Review Article

Micronutrient Biofortification in Pulses: an Agricultural Approach

ABSTRACT

More than two billion global populations are malnourished. In India, 25% of the total children are suffering from protein energy malnutrition. India ranks 103^{rd} among the 119 country in Global Hunger Index (GHI). Though world level hunger declined from 29.2 in 2000 to 20.9 in 2018, the figures still holds a deadly future. Micronutrient malnutrition is a very serious problem mostly affecting children and women in the country. The impact is highly seen in poor and landless rural people who can't afford diverse food or supplement in their diet with needed nutrition. Among consumed food, pulses are the cheapest source of protein, vitamins and micronutrients and can be supplied to the people through daily diet. Biofortification in pulses through agronomic, breeding and microbial intervention can increase the level of bioavailable micronutrients especially Zn and Fe in the final food products. This paper focuses on the role of micronutrients on human health and various mechanisms to get nutrient rich staple food along with main emphasis on biofortification.

Keywords: Biofortification, Pulse, Micronutrient, Malnutrition, Hunger

1. INTRODUCTION

Worldwide more than two billion people or one in every three persons is spotted to be troubled with multiple micronutrient deficiencies (FAO, 2015). Growing children are grievously affected by nutrient deficiency compared to adults as their requirement changes according to growth and developmental phages (Prieto and Cid, 2011). In Kolhapur district, 40% children between the age group of 8-9 years are micronutrient deficient (iron in 38.8% and fluoride in 36.6% respectively) (*Bharati et al 2018*) and globally it is 22% (GNR, 2018). At all India level 18 percent infants had a birth weight of less than 2.5kg, 38% children below five years were under-weight, 28% mild, 29% moderately and 2% severely anaemic (NFHS- 4, 2015-16). Malnutrition caused by vitamins and minerals also known as "Hidden hunger", which don't give any visual symptom usually. As per GHI 2018, India ranked 103^{rd} among 119 countries and world-wide level of hunger declines from 29.2 in 2000 to 20.9 in 2018. Micronutrient deficiencies are the fountainhead of various health issues like poor neurological function, impaired eye sight, diabetes, hypertension, week immunity, diarrhea, food allergies, thinning hair, leaky gut, acne or rashes (Lynch and

Green 2001; Beard 2001; Shankar and Prasad 1998; Gilbert and Foster 2001; Stein *et al*., 2005).Those deficiencies are attributable to less intake of quality diet enriched with protein, vitamins and minerals (Bhatnagar *et al.,* 2011 and Bouis and Saltzman, 2017). Increased price of non staple commodities is one of the important reasons of decreasing dietary quality especially to resource poor people (Bouis *et al*., 2011). In developing countries agricultural products are the prime source of nutrition (Graham *et al.*, 2001; Schneeman, 2001). Main concern of green revolution was laid on yield increase not on quality food production. And it scale down soil productivity accompanied by less nutritive food grain production (Bhatnagar *et al*., 2011). Micronutrient rich vegetables, pulses and animal products have also not been increased in last fifty years (Bouis and Saltzman, 2017). Possible ways to combat those deficiencies encircle dietary diversification (healthy balance diet), food fortification, biofortification and supplementation (Allen *et al*. 2006). Biofortification is the process of increasing nutrient concentration in plant edible parts by fertilization (agronomic intervention), breeding approaches or microbes (White and Broadley, 2005), whereas fortification is nutrient enrichment during processing (https://en.wikipedia.org/wiki/Food_fortification). Biofortification is an effective strategy in long run to overcome the current situation as it is more cost effective, sustainable and practical one to reach poorest of the poor population (Meenakshi *et al*., 2010, Hoddinott *et al*., 2013, Garcia-Banuelos *et al*., 2014). Besides quality enhancement, micronutrient has some added advantages like yield increase, biomass enhancement and disease control in micronutrient deficient soils (Hussain *et al*., 2010). A healthy balance diet must include pulses as they are rich source of energy, protein, dietary fibre and also content considerable amount of vitamins and minerals like thiamin, riboflavin, pyridoxine, folic acid, vitamin E and K, zinc, iron etc (Ofuya and Akhidue, 2005; Thavarajah *et al*., 2011; Johnson *et al*., 2005). So, pulses can be considered as good option for biofortification to provide nutritious food sustainably (Thavarajah *et al*., 2011).

