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Biofortification for Micronutrient in Vegetable Crops

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Introduction

According to Global Nutrition Report, 2018 anemia and stunting are the two forms of malnutrition burdens being experienced by our country. 37.9 per cent of children under-5 are affected by stunting (low height for age). 20.81 per cent of children under-5 are defined as 'wasted' (low weight for height). 51.4 per cent of women in reproductive age were affected by anemia. In India, 17.8 per cent of adult men and 21.6 per cent of adult women are overweight.

1. **CHILD WASTING:** the share of children under the age of five who are wasted (that is, who have low weight for their height, reflecting acute undernutrition);
2. **CHILD STUNTING:** the share of children under the age of five who are stunted (that is, who have low height for their age, reflecting chronic undernutrition).

World status of biofortified crops

150 biofortified varieties of 10 crops have been released in 30 countries. Testing of another 12 crops is being carried out in 25 countries. Harvest plus is the component of the CGIAR Research Program on Agriculture for Nutrition and Health. Launched in 2004, USA. Under joint venture of CIAT and IFPRI. **(HarvestPlus, 2017).**

Biofortification And Zero Hunger Challenge:

- ✓ Second Global Conference on biofortification recommend to the UN to celebrate 2018 - 2020 as the International Year of Biofortified and Underutilized Crops
- ✓ To meet the Zero Hunger Challenge by 2030

Table 1. Recommended dietary allowances for Indians (NIN, Hyderabad)

Minerals	**Daily requirement**	**Functions**	**Deficiency symptoms**
Calcium	500-600 mg	Development of teeth and bones, required for phosphorus absorption.	Causes improper blood clotting and osteomalacia.
Phosporous	1000 mg	Component of nucleic acid and plays vital role in cellular metabolism	Causes weight loss and general weakness.
Iron	10 mg	Formation of haemoglobin and involved in transport of oxygen.	Anaemia, pale lips and spoon shaped nails.
Sodium	4000-6000 mg	To maintain the osmotic balance and keep the cells in proper shape.	Weight loss and nervous breakdown.
Iodine	0.1-0.2 mg	Functioning of thyroid gland and production of thyroxin hormone.	Goiter.
Vitamins	Daily requirement	Functions	Deficiency symptoms
Vit A (Retinol)	5000 IU	For clear vision and increases resistance to infections	Night blindness, xerophthalamia and keratinisation
Vit B1 (Thiamine)	1.2 mg	For proper utilization of carbhohydrates	Beriberi disease
Vit B2 (Riboflavin)	1.7 mg	Oxidation reaction inside the cell	Ulcer in oral cavity.
Vit B12 (Cobalamin)	2.4 µg	Maturation of red cell and proper functioning of CNS	Pernicious anaemia
Vit C (Ascorbic acid)	60-90 mg	For collagen synthesis and calcification of bones and teeth	Scurvy and reduced resistance to diseases
Vit D	10-20 µg	Calcification of bones and teeth	Rickets in children and osteomalacia in adults
Vit E (Tocopherol)	5 mg	Promotion of fertility	Paralysis of eye muscles
Vit K (Anti hemorrhagic vitamin)	0.015 mg	Coagulation of blood and secretion of bile juice from liver	Unusual bleeding from the gums, nose or gastrointestinal tract

There are 4 primary ways a person can get micronutrients into their system

1. **DIETARY DIVERSITY**: Eating a balanced diet.
2. **FOOD FORTIFICATION:** Through the addition of micronutrients to staple products.
3. **SUPPLEMENTATION:** Taking a vitamin pill.
4. **BIOFORTIFICATION:** Biofortification is the process of increasing the density of vitamins and minerals in a crop, through plant breeding or agronomic practices, so that when consumed regularly will generate measurable improvement in vitamin and mineral nutritional status.

Biofortified staple foods cannot deliver high level of minerals and vitamins per day as fortified foods, but increase the daily adequacy of micronutrient intakes among individuals throughout the lifecycle. (Bouis *et al*., 2011)

What is Biofortification?

