Original Research Article

Iron and zinc bio-fortification through agronomic intervention in chickpea (*Cicer arietinum* L.)

Abstract

Essential micronutrients are required for humans to sustain proper physiological body processes and a healthy health condition. Micronutrient deficiencies, such as iron, zinc, and iodine, create global health concerns for people. Zinc and iron deficiency are frequent in chickpea growing regions across the world. Bio-fortification of pulse grains, especially with Fe and Zn by agronomic bio-fortification, is the simplest, most practical, and quickest method. During the *Rabi* season of 2019–20, a field experiment was done at the *Bairiya Dhab Research Farm of Dr. Rajendra Prasad Central Agricultural University*, Pusa, Samastipur, Bihar, India, to find out effect on chickpea genotypes when Zn and Fe were added to the soil and the leaves. Two genotypes (GCP-105 and GNG-2264) and seven fertilization treatments (control, 0.5% Zn, 0.5% Fe, soil application of Zn and Fe, 25 and 15 kg/ha, respectively) were used in a split plot design with three replications. The GNG-2264 variety had a significantly higher plant height, yield and yield characteristics, such as number of pods per plant, test weight, seed and straw yield and economics *viz.* gross return, net return (62860 and 37986 ₹/ha), and B: C ratio (2.52) than GCP-105. Among the fertilization treatments, RDF + Zn (0.5%) and Fe (0.5%) foliar spraying at the pre-flowering and pod development stage recorded significantly higher growth and yield parameters *viz.,* plant height (59.89 cm), number of pods/plant (22.31), seed yield (1283 kg/ha) and straw yields 2433 kg/ha), and quality parameters such as zinc and iron content in seed (31.84 and 17.40 ppm) and straw (72.26, 21.79 ppm, respectively) and economics in term of gross return, net return, and B: C ratio.

Keywords: Fabaceae, foliar spraying, nutritional security, nutrient enrichment, micronutrient and Diara land

1. Introduction

The land used for agricultural production covers 1.6 billion hectares (12%) of the 13.2 billion hectares of global land [1]. In fact, the global population is projected to grow from just over 7.7 billion todays to 10 billion by 2050 [2]. Diara (reverine land) is a place located between river embankments and, depending on the languages and dialects of the locals, is known by various names in different regions [3]. Diaras have several other local names, such as Khadar, Kachhar, Dhab, Dariyari, Kochsr, Nad, Tali and Nadiari. Diara is the area that forms a natural catchment for the monsoon floods and the drainage waters. People often think of Diara land as an ecosystem and think it has a lot of potential for farming [4].

Asian countries have more Zn malnutrition where cereals are the staple food of the people, but cereals contain lower Zn and phytates, which retard the bioavailability of Zn [5]. Zinc deficiency leads to stunting the height of children under five. The gastrointestinal, epidermal, central nervous system, skeletal, and reproductive systems are the three organs most clinically affected by zinc deficiency [6]. Zinc is essential for healthy pregnancies because it supports immune functions and helps people resist infectious diseases like diarrhoea, pneumonia, and malaria [7]. Inadequate dietary intake places 17.3% of the population at risk for zinc deficiency; in some parts of the world, this number rises to as high as 30% [8]. One of the most common causes of anaemia, which is characterised by a deficient amount of haemoglobin, is a lack of iron. Anaemia affects 50% of children under the age of five and 30% of expectant mothers worldwide [9]. Due to the limits of the data that is currently available, estimates of various multi-nutrient deficiencies have proven to be challenging [10]. According to [11] estimation global hidden hunger indices for Fe, vitamin A, and zinc and discovered that near about 20 countries have high levels of hidden hunger. However, a shortage of other micronutrients, including selenium, iron, or vitamin A, might exacerbate iodine insufficiency by altering how the thyroid functions [12].

