Original Research Article

Effect of agronomic biofortification of zinc and iron on plant height and seed yield of chickpea (*cicer arietinum* L.) varieties

Abstract

The present field experiment was conducted to study the effect of agronomic biofortification of zinc and iron on chickpea during *Rabi* season of 2021-22 and 2022-23 at the instructional farm, College of Agriculture, Jodhpur. The field experiment was laid out in split plot design comprised two varieties of chickpea ('GNG-2144' and 'GNG-2171') and three levels of iron fortification treatment including control (F_0), 20 kg $FeSO_4$ (SA) + 0.5 % $FeSO_4$ (F_1) and 25 kg $FeSO_4$ (SA) + 0.5 % $FeSO_4$ (F_3) in the main plot and four-levels of zinc fortification *viz.* control (Z_0), ZSB (SI) + 15 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_1), ZSB (SI) + 20 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_2) and ZSB (SI) + 25 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_3) in sub-plot. These three experimental variables make twenty-four treatment combinations were taken as experimental factors to study their effect on biofortification in chickpea. The results revealed that variety GNG-2144 recorded higher plant height and higher seed yield over variety GNG-2171. Among Iron levels treatment 25 kg $FeSO_4$ (SA) + 0.5 % $FeSO_4$ (F_3) significantly recorded higher seed yield as compare to 20 kg $FeSO_4$ (SA) + 0.5 % $FeSO_4$ (F_1). Moreover, Zinc fortification treatment ZSB (SI) + 25 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_3) was found significantly superior over the treatment ZSB (SI) + 15 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_1) and at par with ZSB (SI) + 20 kg $ZnSO_4$ (SA) + 0.5 % $ZnSO_4$ (Z_2).

Comment [EC1]: Further reflection and improvement is needed

Keywords: Agronomic biofortification, chickpea, zinc, iron

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the most important *rabi* season pulse crop in India. It belongs to sub-family '*Papilionaceae*' under the family '*Fabaceae*'. It is a diploid species having chromosome number 2n=16. It is a self-pollinated legume crop having extensive geographical distribution. It is known by different names in country such as Gram, Chana, Bengal gram etc. Chickpea is a source of amino acid, protein and it plays an crucial role in human nutrition. Chickpea valued for its nutritive seeds with high protein

Comment [EC2]: Replace with a

content 18-22%, fat 4-10%, carbohydrate 52-70%, minerals viz., phosphorus (30 mg/100g), iron (12.8 mg/100g) and calcium (280mg/100g) and vitamins. Chickpea is an important crop for vegetarian people as primary source of protein, it is third most important pulse crop grown in the world after dry beans and peas (Kaur *et al.*, 2020). Biofortification word derived from Greek language "*bios*" means "*life*" and Latin word "*fortificare*" means "*making strong*". It can be defined as the process of increasing the concentrations of certain micronutrients in edible portions of crop plants naturally by application of mineral fertilizers *i.e.* agronomic approaches or through conventional breeding approaches (Majeed *et al.*, 2020). According to the World Health Organization on worldwide prevalence of micronutrient deficiencies, zinc deficiency ranked 11th amongst twenty most important factors in the world, whereas zinc and iron deficiency ranks 5th and 6th, respectively, amongst ten most important factors in developing countries (Cakmak, 2008). The countries suffering from vitamin A, iron and iodine inadequacy are India, Pakistan, China, Bangladesh, Central Africa, Iran and Turkey. However, zinc deficient countries are India, Pakistan, China, Iran and Turkey (Lockyer *et al.*, 2018). Zinc is one of the 8th essential trace elements require for growth and reproduction of plants. Zinc enriched finger proteins are required in signal transduction, regulation and transcription of deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) or other proteins in the plant. Its deficiency causes poor synthesis of phytohormones *viz.* auxins, gibberellins and cytokinins resulted in lesser growth and development of crop (Hassan *et al.*, 2020). Zinc involved in the root nodulation of plant and enables to the pulse crops to fix inert nitrogen in the root nodule. It is also participating in the signal transduction during stress condition in the plant system. Similarly, iron (Fe) plays an important role in chlorophyll synthesis and act as structural component of hemes, hematin and leghaemoglobin involved in the nitrogen fixation in pulses catalysed by an enzyme called 'nitrogenase' (Larson *et al.*, 2018). Moreover, iron is the most essential micronutrient for plant growth especially for chickpea grown on saline and alkaline soils. Although, ubiquitous presence of iron in earth's crust, but low solubility make it lesser availability and finally poor uptake by crops. Similarly, saline and alkaline soils are also deficient in iron, which results in the chlorosis of leaves that reduces photosynthetic potential of chickpea and fails to complete its pod or grain formation ultimately pods may remain empty (Vadlamudi *et al.*, 2020).

