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Agronomic Biofortification of Zinc and Iron on Yield Potential and Zinc Fortification in Chickpea (*Cicer arietinum* L.) Varieties under Arid Western Plains Zone of Rajasthan

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ABSTRACT

Background: 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. Among micronutrients, zinc (Zn) and iron (Fe) deficiency in human nutrition are wide spread in Asian developing countries including India. Therefore, agronomic biofortification is easiest and immediate effective method for enriching seed with zinc and iron, which could relieve malnutrition issues in developing countries, particularly in India.

Methods: A field trial was carried out to study the agronomic biofortification of zinc and iron in chickpea (*Cicer arietinum* L.) varieties under Arid Western Plains Zone of Rajasthan. Growing of two varieties namely GNG-1581 and RSG-974 and seven fortification treatments included various doses of zinc and iron were assigned under FRBD and trial was carried out at Instructional Farm, College of Agriculture, Jodhpur (Rajasthan) during *rabi* season of 2019-20.

Result: The results revealed that significantly higher yield attributes and yield *viz*. pods plant^{-1} and 100-grain weight were produced under the RSG-974, while higher number of seeds pod^{-1} as well as seed, stover and biomass yield along with higher zinc contents and their uptakes were noticed in chickpea variety GNG-1581 under application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA).

Key words: Biofortification, Chickpea, Iron, Yield, Zinc.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) 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). India ranks first in area and production of chickpea followed by Australia, Pakistan and Turkey. As per 4th advance estimates, it accounts an acreage of 10.17 million hectares contributing 11.35 million tonnes of production with an average productivity of 1116 kg ha^{-1} during 2019-20 in India (DES, 2020). 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, zinc deficiency in the soil is more commonly found in cereal based cropping system of the world that affects succeeding pulse crops (Shukla and Mishra, 2020). Though, iron (Fe) also plays an important role in chlorophyll synthesis and act as structural component of hemes, hematin and leghaemoglobin involved in the nitrogen fixation in pulses catalyzed by an enzyme called 'nitrogenase'. Moreover, iron is the most essential micronutrient for plant growth especially for chickpea grown on saline and alkaline soils (Larson *et al*., 2018). 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; Shukla and Mishra, 2018).

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MATERIALS AND METHODS

An experiment was carried out at Instructional Farm, College of Agriculture, Jodhpur (Rajasthan) during *rabi* season of 2019-20. This is situated at a distance of about 10 km from Jodhpur railway station. Geographically, it is located between 26°15′N to 26°45′North latitude and 73°00′E to latitude 73° 29′East longitude at an altitude of 231 meter above mean sea level. The experiment was comprised with two varieties namely GNG-1581 and RSG-974 and seven fortification treatments included various doses and modes of application of zinc (Zn) and iron (Fe) *viz.* control (F_0), $ZnSO_4$ @ 25 kg ha^{-1} (SA) (F_1), $FeSO_4$ @ 25 kg ha^{-1} (SA) (F_2), $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) (F_3), $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) (F_4), $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) (F_5) and $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) (F_6) in total fourteen treatment combinations were designed in factorial randomized block design (FRBD) with three replication that makes forty two plots in total. The soil of experimental plot was sandy-loam in texture, slightly alkaline in soil reaction (pH 8.2), non-saline in conductivity (EC 0.12 d/Sm), low in organic carbon (0.14%) and available nitrogen (176 kg ha^{-1}), whereas medium in phosphorus (22.0 kg ha^{-1}) and high in available potassium (329 kg ha^{-1}). Similarly, the micronutrient analysis of the soil, inferred that the experimental soil was low in available Zn (0.48 mg kg^{-1}) and available Fe (3.21 mg kg^{-1}). The recommended dose of fertilizer (20 N and 40 kg P_2O_5 ha^{-1}) was given in chickpea at the time of sowing. However, zinc sulphate heptahydrate (Zn 21%) and ferrous sulphate (Fe 19.5%) were used as source of zinc and iron and applied as per the treatments as agronomic fortification. All cultural operations were followed as per recommendation package of chickpea. However, zinc and iron were analyzed by wet digestion in di-acid mixture (HNO_3 + $HClO_4$ in the ratio of 3:1) in the plant samples (seed and stover) by following all standard protocols given by Isaac and Kerber (1971) using Atomic Absorption Spectrophotometer at the most sensitive wavelength for zinc (213.7 nm) and iron (248.3 nm).

