



Agronomic Fortification of ZN and Fe in Chickpea an Emerging Tool for Nutritional Security – A Global Perspective

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Abstract

An investigation entitled “Influence of zinc and iron fortification on growth, yield and quality of chickpea (*Cicer arietinum* L.) under rainfed subtropics of Jammu region” was undertaken at Pulse Research Sub Station, Samba of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, during the rabi season of 2015-16. The soil of the experimental field was sandy loam in texture, near neutral in reaction, low in organic carbon, available nitrogen and available Zn but medium in available phosphorus and potassium with sufficient quantity of available iron and electrical conductivity in the safer range. The experiment comprised of two factors (2 chickpea varieties (GNG- 1581 and RSG-963) and 7 fortification treatments of Zinc and Iron viz., T₁: Recommended dose of NPK (Control), T₂: RDF + 0.5% Zn foliar spray, T₃: RDF + 0.05% Fe foliar spray, T₄: RDF + Zn (0.5%) and Fe (0.05%) foliar spray, T₅: RDF + seed treatment 1g Zn/kg of seed, T₆: RDF + soil application of ZnSO₄ @ 25 kg/ha and T₇: RDF + seed treatment + soil application of ZnSO₄ @ 25 Kg/ha) with fourteen treatments combinations was laid out in a Factorial Randomized Block Design in four replications. The recommended dose of N, P₂O₅ and K₂O was applied as basal through diammonium phosphate and muriate of potash at the time of sowing in lines below the seed. The foliar application of zinc and iron through zinc sulphate heptahydrate (ZnSO₄.7H₂O) and iron sulphate heptahydrate (FeSO₄.7H₂O) was given at pre flowering and pod formation stage of chickpea varieties. The crop was sown on 31st of October, 2015 in a crop geometry of 30 cm X 10 cm. The foliar application of Zn and iron was done at pre flowering and pod formation stage.

Significant changes were recorded in all the growth parameters of chickpea varieties at 90 days after sowing and at harvest. The variety GNG-1581 registered better performance over RSG-963 in recording significantly higher values of plant height, leaf area index and dry matter accumulation after 90 DAS and at harvest GNG-1581 variety also took less days to attain 50 per cent flowering and pod formation along with higher values of all the yield attributing characters with significant increase in number of pods/plant, pods/plant and 1000- seed weight as well as higher seed and stover yields as compared to RSG 963 variety of chickpea. Among the Zn and Fe fortification treatments, the treatment RDF followed by foliar application of Zn (0.5%) and Fe (0.05%) at pre flowering and pod formation stages was given to chickpea varieties recorded significantly higher values of plant height, leaf area index, dry matter accumulation at 90 DAS and at harvest. The same treatment recorded minimum days to attain 50% flowering and 50% pod formation along with significant higher number of pods/plant, number of seeds/pod, 1000-grain weight, seed yield, stover yield and Harvest Index over control treatment. There were non-significant differences with plants per square meter in all the treatment. The quality parameters i.e. protein content (%), proline content, chlorophyll content (SPAD value) and imbibition's percentages significantly higher with the treatment where RDF followed by foliar application of Zn (0.5%) and Fe (0.05%) at pre flowering and pod formation stages in both the chickpea cultivars over all the other treatments in comparison. However, the lowest values of seed yield and stover yield, harvest index and all the quality parameters were recorded in control. The chickpea variety GNG-1581 showed superiority in regarding all the quality parameters over RSG-963.

Further, the results of the study indicated the soil parameters after harvest of crop showed non- significant variation with P, K, Zn and Fe expect for available N in soil after harvesting of crop which was significantly influenced by zinc and iron fortification treatments. However, the varieties showed significant positive variations with respect to available N, Zn and Fe and negative variation with available P and K content in the soil.

The economic analysis showed that between the two chickpea varieties, GNG-1581 gave maximum net returns of and B:C ratio over RSG-963. Amongst the zinc and iron fortification treatments, highest gross returns, net returns and B: C ratio were realized with treatment T₄ (RDF + Zn (0.5%) and Fe (0.05%) through foliar application), which was followed by T₇, i.e. RDF+ seed treatment + Soil application of Zn (T₆ + Soil application of Zn) and T₆ (RDF + soil application of ZnSO₄ @ 25 kg/ha (recommended practice). However, lowest net returns were obtained in control treatment.

