Eco. Env. & Cons. 28 (4) : 2022; pp. (1830-1836)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i04.026

Agronomic bio-fortification of Zn and Fe on growth, yield, micronutrient concentration and agronomic use efficiency of chickpea genotypes

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(Received 19 April, 2022; Accepted 26 May, 2022)

ABSTRACT

Zinc (Zn) and Iron (Fe) deficiencies in soil lead to lesser concentration of these micronutrients in seeds and cause physical and mental health problems to human beings. Hence, agronomic bio-fortification of micronutrients is the good remedy for increasing the content of Zn and Fe in a seed. In this experiment two genotypes of chickpea were applied with Zn and Fe through foliage in the form of sulphates at different stages and also tested with the application to soil and via a seed treatment. Genotype GBM-2 recorded higher yield, Zn and Fe content in seed than JG-11. However JG-11 was more efficient in using native Zn and Fe, as it had shown higher Zn and Fe agronomic efficiency under all the methods compared to GBM-2. Foliar application of Zn and Fe proved better method of application compared to soil application or seed treatment of Zn or individual application of Zn or Fe. Yield increase was 20.2 and 19.35% higher in foliar application of Zn and Fe over control respectively in 2016 and 2017. Foliar application of Zn with Fe recorded 22.29 and 11.30% higher Zn accumulation in seed over soil application of micronutrients in 2016 and 2017, respectively.

Key words: *Agronomic efficiency, Bio-fortification, Fe, Zn*

Introduction

Essentiality of Zinc (Zn) and Iron (Fe) for plants has been well established as Zn and Fe are essential micro nutrients involved in number of essential functions. Fe involved in the growth of pulses through enzymatic reactions, photosynthesis and higher dry matter production and in turn it enhances the pod formation and seed setting. Fe is also necessary for symbiotic nitrogen fixation (nitrogenase). Deficiency of Fe leads to interveinal chlorosis (yellowing of interveinal portion with dark green veins). In severe cases, the entire leaf turns into brown and withered. Lesser accumulation of Zn and Fe was observed in the seed of crop which rose on the soils deficit in these micro nutrients. Further, the consumption of Zn and Fe deficit seed by human beings also create deficiency (Zn and Fe) in them.

Zn helps in elongation of internodes, flower initiation, seed production and maturation, protein synthesis. It is one of the essential plant nutrients which plays important role in metabolic, regulatory, and developmental processes (Broadly *et al.* 2007). Zn deficiency led to reduction in pollen viability, changes stigmatic size, morphology and exudations and further inhibiting pollen-stigma interaction (Pandey *et al.* 2009). The Zn deficiency is predicted to worsen due to reducing Zn levels under global

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climate change, intensive cropping and non application of organic manures.

In human, Fe being a co-factor for several enzymes performs basic functions and Zn is involved in normal tissue growth and hormone balance in human body. Inadequate supply of Fe leads to disability, anaemia and stunted mental growth and Zn deficiency enhances the risk of low fertility, poor immune system and depression. For this reason, Zn and Fe deficiency issues have been attracting an increasing focus worldwide.

Production of Zn and Fe fortified crops has been recognized as a tool to cope with the issue of Zn and Fe deficiency. In this direction Shivay *et al* (2014) noticed that the application of Zn brought a positive effect on grain yield and seed Zn concentration, especially under Zn deficient soils. $ZnSO_4$ and $FeSO_4$ are the dominant form of inorganic Zn and Fe which are available for plant uptake in natural condition. It is important to investigate the stage of application and method of application, as they influence the uptake and translocation of Zn and Fe in plants.

Materials and Methods

Experimental site

The experiment was conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during the *winter* season of 2016 and 2017 to know the effect of method of application of Zn and Fe on yield, Zn and Fe content of seed and agronomic use efficiency of Zn and Fe in chickpea genotypes. Physiographic co-ordinates of the location are 15^0 26′N of latitude, 75^0 01′E of longitude and 678 m above mean sea level of longitude. Organic carbon and pH of the soil were 0.52 % and 7.4 respectively. The soil of the experimental field was clay and the soil was low in nitrogen, medium in phosphorus, high in potassium and low in zinc (This classification is specific to Indian situation).

