Current Journal of Applied Science and Technology

35(4): 1-12, 2019; Article no.CJAST.49289
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Micronutrient Biofortification in Pulses: An Agricultural Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author AG chosen the topic, and wrote the first draft of the manuscript. Authors AN and SK helped in literature searches. Authors MHR, SM and VKV helped in final manuscript preparation. Author RN finally checked and approved the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/v35i430194
Editor(s):
(1) Dr. Hamid El Bilali, Centre for Development Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria.
Reviewers:
(1) Kana Sop Marie Modestine, University of Douala, Cameroon.
(2) Florin Sala, Banat University of Agricultural Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49289

Review Article

Received 12 March 2019
Accepted 23 May 2019
Published 30 May 2019

ABSTRACT

Micronutrients are important growth promoting elements not only for crops but also for human being. More than two billion of the global populations are malnourished. For developing countries like India, micronutrient malnutrition among the people of every age is very common. The impact is highly seen in poor and landless rural people who can't afford diverse foods or supplements in their diets with needed nutrients. To alleviate this micronutrient deficiency, biofortification has come to the surface as a potent option. Biofortification of crops can increase the level of micronutrients in final food products. Pulses are the cheapest sources of proteins, vitamins and micronutrients and can be supplied to the people through daily diet. Pulses are irrefutable contender for Biofortification since it is easily available to the each and every group of people. 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.

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Keywords: Biofortification; pulse; micronutrient; malnutrition; hunger.

1. INTRODUCTION

Worldwide, more than two billion of people or one in every three persons is spotted to be troubled with multiple micronutrient deficiencies [1]. Growing children are grievously affected by nutrient deficiencies compared to adults, as their nutrients requirement changes according to growth and developmental phages [2]. In Kolhapur district, 40% children between the age group of 8-9 years are micronutrients deficient (iron in 38.8% and fluoride in 36.6% respectively) [3] and globally it is 22% [4]. In the whole India, 18% of infants had a birth weight of less than 2.5 kg, 38% children below five years were under-weight, 28% mild, 29% moderately and 2% severely anaemic [5]. Malnutrition caused by vitamins and minerals is also known as "Hidden hunger", which don't give any visual symptom usually. As per GHI 2018,[6] India ranked 103rd among 119 countries while 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 [7,8,9,10].Those deficiencies are attributable to low intake of quality diet riched with proteins, vitamins and minerals [11,12]. Increased price of non staple commodities is one of the important reasons of decreasing dietary quality, especially to resource poor people [13]. In developing countries agricultural products are the prime source of nutrients [14,15]. 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 [11]. Micronutrient rich vegetables, pulses and animal products have also not been increased in last fifty years [12]. Possible ways to combat those deficiencies encircle dietary diversification (healthy balance diet), food fortification, biofortification and supplementation [16]. Biofortification is the process of increasing nutrient concentration in plant edible parts by fertilization (agronomic intervention), breeding approaches or microbes, [17] whereas forti-fication 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 [18,19]. Besides quality enhancement, micronutrient has some added advantages like yield increase, biomass enhancement and disease control in micronutrient deficient soils [20]. 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 [21,22,23] So, pulses can be considered as good option for biofortification to provide nutritious food sustainably [22].

2. ROLE OF MICRONUTRIENTS ON HUMAN HEALTH

Iron plays key role in haemoglobin formation and oxygen transport [24]. Iron deficiency exerts influence on learning ability, [25] immune system, [26] ability to work [27] and cognitive development [28]. Its deficiency is also associated with anemia and pregnancy related issues like mortality, low birth weight etc [25].

Zinc requirement get larger during pregnancy and puberty. Zinc deficiency curtails physical growth and development of children [29]. Gastrointestinal, central nervous, epidermal, immune, skeletal, and reproductive systems are known to be affected by zinc deficiency [30]. The daily requirement of Zn and Fe varies with the age of people (Table 1).

Table 1. Daily requirements of Zn and Fe in Indian context [39]

	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

Selenium is a good source of antioxidant which narrow down heart and skin diseases, cancer, alzheimer, [31,32,33,34,35], thyroid [36], asthma [37]. Patients having tuberculosis, influenza and hepatitis C delineated to be benefited by selenium [38].