Henceforth, based on one year study, it is concluded that the treatment, the foliar application of Zn (0.5%) and Fe (0.05%) fortification treatment along with basal recommended dose of fertilizer showed significantly superior values of growth parameters, days to 50 percent flowering and pod formation and quality parameters like proline, protein, chlorophyll content and also helpful in fortification of the Zn and Fe content in seed and stover and gave more economic returns and higher B: C ratio of chickpea variety GNG-1581. Therefore, it can be recommended that an application of recommended dose of fertilizer along with Zn (0.5%) and Fe (0.05%) through foliar application on chickpea variety GNG-1581 have been found an innovative agronomic intervention for zinc and iron fortification in chickpea under sub-tropical rainfed situations of Jammu region for providing an alternative measure for nutritional security to the poor and undernourished masses.

Keywords: Chickpea Varieties; Zn and Fe Fertilization; Net Returns; Gross Returns

Introduction

Unlike plants, humans also require essential micronutrients and protein for normal physiological functions of the body and general health. Due to low concentration of micronutrients and protein in the staple food, billions of population is lacking sufficient daily intake of micronutrient and protein in their diet sometimes called 'hidden hunger' [1,2]. Micro nutrition malnutrition is a serious problem to human health throughout the world, primarily in resource limited countries [3]. The micronutrients most commonly associated with human health problems on a global scale include iron, zinc and iodine. Humans require at least 22 mineral elements for their wellbeing [4-6]. These can be supplied by an appropriate diet. However, it is estimated that over 30 percent of population is zinc (Zn) deficient and rest with other micronutrients. Among micronutrients, Zn deficiency is occurring in both crops and humans [4,7,8]. Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally. According to an estimate of World Health Organization (WHO) during 2015, around two billion people are being suffered with iron and zinc deficiencies and its deficiency ranks 11th among the 20 most important factors in the world and 5th among the 10 most important factors in developing countries like India. Zn deficiency is widespread in chickpea growing region of the world and is most prevalent among the micronutrients. Zinc enhances water use and water use efficiency [9], nodulation and nitrogen fixation. Furthermore, Zn is important for overall plant growth and development. High soil pH, low organic matter content, more sand leads to lower yields due to poor utilization of Zinc. Zinc is involved in a wide variety of metabolic processes, including carbohydrate, lipid, protein and nucleic acid synthesis and degradation. As well it can substantially improve seed germination and seedling vigor [10]. In humans, Zn deficiency leads to diarrhea in infants, dwarfism in adolescents [11,12]. Zn malnutrition is more prevalent in Asian countries where cereals are staple food [11]. Cereals not only contain lesser amounts of Zn but also contain phytates, which reduces the bioavailability of Zn [4].

In the sequence of micronutrient malnutrition; iron is also playing a vital role. Its deficiency is a highly prevalent nutritional disorder afflicting 2.5 to 5 billion people around the globe [13] where poor households and pre-school children are severely affected due to high demand for iron [14]. Iron acts as a co-factor for several enzymes performing basic functions in human body. Inadequate supply of iron contributes to disability, anemia and stunted mental growth [15]. Its malnutrition may be reduced by enhancing the bioavailable iron content through iron supplementation and food fortification [16]. These attempts are usually expensive and difficult to sustain on daily basis particularly in undernourished countries [17]. Therefore, it seems most desirable that crop fortification with iron content would be cost effective approach to overcome the hidden hungers of iron.

Malnutrition of above mentioned nutrients is more prevalent in Asian countries where cereals are staple food. In contrast to food security, nutrition security has traditionally been viewed as being within the realm of health professionals. Yet the entire agri-food chain has a vital role to play in addressing this problem. Producing more nutritious food and feed, or 'farming for health', should therefore be a central objective. This means increasing micronutrient content through fertilization, which holds out the promise of fighting deficiencies in soil, plants, animals and people health. Increasing the micronutrients especially Zn and Fe concentrations of food crops plants like chickpea, resulting in better crop production and improved human health is an important global challenge.

Therefore, with this backdrop, there is need for identification of suitable Zn and Fe agronomic fortification strategies under Jammu subtropics of North western Himalayas to improve micronutrients concentration in crops like chickpea for reducing micronutrients malnutrition and nutritional security of undernourished masses.

