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Bio fortification in cereals is a promising approach to improve nutrition

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Abstract

Global food system is failing to deliver adequate quantities of healthy, nutritionally balanced food, especially to the resource-poor people leading to micronutrient malnutrition. The malnutrition of minerals (Fe, Zn) and vitamin A are major food-related primary health problem among populations of the developing world including India where there is a heavy dependence on cereal-based diets and limited access to meat, fruits and vegetables. Recommended daily intake of vitamin A, Fe and Zn are 600µg, 15mg and 15mg, respectively (Jena *et al.*, 2018). By consuming twice or thrice a day taking 100-150g rice/meal a person can get hardly 2-3mg Fe and 7-8mg Zn which is 1/5th and half of the recommended daily intake of Fe and Zn, respectively.

Bio fortification is one solution among many interventions that are needed to solve the complex problem of micronutrient malnutrition. There are several options for Bio fortification, among which genetic and agronomic Bio fortification are mostly used. Agronomic Bio fortification mainly refers to adequate fertilization using an appropriate method and time of application. It gives immediate results and in, general, goes well along with an increase in yield. In aerobic rice, application of $ZnSO_4$ at 25 kg/ha as basal + foliar spray at 0.5% at three stages reveals significantly highest Zn content in grain (35.09 mg/kg) (Barua and Saikia, 2018). Conventional breeding is the most accepted method of Bio fortification. It offers a sustainable, cost-effective alternative to transgenic and agronomic-based strategies. QPM is the best example for conventional breeding. QPM contain higher lysine and tryptophan than traditional varieties (Vasal *et al.*, 1980). Bio fortification of important crop plants through biotechnological applications is a cost-effective and sustainable solution for alleviating Vitamin A Deficiency. Genetic engineering is the obvious alternative to enhance the β carotene levels in crop plants like Golden rice. Awareness of dietary diversity must be followed up to alleviate micronutrient malnutrition. As people of under developed nations cannot afford to supplemented and diversified foods, Research and development of nutrient enriched bio fortified crops should be carried out to address malnutrition problem. The Bio fortification programme along with conventional breeding and Agronomic aspect will become the first choice of the researchers for crop improvement in future.

Keywords: Bio fortification, vitamin, food, health

Introduction

There was a trending population explosion after independence but no significant increase in food grain production to feed them led to food grain insufficiency. During green revolution era (1965-70) the introduction of high yielding varieties (HYV's) which are highly fertilizer responsive solved the problem of food grain insufficiency and the food grain production has now increased up to 257mt in India from 50.8mt during 1950-51. During pre-green revolution period the poverty was the major issue but it has been shifted to micronutrient malnutrition now-a-days i.e. night blindness, xerophthalmia, Iron deficiency anaemia etc. The main cause for this prevalence may be blamed to rare dietary diversity in under-developed and developing nations.

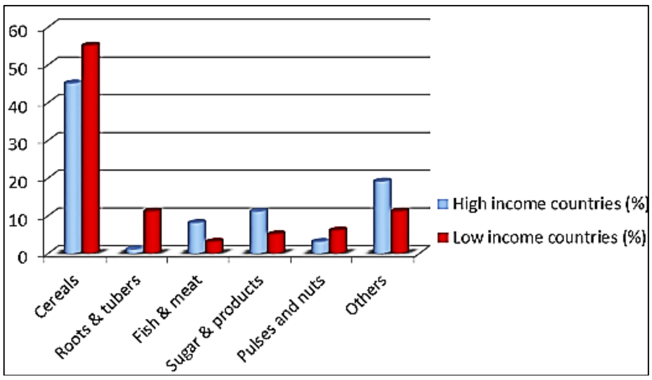


Fig 1: Dietary diversity by sources of dietary energy (FAO, 2008)

From the figure 1 it is clearly observed that in low income countries they consume more cereals but less animal originated products like fish and meats and in other hand high income countries their proportion of energy source from other parts is higher than low income countries showing their diversity in foods**.** This imbalance in diet plan leads to micronutrient deficiency.

What is Bio fortification?

Bio fortification is the process of increasing nutritional value of food crops by increasing the density of vitamins and minerals in a crop through either conventional plant breeding; agronomic practices or biotechnology.

Why Bio fortification?

Bio fortification is one solution among many interventions that are needed to solve the Complex problem of micronutrient malnutrition. Approaches range from the food-based approaches (such as dietary diversification, nutrition education and Bio fortification), to implementing food fortification and supplementation programs of essential nutrients such as vitamin A, iodine zinc, and iron; to inclusion of essential Nutrition Actions in national health and nutrition strategies, to incorporating infant and young child-feeding training into community health extension programs and water and sanitation programs.

Among these interventions Bio fortification is considered one of the most cost-effective interventions for countries to employ in combating micronutrient malnutrition. Bio fortification reaches rural consumers who have limited access to industrially fortified foods, supplementation interventions, and diverse diets. Most rural households already grow and consume staple food crops.