Greek word “**bios**” means “**life**” and Latin word “**fortificare**” means “**make strong**”. It refers to the nutrient enrichment of crops to address the negative economic and health consequences of vitamin and mineral deficiencies in humans (Prasad *et al.,* 2015). Bio-fortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. Developing bio-fortified crops also improves their efficiency of growth in soils with depleted or unavailable mineral composition. It consists of breeding new varieties of staple foods that have higher mineral and vitamin content. It can be described as a complementary, rural- targeted micronutrient program strategy for better reaching remote regions, which often comprise the majority of the malnourished vulnerable populations. Conventional breeding and genetic engineering techniques are the two approaches that may be used to bio-fortify the crops with minerals like iron and zinc (Tiwari *et al.,* 2010). This approach not only will lower the number of severely malnourished people who require treatment by complementary interventions, but also will help them maintain improved nutritional status. Moreover, it provides a feasible means of reaching malnourished rural populations who may have limited access to commercially marketed fortified foods and supplements (Jena *et al.,* 2018).

Advantages of biofortification

Biofortified crops are also a feasible means of reaching rural populations who may have limited access to diverse diets or other micronutrient interventions. Biofortification puts a solution in the hands of farmers, combining the micronutrient trait with other agronomic and consumption traits that farmers prefer. Increment of nutritional quality in daily diets. In the long-term, increasing the production of micronutrient-rich foods and improving dietary diversity will substantially reduce micronutrient

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deficiencies. In the near term, consuming biofortified crops can help address micronutrient deficiencies by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle. Biofortification, however, has two key comparative advantages: its long-term cost-effectiveness and its ability to reach underserved, rural populations. Unlike the continual financial outlays required for supplementation and commercial fortification programs, an upfront investment in plant breeding yields micronutrient-rich biofortified planting material for farmers to grow at virtually zero marginal cost. Once developed, nutritionally improved crops can be evaluated and adapted to new environments and geographies, multiplying the benefits of the initial investment. Once the micronutrient trait has been mainstreamed into the core breeding objectives of national and international crop development programs, recurrent expenditures by agriculture research institutes for monitoring and maintenance are minimal. Improvement of plant or crop quality and increment of variability in germplasm. No dramatic change in food habit, or need to remember to take a pill. Not depend on international organizations or governments. After fulfilling the household's food needs, surplus biofortified crops make their way into rural and urban retail outlets. (Winkler, 2011)

Success of biofortification depends on combination of high nutrient density with high yields and high profitability, sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bioavailable and biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers (Jena *et al.*, 2018).

Methods of Biofortification

1. Agronomic biofortification
2. Gene biofortification
    - Conventional breeding
    - Genetic Engineering

1. Agronomic Biofortification

Agronomic biofortification is the application of micronutrient-containing mineral fertilizer to the soil and/or plant leaves, to increase micronutrient contents of the edible part of food crops (White and Broadley, 2009). Success of agronomic biofortification depends on the presence and bioavailability of soil nutrients for plant uptake (soil to crop). Nutrient allocation within the plant and re-translocation into the edible part (crop to edible part). Bioavailability of nutrients to the human body (food to human). Bioavailability of micronutrients from soil to crop is influenced by many soil factors (i.e., pH, organic matter content, soil aeration and moisture and interactions with other elements). Addition of P also appears to induce Zn deficiency through dilution effects and interference with Zn translocation from

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the roots (Singh *et al.*1988). Degrees of success in agronomic biofortification is directly proportional to mobility of mineral element in soil and plant. (White and Broadley, 2003).

Mobility of nutrients in soil

1. Mobile:	NO_3^-,SO_4^{2-},BO_3^{2-},Cl^- and Mn^{2+}
2. Less mobile:	NH_4^+,K^+,Ca^{2+}, Mg^{2+} and Cu^{2+}
3. Immobile:	$H_2PO_4^-$, HPO_4^{2-} , Fe_2O_3 and Zn^{2+}

Bioavailability from crop to economic part is influenced by the crop (variety) – which defines whether micronutrients are (re-)localized into edible parts of the crop.