2. ROLE OF MICRONUTRIENTS ON HUMAN HEALTH

Iron plays key role in haemoglobin formation and oxygen transport (Underwood and Suttle, 1999). Iron deficiency exerts influence on learning ability (CDC 2010), immune system (Fiall, 2003), ability to work (Viteri, 1974) and cognitive development (Bread and Connor, 2003). Its deficiency is also associated with anemia and pregnancy related issues like mortality, low birth weight etc (CDC 2010).

Zinc requirement get larger during pregnancy and puberty. Zinc deficiency curtails physical growth and development of children (Brown *et al*., 2002). Gastrointestinal, central nervous, epidermal, immune, skeletal, and reproductive systems are known to be affected by zinc deficiency (Hambidge and Walravens, 1982). The daily requirement of Zn and Fe varies with the age of people (Table 1).

Selenium is a good source of antioxidant which narrow down heart and skin diseases, cancer, alzheimer, (Elahi *et al*. 2009; Marksbery and Lovell, 2006; Klaunig and Kamendulis 2004; Cui *et al*., 2012;

Shirley *et al*., 2014), thyroid (Ventura *et al*., 2017), asthma (Norton and Hoffmann, 2012). Patients having tuberculosis, influenza and hepatitis C delineated to be benefited by selenium (Steinbrenner *et al*., 2015).

Table 1. Daily requirements of Zn and Fe in Indian context (ICMR, 2010)

	Group	**Recommended Daily Allowance (mg day^{-1})**	
		Zinc	**Iron**
Adult men		12	21
Adult women	Normal	10	17
	Pregnant	12	35
Children	1-3 Years	5	9
	4-6 Years	7	13
	7-9 Years	8	16
Adolescents	Boys	11-12	21-28
	Girls	9-12	26-27

3. CRITERIA OF BIOFORTIFIED CROP

Bouis and Welch (2010) suggested the following criteria to be a potential biofortified crop.

- High Yielding: Crop productivity must be maintained.
- Effective: The increased level of micronutrient must have significant positive impact on human.
- Stable: Increased level of micronutrients in crop must be stable year after year.
- Good Taste And Cooking Quality

4. POTENTIAL WAYS OF BIOFORTIFICATION

- Agronomic intervention
- Breeding intervention
- Microbial intervention

4.1 AGRONOMIC INTERVENTIONS

Agronomic biofortification is the application of micronutrients via chemical fertilizer with the aid of foliar application, soil application, seed priming and seed coating of fertilizers to increase the bioavailability of nutrients in edible plant parts (De Valença *et al*., 2017). Several factors like source of fertilizer, quantity of fertilizer and time and methods of application regulate the nutrient intake to the edible plant parts and it's bioavailability to the consumer (Singh and Prasad, 2014, Rietra *et al.,* 2015). Micronutrient amendment in soil is a useful strategy to increase micronutrient quantity in crop (Manzeke *et al.,* 2012; Vanlauwe *et al*., 2015 and Voortman and Bindraban, 2015). Among the different methods of application, foliar application is more efficient (Lawson *et al*., 2015) as it can manage soil immobilization (Garcia-Banuelos *et al*., 2014) and quick availability of nutrients to the crop. Hidoto *et al*. (2017) reported 85 g ha^{-1} grain zinc yield with foliar application in chickpea which was significantly higher than soil application (71 g ha^{-1}) and priming (68 g ha^{-1}). Combined application in both soil and foliar often showed better results (Phattarakul *et al*., 2012). Other biofortification methods like seed priming and seed coating are spotted to give very infrequent result (Duffner *et al*., 2014). Johnson *et al*. (2005) found that seed priming with both B and Zn increased the seed Zn and B content of chickpea and lentil respectively (table 2). Zinc and selenium biofortification is most fruitful with agronomic interventions (Cakmak, 2014).