Experimental design and sowing

The experiment was laid out in split plot design. Two genotypes of chickpea were the main plots and six application methods of Zn and Fe were sub plots. Gross plot was 4.5 m x 3.0 m and net plot was 3.9 m x 2.6 m. Zn^- - No Zn and Fe (control), Zn^+ - 0.5% $ZnSO_4$ foliar application, Zn^-+Fe^+ - 0.1% $FeSO_4$ foliar application, Zn^++Fe^+ - 0.5% $ZnSO_4$ and 0.1% $FeSO_4$ through foliar application $Znsd^+$- seed treatment of $ZnSO_4$ 80 g/ha^{-1} Zns^++Fes^+ - Soil application of $ZnSO_4$ 25 kg ha^{-1} and $FeSO_4$ 10 kg ha^{-1}. Recommended dose of nitrogen and phosphorus (25 and 50 kg ha^{-1}) was applied uniformly to all the treatments in the form of di-ammonium phosphate and urea at the time of sowing. The varieties used were GBM-2 and JG-11. The distance between the rows was 30 cm and the gap between the plants on each row was 10 cm.

Soil analysis before sowing

Methods used for analyses are as follows, available nitrogen, phosphorus and potassium were estimated respectively through Alkaline permanganate method (Subbiah and Asija, 1956), Olsen and Sommers's method (Olsen and Sommers1982), Flame photometer method (Jackson, 1967). Available Zn and Fe (mg kg^{-1}), organic carbon (%) pH (1:2.5, Soil: Water) and electrical conductivity (dSm^{-1}) were measured through AAS after DTPA extraction (Lindsay and Norvell,1978), Walkley's procedure (Walkley, 1947), Buckman's pH meter (Piper, 2002) and EC bridge (Jackson, 1973) respectively.

Crop growth parameters

At harvest time (110 days after sowing) number of pods $plant^{-1}$, dry weight $plant^{-1}$ and seed yield $plant^{-1}$ were recorded from 5 randomly selected plants from the net plot area of each plot at maturity. Grain yield was recorded from each net plot and 100 seed weight was recorded.

Agronomic efficiency (AE) of Zn and Fe

AE for Zn and Fe were worked out as suggested by Fageria *et al.* (1990)

$$AE = \frac{(YdZn+) - (YdZn^-)}{ZnS}$$

Where AE = Agronomic efficiency, YdZn+ = grain yield from Zn fertilized plots; YdZn= grain yield from no Zn application, and ZnS = Amount of supplied Zn in kg ha^{-1}.

Similarly AE of Fe was worked out.

Statistical Analysis

The obtained data on chickpea crop of both the years were carried out standard analysis of variance (ANOVA) following standard procedures for Split Plot Design (Gomez and Gomez, 1984). The *F*-test was used to compare significant differences between treatment means with the least significant difference

(LSD) at 5% level. M stat C software was used for ANOVA of data from. Karl Pearson's Coefficient was used for correlating yield parameters with yield.

Results and Discussion

Relation between yield and yield parameters

Karl Pearson's correlation coefficient worked out between important yield parameters, yield and Zn and Fe content in seed (Fig. 1). The correlation heat map indicated that the association between yield v/s important growth, yield parameters, Zn and Fe content was linear and positive. Very strong linear