RESULTS AND DISCUSSION

Effect of varieties on yield attributes and yield

The effect of varieties on yield attributes and yield are presented and summarized in Table 1. Perusal of data showed that significantly higher number of pods $plant^{-1}$ (41.55) and 100-grain weight (14.45 g) were produced under the RSG-974 as compared to GNG-1581 (38.43 pods $plant^{-1}$ and 100-grain weight 13.52 g). However, the number of seeds pod^{-1} (1.99) recorded significantly higher under GNG-1581 as compared to RSG-974 (1.83 seeds pod^{-1}). It was found that variety GNG-1581 recorded significantly higher seed yield (1539 kg ha^{-1}), stover yield (2863 kg ha^{-1}) and biomass yield (4402 kg ha^{-1}) as compared to RSG-974, which recorded seed yield of 1326 kg ha^{-1}, stover yield of 2466 kg ha^{-1} and biomass yield of 3792 kg ha^{-1}. Harvest index of chickpea did not affect by any of the chickpea varieties. However, maximum harvest index (34.96%) was recordedunder the chickpea variety GNG-1581 as compared to RSG-974 (34.95%). Increased in yield attributes of chickpea might be due to formation of more growth attributes which is the individual potential of variety that synthesizes more reproductive parts, which likely to enhances production of yield characters of chickpea ultimately yielded more. The results are also in conformity with the finding of Parmar and Poonia (2020) and Nandan *et al.* (2018).

Effect of varieties on zinc content and uptake

It was found that significantly higher zinc content in seed (30.78 mg kg^{-1}) and stover (22.68 mg kg^{-1}) as well as their uptake by seed (48.84 g ha^{-1}) and stover (656.13 g ha^{-1}) along with total uptake (704.97 g ha^{-1}) were recorded under variety GNG-1581 as compared to RSG-974 (Table 2). The differences in zinc content and their uptake in seed and stover might have been caused due to varietal differences and genetic makeup of individual variety. Similar trends were also observed by Nandan *et al.* (2018) and Prasad and Shivay (2018).

Effect of agronomic biofortification of zinc and iron on yield attributes and yield

Yield attributing characters are outcomes of interaction between crop and applied treatments which synthesize more yield forming attributes. It was found that application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) and $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) significantly recorded higher number of pods $plant^{-1}$ (45.16 and 44.90), seeds pod^{-1} (2.18 and 2.17) and 100-seed weight (15.98 and 15.12 g), which produced significantly higher seed yield (1827 and 1635 kg ha^{-1}) and stover yield (3049 and 2886 kg ha^{-1}) and biomass yield (4876 and 4521 kg ha^{-1}), respectively and showed their significant superiority over rest of the treatments, except stover yield where both of the treatments were statistically at par with each others (Table 1). These theories also supported by Parmar and Poonia (2020) and Shivay *et al*. (2014). Improvements in yield attributes of chickpea varieties might be due to the facts that combined application of Zn and Fe increased availability of major and minor nutrients to plant, which might have enhanced early root growth and cell multiplication leading to more absorption of other nutrients from deeper layers of soil ultimately resulting in increased supply of the major nutrients. This might results more formation of yield attributes as supplies of micronutrients (Zn and Fe) encourage differentiation of cell at reproductive stage. However, harvest index did not influence by any of the biofortification treatments, but maximum harvest index (37.47%) was obtained under the treatment applied with $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) followed by $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA), which recorded harvest index of 36.16 per cent.

Effect of agronomic biofortification of zinc and iron on zinc content and uptake

Among treatments, application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) and $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5%

Table 1: Effect of agronomic biofortification of Zn and Fe on yield attributes and yield of chickpea varieties.