Table 2. Effect of seed priming on Zn, B and Mo content of chickpea and lentil

Treatments	Seed content (mg kg^{-1})					
	Chickpea			Lentil		
	Zn	B	Mo	Zn	B	Mo
(purchased)	40	9	3	50	6	2
water	60	10	4	50	6	2
B	60	100	3	50	100	2
Zn	700	7	3	630	5	2
1/2(B + Zn)**	400	50	2	400	50	2
B + Zn	800	80	3	660	100	2
B, 12 h	40	100	3			
Zn, 12 h	500	8	2			
Mo	60	4	300			

(Source: Johnson *et al.,* 2005) **Priming times were 8 h and 12 h for chickpea and lentil respectively. Solutions used were 0.004M $ZnSO_4 \cdot 7H_2O$ (for Zn), 0.008 M H_3BO_3(for B), 0.0026M $Na_2MoO_4 \cdot 2H_2O$ (for Mo).

4.1.1 ZINC FORTIFICATION

Application of zinc to the pulse crops greatly helps in enhancing the level of zinc in harvested (economic) plant parts. Zinc fertilization increases bioavailability of Zn in human by increasing phytate content (Hussain *et al*., 2013). Guillén-Molina *et al*. (2016) concluded that application of zinc chelate (7 and 14 mM L^{-1} of Zn-EDTA) increase grain zinc and iron concentration in cowpea. Shivay *et al*. (2015) reported that foliar spray of zinc at three different stages of chickpea had significant influence on zinc uptake both in grain and straw during 2011-12 and 2012-13 (Table 3). Foliar spray of Zn-EDTA at active vegetative, flowering and grain filling stages had greatest crop recovery of applied Zn (17.33%) during 2011-12 (table 2). Zinc fertilization improves zinc bioavailability in bean and pea (Cakmak *et al*. 2010, Zhang *et al*. 2010). Zinc content in seed helps in significant liner increase of protein biosynthesis (Martre *et al.* 2003). Maximum Fe content was recorded with application of 50µM Zn-DTPA (183.7±2.16 ppm) and 100 µM $ZnSO_4$ (197.9±3.45 ppm) whereas highest Zn with 100µM Zn-DTPA (46.3±3.87 ppm) and 100 µM $ZnSO_4$ (49.6±2.54 ppm) of bean in hydroponic situation (Table 4). Hidoto *et al*. (2016) stated that maximum grain Zn content and Zn yield in chickpea were noted in soil application of 25 kg ha^{-1} Zn which had an advantage of 7% over control (table 5).

Table 3. Zinc content by grain and straw of Chickpea

Treatment	Zn uptake in grain (g ha^{-1})		Zn uptake in straw (g ha^{-1})	
	2011-12	2012-13	2011-12	2012-13
Check (no Zn)	78.5	71.3	78.0	68.5
ZnSHH soil at 5 kg Zn ha^{-1}	102.3	93.9	104.2	93.9
ZnSHH one spray (V)	96.3	87.9	103.3	92.8
ZnSHH two sprays (V + F)	112.3	103.2	128.6	116.2
ZnSHH, three sprays (V + F + G)	124.9	114.8	166.8	152.0
Zn-EDTA soil at 2.5 kg Zn ha^{-1}	102.7	93.9	114.5	103.5
Zn-EDTA one spray (V)	98.8	90.9	117.0	106.0
Zn-EDTA two sprays (V + F)	125.4	115.8	139.2	126.6
Zn-EDTA three sprays (V + F + G)	162.8	135.4	181.0	148.9
LSD (P = 0.05)	14.93	15.52	10.45	20.25

ZnSHH= Zn sulfate hepta hydrate V= active vegetative stage, F= flowering stage, G= grain filling stage (Source: Shivay *et a.,l* 2015)