Soil zinc deficiency is common in major chickpea-producing countries [13], which is the leading cause of reduced yields in many agronomic crops grown around the world. In addition, Zn is essential for the growth and development of plants. The rate of germination and the final germination can also be improved by making the seeds germinate faster and giving seedlings more strength [14]. Zinc plays a role in metabolic processes, including proteins, carbohydrates, nucleic acids, and lipid synthesis and degradation [15] as well as pollen functionality and fertilization [16]. Zinc improves water use efficiently, enhances the nodulation process and nitrogen fixation [17]. Foliar Zn application after flowering effectively increases Zn content in rice grain [18].

Biofortification by agronomic intevention with Zn-containing fertiliser application to crops is not a permanent solution and a complementary approach to breeding. This approach also improves rhizospheric Zn levels [19]. In the field, foliar spray of zinc or soil with foliar zinc applications are effective and practical ways to get the most zinc into the grain and keep it there [20]. Due to this backdrop, the need is increasing to find a method to bio-fortify nutrient minerals such as Zn and Fe by effective agronomic interventions in riverine (*Dhab*) areas of the world to increase micronutrient content in seeds of crops like chickpea for mitigating micronutrient malnutrition. Therefore, keeping the above given facts in mind, the present research study entitled “Iron and zinc bio-fortification through agronomic intervention in different varieties of chickpea under Riverine lands.”

2. Material and methods

The present field research, conducted at *Bairiya Dhab Research Farm of Dr. Rajendra Prasad Central Agricultural University, Pusa,*is located on the southern bank of the river *Burhi Gandak* in the district of Samastipur, Bihar at 25.98° North latitude and 84.69° East longitude, with an altitude of 52.92 m above the mean sea level. The soil of this riverine (*Dhab*) area was recently developed due to the sedimentation brought about by the *Burhi Gandak* river. The soil of the field was sandy-loam, having medium organic carbon (0.69%), available nitrogen (268.3 kg/ha), phosphorus (20.4 kg/ha), potassium 112.5 kg/ha, zinc 0.54 ppm, iron 23.41 ppm, and EC 0.18 dS/m with pH 7.63.

The experiment, laid out in a split plot design, consisted of two chickpea varieties (V_1: GCP-105 and V_2: GNG-2264) and seven nutrient fortification treatments of Zn and Fe, T_1: Control (recommended dose fertilizer), T_2: RDF + 0.5% Zn foliar spray, T_3: RDF + 0.5% Fe foliar spray, T_4: RDF + Zn (0.5%) and Fe (0.5%) foliar spray, T_5: RDF + soil basal application of $FeSO_4$ (15 kg/ha), T_6: RDF + soil basal application of $ZnSO_4$ (25 kg/ha) + $FeSO_4$ (15 kg/ha), T_7: RDF + soil application of $ZnSO_4$ (25 kg/ha) basal application (recommended practice) laid out in a split plot design with 3 replications.

Carbendazim (3g/kg) was applied to seeds initially, then chlorpyriphos (10EC) (6ml/kg seed), then strains of rhizobium (250g/10kg) to increase atmospheric nitrogen fixation and encourage the development of root nodules in the crop. The recommended dose of nitrogen, phosphorus, and potash (20:45:20 kg/ha, respectively) were given through DAP and MOP as a basal application. Sowing operations were carried out by opening furrows with liners with a keeping distance of 30 cm. Chickpea seeds were sown by the *Kera* method with using seed rate of 80 kg/ha. The bio-fortification treatments of zinc and iron were given by zinc sulphate ($ZnSO_4.7H_2O$) and ferrous sulphate ($FeSO_4.7H_2O$) at pre-flowering and pod development stages of chickpea. For weed management was done by using, pendimethalin 1 kg/ha as pre-emergence after 24 hours of sowing of the experimental crop and one hand weeding was done with *khurpi* 30 days after the crop was planted to keep the plots free of weeds.