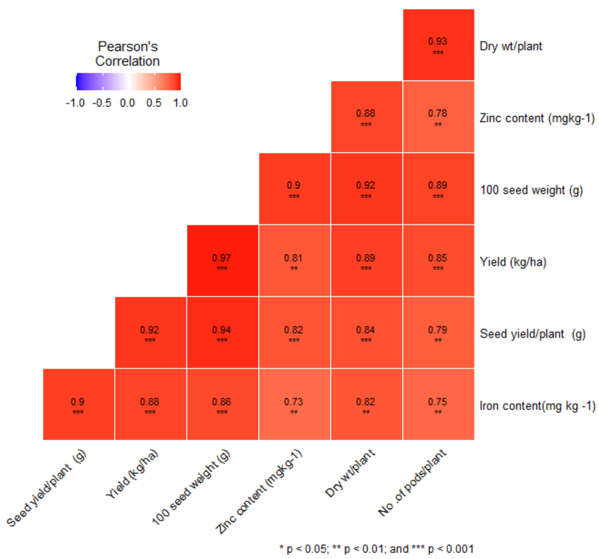

Fig. 1. Heat map showing correlation of important yield parameters, yield and Zn and Fe content in seed

association was observed between yield v/s seed yield per plant (0.92) and 100 seed weight (0.97). Strong correlation was observed between yield v/s number of pods per plant (0.85), dry weight per plant (0.89), Zn (0.81) and Fe (0.88) content in seed.

Effect of genotypes

There was significant difference between the genotypes for growth and yield parameters (Table 1). The genotype GBM-2 recorded 10.03 and 15.54 per cent higher yield than JG 11, respectively in the year 2016 and 2017. In chickpea, yield is highly dependent on number of pods plant^{-1}, seed yield plant^{-1} and 100 seed weight. In this study, these parameters respectively recorded 0.85, 0.92 and 0.97 correlation coefficient for yield (Fig 1). The genotype GBM-2 recorded 11.6 and 23.20 per cent higher number of pods plant^{-1}, 7.62 and 25.44 per cent higher seed yield plant^{-1}, 12.69 and 14.28 per cent higher dry weight plant^{-} and 3.17 and 8.54 per cent higher 100 seed weight than JG-11 in 2016 and 2017 respectively (Table 1). This difference can be attributed to genetic variation and specific physiological trait which makes certain genotypes capable to tolerate the particular environment and produced the yield better than others. Varietal difference with regard to number of pods was also observed by Hideto *et al.* (2016).

In the present investigation GBM-2 recorded significantly higher Zn and Fe content in grain than genotype JG-11 (Table 2). The genotype GBM-2 recorded higher Zn content of 38.52 and 34.67 mg kg^{-1} which was 2.86 and 8.92 per cent higher than JG-11 in 2016 and 2017, respectively. Higher Fe content of

Table 1. Yield and yield parameters ofchickpea as influenced by micro nutrient application methods and genotypes

Genotypes	Dry weight per plant(g) 2017	Dry weight per plant(g) 2018	Number of pods per plant 2017	Number of pods per plant 2018	Seed yield per plant (g) 2017	Seed yield per plant (g) 2018	100 seed weight(g) 2017	100 seed weight(g) 2018	Seed yield (kgha^{-1}) 2017	Seed yield (kgha^{-1}) 2018
GBM-2	21.3 a	20.0 a	35.5 a	35.1a	12.7a	12.9 a	22.8 a	20.46 a	2358 a	1873 a
JG-11	18.9 b	17.5 b	31.8 b	28.5b	11.8b	10.3 b	22.8 b	18.85 b	2190 b	1621 b
Application method of Zn & Fe										
Zn^-	17.8 f	17.2 de	30.9 de	24.78 e	9.4 de	9.07 e	20.2 c	18.47 de	2048 de	1602 de
Zn^+	21.3 b	17.9 cd	34.8 a	32.39 bcd	14.0 ab	11.99 bc	23.6 ab	20.66 ab	2382 ab	1870 ab
$Zn^- + Fe^+$	20.3 c	18.5 b	33.4 c	32.06 bd	12.9 b	12.69 ab	22.9 bc	20.2 a	2340 ab	1851 ab
$Zn^+ + Fe^+$	22.4 a	20.4 a	36.4 a	35.39 a	14.8 a	13.26 a	24.4 a	21.57 a	2462 a	1912 a
$Znsd^+$	19.0 d	18.2 bc	32.4 cd	32.78 ab	10.3 d	10.22 d	21.5 d	19.46 c	2175 cd	1581 e
$Zns^+ + Fes^+$	19.6 de	20.2 a	34.5 ab	33.44 a	12.1 bc	11.42 bc	22.3 cd	19.14 cd	2238 bc	1664 c