Comment [EC3]: Provide Citation

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Comment [EC5]: Provide citation

Comment [EC6]: This sentence needs further improvements

Comment [EC7]: Provide citation

Materials and Methods

The field experiment was conducted at Instructional farm, college of Agriculture, Jodhpur. The field experiment was laid out in split plot design with three replications. It comprised 24 treatment combinatioins with three replications. investigation comprised with two varieties of chickpea ('GNG-2144' and 'GNG-2171') and three levels of iron fortification treatment including control (F_0), 20 kg $FeSO_4$ (SA) + 0.5% $FeSO_4$ (F_1), 25 kg $FeSO_4$ (SA) + 0.5% $FeSO_4$ (F_2) in the main-plot and four-levels of zinc fortification *viz.* control (Z_0), ZSB (SI) + 15 kg $ZnSO_4$ (SA) + 0.5% $ZnSO_4$ (Z_1), ZSB (SI) + 20 kg $ZnSO_4$ (SA) + 0.5% $ZnSO_4$ (Z_2) and ZSB (SI) + 25 kg $ZnSO_4$ (SA) + 0.5% $ZnSO_4$ (Z_3) in sub-plot. These three experimental variables make twenty-four treatments combinations were taken as experimental factors to study their effect on biofortification in chickpea. The different doses of $FeSO_4$ were applied at the time of sowing into the soil and foliar application (0.5% $FeSO_4$) was done at 50 DAS of experimental crop. In zinc fortification treatment, the seed was inoculated with ZSB and the different doses of $ZnSO_4$ was also applied at the time of sowing, however, the foliar spray of 0.5% $ZnSO_4$ was done at flower initiation stage of chickpea during *Rabi* season 2021-22 and 2022-23.

Comment [EC8]: Summaries this sentences

2.1 Plant height (cm)

Height of the plant is one of the major growth attributes and is measured from base of soil to the toped leaf. Accordingly, the height of five tagged plants was measured in centimeter (cm) from ground level to the tallest leaf of the plant at 30, 60, 90 DAS and at harvest, finally average mean of height was recorded.

Comment [EC9]: What was used to measure the height of the plant?

2.2 Seed yield (kg/ha)

After winnowing, cleaned seeds were weighed to record seed yield per plot. The moisture percentage in 100 g samples drawn from each treatment were recorded with the help oven dry method and thereafter, the yield thus obtained was adjusted to 12 per cent moisture and finally the seed yield of net plot (3.0 m × 4.0 m) was converted into kg/ha.

Comment [EC10]: This sentence is not clear

Comment [EC11]: What was used for weighing?

Results

3.1 Plant height (cm)

The data on mean plant height pertaining to the different treatments recorded at 30, 60, 90 DAS and at harvest stage of chickpea were significantly affected by variety and zinc fortification, while iron fortification did not affect plant height at 30 and 60 DAS and zinc fortification at 30 DAS did not affect plant height significantly during the years as well as pooled analysis. (Table 1) It is clear from the that plant height at 30, 60, 90 DAS and at harvest significantly influenced by chickpea varieties in either years of study and in pooled analysis during experimentation. Data indicated that chickpea variety 'GNG-2144' (V_1) attained highest plant height (19.2, 38.6, 68.6 and 71 cm) at 30, 60, 90 DAS and at harvest, which was significantly higher as compared to 'GNG-2171' (V_2) with the magnificent increments of (4.91, 3.76, 3.93 and 4.87 per cent) on pooled mean basis during experimentation, respectively. Conspectus of pooled data, it was observed that significantly taller plant (69.3 and 71.5 cm) at 90 DAS and at harvest stage of chickpea was recorded under the treatment fortified, soil application 25 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_2) followed by soil application 20 kg $FeSO_4$/ha and foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_1) which recorded plant height of (67.8 and 69.8 cm) at 90 DAS and at harvest stages of chickpea over control (F_0), but these treatments (F_2 and F_1) remained at par with each others during experimentation. Moreover, increments in plant height of chickpea by (4.62 and 6.94 per cent) at 90 DAS, while (4.33 and 6.88 per cent) at harvest stage due to soil application 20 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_1) and soil application 25 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_2), respectively over control (F_0) in pooled analysis.