Bio fortification combines increased micronutrient content with preferred agronomic, quality, and market traits and therefore bio fortified varieties will typically match or outperform the usual varieties that farmers grow and consume. Poor people often get 60-70% of their calories from staple food crops. Hence, Bio fortification targets the poorest consumers. In the long-term, dietary diversification is likely to ensure a balanced diet that includes the necessary micronutrients needed by rural poor populations.

What are micronutrients?

Micronutrients are a group of compounds that are needed in small amounts by human bodies for a wide range of essential functions and for proper growth and development (for example, vitamin A, iron, folate, or zinc). Healthy diets contain a balanced and adequate combination of macronutrients (carbohydrates, fats, and protein) and essential micronutrients.

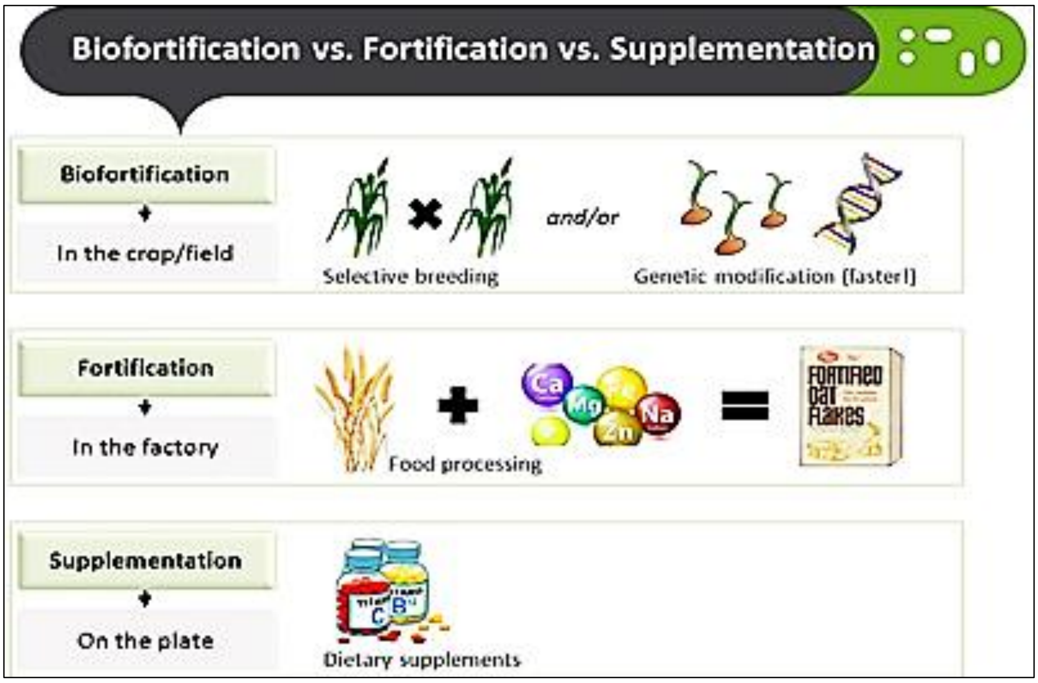
What is micronutrient malnutrition or hidden hunger?

Micronutrient malnutrition or hidden hunger is caused by a chronic or prolonged lack of essential minerals and vitamins required for proper growth and development of the body. Micronutrient malnutrition is a major risk factor for increased incidence of illness and low productivity and in young children, poor growth and even death. Deficiencies in different Micronutrients have different effects.

The Factors that contribute to micronutrient malnutrition include poor diet, increased micronutrient needs during certain life stages, and health problems such as diseases, infections, and parasites. However, unlike wasting (severe underweight), symptoms of micronutrient malnutrition are not necessarily visible to the naked eye even when they are affecting health-hence, micronutrient malnutrition is referred to as the hidden hunger.

How does Bio fortification differ from food fortification?

Bio fortification has the increased nutritional micronutrient content bred into the crop being grown. Food fortification increases the nutritional value of foods by adding trace amounts of micronutrients to foods during processing.



What are the benefits of consuming bio fortified foods as compared to other non-bio fortified foods?

Consuming bio fortified staple foods results in higher intakes of targeted micronutrients, which depending on the health status of the individual, can result in improved micronutrient status, thus avoiding the negative effects. Bio fortification does not treat acute deficiencies, but contributes to the prevention of micronutrient deficiencies, thereby promoting healthy growth and development.

Different approaches of Bio fortification

There are several options for Bio fortification, among which genetic and agronomic Bio fortification are mostly used.