Note: Zn^- : RDF (control), Zn^+ : RDF+ 0.5% Zn foliar application, $Zn^- + Fe^+$:RDF+0.1%Fe foliar application, $Zn^+ + Fe^+$: RDF+0.5% Zn and 0.1% Fe through foliar application,$Znsd^+$: RDF + seed treatment 5 g Zn/kg of seeds, $Zns^+ + Fes^+$: Soil application of 25 kg/ha $ZnSO_4$ and 10 kg/ha$FeSO_4$ RDF: Recommended dose of fertilizer (25:50:0 N:P_2O_5:K_2O)

26.43 and 27.58 mg kg^{-1} also recorded GBM-2 (10.54 and 6.69 per cent higher than JG-11) in 2016 and 2017, respectively. The variation in seed Zn and Fe content of the chickpea genotype GBM-2 could be due to difference in physiological mechanism, seed physiology, morphology and Zn accumulation which influenced by genetic character (Ariza Nieto *et al.*, 2007 and Norton *et al.*, 2014).

Genotype JG-11 recorded significantly higher agronomic efficiency of Zn (65.2 and 64.1 kg kg^{-1}) and Fe (32.2 and 34.6 kg kg^{-1}) in 2016 and 2017, respectively compared to GBM-2 (Zn: 58.7 and 56.6 kg kg^{-1} and Fe: 27.6 and 19.6 kg kg^{-1}, respectively in 2016 and 2017) and indicated that JG-11 was more responsive to Zn application compared to GBM-2.

Effect of application methods of Zn and Fe

Zn and Fe application methods had significant effect on growth of chickpea. Foliar application of Zn and Fe produced significantly higher dry weight (22.4 and 20.4 g), seed yield $plant^{-1}$ (14.80 and 13.26 g), test weight (24.4 and 21.57 g) and higher yield (2462 and 1912 kg ha^{-1}) respectively in 2016 and 2017 over others (Table 1).

Foliar application of Zn and Fe recorded significantly higher yield than control (20.21 and 19.35%,), seed treatment of Zn (15.32 and 20.94%) and soil application of Zn and Fe (10.01 and 14.90%) in 2016 and 2017, respectively. The higher grain yield of foliar feeding may attribute to better availability of micro nutrients in foliar application compared to soil application due to their lack of mobility in calcareous and alkaline soils. These nutrients have specific physiological and biochemical roles of in plant growth (Putra *et al.*, 2012; Narimani *et al*. 2010). Since the application of Fe successfully prevented occurrence of chlorosis and the application of Zn improved the pollen formation and fertilization which improved the number of pods $plant^{-1}$ (17.80 and 27.4%), seed yield $plant^{-1}$ (57.45 and 46.20%) and 100 seed weight (20.79 and 16.78%) than the control in 2016 and 2017, respectively, which helped to get higher yield ha^{-1}. Mousavi (2011) indicated the easiness, rapid availability and reduced toxicity (accumulation and element stabilization) of foliar application of micro nutrients compared to soil application. Zn application increased chickpea growth (Khan *et al*. 2000) and plants fertilized with Zn had a greater total dry weight (Brennan *et al.*, 2001).