Mobility of nutrients in plants

1. Highly mobile:	N, P and K.
2. Moderately mobile:	Zn
3. Less mobile:	S, Fe, Mn, Mo and Cl
4. Immobile:	Ca and B.

Bioavailability of micronutrients in the food for the human body is influenced by many factors that can be either food or host related, micronutrient deficient people absorb micronutrient fastly(Gibson, 2007). Enhancers like ascorbic acid can increase iron bioavailability, while polyphenols and especially phytate or phytic acid are major inhibitors that form complexes with Fe and Zn and limit uptake in the human body. When calcium is present in the form of calcium oxalate the bioavailabilty will be 5 per cent but if calcium alone is present bioavailabilty will be 30 per cent (Clemens, 2014). Foliar pathways are generally more effective in ensuring uptake into the plant because immobilization in the soil is avoided (Garcia-Banuelos *et al.*, 2014). Foliar fertilization with micronutrients often stimulates more nutrient uptake and efficient allocation in the edible plant parts than soil fertilization for leafy vegetables (Lawson *et al.*, 2015). Agronomic biofortification has so far been most effective with Zn and Se then comes the iron which has low mobility in the plants (Cakmak 2014). Agronomic biofortification not only increases the particular mineral content in the plant it also improves quality, in capsicum, low-moderate (0.25-1.0 mg/L) of KI application improved the fruit quality by enhancing the ascorbic acid and soluble sugar content in addition to increased level of iodine to 350-1330 μg/kg FW. (Li *et al.*, 2017).

SWOT analysis of agronomic Bio fortification

STRENGTHS:	Comparatively simple method than other methods and suitable for immediate results.
WEAKNESS:	Success limited to minerals and dependent on several factors, needs regular application of nutrients, expensive and difficult to distribution.
OPPORTUNITIES:	Often used as a compliment to other strategies
THREATS:	Negative environmental impact, reverse exhaustion (Eg: Se)

Conventional plant breeding

Traditional breeding mainly focused on yield attributes and resistance breeding from last four decades and lack of priority on nutritional aspects lead's to decreased amount of nutrient status in the existed varieties. Examples of minerals that their mean concentration in the dry matter has declined in several plant-based foods are Fe, Zn, Cu and Mg (Susana *et al.*, 2013). Recent progress in conventional plant breeding has given emphasis on fortification of important vitamins, antioxidants and micronutrients. The potential to increase the micronutrient density of staple foods by conventional breeding requires adequate genetic variation in concentrations of β-carotene, other functional carotenoids, iron, zinc, and other minerals exists among cultivars, making selection of nutritionally appropriate breeding materials possible. Fe and Zn levels and up to a 6.6-fold variation has been reported in beans and peas (Gregorio *et al.*, 2000) Steps in biofortification by conventional plant breeding are

Discovery	Identify target populations
	Set nutrient target levels
	Screen germplasm and gene
Development	Breed biofortified crops
	Test performance of new crop varieties
	Measure nutrient retention in crops/food
	Evaluate nutrient absorption and impact
Dissemination	Develop strategies to disseminate seeds
	Promote marketing & consumption of biofortified food
Outcomes	Improve nutritional status of target populations

Precedent plant breeding was based on yield attributes and resistance breeding and prominence was not given on nutritional aspects but present plant breeding is concentrated on fortification of vitamins, antioxidants and micronutrients in edible parts **(Prasad *et al*., 2015).**

Diets rich in broccoli (*Brassica oleracea* var *italica*) have been associated with maintenance of cardiovascular health and reduction in risk of cancer. These health benefits have been attributed to glucoraphanin that specifically accumulates in broccoli. The development of broccoli with enhanced concentrations of glucoraphanin may deliver greater health benefits. Three high-glucoraphanin F^1 broccoli hybrids were developed in independent programmes through genome introgression from the wild species *Brassica villosa*. Glucoraphanin and other metabolites were quantified in experimental field trials. Global SNP analyses quantified the differential extent of *Brassica villosa* introgression. The high-glucoraphanin broccoli hybrids contained 2.5-3 times the glucoraphanin content of standard hybrids due to enhanced sulphate assimilation and modifications in sulphur partitioning between sulphur-containing metabolites. All of the high-glucoraphanin hybrids possessed an introgressed *Brassica villosa* segment which contained a *Brassica villosa* Myb28 allele. Myb28 expression was increased in all of the high-glucoraphanin hybrids. Two high-glucoraphanin hybrids have been commercialised as Beneforté broccoli (Traka *et al*., 2013)

SWOT analysis

STRENGTHS:	Successful for minerals and vitamins, one-off cost, easier distribution, long term strategy
WEAKNESS:	Long development time, success limited to minerals available in the soil.
OPPORTUNITIES:	Wide public acceptance, simple legal frame work
THREATS:	Requires genetic variation

Genetic engineering

Lack of sufficient variation among the genotypes for the desired character/trait within the species, or when the crop itself is not suitable for conventional plant breeding (due to lack of sexuality) then genetic engineering offers a valid alternative for increasing the concentration and bioavailability of micro nutrients in the edible crop tissues. One of the main concerns is these-called 'gene flow' environmental problem, i.e. the concern of transfer of foreign genes to non-target species (Winkler, 2011). Targets for transgenes include, redistributing micronutrients between tissues, increasing the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways.