Moreover On pooled data basis, seed inoculation of chickpea with ZSB (SI) + 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_3) and ZSB (SI) + 20 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_2) significantly improved plant height by (39.2 and 39.1cm) at 60 DAS, (73.0 and 71.9 cm) at 90 DAS and (75.1 and 73.8 cm) at harvest stages as compared with seed inoculation with ZSB (SI) + application of 15 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_1) and control (Z_0), respectively. However, both the treatments (Z_2 and Z_3) were remained statistically similar subjected to increasing plant height. The increments in plant height due to seed inoculation of chickpea with ZSB + 20 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_2) and ZSB (SI) + 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_3) were, whereas 9.52 and 9.80 per cent at 60 DAS, 27.27 whereas 24.39 and 26.29 per cent at 90 DAS while 22.38 and 24.54 per cent at harvest stage of chickpea over ZSB + 15 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_1) and control (Z_0), respectively during experimentation.

3.2 Seed yield (kg/ha)

It is apparent from the data that seed yield significantly influenced by chickpea varieties in individual year as well as pooled analysis (Table .2). Mean data of two years revealed that variety 'GNG-2144' (V_1) significantly produced higher seed yield (2159 kg/ha) compared with 'GNG-2171' (V_2) variety (1907 kg/ha) during field trial. The magnitude of improvement pertained to seed yield was 13.21 per cent recorded by 'GNG-2144' (V_1) over 'GNG-2171' (V_2) variety during pooled analysis. According to mean data of two years, soil application 25 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_2) significantly improved seed yield (2282 kg/ha) followed by the treatment sprayed with soil application 20 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_1), which also recorded good tonnage of harvest in terms of seed yield (2140 kg/ha). However, these treatments (F_2 and F_1) showed similar relationship in improving seed yield as compared to rest of the experimentation. Further, analysis of data revealed that lowest quantity of seed yield (1677 kg/ha) was produced under control (F_0) on pooled data basis. However, both the treatments (F_1 and F_2) which recorded magnificent increments by 36.07 and 27.60 per cent over control (F_0), respectively in pooled analysis. Pooled results indicate that seed inoculation with ZSB + 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_3) significantly harvest huge tonnage of seed yield (2296 kg/ha) followed by the treatment integrated as seed inoculation with ZSB + 20 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_2), which accounted with the production of 2253 kg seeds/ha during investigation. Both the treatments (Z_3 and Z_2) proved their significant superiority over rest of the treatments and were found statistically at par with each others in obtaining similar grain yield. Wherein, magnitudes of increment subjected to grain yield by 14.68 and 45.40 per cent over ZSB (SI) + 15 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_1), while 12.53 and 42.68 per cent over control (Z_0), respectively were recorded due to the treatment integrated with seed inoculation with ZSB + 20 kg $ZnSO_4$/ha + + 0.5% $ZnSO_4$ (Z_2) and ZSB (SI) + application of 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_3) during investigation. Furthermore, when seed inoculated with ZSB + 15 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_1) also caused significant improvement in producing a better harvest of seed yield (2002 kg/ha) by 26.78 per cent higher over control (Z_0). However, the lesser quantity of seed yield (1579 kg/ha) was produced under control (Z_0) in pooled analysis during field trial.

Comment [EC12]: Present this information in a table.