Materials and Methods

The experiment was conducted at Pulse Research Sub Station, Samba of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, during the rabi season of 2015-16. The soil of the experimental field was sandy loam in texture, nearly neutral in reaction, low in organic carbon, available nitrogen and available Zn but medium in available phosphorus and potassium with sufficient quantity of available iron and electrical conductivity in the safer range. The experiment comprised of two factors (2 chickpea varieties (GNG-1581 and RSG-963) and 7 fortification treatments of Zinc and Iron viz., T₁: Recommended dose of NPK (Control), T₂: RDF + 0.5% Zn foliar spray, T₃: RDF + 0.05% Fe foliar spray, T₄: RDF + Zn (0.5%) and Fe (0.05%) foliar spray, T₅: RDF + seed treatment 1g Zn/kg of seed, T₆: RDF + soil application of ZnSo₄ @ 25 kg/ha and T₇: RDF + seed treatment + soil application of ZnSO₄ @ 25 Kg/ha) with fourteen treatments combinations was laid out in a Factorial Randomized Block Design in four replications. The recommended dose of N, P₂O₅ and K₂O was applied as basal through diammonium phosphate and muriate of potash at the time of sowing in lines below the seed. The foliar application of zinc and iron through zinc sulphate heptahydrate (ZnSO₄·7H₂O) and iron sulphate heptahydrate (FeSO₄·7H₂O) was given at pre flowering and pod formation stage of chickpea varieties. The crop was sown on 31st of October, 2015 in a crop geometry of 30 cm X 10 cm. The foliar application of Zn and iron was done at pre flowering and pod formation stage of chickpea crop DTPA was prepared using 0.005M diethylene triamine penta acid (DTPA) + 0.1M triethalamine (TEA) buffer + 0.1 M CaCl₂·H₂O adjusted to pH 7.3 was used as extractants at a ratio of 10:20. The sample was shaken for two hours at a mechanical shaker and filtered through Whatman No 42 filter paper. The DTPA extractable Zn and Fe was determined through Atomic Adsorption Spectrophotometer (AAS) model Z.2300 (Hitachi, Japan) (Lindsay and Norvell, 1978).

Uptake studies in crop

The seeds of chickpea were also taken for uptake studies from each plot. The samples were oven dried, then finely ground with electric grinder and analyzed for nitrogen, zinc and iron concentration. One gram seed and stover sample was digested in di-acid mixture of HNO₃ and HClO₄ in the ratio of 3:1 (Piper, 1966).

The digested material was transferred to 100 ml. volumetric flask and the volume made with double distilled water. The solution filtered and analysed for Zn and Fe using atomic absorption spectrophotometer. Nitrogen was determined by Kjeldahl procedure. Nitrogen, zinc and iron uptake in seed and stover samples were calculated by multiplying per cent nutrient content with their respective dry matter accumulation as per the formula given below:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%)} \times \text{seed and stover yield (kg/ha)}}{100}$$

To determine the Zn and Fe in the plant and seed samples, the samples after being prepared were digested by tri acid method using HNO₃, HClO₄ and H₂SO₄ in the ratio of (10:4:1) Jackson (1973). A triple acid digestion destroys the organic matter in a shorter time. 0.5 g sample was taken and kept overnight in 150 ml conical flask. The sample was then heated on a hot plate till the acid solution evaporated and a pale white digest remained. The digest after cooling was filtered. The filtrate was transferred in a 50 ml volumetric flask and volume was made by de-ionised water and the filtrate was run through AAS to determine the concentrations of Zn, and Fe. Standards were prepared using ZnSO₄·7H₂O and FeSO₄·7H₂O (Jackson, 1973).

Qualitative Studies

Protein Content

Protein content of chickpea seeds were estimated by micro Kjeldahl digestion to determine nitrogen content, which is then converted to protein by multiplying respective nitrogen content in chickpea seed with a factor 6.25 (A.O.A.C, 1970).

Chlorophyll content was measured in the field and chlorophyll content was estimated with the help of SPAD meter (Soil Plant Analysis Development chlorophyll meter). The Zn content in seed and stover was determined using with DTPA-extractable as described by Lindsay and Norvell (1978) and zinc was determined by atomic absorption spectrophotometer and expressed as mg Zn/kg of seed and stover to see the fortification effect of applied Zn on chickpea crop. however, the Fe content was examined using with DTPA-extractable as described by Lindsay and Norvell (1978) and Iron was determined by atomic absorption spectrophotometer and expressed as mg Fe/kg of seed and stover. The studies related to stress protein Proline (µmoles/g) was conducted through standard procedure by taking 0.5g of fresh leaf material was homogenized in 10 ml of 3% sulphosalicylic acid and then filtered through Whatman No. 2 filter paper. The filtrate was centrifuged at 10,000 rpm for 10 minute. To 2 ml of supernatant, 2 ml glacial acetic acid and 2 ml acid ninhydrin reagent were added in a test tube. This mixture was incubated for 1 hour at 100°C to complete the reaction. The reaction was terminated by putting the tubes in ice bath. After the completion of incubation, the mixture was extracted with 4 ml of toluene. The extract was vigorously vortexed for 20 seconds. The

chromatophore containing fraction was then aspirated from the aqueous phase and its absorbance determined at 520 nm using spectrophotometer. L-proline was processed in the same manner to prepare the standard curves (Bates., *et al.* 1973).