Significant variation in Zn and Fe content was observed between the micro nutrient application methods. Among the micro nutrient application methods foliar application of Zn and Fe at flowering and pod initiation stage recorded significantly higher Zn (42.40 and 37.12 mg kg^{-1}) and Fe (28.06 and 29.78 mg kg^{-1}) content in grain compared to control (Table 2). This treatment recorded 11.29 and 11.30% higher Zn content and 15.00 and 14.98% higher Fe content than the soil application in 2016 and 2017, respectively. Nutrients applied through foliage usually penetrate the leaf cuticle or stomata and enter the cells, facilitating easy and rapid utilization of nutrients for photosynthetic pigments, growth and yield of crop (Kandoliya and Kunjadia 2018). The different transporters and chelators involved in the uptake and transport of Fe and Zn are

Table 2. Zn and Fe content of seed and agronomic efficiency of Zn and Fe as influenced by micro nutrient application methods and genotypes of chickpea

Genotypes	Zn content (mg kg^{-1})		Fe content (mg kg^{-1})		Agronomic efficiency of Zn(kg kg^{-1})		Agronomic efficiency of Fe (kg kg^{-1})	
	2017	2018	2017	2018	2017	2018	2017	2018
GBM-2	38.52 a	34.67 a	26.43 a	27.58 a	58.7 a	18.6 b	27.6 a	19.6 a
JG-11	37.47 b	31.83 b	23.91 b	25.85 b	45.2 b	31.1 a	32.2 b	34.6 b
Application method of Zn & Fe								
Zn^-	35.6 d	31.21 d	23.28 d	24.50 cd	-	-	-	-
Zn^+	38.95 b	34.10 b	25.16 c	26.56 b	33.45 b	26.85 bc	-	-
$Zn^- + Fe^+$	36.65 c	32.08 bcd	27.54 ab	29.07 a	41.4 b	31.0 b	29.2 b	24.95 b
Zn^++Fe^+	42.4 a	37.12 a	28.06 a	29.78 a	-	-	41.4 a	31.0 a
$Znsd^+$	36.15 c	31.64 cd	23.42 d	24.86 cd	158.75 a	61 a		
Zns^++Fes^+	38.1 b	33.35bc	24.10 c	25.90 bc	7.6 c	7.53 d	19 bc	25.35 b

Note: Zn^- : RDF (control), Zn^+ : RDF+ 0.5% Zn foliar application, Zn^- + Fe^+ :RDF+0.1%Fe foliar application, Zn^++Fe^+ : RDF+0.5% Zn and 0.1% Fe through foliar application,$Znsd^+$: RDF + seed treatment 5 g Zn/kg of seeds, Zns^+ + Fes^+: Soil application of 25 kg/ha $ZnSO_4$ and 10 kg/ha$FeSO_4$ RDF: Recommended dose of fertilizer (25:50:0 N:P_2O_5:K_2O)

also the same (Haydon and Cobbett, 2007), hence it enhanced the uptake of both nutrients by the plants.

Among the application methods, seed treatment recorded higher agronomic efficiency of Zn (214 kg kg^{-1}) than other application methods (Table 2). This was due to the lower amount of Zn (250 g ha^{-1}) applied in the seed treatment compared to soil application (25 kg ha^{-1}) and foliar application (10 kg ha^{-1} for two sprays each 5 kg ha^{-1}). However, between the soil and foliar applications, foliar application of Zn and Fe recorded 4-7 times higher Zn agronomic efficiency than soil application.

Effect of interaction of genotypes and micro nutrient application methods

In the present study interaction of GBM-2 with foliar application of Zn and Fe recorded significantly higher dry weight, number of pods plant, seed yield plan^{-1}, 100 seed weight and yield ha^{-1} than JG-11 and GBM-2 with other methods of applications (Table 3).

Genotype GBM-2 with Zn and Fe foliar spray recorded significantly higher yield (2517 and 2005 kg ha^{-1}, respectively in 2016 and 2017) than both genotypes without Zn and Fe (2144 and 1725 kg ha^{-1} for GBM-2 and 1952 and 1422 kg ha^{-1} for JG-112 in the year 2016 and 2017, respectively) and seed treatment with Zn for both the genotypes (2156 and 2054 kg ha^{-1} for GBM-2 and 1735 and 1426 kg ha^{-1} for JG-11 (Table 3).