Table 4. Iron and zinc concentration of bean in hydroponic situation

Dose	Micronutrient concentration	
Zn-DTPA (μM)	Fe	Zn
0	146.5±0.41	28.4±1.12
25	174.4±1.45	45.7±2.35
50	183.7±2.16	42.8±3.55
100	153.0±1.63	46.3±3.87
$ZnSO_4$ (μM)	Fe	Zn
0	146.5±0.41	28.4±1.12
25	189.2±2.89	42.3±3.11
50	162.1±2.03	42.6±2.87
100	197.9±3.45	49.6±2.54

Source: (Sida-Arreola *et al.,* 2017)

Table 5. Effect of zinc sulphate soil application on Chickpea

Zn rate	Straw Zn	Grain Zn	Zn yield
$ZnSO_4.7H_2O$ (kg ha^{-1})	(mg kg^{-1})	(mg kg^{-1})	(g ha^{-1})
0	20.63	37.05	91.0
5	20.48	37.54	98.3
10	23.24	34.20	87.7
15	22.15	33.11	86.2
20	21.82	35.52	86.3
25	21.57	39.55	99.7
30	22.31	39.18	98.0

Source: Hidoto *et al*., 2016.

4.1.2 IRON FORTIFICATION

Iron is another most important micronutrient which improves human health. Supply of iron through fortification of pulses is helpful and economic for major portion of Indian population. Iron content of cowpea bean seed increased 29.4% with application of 100μM L^{-1} ferrous sulphate and 32% with 50μM L^{-1} ferrous chelate over control (Mirquez- Quiroz *et al.,* 2015). Ali *et al*. (2014) observed that application of 1.5% $FeSO_4$ at branching and flowering resulted 55%, 66% and 81% increase in iron content in leaf, stem and grain in mungbean over control respectively (Table 6). Khalid *et al*. (2015) reported that application of PGPR along with iron (@ 5.6 kg ha^{-1}) resulted grain, root and shoot iron content 4.6 mg, 3.16 mg and 1.7 mg in 100 g chickpea seed respectively (Table 7). According to Salih (2013), foliar fertilization of 2 ppm Fe and 2 ppm Zn reported maximum increase in Fe (154 mg kg^{-1}) and Zn (42 mg kg^{-1}) content of cowpea seed respectively (Table 8). Nandan *et al*. (2018) pointed out that foliar spray of Fe @ 0.05% along with recommended dose of fertilizer resulted significantly higher iron content in seed (66.46 mg kg^{-1}) and stover (66.83 mg kg^{-1}) whereas, maximum zinc content in seed (44.98 mg kg^{-1}) and straw (44.08 mg kg^{-1}) was noted with Zn (0.5%) and Fe (0.05%).

Table 6. Iron content in leaves, stems and grains in mungbean

Treatment	Iron content (mg kg^{-1})		
	Leaves	Stems	Grains
Control	511.37	380.07	78.50
0.5% $FeSO_4$ at branching	601.73	470.42	90.43
0.5% $FeSO_4$ at flowering	623.70	488.17	96.10
0.5% $FeSO_4$ at branching + 0.5% $FeSO_4$ at flowering	675.43	520.24	101.50
1.0% $FeSO_4$ at branching	654.07	515.22	96.83
1.0% $FeSO_4$ at flowering	668.37	505.16	99.60
1.0% $FeSO_4$ at branching + 1.0% $FeSO_4$ at flowering	717.17	585.54	127.80
1.5% $FeSO_4$ at branching	672.60	550.33	115.73
1.5% $FeSO_4$ at flowering	698.70	559.51	121.43
1.5% $FeSO_4$ at branching + 1.5% $FeSO_4$ at flowering	794.90	634.27	146.43

Source: Ali *et al*., 2014

Table 7. Iron uptake in different plant parts of chickpea

Treatment	Fe Concentration (mg 100 g^{-1})		
	Grains	Shoot	Root
Absolute control	1.20	0.66	0.14
Fe @ 5.6 kg ha^{-1}	2.40	1.80	0.86
S1	3.26	2.23	1.40

