-6.1 mg/100g), zinc (0.64-0.69 mg/100g) and vitamin C (19.4 -19.8 mg/100 g) in fresh arils in comparison to 2.7-3.2 mg/ 100g, 0.50- 0.54 mg/100g and 14.2-14.6 mg/100g, respectively in popular variety Ganesh

Source: ICAR Publication

in Finland; Zn fertilization of wheat in Turkey; I fertilization in China.

Limitations

- Need for continuous application of fertilizers, which may be detrimental to soil health.
- Physiological differences between plants that could affect effectiveness of absorption into grains.
- Geographical variations of soil micronutrient deficiencies.
- Uncontrollable factors that could affect the application of fertilizers e.g., weather conditions.

2. Conventional or traditional plant breeding

- It involves identifying and developing parent lines with high vitamin or mineral levels and crossing them over several generations to produce plants with the desired nutrient and agronomic traits.
- It improves the acquisition, utilization or accumulation of mineral elements available to the crop.

Limitations

- Biological boundaries, time-consuming, reduced expression of desirable traits due to uncontrolled gene interactions, loss of hybrid vigor, buy new seeds every season (for hybrid seed).

3. Transgenic/Genetic engineering

- Use in crops where the target nutrient does not naturally exist at the required levels.
- It includes genetic modification and transgenesis.

Limitations

- Need to advance understanding of the regulation of the endogenous metabolic pathways involved.
- Food and health safety issues and ethical concerns related to the environment and conservation of genetic resources.
- Labeling of products with genetically modified crops as ingredients, and to address issues related to intellectual property rights.

Cost-effectiveness of biofortification

Cost benefit analysis has been done by the World Health Organization's CHOICE (Choosing Interventions that are Cost Effective), Birol et al. (2014); Fiedler and Macdonald (2009) and observed that compare to fortification and supplementation, biofortification is found to be more cost-effective for all crop-country-micronutrient combinations but a combination of biofortification, supplementation and fortification may be best for achieving cost-effective and large scale impact.

For more information contact: Email id: jayprakashaditya@gmail.com & Mob: 5962230208.