Results

Seed yield (kg/ha)

The data on seed yield under the influence of varieties and various zinc and iron fortification treatments are presented in table given below and depicted in figure 1. The data indicated that two chickpea varieties significantly differ from each other with respect to recording seed yield. Between the varieties GNG-1581 recorded significantly higher grain yield (1590 Kg/ha) with a magnitude of superiority of 29.90 percent over RSG-963.

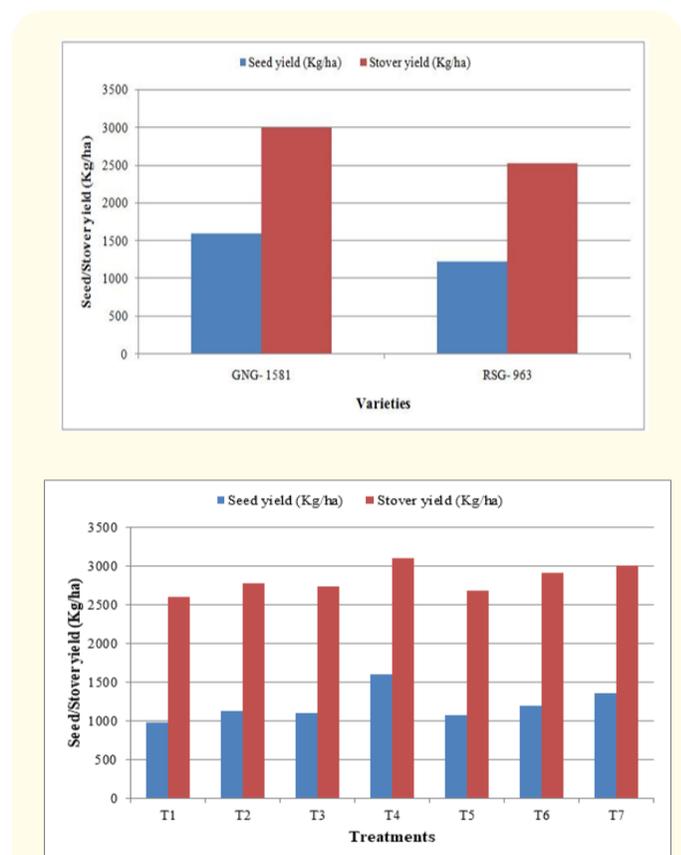


Figure 1: Effect of zinc and iron fortification on seed and stover yields of chickpea varieties.

Seed yield of chickpea was also significantly influenced under all zinc and iron fortification treatments. Among various fortification treatments, the foliar application of zinc and iron at pre flowering and pod formation stage recorded significantly higher seed yield over all the treatments. All the zinc and iron. All the zinc and iron fertilization treatments significantly increased the seed yield over control. The highest seed yield of 1598 Kg/ha was recorded with T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over rest of the Zn and Fe fortified treatments including control and the extent of yield advantage of treatment T₄ over T₁, T₂, T₃, T₅, T₆ and T₇ treatments were to the tune of 62.39, 41.91, 44.09, 47.82, 33.72 and 17.67 per cent, respectively.

Stover yield (kg/ha)

The data on stover yield given in table 1 and depicted in figure 1, revealed that stover yield was significantly influenced by chickpea varieties. The chickpea varieties GNG-1581 produced signifi-

cantly more stover yield (2988 Kg/ha) as compared to that of as realized in RSG 963 treatments with 2525 kg/ha. Chickpea variety GNG-1581 recorded higher stover yield with superiority of 18.33 percent over RSG-963.

Treatments	Seed Yield (Kg/ha)	Stover Yield (Kg/ha)	Harvest Index (%)
Varieties			
GNG- 1581	1590	2988	34.73
RSG- 963	1224	2525	32.64
SEm±	9.95	15.76	0.25
CD (0.05)	28.56	45.25	0.72
Zinc and Iron fortification			
Recommended dose of NPK (control)	984	2602	27.44
RDF + 0.5% Zn* foliar spray	1126	2770	28.90
RDF + 0.05% Fe* foliar spray	1109	2742	28.79
RDF + Zn* (0.5%) and Fe* (0.05%) foliar spray	1598	3102	34.00
RDF + seed treatment 1g Zn/kg of seed	1081	2683	28.71
RDF + soil application of ZnSO ₄ @ 25 kg/ha	1195	2915	29.07
RDF + seed treatment 1g Zn/kg + soil application of ZnSO ₄ @ 25 kg/ha	1358	3011	31.08
SEm±	18.60	29.49	0.66
CD (0.05)	53.43	84.67	1.90
Interaction	NS	NS	NS

Table 1: Effect of zinc and iron fortification on seed yield, stover yield and Harvest index of chickpea cultivars.