Agronomic Bio fortification

Agronomic Bio fortification mainly refers to adequate fertilization using an appropriate method and time of application. Agronomic Bio fortification alternatively termed as ferti-fortification. Ferti-fortification, a term coined by Prasad (2009) [10] involves fertilizing crops with micronutrients. It gives immediate results and in, general, goes well along with an increase in yield. Bio fortification may therefore present a way to reach populations where supplementation and conventional fortification activities may be difficult to implement and/or limited.

Also necessary is the use of Zn and Fe efficient genotypes. Plant genotypes differ widely in their tolerance to Zn–deficient soils, both in uptake and utilization. An explanation of differential Zn efficiency of crop plants/genotypes is still missing; however it may be presumed that:

1. Efficiency mechanisms differ among crops/varieties.
2. More than one mechanism is often responsible for their efficiency level in a particular type of genotype.
3. Efficiency of one genotype in relation to Zn stress–release phytosiderophores and field assessed Zn efficiency is poor (Graham and Rengel, 1993).

Growing Zn efficient plants in deficient soils represents the strategy of 'tailoring the plant to fit the soil' in contrast to old strategy 'tailoring the soil to fit the plant' (Foy, 1983). Currently, the combination of both strategies (i.e., growing Zn efficient cultivars and fertilizing them with smaller quantities of Zn and less frequently) may be the most realistic approach under nutrient stressed conditions. Despite the fact that the efficiency of Zn fertilizers is good, there are situations in which fertilizers are ineffective and there is scope for developing Zn efficient cultivars (Graham *et al*., 1992).

The major advantages of agronomic Bio fortification include the following:

1. It is done on crop cultivars already being cultivated by the farmers and the produce is acceptable to the consumers.
2. The farmer is saved from investment in new seeds.
3. The gain in the micronutrient concentration in grain or other food products is obtained in the same year.
4. Application rates of mineral micro–nutrients (MMNs) are much smaller when these are applied to foliage.

Breeding approach

Plant breeding programs focus on improving the level and bioavailability of minerals in staple crops using their natural genetic variation (Welch and Graham 2005) [13]. Breeding approaches include the discovery of genetic variation affecting heritable mineral traits, checking their stability under different conditions, and the feasibility of breeding for increasing mineral content in edible tissues without affecting yields or other quality traits. Breeding for increased mineral levels has several advantages over conventional interventions (e.g., sustainability); no high mineral varieties produced by this method have been introduced onto the market thus far. This reflects long development times, particularly if the mineral trait needs to be intro gressed from a wild relative. Breeders utilize molecular biology techniques such as quantitative trait locus (QTL) maps and marker-assisted selection (MAS) to accelerate the identification of high-mineral varieties, but they have to take into account differences in soil properties (e.g., pH, organic composition) that may interfere with mineral
uptake and accumulation.

Genetic modification technology

Biotechnology is the process of inserting the specific genes responsible for a desired micronutrient from one variety into the DNA of another variety lacking any of the desired micronutrient. The capacity of genetic modification to produce plants with useful traits such as increased pest resistance, reduced post–harvest losses, increased yield, reduced labour requirements, or enhanced content of particular desirable constituents is readily apparent (Tripp, 2001). Enhanced nutritional quality of crops may be achieved by enabling the capacity of the plant to synthesize vitamins, to take up minerals with greater efficiency, or by reducing anti–nutrient factors such as phytates or tannins that can make nutrients unavailable as well as lower food palatability (Raboy, 2002; Bouis *et al*., 2003). As per published facts, forage crops showed potential for nutritional quality enhancement with positive impact on livestock nutrition and productivity. Because animal food–sources are richer in available Fe, vitamin A, and protein than plants, even minor increases in intake lead to real benefits to the majority of the malnourished (Allen, 2003; Murphy and Allen, 2003).

Animal food sources are more expensive and increased production can have negative environmental impact. However, in nutritional terms, considerable benefit will occur from increasing animal productivity and consequently animal–source foods in the diet. Nonetheless, for poor households, keeping livestock is an important economic and dietary asset. Increasing consumption of animal–source foods in poor communities using improved fodders and other innovative means contributes to the economic benefits of animal ownership and is a positive example of programs that have increased local production and consumption (Allen, 2003; Rahal and Shivay, 2016). While the technical achievements of nutritional enhancement of human foods and animal fodders may be analogous, the impacts on nutrition and health are not. The objectives of each exercise differ, as do the measures of health. In the case of animal fodder, the diet is consumed under controlled situations with no or little choice offered. Moreover, nutritional physiology and behaviour of omnivorous humans are considerably more complex than herbivorous and provisioned animals. With respect to human populations, the benefits of consuming phytochemically rich foods for individual or public health have not been demonstrated. Thus, interventions with nutrient enriched foods in undernourished populations would have shorter-term impact but demonstrating the effectiveness and long–term sustainability of these interventions will also be challenging (Allen and Gillespie, 2001; King, 2002; Bouis *et al*., 2003; Rahal and Shivay, 2016). A number of projects on genetic Bio fortification of food crops are under way and some are listed below. In addition to this, Harvest Plus, a Global Challenge Program of the Consultative (now Consortium) Group of the International Agricultural Research (CGIAR) focuses on breeding for higher levels of Fe, Zn, and beta–carotene in the major staple crops in developing countries.