Treatment	Number of pods plant^{-1}	Number of seeds pod^{-1}	100-grain weight (g)	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Biomass production (kg ha^{-1})	Harvest index (%)
Varieties							
C_1: GNG-1581	38.43	1.99	13.52	1539	2863	4402	34.96
C_2: RSG-974	41.55	1.83	14.45	1326	2466	3792	34.95
SEm±	0.97	0.05	0.22	26.47	43	51.39	0.57
CD (P= 0.05)	2.82	0.14	0.65	76.95	124	149.38	NS
Zinc and Iron levels							
F_0: Control	34.95	1.67	12.70	1108	2226	3334	33.23
F_1: $ZnSO_4$ @ 25 kg ha^{-1} (SA)	38.34	1.80	13.36	1311	2554	3865	33.92
F_2: $FeSO_4$ @ 25 kg ha^{-1} (SA)	37.57	1.73	13.21	1248	2489	3737	33.40
F_3: $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA)	44.90	2.17	15.12	1635	2886	4521	36.16
F_4: $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA)	45.16	2.18	15.98	1827	3049	4876	37.47
F_5: $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA)	39.58	1.91	13.79	1535	2787	4322	35.52
F_6: $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA)	39.44	1.90	13.75	1363	2661	4024	33.88
SEm±	1.82	0.09	0.42	49.52	80	96.14	1.06
CD (P= 0.05)	5.28	0.25	1.21	143.96	232	279.47	NS
Interaction (C × F)							
SEm±	2.57	0.12	0.59	70.04	112.75	135.96	1.50
CD (P= 0.05)	NS	NS	1.73	203.60	NS	NS	NS

*(SA = Soil application and FA = Foliar application).

Table 2: Effect of agronomic biofortification of Zn and Fe on Zn contents and uptakes in seed and stover of chickpea varieties.

Treatment	Zn content (mg kg^{-1})		Zn uptake (g ha^{-1})		Total Zn uptake (g ha^{-1})
	Seed	Stover	Seed	Stover	
Varieties					
C_1: GNG-1581	30.78	22.68	48.84	656.13	704.97
C_2: RSG-974	28.53	18.84	39.16	466.95	506.12
SEm±	0.71	0.124	1.16	8.82	9.11
CD (P= 0.05)	2.08	0.359	3.36	25.64	26.49
Zinc and Iron levels					
F_0: Control	20.69	16.78	23.04	372.86	395.90
F_1: $ZnSO_4$ @ 25 kg ha^{-1} (SA)	23.27	20.42	31.08	525.04	556.11
F_2: $FeSO_4$ @ 25 kg ha^{-1} (SA)	21.53	19.68	26.99	493.89	520.87
F_3: $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA)	37.46	22.95	60.98	667.03	728.01
F_4: $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA)	36.19	22.31	66.29	685.64	751.93
F_5: $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA)	35.12	21.97	54.29	618.44	672.73
F_6: $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA)	33.32	21.21	45.34	567.90	613.24
SEm±	1.34	0.23	2.16	16.50	17.05
CD (P= 0.05)	3.89	0.67	6.29	47.97	49.56
Interaction (C × F)					
SEm±	1.89	0.33	3.06	23.34	24.11
CD (P= 0.05)	5.50	0.95	8.90	67.85	70.08

*(SA = Soil application and FA = Foliar application).

Table 3: Interaction effects of varieties and agronomic biofortification of zinc and iron on 100-seed weight (g) and seed yield (kg ha^{-1}) of chickpea.

C × F	Varieties					
	100-seed weight (g)			Seed yield (kg ha^{-1})		
	C_1	C_2	Mean	C_1	C_2	Mean
F_0	12.67	12.73	12.70	1096	1120	1108
F_1	13.54	13.18	13.36	1487	1135	1311
F_2	12.89	13.53	13.21	1313	1183	1248
F_3	14.75	15.49	15.12	1736	1533	1635
F_4	15.36	16.60	15.98	1939	1716	1827
F_5	11.73	15.85	13.79	1768	1303	1535
F_6	13.72	13.79	13.75	1433	1292	1363
Mean	13.52	14.45		1539	1326	
SEm±		0.59			70.04	
CD (P=0.05)		1.73			203.60	

$FeSO_4$ (FA) significantly recorded higher zinc contents in seed (37.46 and 36.19 mg kg^{-1}) and stover (22.95 and 22.31 mg kg^{-1}), while owing to higher yield obtained by the application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) and $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) that significantly improved zinc uptake by seed (66.29 and 60.98 g ha^{-1}) and stover (685.64 and 667.03 g ha^{-1}) of chickpea as well as their total uptake (751.93 and 728.01 g ha^{-1}), respectively, however, both the treatments were at par with each others with respect to zinc contents and uptakes (Table 2). Zinc treated as hyper-accumulators in seed and this might be possible due to Zn transporters, vascular sequestration and detoxification mechanisms that maintained Zn-homeostasis results in higher accumulation of zinc in plant body (Nandan *et al*., 2018 and Shivay *et al*., 2014) and also regulates respiration, photosynthesis, nitrogen fixation, reduction of nitrates and sulphates might be have positive response to enhance its contents and uptake in crop (Pal *et al*., 2019 and Nandan *et al*., 2018).