Among the various zinc and iron treatments significantly influenced the stover yield of chickpea crop. The data revealed that among all the fertilization treatments, T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) registered significantly higher stover yield of 3102 Kg/ha over control. The treatment T₇ i.e. RDF+ seed treatment + soil application of ZnSO₄ @ 25 Kg/ha) and T₆ (RDF + soil application of ZnSO₄ @ 25 kg/ha (recommended practice) was found to be the second best treatment in recording the stover yield over rest of the treatments. Among the different treatments, the lowest stover yield of 2602 Kg/ha was recorded in T₁ (Recommended dose of NPK (control)). The yield advantage in treatment T₄ over treatment T₇, T₆ and T₁ was to the tune of 3.02, 6.41 and 19.21 per cent, respectively.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Harvest Index (%)

The data depicted in table 1 revealed that harvest index affected by both the chickpea varieties. Chickpea variety GNG-1581 recorded significantly higher values of harvest index (34.73%) which was significantly superior over RSG-963.

From the table 1, it concluded that different zinc and iron fortification treatments had significant influence on the harvest index. Significantly highest harvest index (34.00%) was recorded with T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over rest of treatments including control treatment.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Effect of zinc and iron fortification on quality parameters of chickpea varieties

Crude Protein (%)

Crude protein is one of the most important determinants of seed quality in chickpea. Data pertaining to crude protein (%) as influenced by chickpea varieties is given in table 2 and showed that protein content was significantly influenced by chickpea varieties. Highest protein content was recorded in variety GNG-1581 (20.15%) which is statistically superior over chickpea variety RSG-963. The percent increase in protein content of chickpea varieties GNG-1581 was to the tune of 3.33 over RSG-963.

Treatments	Chlorophyll content (SPAD)	Proline content (µmoles/g)	Crude Protein (%)
Varieties			
GNG- 1581	43.20	1.38	20.15
RSG- 963	39.71	1.29	19.50
SEm±	0.15	0.03	0.07
CD (0.05)	0.44	0.07	0.21
Zinc and Iron Fortification			
Recommended dose of NPK (control)	39.25	1.03	18.96
RDF + 0.5% Zn* foliar spray	39.90	1.10	19.40
RDF + 0.05% Fe* foliar spray	39.77	1.08	19.34
RDF + Zn* (0.5%) and Fe* (0.05%) foliar spray	42.51	1.59	20.95
RDF + seed treatment 1g Zn/kg of seed	39.40	1.06	19.28
RDF + soil application of ZnSO ₄ @ 25 kg/ha	40.76	1.25	19.82
RDF + seed treatment 1g Zn/kg + soil application of ZnSO ₄ @ 25 kg/ha	41.66	1.43	20.48
SEm±	0.29	0.05	0.14
CD (0.05)	0.83	0.14	0.40
Interaction	NS	NS	NS

Table 2: Effect of zinc and iron fortification on chlorophyll content, proline content and protein content in chickpea varieties.

Among the various zinc and iron fortification treatments, significantly highest protein content (20.95%) was recorded in T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over control treatment (18.96%). The protein advantage in treatment T₄ over treatment T₇, T₆ and T₁ was to the tune of 2.29, 5.70 and 10.50 per cent, respectively.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Chlorophyll Content

Chlorophyll content in chickpea seed was significantly influenced by chickpea varieties as is evident from the table 2. Chlorophyll content recorded by SPAD meter showed significantly higher values in variety GNG-1581 (43.20) over RSG-963 (39.71) with a superiority of 8.78 per cent.

It is apparent from the table 2 that chlorophyll content recorded with SPAD meter showed significant variations by different zinc and iron fertilization treatments. Being significant, highest content of chlorophyll (42.51) was recorded with T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over rest of the treatments. The next superior treatment in recording chlorophyll content was T₇, i.e. RDF+ seed treatment + soil application of ZnSO₄ @ 25 Kg/ha) followed by T₆ (RDF + soil application of ZnSO₄ @ 25 kg/ha. While the treatment T₁ (Recommended dose of NPK (control) recorded the lowest value of chlorophyll content of 39.25 as recorded with SPAD meter.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Proline Content (%)

The data presented in table 2 revealed that different varieties had significant influence on proline content. Proline content recorded significantly higher values with GNG-1581 (1.38 μmoles/g) over the variety RSG-963 (1.29 μmoles/g). GNG-1581 recorded a significant increase of 6.97 per cent in proline content over RSG-963.