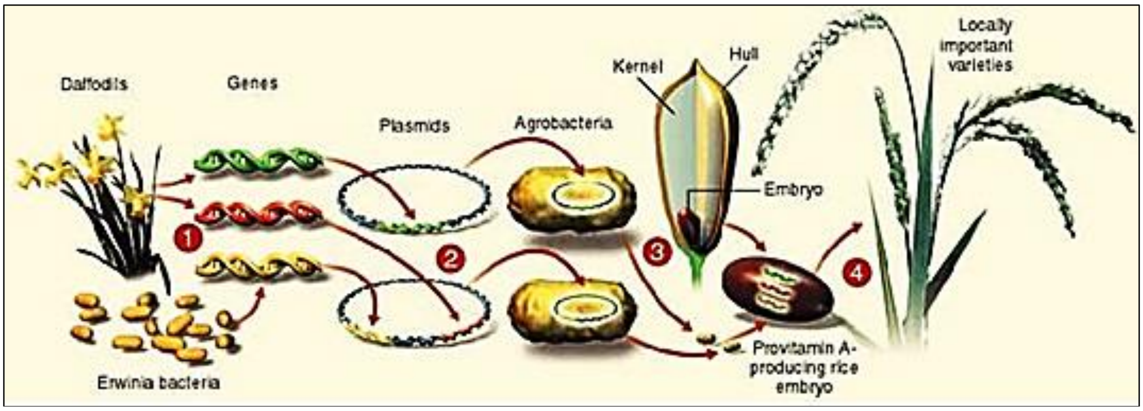
- African Bio fortified Sorghum Project to fortify sorghum with Fe, Zn, Vitamin A and E.

- The Golden Rice Project bio fortify rice with beta–carotene, Fe, Zn, Vitamin E, and protein under the 'Great Challenges in Global Health'.

Golden rice-A GM food crop

It is a genetically modified provitamin A (β-carotene) enriched rice genome. All the credits of golden rice go to Rockefeller foundation, EU and the Swiss federal institute of technology**.** Professor Ingo Potrycus and Dr. Peter Beyer considered as the founder of β-carotene enriched golden rice. They used *crtl gene* from soil bacterium (*Agrobacterium tumifascience)* and *Daffodil* gene for modification of the genetic makeup. Golden rice cannot be achieved by breeding. There are two grades of golden rice;

- **Golden rice1 (SGR1):** Promoter is modified here and it contains 5-7µg β-carotene per gram of rice.
- **Golden rice2:** Replacement of daffodil Pys with maize gene and contain 31µg β-carotene per gram of rice.



1. The genes that given golden rice its ability to make beta carotene in its endosperm comes from daffodils and a bacterium called *Erwinia uredovor.*
2. These genes, along with promoters inserted into plasmids that occur inside a sps of bacterium known as *Agrobacterium tumefaciens.*
3. These *Agrobacteria* are then added to a petri dish containing rice embryos. As they also transfer the genes that encode the instructions for making Beta carotene.
4. The transgenic rice plants must now be crossed with strains of rice that are locally and are suited to a particular regions climate and growing conditions.

Some β-carotene enriched popular rice varieties are

- **IR 64, IR 36:** Mega varieties with broad Asian coverage.
- **BRRI dhan 29:** The most popular *boro* rice variety in Bangladesh.
- **PSB Rc 82:** The most popular Rice variety of Philippines.
- **OS 6561:** Most popular in Vietnam.
- **Chehirang:** Leading variety in Indonesia.
- **Swarna:** Important in India.

Table 1: Recent Bio fortified varieties

Crop	Variety	Specification	Approach followed
Rice (2016)	CR Dhan 310	Protein 10.3%	Pure line variety
Rice (2016)	DRR Dhan 45	Zn 22.6 ppm	Pure line variety
Wheat (2017)	WB 02	Zn 42.0 ppm	Pue line variety
		Fe 40.0 ppm	
Wheat (2017)	HPBW 01	Zn 40.6 ppm	Pue line variety
		Fe 40.0 ppm	
Maize (2017)	Pusa vivek QPM9 Improved	Provitamine-A 8.15 ppm,	Hybrid
Maize (2017)	Pusa HM4 Improved	Tryptophan 0.91%	Hybrid
		Lysine 3.62%	
Maize (2017)	Pusa HM8 improved	Tryptophan 1.06%	Hybrid
		Lysine 4.18%	
Maize (2017)	Pusa HM9 Improved	Tryptophan 0.68% Lysine 2.97%	Hybrid

Source: Yadav *et al. (*2017)

Quality protein maize (QPM)

Nutritional quality of maize protein

Human beings require 0.66 g protein/kg body weight/ day to meet the requirement for proper growth and development (WHO/FAO/UNU, 2007). Essential amino acids such as lysine and tryptophan are not synthesized in human body and other monogastric animals; thus are required to be provided through diet.

