

Original Research Article

Iron and zinc bio-fortification through agronomic intervention in chickpea

Abstract

Bio-fortification, or the practice of incorporating nutrients into food crops, is a long-term and cost-effective technique to boost micronutrient density in critical staple crops. For 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 *BairiyaDhab 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 chickpea variety had a significantly higher plant height (56.12 cm) than GCP-105. GNG-2264 had the highest yield and yield characteristics, such as number of pods per plant, test weight, and higher seed and straw yield. 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 (1283kg/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, 21.79ppm, respectively). However, the control treatment recorded the lowest values of plant growth, yield and quality parameters. Variety GNG-2264 outperformed over the variety of GCP-105 in terms of gross return, net return (62860 and 37986 ₹/ha), and B: C ratio (2.52). The treatment RDF + Zn (0.5%) and Fe (0.5%) foliar spraying at the pre-flowering and pod development stages had the highest gross return, net return, and B: C ratio, followed by RDF + soil application of ZnSO₄ 25 kg/ha + FeSO₄ 15 kg/ha.

Keywords: Bio-fortification, foliar spraying, Zn and Fe, chickpea, FeSO₄, ZnSO₄

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 today to 10 billion by 2050 [2]. *Diara* lands are located between the levees of the river and are created by regular erosion and the deposition of sediments (alluvium) caused by the meandering and changes in the actions of the rivers. Riverine lands are known by many local names like *dhab-land*, *char-land*, *daryayi-*

Comment [U1]: abstract is still long, generally 250-300 words

Comment [U2]: Describe the formulation of the problem, so that it can be seen the importance of this research

land, majha-land, and kachhar-land in different regions of India. This land is considered one of the most useful natural resources by properly managed, such areas can make a significant contribution to the economies of the people who are living in such riverine (*Dhab*) areas and also the economy of the country. Chickpea (*Cicerarietinum* L.) is a cool-season crop and the most important leguminous pulse crop widely grown for human consumption. Essential micronutrients are also needed for human beings to have full normal physiological body functions and to maintain a healthy health status. Micronutrients deficiency such as iron, zinc, and iodine cause health problems for humans at a global level. For human well-being, 22 mineral elements are required. Deficiency of zinc is common in the world's chickpea cultivating areas and is the most severe among the different micronutrients. 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. 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 [3]. Iron has an important role in the sequence of micronutrient malnutrition. Iron deficiency as a prevalent nutritional disorder impacting 2.5 to 5 billion people worldwide [4]. Iron deficiency reduces the amount of oxygen delivered to cells, resulting in weariness, poor work performance, lowered immunity, and mortality [5]. In addition, Zn is essential for the growth and development of plants. Germination of seed and vigour of seedlings can also be significantly increased [6]. Zinc plays a role in metabolic processes, including proteins, carbohydrates, nucleic acids, and lipid synthesis and degradation. Zinc improves water use efficiently, enhances the nodulation process and nitrogen fixation [7].

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 *BairiyaDhab Research Farm of Dr. Rajendra Prasad Central Agricultural University, Pusa*, is located on the southern bank of the river *BurhiGandak* 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 *BurhiGandak* 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

Comment [U3]: literature source

dose fertilizer), T₂: RDF + 0.5% Zn foliar spray, T₃: RDF + 0.5% Fe foliar spray, T₄: RDF + Zn (0.5%) and Fe (0.5%) foliar spray, T₅: RDF + soil basal application of FeSO₄ (15 kg/ha), T₆: RDF + soil basal application of ZnSO₄(25 kg/ha) + FeSO₄(15 kg/ha), T₇: RDF + soil application of ZnSO₄ (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 chlorpyrifos (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 (ZnSO₄.7H₂O) and ferrous sulphate (FeSO₄.7H₂O) 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 T₄(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.

Although the pattern of test weight (g) for different varieties of chickpea was found to be significant, GNG-2264 produced significantly higher pods/plant (20.48), test weight (297 g), seed (1199kg/ha), and straw yield (2194 kg/ha) than GCP-105. Application of different zinc and iron treatments didn't make a big difference on test weight (g) of chickpea. However, T₄treatment (RDF + Zn (0.5%) and Fe (0.5%) foliar spray produced significantly higher test weight (290.5g), seed (1283 kg/ha), and straw yield (2433 kg/ha) than either soil or foliar spray alone during the pre-flowering and pod development stages.