3. Result and discussion

3.1 Effect of Fe and Zn fortification on growth and yield attributes of chickpea

Chickpea varieties differed significantly in terms of their growth and yield parameters like plant height, pods/plant, test weight, seed and straw yield (**Table 1**). Plant height and the number of branches/plant were significantly higher in GNG-2264 than in GCP-105. Among the different treatments for fortification of zinc and iron, the treatment T_4 (RDF + Zn (0.5%) and Fe (0.5%) foliar spray at pre-flowering and pod development stage significantly increases the height of the plant at harvest stage and the number of branches/plant over the all other treatments. The positive effects of foliar Zn application on chickpea higher branch number and seed yield were noted by Hadi *et al*. [21]. Kayan *et al.* [22] compared the comparative effects of 0, 0.2, 0.4, 0.6, and 0.8% Zn levels of zinc chelate (Zn-EDTA; 8% Zn) and zinc sulphate (23% Zn) applied as foliar sprays for treating zinc deficiency in chickpea and found that 0.8% Zn levels of zinc recorded the highest plant height (41.3 cm), but the control recorded the lowest (40.7 cm).

Although the pattern of test weight (g) for different varieties of chickpea was found to be significant, GNG-2264 produced significantly higher pods/plant, test weight, seed, and straw yield than GCP-105. Application of different zinc and iron treatments didn't make a big difference on test weight (g) of chickpea. However, T_4 treatment (RDF + Zn (0.5%) and Fe (0.5%) foliar spray produced significantly higher test weight, seed, and straw yield than either soil or foliar spray alone during the pre-flowering and pod development stages. Purushottam *et al*. [23] found that applying 0.5% Zn foliar spray to chickpea increased grain yield by 40.9% when compared to the control.

The above result might be due to foliar spray of Zn and Fe that is highly bioavailable by the aerial portion of the plant and is translocated to the growing tip, stimulating auxin activity at the crop's growing point and improving shoot development. Because of its role in controlling chlorophyll and carotenoid synthesis [24], zinc is an essential element for the optimum functioning of the photosynthetic system. Foliar application of zinc sulphate significantly increases the yield attributes in lentil [25] and iron sulphate in pigeon pea [26].

3.2 Effect of Fe and Zn fortification on Zn concentration of chickpea seed and straw

Varieties were shown a significant difference on the zinc content in seed and straw. Between the two varieties, GNG-2264 registered significantly higher zinc content in seed and straw than GCP-105. Significantly higher values of zinc content in seed and straw were observed under treatment (RDF + Zn (0.5%) and Fe (0.5%) foliar spray at pre-flowering and pod development stages) compared to the other treatments. Foliar Zn application (0.5%) increased chickpea grain Zn content by 27.5% as compared to control [23]. Ram *et al*. [27] also reported that foliar application of Zn on common bean (*Phaseolus vulgaris* L.) increased from 68 to 78 mg kg^{-1} Zn concentration in grain. The treatments $T_{6,}$ *i.e*., (RDF + soil application of $ZnSO_4$ 25 kg/ha + $FeSO_4$ 15 kg/ha) and T_7 (RDF + soil application of $ZnSO_4$ 25 kg/ha) were at par with the treatment T_4 in realizing the content of Zn in seed and straw over control.

In the above-mentioned treatment, there was more zinc in the root zone, which may have increased nutrient uptake, improved nutrient absorption, and zinc's beneficial role in increasing the roots' cation exchange capacity and stimulating most of the plant's physiological and metabolic processes. It was found that the effect of zinc and iron on both varieties and fortification treatments did not make a difference.

3.3 Effect of Fe and Zn fortification on Fe concentration of chickpea seed and straw

Due to soil and foliar treatment of Zn and Fe, Fe concentrations in chickpea seeds varied significantly (**Table 2**). Between the two varieties, GNG-2264 had a significantly higher content of iron in seed and straw. RDF+ Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development stage) had the highest iron content in seed and straw, while T_1 treatment (recommended NPK dose) had the lowest iron content in seed and straw. This could be due to increased iron supply through soil and foliar application, establishment of Fe pools in soil, and foliar spraying of Fe, which easily penetrates through leaves either by transportation or via stomatal pathway, explaining the higher Fe content in seeds in these treatments. Sharma *et al*. [28] also reported that foliar application of iron sulphate significantly increases the consentration in pigeon pea grains.