Interaction effects of varieties and agronomic biofortification of zinc and iron (C × F)

Among treatment combinations, RSG-974 under the treatment application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) recorded significantly higher 100-seed weight (16.60 g), while GNG-1581 gave significantly higher seed yield (1939 kg ha^{-1}) as compared to rest of treatments (Table 3), but it was at par with $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) and $FeSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA). This might be due to higher photosynthesis rate that improved

Table 4: Interaction effects of varieties and agronomic biofortification of zinc and iron on zinc contents in seed and stover of chickpea.

C × F	Varieties					
	Zn content in seed (mg kg^{-1})			Zn content in stover (mg kg^{-1})		
	C_1	C_2	Mean	C_1	C_2	Mean
F_0	22.84	18.54	20.69	16.63	16.93	16.78
F_1	28.17	18.37	23.27	23.17	17.66	20.42
F_2	22.98	20.08	21.53	21.97	17.40	19.68
F_3	35.58	39.35	37.46	25.59	20.32	22.95
F_4	38.76	33.62	36.19	24.39	20.22	22.31
F_5	37.07	33.16	35.12	24.01	19.94	21.97
F_6	30.08	36.56	33.32	23.03	19.39	21.21
Mean	30.78	28.53		22.68	18.84	
SEm±		1.89			0.33	
CD (P=0.05)		5.50			0.95	

Table 5: Interaction effects of varieties and agronomic biofortification of zinc and iron on zinc uptakes by seed and stover of chickpea.

C × F	Varieties								
	Zn uptake in seed (g ha^{-1})			Zn uptake in stover (g ha^{-1})			Total uptake (g ha^{-1})		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
F_0	25.05	21.04	23.04	391.77	353.94	372.86	416.82	374.98	395.90
F_1	41.35	20.80	31.08	626.14	423.93	525.04	667.49	444.73	556.11
F_2	30.09	23.89	26.99	581.58	406.20	493.89	611.66	430.08	520.87
F_3	61.65	60.30	60.98	782.69	551.38	667.03	844.34	611.68	728.01
F_4	75.10	57.49	66.29	808.65	562.62	685.64	883.75	620.11	751.93
F_5	65.39	43.19	54.29	744.95	491.94	618.44	810.33	535.13	672.73
F_6	43.24	47.44	45.34	657.15	478.65	567.90	700.39	526.09	613.24
Mean	48.84	39.16		656.13	466.95		704.97	506.12	
SEm±		3.06			23.34			24.11	
CD (P=0.05)		8.90			67.85			70.08	

the process of formation of growth and yield attributing characters in the variety GNG-1581 resulted in higher seed yield. Treatment combination of RSG-974 under application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) significantly recorded higher zinc contents (39.35 mg kg^{-1}) in seed, while higher concentration of zinc was recorded in stover (25.59 mg kg^{-1}) of GNG-1581 (Table 4). Furthermore, the results revealed that chickpea variety GNG-1581 under application of $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $FeSO_4$ (FA) significantly recorded higher zinc uptakes by seed (75.10 g kg^{-1}) and stover (808.65 g ha^{-1}) along with total uptake (883.75 g ha^{-1}) as compared to variety RSG-974 under similar combination (Table 5). The results are also in conformity of the findings of Nandan *et al.* (2018), Pal (2018) and Kayan *et al.* (2015). This was might be due to positive correlation of iron and zinc that stimulates various enzymes to form signal transduction through transcription factor for acquisition of more zinc and iron from roots to shoot (Pal, 2018 and Pingoliya *et al.,* 2015).

CONCLUSION

Based on experimental results, it may concluded that growing of chickpea variety GNG-1581 under $ZnSO_4$ @ 25 kg ha^{-1} (SA) + 0.5% $ZnSO_4$ (FA) showed better agronomic biofortification treatment which interactively and significantly produced higher yield attributes and seed yield (1,539 kg ha^{-1}) and also, enriched the seed with higher concentration of zinc as compared to variety RSG-974.

Conflict of interest

All authors declared that there is no conflict of interest.

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