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. Verma *et al.* [8] discovered similar results. Also zinc is involved in the photosynthetic process, which provides a substrate for growth and development, both directly and indirectly as a coenzyme. Sharma *et al.* [9] found the same thing with pigeonpea and groundnut, respectively.

Comment [U4]: give an analysis, why the result like this?

Next, give a comparison with the results of other researchers' research

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

Comment [U5]: recheck the way the table is presented

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 developm ent stage	53.48	18.2	287	1115	2095
T ₃ - RDF+Fe (0.5%) foliar spray at pre- flowering and pod developm ent stage	53.25	17.6	289	938	2005
T ₄ - RDF+Zn(0.5%)+ Fe(0.5%) foliar spray at pre- flowering and pod developm ent 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 (25kg/ha) + FeSO ₄ (15kg/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 x Fortification treatments					
SEM_±	3.29	2.23	1.96	152.12	263.08

<i>CD (P = 0.05)</i>	NS	NS	NS	NS	NS
----------------------	----	----	----	----	----

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 (33.98 ppm) and straw (17.54 ppm) than GCP-105. Significantly higher values of zinc content in seed (31.84 ppm) and straw (17.40 ppm) 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. Ram *et al.* [10] got the same results with the common bean (*Phaseolus vulgaris* L.). The treatments T₆, *i.e.*, (RDF + soil application of ZnSO₄ 25 kg/ha + FeSO₄ 15 kg/ha) and T₇ (RDF + soil application of ZnSO₄ 25 kg/ha) were at par with the treatment T₄ in realising 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 (88.97 ppm) and straw (25.12 ppm). RDF+ Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development stage) had the highest iron content in seed (72.26 ppm) and straw (21.79 ppm), while T₁ treatment (recommended NPK dose) had the lowest iron content in seed (67.99 ppm) and straw (18.77 ppm). 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.

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

Treatments	Zncontent (ppm)		Fecontent (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

Comment [U6]: recheck the way the table is presented

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 (62860 ₹/ha), net returns (37986 ₹/ha), and B: C ratio (2.52) while GCP-105 produced the lowest gross returns (51499 ₹/ha), net returns (26624 ₹/ha), and B: C ratio (2.06).

When compared Fe and Zn fortification treatments, treatment T₄ (RDF + Zn (0.5%) + Fe (0.5%) foliar spray at pre-flowering and pod development) had the highest gross returns (67419₹/ha), net returns (42926₹/ha), and B: C ratio (2.75), followed by treatment T₆ (RDF + soil application of ZnSO₄ 25 kg/ha + FeSO₄ 15 kg/ha). Higher yield under this treatment was due to soil fertiliser application, which may have

resulted in a higher nutrient concentration in the root zone. While direct foliar fertiliser 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.

Table 3. Effect of Fe and Zn fortification on economics

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

4. Conclusion

The study indicated that for the GNG-2264 variety of chickpea, applying the recommended dosage of fertilisers (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.

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. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from human nutrition prospective. *Journal of Experimental Botany*, **55**:353-364; 2004.
4. Yip R. Prevention and control of iron deficiency: policy and strategy issues. *Journal of Nutrition*, **132**(4): 802-805; 2002.
5. Jones DL, Darah PR, Kochian LV. Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. *Plant and soil*. **180**: 57-66; 1996.
6. Auld DS. Zinc coordination sphere in biochemical zinc sites. *Biometals*, **14**: 271-313; 2001.
7. Khan HR, McDonald GK, Rengel Z. Zinc fertilization and water stress affects plant water relations, stomatal conductance and osmotic adjustment in chickpea (*Cicerarietinum* L). *Plant and Soil*, **267**(1–2): 271–284; 2004.
8. 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. *Agricways*. **5** (2): 123-127; 2017.
9. Sharma A, Nakul HT, Jelgeri BR, Surwenshi A. Effect of micronutrients on growth, yield and yield components in pigeonpea [*Cajanuscajan* (L.) Millsp.]. *Research Journal of Agricultural Sciences*. **1**: 142- 144; 2010.

Comment [U7]: Reference has not used the medeley or zatero application. the number of references used is also very small

Comment [U8]: use the latest literature, last five years will be better

Comment [U9]: use the latest literature, last five years will be better

10. 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*, **403**: 389-401; 2016.

UNDER PEER REVIEW