The daily requirement of lysine is 30 mg/kg body weight/day for adults, while it is 35 mg/kg body weight/ day for children of 3 to 10 years of age. Tryptophan is required at the rate of 4 mg/kg body weight/day and 4.8 mg/kg body weight/day in adults and children, respectively (WHO/FAO/UNU, 2007). Besides role in protein synthesis, lysine and tryptophan serve as precursors for several neuro-transmitters and metabolic regulators, and their deficiency leads to reduced appetite, delayed growth, impaired skeletal development and aberrant behaviour (Tome and Bos, 2007; Moehn *et al*. 2012) [11, 7].

A maize kernel generally contains 8-10% protein, but is deficient in essential amino acids like lysine and tryptophan. Maize protein contains 1.5-2.0% lysine, which is less than half of the recommended dose specified for human nutrition (Young *et al*. 1998).

History of QPM

QPM development dates back to the 1920s when a natural spontaneous mutation of maize with soft and opaque grains was discovered in a maize field in Connecticut, USA. The salient events of this discovery (Prasanna *et al.*, 2001; Vasal, 2000) are summarized as follows:

- Kernels of the mutant maize were delivered to the Connecticut Experiment Station and the mutant was eventually named *opaque2* (*o2*) but received little further attention.
- In 1961, researchers at Purdue University, USA, discovered that maize homozygous for the *opaque2* (*o2o2*) recessive mutant allele had substantially higher levels of lysine and tryptophan in the endosperm, compared to CM with the dominant *O2* allele (*O2O2* or *O2o2*).
- Further experimentation in the 1980s demonstrated that the increased tryptophan content in *o2* maize effectively doubled the biological value of the maize protein, thus reducing by half the amount of maize that needs to be consumed to get the same amount of biologically usable protein in a maize diet.

India's first bio fortified Jowar (Sorghum) developed by ICRISAT

- The ICSR 14001 variety was taken up under the sorghum Bio fortification project Harvest Plus.
- Officially launched on July 5, 2018
- The sorghum variety has been named as 'Parbhani Shakti'
- It is a double fortified variety that gives more iron and zinc

Table 2: Agronomic Bio fortification in rice varieties through Zinc fertilization under aerobic condition

Treatments	Zn content (mg/kg)	
	Grain	Brown rice (Kernel)
Varieties		
Dishang	26.36	20.03
Banglami	28.44	22.24
Inglongkiri	32.03	26.81
Zn fertilizer schedules (Zn)		
Control	20.50	16.27
$ZnSO_4$ at 25 kg ha^{-1} as basal	27.89	20.85
$ZnSO_4$ at 25 kg ha^{-1} as basal + seed priming with 2% $ZnSO_4$	32.24	26.57
$ZnSO_4$ at 25 kg ha^{-1} as basal + foliar spray 0.5% at Tillering, Panicle initiation and Milking stage	35.09	28.31
Seed priming with 2% $ZnSO_4$ + foliar spray 0.5% at 3 stages	29.01	23.12
S.Em ±	0.84	1.06
CD(P=0.05)	2.44	3.09

Source: *Barua and Saikia, 2018*

Highest grain Zn content (32.03 mg/kg) and Zn content of brown rice (without polish) (26.81 mg/kg) was noticed in Inglongkiri. Application of $ZnSO_4$ at 25 kg/ha as basal + foliar spray at 0.5% at three stages reveals significantly highest Zn content in grain (35.09 mg/kg) and brown rice (28.31mg/kg).

Table 3: Effect of sources, time and method of zinc application on Zn concentration in grain and straw of Basmati rice