The data pertaining to proline content as influenced by different treatments of zinc and iron fertilization presented in table 2 and depicted in figure 2 showed that the zinc and iron fertilization treatments had high proline content values as compared to rest of the treatments. Data revealed that significantly superior values of proline content (1.59 μmoles/g) was recorded T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over the treatment T₁ (Recommended dose of NPK (control) which recorded the lowest value of proline content (1.03 μmoles/g).

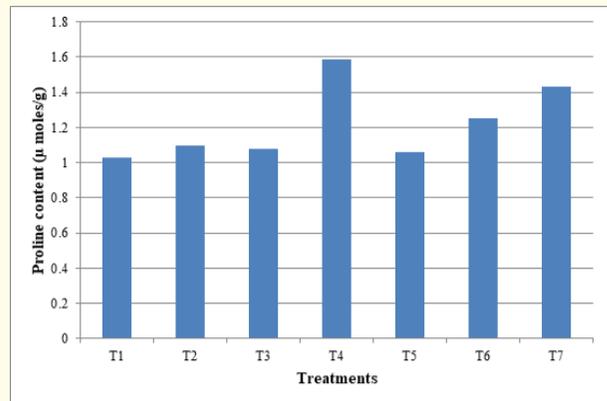
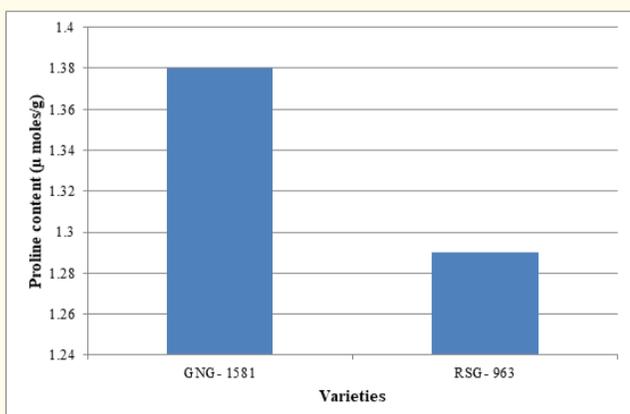


Figure 2: Effect of zinc and iron fortification on proline content in chickpea varieties.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Zn content in seed and stover (mg/kg)

The data depicted in table 3 and graphically presented in figure 3 showed varieties had significant influence on zinc content in seed and stover. Between the two varieties, GNG-1581 recorded higher zinc content in seed (43.26 mg/kg) and stover (40.86 mg/kg) which is statistically superior over variety RSG-963. Chickpea variety GNG-1581 recorded zinc content with a magnitude of superiority of 4.87 percent in seed and 5.34 per cent in stover over RSG-963.

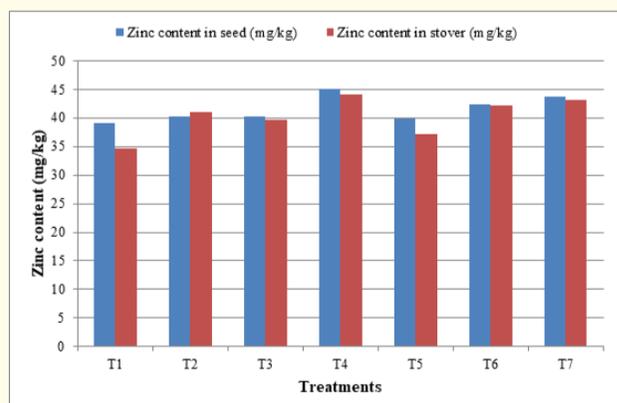
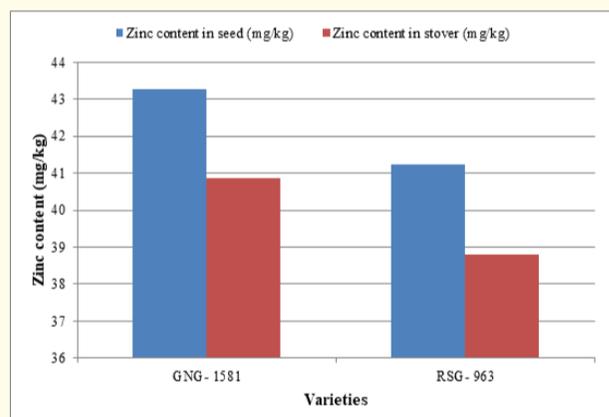


Figure 3: Effect of zinc and iron fortification on Zn content of seed and stover of chickpea cultivars.