Table 1. Effect of Fe and Zn fortification on growth and yield attributes of chickpea

Sr. No.	Treatments	Plant height at harvest (cm)	Pods/plant	Test weight (g)	Seed yield (kg/ha)	Straw yield (kg/ha)
	Variety					
V_1	GCP-105	53.78	17.92	279	973	2038
V_2	GNG-2264	56.12	20.48	297	1199	2194
	SEm±	0.08	0.04	1.24	32.71	1.67
	CD (*P = 0.05*)	0.50	0.28	8.16	214.26	10.14
	Fortification treatments					
T_1	Recommended dose of fertilizer NPK	50.15	15.8	285	845	1680
T_2	RDF + Zn (0.5%) foliar spray at pre-flowering and pod development stage	53.48	18.2	287	1115	2095
T_3	RDF + Fe (0.5%) foliar spray at pre-flowering and pod development stage	53.25	17.6	289	938	2005
T_4	RDF + Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development stage	59.89	22.31	290.5	1283	2433
T_5	RDF + $FeSO_4$ (15 kg/ha) soil application	52.50	19.05	287	1068	2049
T_6	RDF + $ZnSO_4$ (25 kg/ha) + $FeSO_4$ (15 kg/ha) soil application	58.24	21.16	289	1211	2351
T_7	RDF + $ZnSO_4$ (25 kg/ha) soil application	57.18	20.3	289	1143	2197
	SEm±	0.26	0.13	7.59	49.15	3.38
	CD (*P = 0.05*)	0.77	0.39	NS	144.30	9.86
	Variety × Fortification treatments					
	SEm±	3.29	2.23	1.96	152.12	263.08
	CD (*P = 0.05*)	NS	NS	NS	NS	NS

Table 2. Effect of Fe and Zn fortification on Zn and Fe concentration of chickpea seed and straw

Sr. No.	Treatments	Zn content (ppm)		Fe content (ppm)	
		Seed	Straw	Seed	Straw
	Variety				
V_1	GCP-105	26.78	13.61	51.30	15.07
V_2	GNG-2264	33.98	17.54	88.97	25.12
	SEm±	0.12	0.09	0.23	0.05
	CD (*P = 0.05*)	0.75	0.55	1.43	0.34
	Fortification treatments				
T_1	Recommended dose of fertilizer NPK	29.45	13.29	67.99	18.77
T_2	RDF + Zn (0.5%) foliar spray at pre-flowering and pod development stage	30.45	14.78	68.25	19.35
T_3	RDF + Fe (0.5%) foliar spray at pre-flowering and pod development stage	29.10	15.21	71.14	20.39
T_4	RDF + Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development stage	31.84	17.40	72.26	21.79
T_5	RDF + $FeSO_4$ (15 kg/ha) soil application	29.22	15.41	71.24	20.04
T_6	RDF + $ZnSO_4$ (25 kg/ha) + $FeSO_4$ (15 kg/ha) soil application	31.26	16.69	71.97	21.00
T_7	RDF + $ZnSO_4$ (25 kg/ha) soil application	31.36	16.25	68.1	19.34
	SEm±	0.29	0.09	0.32	0.07
	CD (*P = 0.05*)	0.84	0.29	0.95	0.20
	Variety × Fortification treatments				
	SEm±	0.98	1.43	1.47	1.09
	CD (*P = 0.05*)	NS	NS	NS	NS

3.4 Effect of Fe and Zn fortification on economics

The economics of different treatments were worked out in the cultivation cost, gross return, net return, and benefit to cost ratio (B: C ratio) for chickpea varieties. The data obtained has been given in Table 3 showed that variety GNG-2264 produced the highest gross returns, net returns, and B: C ratio while GCP-105 produced the lowest gross returns, net returns, and B: C ratio.