S2	3.30	2.50	1.30
S3	3.36	2.26	1.33
S4	3.20	2.36	1.36
S5	3.40	2.40	1.30
S1+Fe @ 5.6 kg ha^{-1}	3.60	2.73	1.70
S2+Fe @ 5.6 kg ha^{-1}	4.36	3.16	1.56
S3+Fe @ 5.6 kg ha^{-1}	3.50	2.80	1.50
S4+Fe @ 5.6 kg ha^{-1}	3.53	2.70	1.50
S5+Fe @ 5.6 kg ha^{-1}	3.63	2.63	1.46

Source: Khalid *et al*., 2015

Table 8: Effect of foliar fertilization on Fe, B and Zn content of cowpea

	Treatment	**Fe**	**B**	**Zn**
		Mg kg^{-1}		
	Control, 0 pmm	40.00	16.00	8.00
	Fe, 1 ppm	90.00	31.00	25.00
	Fe, 2 ppm	154.00	47.00	42.00
	B, 1 ppm	51.00	31.00	18.00
	B, 2 ppm	58.00	40.00	24.00
	Zn, 1 ppm	47.00	26.00	13.00
	Zn, 2 ppm	50.00	37.00	17.00
Tukey's HSD	Treatment and concentration	1.28	1.35	1.35
	Interaction	2.61	2.94	2.94

Source: Salih, 2013

4.1.3 SELENIUM FORTIFICATION

Selenium fertilization by means of inorganic fertilizer results increased selenium concentration in diet (White and Broadley, 2009; Alfthan *et al*., 2015). Unlike selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) provides immediate availability to plants when added to soil (Broadley *et al*., 2006; Fordyce, 2013; Pilbeam *et al*., 2015). Selenium foliar application increases concentration in pea and common bean from 21 µg kg^{-1} to 743 µg kg^{-1} (Smrkolj *et al*., 2005) and 30 to 2379 µg kg^{-1} (Smrkolj *et al.,* 2007) respectively.

Further credibility of agronomic biofortification requires much more research on micronutrient bioavailability, including metabolic pathways that affect absorption and health benefits of different chemical forms of micronutrients.

4.2 BREEDING INTERVENTIONS

When utilizable genetic variability is present in a species then genetic biofortification is conductible, but when there is no variability, transgenic approaches are well qualified (Garg *et al*., 2018). Initially reduction of Phytic acid and polyphenols are used to be the fundamental approach of biofortification as these compounds are known to narrow down iron bioavailability. But recent studies implies that priority should be given to increase iron concentration rather than Phytic acid and Plyphenol reduction because those also have some beneficial properties and resist cancer cell (Pixley *et al*., 2011, Murgia *et al*., 2012). Zein protein over expression on soybean increases methionine and cysteine content (Dinkins *et al*., 2001) and methionine content by cystathionine γ-synthase (Song *et al*., 2013, Hanafy *et al*., 2013). Increase in beta carotene and oleic acid in soybean has been attended by introducing bacterial PSY gene (Schmidt *et al*., 2015) and siRNA-mediated gene silencing had been used to reduce α-linolenic acids (Flores *et al*., 2008). Similarly, linoleic acid and palmitic acid content of soybean was reduced by antisense RNA technology (Zhang *et al*., 2014). Storage albumin of Brazil nut which is rich source of methionine has been used to increase common bean methionine content (Aragao *et al*., 1999) whereas, lupines methionine has been intensified by albumin of Sunflower (Molvig *et al*., 1997). A sensitive approach to understand the escalated zinc uptake is DNA strand breakage (King *et al*., 2015).