Bring the tables from appendices to this section

Discussion

It is evident from the data clearly showed that plant population was not affected by chickpea varieties. On pooled basis, among chickpea varieties, 'GNG-2144' variety registered significantly higher values of plant height (19.2, 38.6, 68.6, 71.0 cm), The significant variations in plant height among the varieties may be due to their genetic variability for this trait. Variations in dry matter production of chickpea among genotypes could be attributed to genetic variation branching is an important character of crop which is directly releated with the number of pod formation per plant and ultimately the productivity of crop. Growth pattern of a crop in its vegetative phase mainly determines the formation of number and size of sink, which ultimately serves as the base for developing yield attributes. Thus, the yield attributing characters of a plant are closely correlated with growth characters emerged in vegetative phase (Bouis and Saltzman, 2017). Improvement in yield attributing characters due to differential performance of chickpea varieties that significantly increases seed yield. seed yield of crop are multiplication of yield attributes potentially produced by the plant under good agronomic practices (GAPs). It is also regulated by how efficiently assimilates are transfer from the source to sink in the crop. It is quite evident from the data that chickpea varieties ('GNG-2144' and 'GNG-2171') significantly improved seed yield, Also chickpea variety 'GNG-2144' has capacity to utilize all agronomic inputs in efficient way and has potential to divert energy from source to sink. The results are also in conformity with the finding of Choudhary *et al.* (2020), Parmar and Poonia (2020). It was observed that fortification of iron played imperative role to increase plant height (cm) Such enhancement effect might be also attributed to the favorable influence of these nutrients on metabolism and biological activity and stimulatory effect on photosynthetic pigments and enzymatic activity which in turn increases vegetative growth of plants (Choudhary *et al.*, 2018, Thalooth *et al.*, 2006). Higher plant height with the application of iron might be due to the role of iron in starch formation and protein synthesis (Rout and Sahoo 2015) as well as maintenance and synthesis of chlorophyll in plants (Rout and Sahoo2015). The increase in the availability of iron to the plant might have stimulated the metabolic and enzymatic activities thereby increasing the growth and ultimately the plant height of the crop. These results are in agreement with the findings of Pingoliya *et al.* (2014). most of Indian soils are deficient in micronutrients (Zn and Fe) that could mark in lower yield. It is clearly observed in the experimental findings that soil application and foliar spray of iron remarkably increased yield of chickpea. to iron play important role in various physiological and biochemical pathways in plants particularly in biosynthesis of chlorophyll in leaves and essential for the maintenance of chloroplast structure and function. It also participate as a component of various catalyzing enzymes namely cytochromes of the electron transport chain which involves in fixing assimilates through photosynthesis results in development of yield attributing characters in crops (Larson *et al.,* 2018), Banjara and Majgahe (2019), Deshlahare *et al.* (2019) and Nandan *et al.* (2018). Increments in plant height in relation to the application of zinc is might attributed by the formation of auxins and also to ease in availability of zinc to plant leaves in the apical portion of the plant, which promotes cell division results in taller plant. The higher dry matter production might be due to the application of zinc is ascribed to the vigorous and enhanced plant growth that caused more leaf area development which aided in the effective interception of light, thus leading to higher dry matter production per unit area of leaf. These findings were correlated with the findings of Pal *et al.* (2019), Habib *et al.* (2018) and Pal (2018). According to pooled analysis of data, seed inoculation with ZSB + 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ at 50 DAS of crop (Z_3) significantly increased seed yield (2296 kg/ha). it was found that inoculation of ZSB along with different zinc fertilization enhanced the seed yield of chickpea by enhancing the availability of zinc during field trials. Zinc solubilizing bacteria increased the seed yield by increasing the zinc mobilization and uptake by the plant (Gandhi and Muralidharan, 2016), which plays an important role in the biosynthesis of auxins and carbohydrate as well as participate in nitrogen and protein metabolism, these physiological process stimulate efficient metabolic reactions within the plant (Rehman *et al.*, 2008) and yielded more outputs.

Comment [EC13]: Discuss your results based on the two subsection thus plant height and seed yield.

CONCLUSIONS

In conclusion, based on pooled analysis of two years experimental results it may be concluded that growing of chickpea variety 'GNG-2144' significantly produced higher plant height and seed yield (2159 kg/ha). Among agronomic biofortifications treatment, soil application 25 kg $FeSO_4$/ha and one foliar spray of 0.5% $FeSO_4$ at 60 DAS of crop (F_2) and ZSB (SI) + application of 25 kg $ZnSO_4$/ha + 0.5% $ZnSO_4$ (Z_3) in chickpea gave significantly higher seed yield (2282 and 2296 kg/ha).

Comment [EC14]: Include the mean value of plant height too

Comment [EC15]: Include recommendation based on your finds

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Table .1 Effect of agronomic biofortification of zinc and iron on plant height (cm) of chickpea varieties