Treatments	Zn content in Seed (mg/kg)	Zn content in Stover (mg/kg)	Fe content in Seed (mg/kg)	Fe content in Stover (mg/kg)
Varieties				
GNG- 1581	43.26	40.86	65.10	65.28
RSG- 963	41.25	38.79	64.20	64.11
SEm±	0.24	0.17	0.20	0.17
CD (0.05)	0.69	0.49	0.58	0.48
Zinc and Iron Fortification				
Recommended dose of NPK (control)	39.05	34.61	63.69	63.68
RDF + 0.5% Zn* foliar spray	40.30	41.06	64.10	65.14
RDF + 0.05% Fe* foliar spray	40.29	39.70	66.46	66.83
RDF + Zn* (0.5%) and Fe* (0.05%) foliar spray	44.98	44.08	65.30	65.71
RDF + seed treatment 1g Zn/kg of seed	39.79	37.18	64.09	64.09
RDF + soil application of ZnSO ₄ @ 25 kg/ha	42.31	42.08	65.25	65.18
RDF + seed treatment 1g Zn/kg + soil application of ZnSO ₄ @ 25 kg/ha	43.62	43.10	65.70	65.79
SEm±	0.45	0.32	0.38	0.32
CD (0.05)	1.28	0.92	1.09	0.90
Interaction	NS	NS	NS	NS

Table 2: Effect of zinc and iron fortification on seed and stover of chickpea varieties.

The data presented in table 3 and depicted graphically in figure 3 related to zinc content in seed and stover revealed that zinc and iron fortified treatments significantly increased both the contents. The significantly higher values of zinc content in seed (44.98 mg/kg) and stover (44.08 mg/kg) was observed under T₄ treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over rest of the treatments including control (Recommended dose of NPK) with Zn content in seed (39.05 mg/kg) and stover (34.61 mg/kg). The treatment T₇, i.e. RDF+ seed treatment + soil application of ZnSO₄ @ 25 kg/ha and T₆ (RDF + soil application of ZnSO₄ @ 25 kg/ha) were next to treatment T₄ in realizing the Zn content in Seed and stover over control. The data clearly indicates that fortification of Zn and Fe treatments through foliar as well as soil application had positive bearing on the Zn content in seed and stover. The per cent increase in Zn content with treatment T₄ over the control was to the tune of 15.18 in seed and 27.36 in stover.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Fe content in seed and stover (mg/kg)

The data depicted in table 3 and graphically presented in figure 3 showed varieties had significant influence on iron content in seed and stover. Between both the varieties, the chickpea varieties GNG-1581 recorded significantly higher iron content in seed (65.10 mg/kg) and stover (65.28 mg/kg) with a magnitude of superiority of 1.40 percent in seed and 1.82 per cent iron content in stover over RSG-963.

The data given in table 3 and presented in figure 3 in respect of iron content in seed and stover revealed that significantly higher value of iron content in seed (66.46 mg/kg) and stover (66.83 mg/kg) was observed under T₃ treatment (RDF + Fe (0.05%) foliar spray) over control treatment (Recommended dose of NPK) with iron content in seed (63.69 mg/kg) and stover (63.68 mg/kg). The treatment T₄ (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) and T₇ (RDF + soil application of ZnSO₄ @ 25 kg/ha) also recorded superior values of Fe content in seed and stover over control. The data clearly indicates that fortification of Zn and Fe treatments through foliar as well as soil application has positive bearing on the seed and stover content. The per cent increase in Fe content with foliar application of Fe treatment along with RDF was to the tune of 4.34 in seed and 4.94 in stover over control.

The interaction effect of varieties as well as zinc and iron fortification treatments was found non-significant.