Treatment	Zn concentration in rice grain (mg /kg)		Zn concentration in rice straw (mg /kg)	
	2010	2011	2011	2012
Absolute control	20.7	21.2	74.7	76.2
NPK (120 kg N+ 26.2 kg P + 60 kg K)	23.1	23.6	79.8	81.3
NPK + 5 kg Zn /ha through ZnSHH as SA	26.4	26.9	87.4	88.9
NPK +ZnSHH 0.2% FSAT	24.8	25.3	84.8	86.3
NPK + ZnSHH 0.2% FSAT + B stages	26.3	26.8	86.9	88.4
NPK + ZnSHH 0.2% FSAT +B +GF stages	26.8	27.3	88.6	90.1
NPK +ZnSHH 0.5% FSAT	25.4	25.9	85.7	87.2
NPK +ZnSHH 0.5% FSAT + B stages	26.6	27.1	90.3	91.8
NPK + ZnSHH 0.5% FSAT + B + GF stages	28.2	28.7	92.8	94.3
NPK + 5 kg Zn ha^{-1} through Zn–EDTA as SA	27.8	28.3	92.0	93.5
NPK + Zn–EDTA 0.2% FSAT	24.7	25.2	84.6	86.1
NPK + Zn–EDTA 0.2% FSAT + B stages	26.6	27.1	87.8	89.3
NPK + Zn–EDTA 0.2% FSAT + B + GF stages	27.7	28.2	92.3	93.8
NPK + Zn–EDTA 0.5% FSAT	25.8	26.3	87.7	89.2
NPK +Zn–EDTA 0.5% FSAT + B stages	28.2	28.7	92.7	94.2
NPK + Zn–EDTA 0.5% FSAT + B +GF stages	29.8	30.3	96.0	97.5
SEm±	0.74	0.58	1.08	0.60
CD (P = 0.05)	2.15	1.68	3.10	1.73

(***Source***: Shivay *et al*, 2015) [20]

* ZnSHH-zinc sulphate heptahydrate, SA -soil application, FSAT -foliar spray at tillering, B-booting, GF-grain filling

The highest Zn concentration in grains of Basmati was recorded with three foliar applications of 0.5% solution of Zn–EDTA, significantly more than soil application of ZnSHH or Zn–EDTA and most other foliar application treatment.

Table 4: Effect of different treatments of zinc impregnated urea the root, shoot and grain Zn and phytate concentration of rice

Treatments	Root Zn concentration (µg /g)	Shoot Zn concentration (µg/g)	Grain Zn concentration (µg /g)	Grain Phytate concentration (µg /g)
Control	14.5	9.45	17.4	1100
Recommended Zn (ZnSO4)	22.8	15.67	35.08	436.67
Zn solubilizing bacteria	16.58	13.42	21.83	900
0.5% Zn coated urea	17.08	13.48	24.5	1095

1% Zn coated urea	18.58	14.32	28.5	1009.3
1.5% Zn coated urea	21.62	15.8	32.5	400
0.5% bio-activated Zn coated urea	17.5	12.08	39.8	1066.7
1% bio-activated Zn coated urea	21.16	14.4	42.5	736.67
1.5% bio-activated Zn coated urea	25.16	18	43	326.67
0.5% Zn blended urea	16.83	12.08	22.5	960
1% Zn blended urea	18.81	13.4	27.58	766.67
1.5% Zn blended urea	21.5	14.7	30.5	426.67
LSD	0.9657	0.6100	0.7626	76.570

Source: Nazir *et al*. (2016) [9]

Foliar application of 1.5% bio-activated Zn (ZnO) coated urea showed maximum increase in Zn acquisition in root, shoot and grains i.e. 9.3, 13 and 18% increase, respectively as compared to recommended Zn ($ZnSO_4$).

Table 5: Bio fortification of Maize Grain with Zinc and Iron by Using Fertilizing Approach

Treatments	Fe Content (mg kg-1)	% increase	Zn Content (mg kg-1)	% increase
Control (No zinc & Fe)	74.1	-	14.3	-
Soil $ZnSO_4$ & $FeSO_4$ (each at 10 kg ha^{-1})	91.6	23.6	18.3	28.0
Soil $ZnSO_4$ & $FeSO_4$ (each at 20 kg ha^{-1})	107.6	45.2	23.2	62.2
Soil $ZnSO_4$ & $FeSO_4$ (each at 30 kg ha^{-1})	122.7	65.6	25.1	75.5
Foliar $ZnSO_4$ & $FeSO_4$ spray (each at 0.1%)	153.6	107.3	31.8	122.4

Foliar application: silking and grain filling stage *Source*: Saleem *et al.* (2016) [4]

The maximum accumulation of iron and zinc was in grains when foliar application of Zn & Fe at 0.1%

Table 6: Effect of source and method of Zn application on Zn concentration in grain and Stover of corn and its Zn uptake by corn

Treatment (all values are quantities of Zn/ha)	Zn concentration		Zn uptake		
	Corn grain(mg kg^{-1} grain)	Corn stover (mg kg^{-1} DM)	Corn grain (g ha^{-1})	Corn Stover (g ha^{-1})	Total (g ha^{-1})
control (no added Zn)	40.2	45.0	160.8	274.5	435.3
5 kg to soil	44.2	49.2	207.7	328.5	536.4
1 kg foliar spray	46.0	59.2	203.2	384.8	588.0
5 kg to soil + 1 kg foliar spray	49.2	64.5	250.9	453.4	704.3
2.83 kg through Zn coated urea (to soil)	45.8	58.2	219.8	401.6	621.4
S.Em ±	0.6	0.8	3.4	3.4	3.8
LSD (for p=0.05)	2.0	2.7	11.1	11.1	12.5

Source: Shivay & Prasad, (2014)

Zn concentrations and uptake were all highest for the combined soil + foliar treatment, and always significantly superior to all other treatments. Higher mean values were always seen for foliar than soil application, often significantly so. Soil application as Zn-coated urea was nearly always statistically higher than application as Zn sulphate. All Zn treatments for agronomic Bio fortification of Zn in corn grain as well as in Stover were in the following order: 5 kg soil + 1 kg foliar > 1 kg foliar > 2.83 kg as Zn-coated urea to soil > 5 kg soil.