Treatment	Plant height (cm)											
	30 DAS			60 DAS			90 DAS			At harvest		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Varieties (V)												
V_1: GNG-2144	18.75	18.76	18.76	38.3	38.9	38.6	68.4	68.8	68.6	70.8	71.3	71.0
V_2: GNG-2171	17.71	17.73	17.72	37.0	37.5	37.2	65.7	66.3	66.0	67.5	67.9	67.7
SEm±	0.27	0.29	0.20	0.39	0.42	0.29	0.79	0.80	0.56	0.90	0.92	0.64
CD (P= 0.05)	0.84	0.91	0.58	1.23	1.31	0.84	2.50	2.52	1.66	2.85	2.90	1.90
Iron fortification												
F_0: Control	18.21	18.23	18.22	37.2	37.9	37.6	64.5	65.1	64.8	66.6	67.1	66.9
F_1:20 kg $FeSO_4$ /ha (SA) + 0.5% $FeSO_4$ (FA)	18.23	18.25	18.24	37.8	38.2	38.0	67.4	68.1	67.8	69.5	70	69.8
F_2:25 kg $FeSO_4$ /ha (SA) + 0.5% $FeSO_4$ (FA)	18.25	18.26	18.25	38.0	38.6	38.3	69.2	69.4	69.3	71.3	71.8	71.5
SEm±	0.33	0.35	0.24	0.48	0.51	0.35	0.97	0.98	0.69	1.11	1.13	0.79
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	3.06	3.08	2.03	3.49	3.55	2.33
Zinc fortification												
Z_0: Control	18.18	18.18	18.21	35.3	36.0	35.7	57.9	57.7	57.8	60.1	60.5	16.2
Z_1: ZSB (SI) + 15 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	18.22	18.22	18.24	37.5	38.0	37.8	66.1	66.8	66.4	68.3	68.7	18.4
Z_2: ZSB (SI) + 20 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	18.24	18.24	18.26	38.8	39.3	39.1	71.4	72.4	71.9	73.5	74	73.8
Z_3: ZSB (SI) + 25 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	18.26	18.26	18.27	39.0	39.5	39.2	72.7	73.3	73.0	74.9	75.3	75.1
SEm±	0.21	0.21	0.23	0.42	0.43	0.30	0.81	0.84	0.59	0.76	0.77	0.54
CD (P= 0.05)	NS	NS	NS	1.21	1.22	0.85	2.33	2.42	1.65	2.17	2.18	1.51
Interaction (V × Fe)												
SEm±	0.46	0.50	0.34	0.67	0.72	0.49	1.37	1.38	0.98	1.56	1.59	1.11
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction (V × Zn)												
SEm±	0.30	0.32	0.22	0.60	0.61	0.42	1.37	1.38	0.98	1.07	1.08	0.75
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction (Fe × Zn)												
SEm±	0.37	0.39	0.13	0.73	0.74	0.26	1.41	1.46	0.51	1.31	1.32	0.52
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: SA: Soil application; FA: Foliar application and SI: Seed inoculation

Table .2 Effect of agronomic biofortification of zinc and iron on plant height (cm) of chickpea varieties

Treatment	Seed yield (kg/ha)		
	2021-22	**2022-23**	**Pooled**
Varieties (V)			
V_1: GNG-2144	2144	2173	2159
V_2: GNG-2171	1899	1915	1907
SEm±	22.86	23.44	16.37
CD (P= 0.05)	72.03	73.87	48.30
Iron fortification			
F_0: Control	1667	1687	1677
F_1: 20 kg $FeSO_4$/ha (SA) + 0.5% $FeSO_4$ (FA)	2124	2155	2140
F_2: 25 kg $FeSO_4$/ha (SA) + 0.5% $FeSO_4$ (FA)	2274	2290	2282
SEm±	28.00	28.71	20.05
CD (P= 0.05)	88.22	90.47	59.15
Zinc fortification			
Z_0: Control	1566	1593	1579
Z_1: ZSB (SI) + 15 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	1990	2015	2002
Z_2: ZSB (SI) + 20 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	2244	2263	2253
Z_3: ZSB (SI) + 25 kg $ZnSO_4$/ha (SA) + 0.5% $ZnSO_4$ (FA)	2287	2306	2296
SEm±	23.09	24.46	16.82
CD (P= 0.05)	66.23	70.14	47.41
Interaction (V × Fe)			
SEm±	39.59	40.60	28.36
CD (P= 0.05)	124.76	127.94	83.65
Interaction (V × Zn)			
SEm±	32.66	34.59	23.78
CD (P= 0.05)	93.66	99.20	67.05
Interaction (Fe × Zn)			
SEm±	39.99	42.36	14.56
CD (P= 0.05)	NS	NS	NS

Note: SA: Soil application; FA: Foliar application and SI: Seed inoculation

Comment [EC16]: Take this tables to results section