Field trials regarding genetic effect on selenium concentration reported significant difference among genotypes (Thavarajah *et al*., 2010; Garrett *et al*., 2013; Ray *et al*., 2014). 94 pea genotypes were grown in Saskatchewan field (University of Saskatchewan) and not a single nucleotide polymorphism (SNP) marker was noted to affect seed Se concentration (Diapari *et al*., 2015). In contrast, lentil and chickpea revealed genotypic variation associated with selenium concentration in Saskatchewan (Thavarajah *et al*., 2008 ; Thavarajah, 2012; Ray *et al*., 2014; Rahman *et al*., 2015). Field experiments conducted in Morocco, Nepal, Syria, Australia and Turkey were also ensured significant genetic variance in lentil Se concentration (Thavarajah *et al*., 2011). Mungbean (Nair *et al*., 2015) and soybean (Yang *et al*., 2003) also shown genetic variation. Bean has a potential to increase zinc content by 50% and iron by 60-80% as it evidence high heritability in zinc and iron content (Blair *et al*., 2009; Beebe *et al*., 2000; Petry *et al*., 2015).

4.3 MICROBIAL INTERVENTIONS

Phytoavailability of micronutrients can be increased by soil microorganisms like *Rhizobium*, *Bacillus*, *Pseudomonas* etc (Rengel *et al*., 1999; Smith, 2007). PGPR can be an alternate approach to biofortify pulses as it increases disease resistance (Phi *et al*., 2010; Dary *et al*., 2010), solubility of phosphorus (Richardson, 2001; Wani, 2007) and root growth (Glick, 1995, Zhang *et al*., 2010). But the implication of PGPR and other microorganisms in biofortification of pulses are sparse (De *et al*., 2011). Rhizobacteria produce siderophores which promote iron fortification in crop as well as revamps soil fertility directly by enhancing iron availability at rhizosphere or indirectly by reducing pathogen effect (Rana *et al*., 2012; Srivastava *et al*., 2013).

Grain protein concentration of chickpea ranged from 180 to 309 mg g^{-1} with inoculation of *Bacillus* PSB1 and *M. ciceri* RC3 + *A. chroococcum* A4 + *Bacillus* PSB10 respectively with 25% yield advantage (Wani, 2007).

Fungi and bacteria improves bioavailability of zinc at rhizosphere zone (Fasim *et al*., 2002; Biari *et al*., 2008) due to decline in soil pH (Koide and Kabir, 2000; Subramanian *et al*., 2000), chelation (Whiting *et al*., 2001) and increased root sphere (Burkert and Robson, 1994).

Some biofortified pulse crop varieties were released across the world helping to combat the present situation of malnutrition and hidden hunger of mineral nutrients among the people (table 9 and 10).

Table 9. Several Lentil released varieties that possess high iron and zinc levels (The 2nd Global Conference on Biofortification: Getting Nutritious Foods to People, Ashutosh Sarker (ICARDA))

Country	Variety	Content (ppm)	
		Fe	Zn
Bangladesh	Barimusur-4	86.2	---
	Barimusur-5	86	59
	Barimusur-6	86	63
	Barimusur-7	81	---
Nepal	Sisir	98	64
	Khajurah-2	100.7	59
	Khajurah-1	---	58
	Shekhar	83.4	---
India	Pusa Vaibhav	102	---
	L4704	125	74
	IPL 220	73-114	51-64

	Pusa Ageti Masoor	65.0	---
Syria	Idlib-2	73	---
	Idlib-3	72	---
Ethiopia	Alemaya	82	66

Table 10. Iron biofortified bean variety released by Harvest Plus (Garg *et al*., 2018)

Rwanda	Democratic Republic of Congo
RWR 2245, RWR 2154, MAC 42, MAC 44, CAB 2, RWV 1129, RWV 3006, RWV 3316, RWV 3317, and RWV 2887	COD MLB 001, COD MLB 032, HM 21-7, RWR 2245, PVA 1438, COD MLV 059, VCB 81013, Nain de Kyondo, Cuarentino, Namulenga.

5. CONCLUSION

Largest number of hungry people especially children and women live in India which is quite alarming. In a developing country like India, where maximum people does not have sufficient access to afford commercially fortified food, diversified diet and food suppliments, biofortification is an acceptable cost effective way to eliminate malnutrition. And evidences revealed that a nutritious food like pulse is one of the good options to fortify.

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