Table 7: Effect of various Zn treatments on the nutritional attributes in maize grain

Treatment	Zinc concentration in grain (mg /kg)	Zinc content in Grain (µg /seed)	Zinc concentration in Stover (mg /kg)
Control (without Zn)	22.3 ± 0.37 F	5.2 ± 0.12 F	13.9 ± 0.08 F
surface broadcasting (16 kg Zn/ha before sowing of crop)	26.7 ± 0.15 E	6.9 ± 0.09 E	22.9 ± 0.05 D
Zn foliar (0.5% w/v Zn sprayed at 25 days after sowing and 0.25% w/v at tasseling stage),	30.1 ± 0.14 D	7.6 ± 0.05 D	19.0 ± 0.07 E
subsurface banding (16 kg Zn/ha at the depth of 15 cm)	34.0 ± 0.11 C	9.0 ± 0.08 C	24.6 ± 0.10 C
Surface broadcasting + foliar	37.4 ± 0.17 B	10.4 ± 0.04 B	28.0 ± 0.15 B
Subsurface banding + foliar	41.9 ± 0.15 A	12.3 ± 0.11 A	30.3 ± 0.09 A

Source: Imran & Rehim, (2016)

Zinc treatments included: control (without Zn), Zn foliar (0.5% w/v Zn sprayed at 25 days after sowing and 0.25% w/v at tasseling stage), surface broadcasting (16 kg Zn ha^{-1} before sowing of crop), subsurface banding (16 kg Zn ha^{-1} at the depth of 15 cm), broadcasting + foliar and banding + foliar. Different letters in the same column indicate significant differences by LSD at $P \leq 0.05$ and ± indicate standard error (n = 3).

The maximum increase (136%) in grain Zn content (µg seed-1) was achieved with banding + foliar Zn fertilization, 100% by broadcasting + foliar, 72%by banding, 45% by foliar and 33% by broadcasting Zn application. However, Zn concentration in stover was also statistically significant in all Zn treatments. Zinc concentration in stover was found less as compared to Zn concentration in grain.

Table 8: Bio fortification of post-rainy sorghum (*Sorghum bicolor) with zinc and iron* through fertilization strategy

Treatment	Fe content (mg/kg) in grain	Zn content (mg/kg) in grain
Micronutrients		
RDF(80:40:40 kg NPK/ha)	35.00	23.71
RDF+ $ZnSO_4$ at 50 kg/ha soil application	38.75	25.34
RDF+ $FeSO_4$ at 50 kg/ha soil application	34.68	25.26
RDF+$ZnSO_4$+$FeSO_4$ SA fb foliar application(0.5%+ 1.0%) at 45DAS	44.06	27.47
LSD(P = 0.05)	5.7	2.76
Cultivars		
CSH 15R	39.22	26.29
M 35-1	34.37	24.42
Phule chitra	39.00	25.16
Phule Maulee	41.59	26.42
Phule Yashoda	36.42	24.93
LSD(P = 0.05)	6.40	NS

Source: Mishra *et al*. (2015) [6]

Sorghum genotypes differed significantly for Fe and Zn content in grains. Among genotypes, Phule Maulee had the highest Fe (41.59 mg/kg) and Zn (20.80 and 26.42 mg/kg). Sorghum cultivar Phule Maulee with soil application of ZnSO4 + FeSO4 each at 50 kg/ followed by foliar application (0.50%+1.0%) at 45 DAS along with recommended dose of fertilizer (80:40:40kg NPK/ha) is recommended for producing micronutrient (Fe and Zn) rich post-rainy sorghum.