Among the interactions, genotype GBM-2 with foliar application of Zn and Fe recorded significantly higher zinc content than other methods in both the years. Fe content was significantly superior in GBM-2 with foliar application of Zn and Fe and followed by application of Fe alone. However, it was on par with JG-11 applied with foliar application of Fe alone and Zn and Fe (Table 4). This genotype also recorded higher agronomic efficiency under controlled condition and indicated that it was more efficient in using native Zn and Fe compared to GBM-2 as it recorded very low yield (1687 kg ha^{-1}) under control condition (no Zn) compared to com-

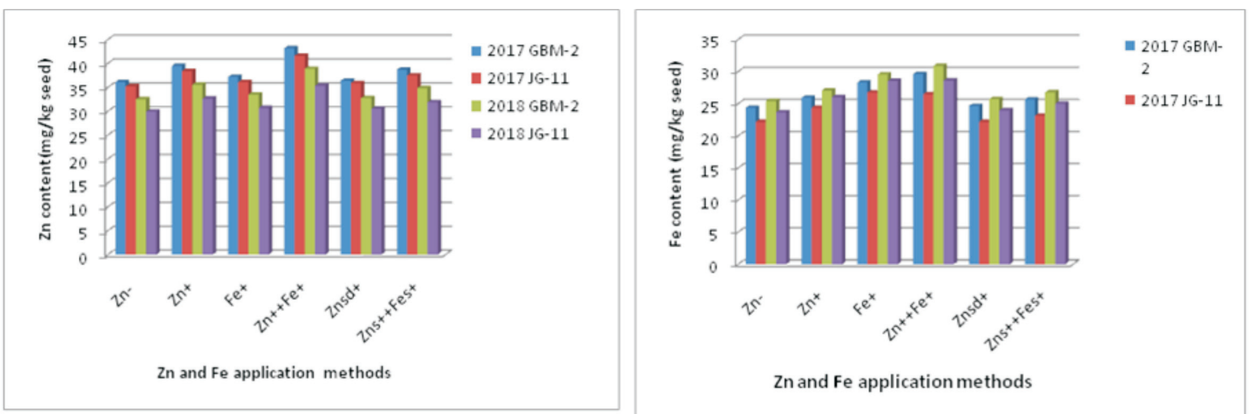

Fig.2a. Zn and Fe content of chickpea seed as influenced by micronutrient application methods and genotyhpes

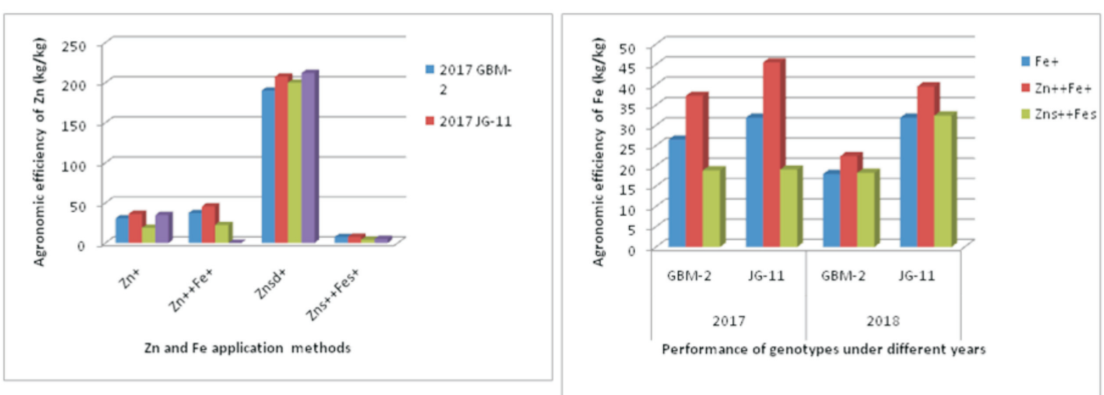

Fig.2b. Agronomic efficiency of Zn and Fe in chickpea seed as influenced by micronutrient application methods and genotypes

Table 3. Growth and yield parameters as influenced by interaction of micro nutrient application methods and genotypes of chickpea