Some strategies involved in the removal of 'antinutrients'. For Instance, one of the first biofortified crops was golden rice, which was engineered to produce beta-carotene or provitamin A in the edible portion of the grain (Eenennaam *et al.*, 2003).Since then, there have been similar successes with other crops, giving us a variety of carotenoid-enriched foods as well as crops enriched with other micronutrients such as vitamin E and folate (Bekart *et al.*, 2008). Micronutrient powders, popularly known under their original name of Sprinkles, are a form of 'home fortification' that also provide several nutrients at once. In sachets for a single serving, they are sprinkled on top of normal foods. Beginning with just, encapsulated iron, they have now developed numerous varieties, with as many as 15 vitamins and minerals, appropriate for the nutritional problems of specific areas.

Cauliflower is grown and consumed extensively all year round in India, but is deficient in vitamin A, it was identified as a preferred crop for biofortification in order to address a malnutrition problem in the country. Report on the introgression of the β-carotene (pro-vitamin A)-enhancing Or gene from donor line EC625883 into Indian cauliflower 'Pusa Sharad' (DC 309) and the parents of 'Pusa Hybrid-2' (CC-35 and DC 18-19). The sequence-characterized amplified region (SCAR) marker SA4 was utilized for foreground selection in the 'Pusa Sharad' (DC 309) × EC625883 BC1F1 cross. A total of 1013 PCR-based markers were used for a polymorphic survey among parents, with 6.81% being polymorphic between 'Pusa Sharad' and EC625883, 6.91% between CC35 and EC625883, and 6.71% between DC 18-19 and EC625883. Background analysis showed a maximum recovery of the recurrent parent genome (RPG) to the level of 72%. The β-carotene content of BC1F1 plants of the dark-orange category was 2.0314.64 µg g-1 (DC 309 × EC625883), 7.03-15.99 µg g-1 (CC-35 × EC625883) and 4.5218.60 µg g-1 (DC 18-19 × EC625883). The combination of high RPG and β-carotene content was recorded in two plants, 18-19-1-7-7 (18.60 µg g-1) and 18-19-1-3-6 (17.94 µg g-1). Promising lines showing more than 10 µg g-1 β-carotene in BC1F1 were advanced to BC1F2 and BC2F1 (Kalia *et al.*, 2018)

SWOT analysis

STRENGTHS:	Successful for minerals and vitamins, one-off cost, easier distribution, long term strategy,speed up process of conventional plant breeding.
WEAKNESS:	Long development time,Success limited to minerals available in the soil,interactions among transgenes(may limit the process)
OPPORTUNITIES:	Fast 'omics'developments
THREATS:	Low public acceptance,high sociopolitical problems,Environmental impact(gene flow)

Success Story

In Rwanda, iron-depleted university women showed a significant increase in hemoglobin after consuming biofortified beans for 4.5 months. Randomized controlled trial was conducted to determine the efficacy of iron biodortified beans (*Phaseolus vulgaris*) on improving iron status in women of reproductive age in Huye, Rwanda. A total of 195 female university students (aged 18-27 years) who were iron insufficient (serum ferritin <20.0 µg/L) at baseline were randomly assigned to receive either iron-biofortified beans containing 86 mg of iron per kilogram, or standard unfortified beans, containing 50 mg of iron per kilogram, two times per day for 128 days. Random serial sampling was used to collect blood during each of the eight middle weeks of the randomized feeding trial. A total of 86 (%) of participants were iron defecient and 36 (%) were anemic (haemoglobin < 120 g/L) at baseline. The intervention group receiving the iron biofortified beans consumed 14.5 mg of iron per day, whereas the control group receiving the conventional beans consumed 8.6 mg of iron per day. The intervention group receiving iron biofortified beans had significantly greater increases in hemoglobin content of blood (138 g/L), compared to the group consuming conventional beans (90 g/L) after 128 days of follow up (Haas *et al.*, 2017).

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