Discussion

Yield attributes and yields of chickpea crop

The data on yield attributes have been presented in above tables revealed that varieties had significant influence on yield attributes. Variety GNG-1581 registered highest plant/meter² as compared to variety RSG-963. Significantly higher number of pods/plant was recorded in GNG-1581 among the varieties. The differences in number of pods/plant might have been caused due to varietal differences. In line with this result, Tripathi, *et al.* [18] reported significant differences among genotypes of chickpea for number of pods/plant. Whereas, higher number of seeds/pod were also recorded in GNG-1581. This might be attributed to genetic makeup of different chickpea varieties. 1000-seed weight was recorded higher in GNG-1581 as compared to RSG-963, might be due to ability of varieties to higher dry matter partitioning towards seeds and greater sink capacity. These results corroborate to the findings of Shamsi [19] and Tripathi, *et al.* [18] who reported significant differences among genotypes of chickpea on hundred seed weight. Crop yield in terms of grain and stover is the resultant of better growth and development of the higher rate of photosynthesis, better translocation and photosynthates in a good source-sink association, better association of yield attributes viz., number of pods per plant, number of seeds per pod and 1000-seed weight. Due to the increased yield attributes, variety GNG-1581 recorded significantly higher seed yield, stover yield and harvest index over RSG-963.

Zinc and iron fortification treatments had significant influence on yield attributes and yield of chickpea varieties as indicated in table 1 and 2 and figure 1, except for number of plants/m² which showed non-significant variation with these zinc and iron fertilization treatments. The higher number of pods/plant, number of seeds/pod and 1000-seed weight were recorded with T₄ (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) followed by treatment T₇,

i.e. RDF+ seed treatment + soil application of $ZnSO_4$ @ 25Kg/ha) and T_6 (RDF + soil application of $ZnSO_4$ @ 25 kg/ha. The reason for these variations in yield attributing characters and yield of chickpea varieties might be due to the fact that the combined application of NPK, 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. The increased yield attributes and yield might be due to the increased supply of the major nutrients by translocation of photosynthates accumulated under the influence of nutrients. Further, the translocation and accumulation of photosynthates in the economic sinks resulted in increased seed, stover and biological yields. The results of the investigation are in line with those of Valenciano, *et al.* [20], Pathak, *et al.* [21], Pandey, *et al.* [22], Shivay, *et al.* [23], Jha, *et al.* [24], Parimala, *et al.* [25] and Sajid, *et al.* [26].

Quality parameters

The data presented in previous tables revealed that quality parameters viz. chlorophyll content, imbibition percentage, Zn and Fe content in seed and stover were significantly affected by different chickpea varieties. Variety GNG-1581 recorded higher chlorophyll content as compared to RSG-963. The reason being for decrease in total chlorophyll might be due to the varied capacity of chickpea varieties for light interception [27]. Zn and Fe content in seed and stover were also higher in GNG-1581 as compared to RSG-963. The differences in zinc and iron content of seed and stover might have been caused due to varietal differences and genetic makeup of individual variety. In line with this result, Tripathi, *et al.* [18] reported similar results among the varieties. With regard to protein content and proline content, chickpea variety GNG-1581 showed supremacy over RSG-963. This might be due to differential ability of chickpea varieties to uptake nitrogen in seeds. These results are in agreement with previous studies [28,29].

Quality of chickpea in terms of protein content, proline content, as reported in table 2 and 3 and graphically presented in figure 2 and 3 revealed that in case of quality parameters, chlorophyll content, proline content and protein content. Significantly superior values of proline content was recorded T_4 treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over the treatment T_1 (control) which recorded the lowest value of chlorophyll, proline and protein content. These results are in close conformity with the finding of Dadkhah, *et al.* [28] who studied the effect of foliar application of zinc on physiological characteristics of chickpea. The results showed increased proline and protein content due to enhanced protease enzyme activity. Pingoliya, *et al.* [30] observed that application of iron significantly increased the chlorophyll content in leaf. It enhanced the photosynthates rates of plant and also root exudates.

Zn and Fe content in seed and stover, and imbibition effect on seed was significantly influenced by different Zn and Fe fortification treatments. Significantly higher value of zinc and iron content in seed and stover was observed under T_4 treatment (RDF + Zn (0.5%) and Fe (0.05%) foliar spray) over control treatment. The data clearly indicates that fortification of Zn and Fe treatments through foliar as well as soil application has positive bearing on the seed and stover content of the chickpea varieties. Marschner [31] reported that zinc and iron have imperative roles in normal growth. Zn is an important component of lots of enzymes and plays an important role in metabolism of nitrogen, synthesizing proteins,

nucleic acids and precursor of auxin. Imsande, [32] stated that iron has an important role in the synthesis of chlorophyll and also helps in the absorption of other nutrients. It is an important constituent of chlorophyll and it regulates respiration, photosynthesis, nitrogen fixation, reduction of nitrates and sulphates. Zinc into seeds might be related to significant increase in protein biosynthesis [33-45].

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