Treatments	Dry weight/plant(g)				No .of pods/plant				seed yield/plant (g)			
	2017		2018		2017		2018		2017		2018	
	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11
Zn^-	19.2c-e	16.4f	18.1cd	16.3d	32.5cf	29.2f	24.78 e	24.78e	9.7ef	9.2f	9.68cd	8.46d
Zn^+	22.6ab	20b-e	19.2c	16.6d	36.9ab	32.6bf	34.44 bc	30.33 d	14.6ab	13.4bc	12.62ab	11.36bc
Fe^+	21.2bc	19.3c-e	19.4c	17.5cd	35.7ad	31.2 ef	36.22 ab	27.89 de	13.2bc	12.6b-d	13.09ab	12.28ab
Zn^++Fe^+	23.7a	21.2bc	21.6ab	19.2c	38.1a	34.7 ae	39.44 a	31.33cd	15.5a	14ab	13.89a	12.62ab
$Znsd^+$	20.3b-e	17.7ef	19.6bc	16.8d	34 de	30.8ef	37.67 ab	25.89 e	10.6d-f	10.1ef	11.44bc	8.9d
Zns^++Fes^+	20.7b-d	18.5d-f	22.1a	18.3cd	36.8ac	32.3df	36.11 ab	30.78 cd	12.6b-d	11.5c-e	12.69ab	10.15cd

Table 3. Continued……..

Treatments	100 seed weight(g)				Yield (kg/ha)			
	2017		2018		2017		2018	
	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11	GBM-2	JG-11
Zn^-	20.8bc	19.6c	19.94b-d	17.01e	2144b-d	1952d	1725b	1422b
Zn^+	24a	23.1ab	21.2ab	20.13b-d	2450ab	2315a-c	1970a	1770a
Fe^+	23ab	22.7ab	21.21ab	19.18b-e	2409ab	2271a-d	1961a	1741a
Zn^++Fe^+	24.5a	24.3a	22.84a	20.3b-d	2517a	2407ab	2005a	1818a
$Znsd^+$	21.9a-c	21bc	20.72a-c	18.2de	2296b-c	2054cd	1737b	1426b
Zns^++Fes^+	22.6ab	22a-c	19.89b-d	18.4c-e	2333a-c	2143b-d	1781a	1746a

Note: Zn^- : RDF (control), Zn^+ : RDF+ 0.5% Zn foliar application, Zn^- + Fe^+ :RDF+0.1%Fe foliar application, Zn^++Fe^+ : RDF+0.5% Zn and 0.1% Fe through foliar application,$Znsd^+$: RDF + seed treatment 5 g Zn/kg of seeds, Zns^+ + Fes^+: Soil application of 25 kg/ha $ZnSO_4$ and 10 kg/ha$FeSO_4$ RDF: Recommended dose of fertilizer (25:50:0 N:P_2O_5:K_2O)

bined foliar application of Zn and Fe (2112 kg ha^{-1}). It also indicated that JG-11 was efficient in using native Zn and Fe compared to GBM-2. Shivay *et al.* (2014) also concluded that growing of 'Pusa 372' chickpea variety in conjunction with application of 5.0 kg Zn ha^{-1} is most efficient for increased productivity, nutrient-use efficiency and nutrition quality of the chickpea compared to other genotypes and other levels of Zn.

Conclusions

Grain Zn content, agronomic efficiency, growth and yield of chickpea genotypes varied in this study. Since the genotypes had shown significant difference in Zn content, genotype with high Zn content can be used under Zn deficiency condition to have higher Zn content in seed. Among the application methods, Zn and Fe foliar spray at the time of flower initiation and pod development stage increased both the yield and Zn and Fe content of seed. Thus, this study provided a possibility of Zn and Fe biofortication through foliar application.

Acknowledgements

This study was financially supported by Indian Council of Agriculture Research (ICAR) through AICRP of chickpea of the Government of India. The authors wish to thank Dr. K.K. Math of the University of Agricultural Sciences for micro nutrient analysis using AAS.

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