Table 9: Effect of levels, sources and methods of Zn application on Zn uptake in grain and straw and total by oats

Treatment	Zn uptake (g ha^{-1})		
	Grain	Straw	Total
Control (no Zn only NPK)	71.1	279.6	350.7
2 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ deep placed at sowing	96.2	445.9	542.1
2 kg Zn ha–1 as ZnSO4.7H_2O ha^{-1} broadcast at sowing	86.3	397.0	482.3
2 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ (used coating of 100 kg seed)	130.6	573.5	704.1
2 kg Zn ha^{-1} as ZnO deep placed at sowing	89.8	411.7	501.5
2 kg Zn ha^{-1} as ZnO broadcast at sowing	79.7	377.7	457.4
2 kg Zn ha^{-1} as ZnO (used for coating of 100 kg seed)	126.1	545.3	671.4
5 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ broadcast at sowing	114.5	517.1	631.6
5 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ band placed at CRI before irrigation	104.1	425.9	530.0
5 kg Zn ha^{-1} as $ZnSO_4.7H_2O$ band placed at CRI after irrigation	97.8	411.3	509.1
5 kg Zn ha^{-1} as ZnO broadcast at sowing	108.5	481.1	589.6
5 kg Zn ha^{-1} as ZnO band placed at CRI before irrigation	98.7	420.9	519.6
5 kg Zn ha^{-1} as ZnO band placed at CRI after irrigation	94.8	410.8	505.6
SEm ±	3.04	11.50	11.99
LSD (P = 0.05)	8.87	33.57	35.02

Source: Shivay *et al*. (2015) [20]

The Deep placement of Zn sulphate or ZnO significantly increased Zn uptake in grain than broadcast Zn application. Coating of Zn sulphate or ZnO onto seeds at 2 kg Zn ha^{-1} resulted in the highest Zn uptake by grain, which was significantly more than 5 kg Zn ha^{-1} soil application.

Zn uptake in straw was significantly increased by Zn application of 5 kg Zn ha^{-1} either as Zn sulfate or ZnO compared to their application at 2 kg Zn ha^{-1}. When deep placed or coated onto seeds at 2 kg Zn ha^{-1} or when broadcast at 5 kg Zn ha^{-1}, Zn sulfate recorded significantly more Zn uptake in straw than ZnO.

The highest Zn uptake (704 g ha^{-1}) was obtained by coating seeds with Zn sulphate and was significantly superior to coating with ZnO (671 g ha^{-1}). Coating of oats seeds with Zn sulfate or ZnO at 2 kg Zn ha^{-1} was significantly superior to deep placement (501 to 542 g ha^{-1}), which was followed by broadcast application (378–482 g ha^{-1}).

Advantages and limitations of Bio fortification

Bio fortification have certain advantages

1. Increase in nutritional value, reduced adult and child micronutrient caused mortality,
2. Reduced dietary deficiency diseases and healthier population with strong and quick immune responses to infections.
3. Scope-reaching rural communities without access to pharmaceutical supplements or fortified food and improving life-time nutritional status;
4. cost effectiveness-the potential to impact a large number of people at a low cost per person

The possible limitations include

- **Narrow focus**: Increasing any single micronutrient in the diet is unlikely to address the whole problem.
- **Allergenicity and toxicity:** Increasing the amount or incidence of certain plant products in the diet could have a negative impact on some people's health.
- **Top-down approach:** A technological solution alone will not address root causes of the problem, such as social inequality, lack of education and poverty.
- **Lack of capacity:** Plant breeding is an ongoing exercise requiring continued effort and financial support, at a regional level, with local farmer engagement.
- **Technical Considerations:** Bio fortified crop varieties must be shown to have increased nutritional value in the environments in which they will be grown. These

varieties must also perform well for yield and pest resistance to meet with farmer's approval. The nutrients in the crop must withstand post-harvest processing such as milling, storage and cooking and must also be bioavailable (in a form that can be absorbed by the body).

- **Social and Economic Implications:** The introduction of new varieties into countries or regions must take into account the possible impacts on local markets. For example, is there effective infrastructure for delivering improved varieties to local farmers? Are these crops already being grown or will they have an impact on local agricultural systems? Bio fortified varieties may attract a market price premium that may encourage farmers to adopt the bio fortified variety as a marketable commodity. However, they should not price the poor out of the market.
- **Regulatory Approval:** Bio fortified varieties are subject to regulatory approval prior to release. Selectively bred varieties require relatively little testing prior to release. GM varieties must undergo compositional, allergenicity and toxicity testing, assessment of the molecular characteristics and potential environmental impact of the crop. GM technology has been used to produce disease-resistant, herbivore-resistant and herbicide-resistant crops that are now being grown in 25 countries in both the developed and developing world. No bio-fortified GM crops have yet been commercialised.
- **Public Acceptance of Products:** Public acceptance is critical for Bio fortification to succeed. If flavour or texture is altered consumers must be willing eat the altered variety. If flavour or texture is not altered consumers must be able to identify the product.

Conclusion
Awareness of dietary diversity must be followed up to alleviate micronutrient malnutrition. As people of under developed nations cannot afford to supplemented and diversified foods, research and development of nutrient enriched bio fortified crops should carried out to face this problem. There are several aspects of Bio fortification but agronomic aspect (Ferti-fortification) is simpler one and is mostly followed. This bio-fortification programme along with conventional breeding will become the first choice of the researchers for crop improvement in future.

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