3. CRITERIA OF BIOFORTIFIED CROP

Bouis and Welch [40] 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.

Quality: Good Taste and Cooking Quality

4. POTENTIAL WAYS OF BIOFORTIFICATION

Biofortification of crop can be done through agronomic, breeding and microbial interventions.

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 [41]. 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 [42,43]. Micronutrient amendment in soil is a useful strategy to increase micronutrient quantity in crop [44,45,46]. Among the different methods of application, foliar application is more efficient [47] as it can manage soil immobilization [11] and quick availability of nutrients to the crop. Hidoto et al. [48] 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 [49]. Other biofortification methods like seed priming and seed coating are spotted to give very infrequent result [50,23] 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 [51].

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 [52]. Molina et al. [53] 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. [54] 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 [55,56]. Zinc content in seed helps in significant liner increase of protein biosynthesis [57]. 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. [58] 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).

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 [59]. Ali et al. [60] 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. [61] 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 [63] 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. [64] pointed out that foliar spray of 0.05% Fe 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 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)[23] **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)*

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 al. 2015 [54]

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) [62]

4.1.3 Selenium fortification

Selenium fertilization by means of inorganic fertilizer results increased selenium concentration in diet [65,66]. Unlike selenite (SeO_3^{2-}), selenite (SeO_4^{2-}) provides immediate availability to plants when addedtosoil [67,68,69]. Selenium foliar application increases concentration in pea and common bean from 21 μg kg^{-1} to 743 μg kg^{-1} [70] and 30 to 2379 μg kg^{-1} [71] 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.

Table 5. Effect of zinc sulphate soil application on chickpea

Zn rate $ZnSO_4.7H_2O$ (kg ha^{-1})	Straw Zn (mgkg^{-1})	Grain Zn (mgkg^{-1})	Zn yield (gha^{-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 [58]

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[60]

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 [61]

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 [72]. 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 [73,74]. Zein protein over expression on soybean increases methionine and cysteine content [75] and methionine content by cystathionine γ-synthase, [76,77]. Increase in beta carotene and oleic acid in soybean has been attended by introducing bacterial PSY gene

[78] and siRNA-mediated gene silencing had been used to reduce α-linolenic acids [79]. Similarly, linoleic acid and palmitic acid content of soybean was reduced by antisense RNA technology [80]. Storage albumin of Brazil nut which is rich source of methionine has been used to increase common bean methionine content [81] whereas, lupines methionine has been intensified by albumin of Sunflower [82]. A sensitive approach to understand the escalated zinc uptake is DNA strand breakage [83].

Field trials regarding genetic effect on selenium concentration reported significant difference among genotypes [84,85,86]. 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 [87]. In contrast, lentil and chickpea revealed genotypic variation associated with selenium concentration in Saskatchewan [88,89,86,90]. Field experiments conducted in Morocco, Nepal, Syria, Australia and Turkey were also ensured significant genetic variance in lentil Se concentration [22]. Mungbean [91] and soybean [92] 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 [93,94,95].

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

	Treatment	Fe	B	Zn
		Mg kg^{-1}		
	Control, 0 ppm	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	Treatment and concentration	1.28	1.35	1.35
HSD	Interaction	2.61	2.94	2.94

Source: Salih, 2013[63]

Table 9. Several lentil released varieties that possess high iron and zinc levels (The 2nd Global Conference on Biofortification: Getting Nutritious Foods to People, AshutoshSarker (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	PusaVaibhav	102	---
	L4704	125	74
	IPL 220	73-114	51-64
	PusaAgetiMasoor	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. [72]

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.

4.3 Microbial Interventions

Phytoavailability of micronutrients can be increased by soil microorganisms like *Rhizobium*, *Bacillus*, *Pseudomonas*etc [96,97]. PGPR can be an alternate approach to biofortify pulses as it increases disease resistance [98,99], solubility of phosphorus [100,101] and root growth, [102,56]. But the implication of PGPR and other microorganisms in biofortification of pulses are sparse [103]. 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 [104,105].

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 [101].

Fungi and bacteria improves bioavailability of zinc at rhizosphere zone [106,107] due to decline in soil pH [108,109], chelation [110] and increased root sphere [111].

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).

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 supplements, 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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