When compared Fe and Zn fortification treatments, treatment T_4 (RDF + Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development) had the highest gross returns, net returns, and B: C ratio followed by treatment T_6 (RDF + soil application of $ZnSO_4$ 25 kg/ha + $FeSO_4$ 15 kg/ha). Higher yield under this treatment was due to soil fertilizer application, which may have resulted in a higher nutrient concentration in the root zone. While direct foliar fertilizer spraying might have led to more nutrients being absorbed, better photosynthetic activity and its spread to different parts of the plant, more growth and yield-related traits, and, in the end, higher yields, gross returns, net returns, and the B:C ratio. Rathore *et al.* [29] observed that biofortification with Zn (0.5%) + Fe (0.1%) treatment, was recorded higher gross return (52829 ha), net return (31179 ha) and benefit cost ratio (2.44) in Chickpea.

4. Conclusion

The study indicated that for the GNG-2264 variety of chickpea, applying the recommended dosage of fertilizers (RDF) together with a foliar spray of 0.5% Zn and 0.5% Fe was the best strategy for increasing nutrient concentration in chickpea grains as well as increasing yield, net returns and B: C ratio.

Table 3. Effect of Fe and Zn fortification on economics

Sr. No.	Treatments	Cost of cultivation (₹/ha)	Gross return (₹/ha)	Net return (₹/ha)	B:C ratio
	Variety				
V_1	GCP-105	24875	51499	26624	2.06
V_2	GNG-2264	24875	62860	37986	2.52
	SEm±	-	562.72	562.72	0.02
	CD (*P = 0.05*)	-	3424.11	3424.11	0.13
	Fortification treatments				
T_1	Recommended dose of fertilizer NPK	24217	44555	20337	1.83
T_2	RDF + Zn (0.5%) foliar spray at pre-flowering and pod development stage	24342	58539	34197	2.40
T_3	RDF + Fe (0.5%) foliar spray at pre-flowering and pod development stage	24367	49743	25376	2.04
T_4	RDF + Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development stage	24492	67419	42926	2.75
T_5	RDF + $FeSO_4$ (15 kg/ha) soil application	25117	56141	31023	2.23
T_6	RDF + $ZnSO_4$ (25 kg/ha) + $FeSO_4$ (15 kg/ha) soil application	26242	63738	37495	2.42
T_7	RDF + $ZnSO_4$ (25 kg/ha) soil application	25342	60122	34780	2.37
	SEm±	-	898.29	898.29	0.03
	CD (*P = 0.05*)	-	2621.93	2621.93	0.10
	Variety × Fortification treatments				
	SEm±	-	7762.87	7603.17	0.37
	CD (*P = 0.05*)	-	NS	NS	NS

References:

[1] FAO, World Agriculture: Towards 2015/2030, Summary Report, Food and Agriculture Organization, Rome;2015.

[2] USCB. U.S. Census Bureau, international Data Base, June 2019 update. 2019.

[3] Kumari R, Sharma A, Shikha B, Kumar R. River Bed Cultivation: A Kind of Vegetable Forcing for Remunerative Returns. International Journal of Current Microbiology and Applied Sciences. 2018;7(04):2319-7706.

[4] Project Proposal on Scaling up Climate Smart Agriculture (CSA) through mainstreaming Climate Smart Villages (CSVs) in Bihar, (November, 2016); Department of Agriculture, Government of Bihar.

[5] Welch RM, Graham RD. Breeding for micronutrients in staple food crops from human nutrition prospective. Journal of Experimental Botany. 2004;55:353-364.

[6] Roohani N, Hurrell R, Kelishadi R, Schulin, R. Zinc and its importance for human health: An integrative review. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2013;18(2):144.

[7] Ackland ML, Michalczyk AA. Zinc and infant nutritionexternal icon. Arch Biochem Biophys. 2016;611:51-57.

[8] Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stuntingexternal icon. *PLoS One*. 2012;7(11):e50568.

[9] World Health Organization. WHO global anaemia estimates, 2021 editionexternal icon. Accessed June 3, 2021.

[10] Bailey RL, West Jr. KP, Black RE. The Epidemiology of Global Micronutrient Deficiencies. Annals of Nutrition and Metabolism. 2015;66(2):22-33.

[11] Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: an advocacy tool for action. PLoS One. 2013;8:e67860.

[12] Zimmermann MB, Jooste PL, Mabapa NS, *et al*. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. Am Journal of Clinical Nutrition. 2007;86:1040-1044.

[13] Ullah A, Farooq M, Hussain M, Ahmad R, Wakeel A. Zinc seed coating improves emergence and seedling growth in desi and kabuli chickpea types but shows toxicity at higher concentration. International Journal of Agriculture and Biology. 2019;21:553–559.

[14] Rehman A, Farooq M; Ahmad R, Basra SMA. Seed Sci. & Technol. Seed priming with zinc improves the germination and early seedling growth of wheat. 2015;43:262-268.

[15] Nishizawa NK. The uptake and translocation of minerals in rice plants. In 'Rice is life: scientific perspectives for the 21st century. Proceedings World Rice Research Conference'. 4–7 November 2004, Tsukuba, Japan. 2005;90–93 (International Rice Research Institute: Manila).

[16] Pandey N, Pathak GC, Sharma CP. Zinc is critically required for pollen function and fertilisation in lentil. Journal of Trace Elements in Medicine and Biology. 2006;20:89–96.

[17] Khan HR, McDonald GK, Rengel Z. Zinc fertilization and water stress affects plant water relations, stomatal conductance and osmotic adjustment in chickpea (*Cicer arietinum* L). Plant and Soil. 2004;267(1-2):271-284.

[18] Boonchuay P, Benjavan I, Chanakan R, Thai P. Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. Soil Science and Plant Nutrition. 2013;59(2):180-188.

[19] Ullah A, Farooq M, Rehman A, Hussain M, Siddique HM. Zinc nutrition in chickpea (*Cicer arietinum*): a review. Crop & Pasture Science. 2020,;71:199–218.

[20] Tabassum S, Jeet S, Kumar R, Dev C, Kumar P, Rehana R. Effect of organic manure and zinc fertilization on zinc transformation and biofortification of crops in vertisols of central India. The Journal of Agricultural Science. 2014;6:221.

[21] Hadi MRHS, Bazargani P, Darzi MT. Effects of irrigation treatment and zinc foliar application on yield and yield components of chickpea (*Cicer arietinum* L.). International Journal of Farming and Allied Sciences. 2013;2:720–724.

[22] Kayan N, Gulmezoglu N, Kaya MD. The optimum foliar zinc source and level for improving Zn content in seed of chickpea. Legume Research. 2015;38:826–831.

[23] Purushottam B, Puhup K, Kumar C, Sodi B. Effect of irrigation scheduling and zinc application on chlorophyll content, zinc content, uptake and yield of chickpea (*Cicer arietinum* L.). Journal of Pharmacognosy and Phytochemistry. 2018;7:1834–1837.

[24] Rehman A, Farooq M, Asif M, Ozturk L. Supra-optimal temperature exacerbates adverse effects of low Zn supply in wheat. Journal of Plant Nutrition and Soil Science. 2019;182:656–666.

[25] Verma CB, Pyare R, Aslam M, Verma VK, Singh V, Sharma H. Enhancing growth, yield and quality of lentil through foliar spray of zinc, urea and thiourea under rainfed condition. *Agriways*. 2017;5(2):123-127.

[26] Sharma A, Nakul HT, Jelgeri BR, Surwenshi A. Effect of micronutrients on growth, yield and yield components in pigeon pea [*Cajanus cajan* (L.) Millsp.]. *Research Journal of Agricultural Sciences.* 2010;1:142- 144.

[27] Ram H, Rashid A, Zhang W, Duarte AP, Phattarakul N, Simunji S, *et al.* Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. Plant and Soil. 2016;403:389-401.

[28] Sharma A, Nakul HT, Jelgeri BR, Surwenshi A. Effect of micronutrients on growth, yield and yield components in pigeon pea [*Cajanus cajan* (L.) Millsp.]. Research Journal of Agricultural Sciences. 2010;1:142- 144.

[29] Rathore PS, Patil DS, Bellad SB, Haveri RV. Biofortification of Zn and Fe in Chickpea through Agronomic Intervention in Medium Black Soils of Karnataka. Legume Research. 2021; 10.18805/LR-4341.

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