



**Full Length Article**

## Growth and Yield Patterns of Chickpea Genotypes Differing in Zinc-accumulating Capacity

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### Abstract

This study compares the yield and growth patterns of a high zinc-accumulating genotype (HZnG) and a low zinc-accumulating genotype (LZnG) of chickpea (*Cicer arietinum* L.) grown for 120 days in earthen pots filled with soil at four zinc regimes (0, 2.5, 5 and 10 mg ZnSO<sub>4</sub> kg<sup>-1</sup>) under naturally illuminated field conditions. Growth parameters were monitored at pre-flowering, flowering and post-flowering stages of the crop. Plants grown in Zn-deficient soil showed suppressed growth, as compared with those grown in soil with Zn (2.5, 5 or 10 mg kg<sup>-1</sup> soil) supplied as zinc sulphate. Increase in Zn supply enhanced the growth parameters significantly; however, highest dose was inhibitive in both the genotypes. All growth parameters evinced improvement from pre-flowering stage to post-flowering stage, except for leaf area index, which declined at the post-flowering stage. There were significant genotypic differences for yield parameters; HZnG performed better than LZnG at deficient levels of zinc supply. The 5 mg Zn kg<sup>-1</sup> supply was most effective in causing significant differences for the parameters studied. Cultivation of hyperaccumulating chickpea genotypes seems to be a sustainable and cost-effective approach to combat the increasing menace of Zn-deficiency in chickpea production. © 2016 Friends Science Publishers

**Keywords:** Chickpea; Crop production; Growth; Yield; Zn accumulation; Zn fertilization

### Introduction

Chickpea (*Cicer arietinum* L.), a leguminous crop, is an important source of high quality proteins, micro- and macro-nutrients as well as health-promoting secondary metabolites (White and Brown, 2010). This may be an important target crop to fulfil the nutritional and food demands of ever-rising world population. However, productivity of chickpea, like other grain legumes, has been low and variable. Although low-fertility soils are suitable for pulse production, chickpea (*Cicer arietinum*) productivity is often limited by mineral-nutrient deficiencies (Ahlawat *et al.*, 2007). Chickpea is more sensitive to zinc deficiency (Tiwari and Dwivedi, 1990; Ahlawat *et al.*, 2007), which becomes a key constraint in grain yield and limits the crop productivity. Zinc management has thus emerged as an obvious strategy to increase chickpea productivity.

Being an essential trace element for plants (Broadley *et al.*, 2007), Zn plays a central role in plant metabolism and growth processes (Zhao *et al.*, 2012; Xu *et al.*, 2015). Zinc content of plants may differentially influence plant performance under Zn-deficient conditions. Soils with zinc deficiency are widespread in the world and now considered as the most common crop micronutrient deficiency (White

and Broadley, 2009; Rehman *et al.*, 2012). Globally, more than 30% of soils are low in plant-available Zn (Hacisalihoglu and Kochian, 2003; Alloway, 2008a), causing considerable reduction in yield and nutritional quality of grain. According to Alloway (2004), Zn-deficiency has spread to 49 countries of the world, including even the technologically developed countries like Australia, China and USA. Soils in India are generally alkaline and calcareous in nature and prone to Zn deficiency. The arid and semi-arid climate of India further aggravates the Zn-deficiency problem due to salt stress. Zn-deficiency is widespread in the chickpea-growing areas, including India (Saxena, 1993; Singh, 2008). Up to 50% of the agricultural land in India is reportedly Zn-deficient (Sillanpaa, 1990; Tandon, 1993; Singh, 2008; Alloway, 2009), including 50% of the pulse-growing districts of the country (Ganeshmurthy *et al.*, 2006) and 50-70% agricultural soils of Indo-Gangatic plains, the major food-crop-growing regions of the country (Alloway, 2008b). Given this, zinc supply to the affected soils could be an effective strategy to compensate the zinc loss from the soil and ensure a good quality and quantity of plant product.

Although application of zinc fertilizers is indispensable for overcoming Zn-related nutritional disorders in economically important crop plants, it is not

always a successful strategy. Zinc fertilizers are expensive inputs used by farmers and need to be used optimally for the optimal production from each unit of fertilizer applied. Besides, the enhanced use of fertilizers causes adverse effects on the environment in terms of eutrophication of surface waters, pollution of drinking water and toxicity of the air. The innate potential of any crop variety to attain the maximum-yield in response to fertilizer is governed by its genetic makeup. It is worth mentioning that improper nutrient management, along with lack of concern for genotypes with high tolerance for nutrient deficiencies or toxicities (Malakouti and Balali, 2004), is a major constraint in achieving the desired yield. Therefore, identification and cultivation of Zn-efficient genotypes, which could use the soil- or tissue-Zn efficiently, is a realistic approach to ensure food security in sustainable way. Efficient genotypes greatly enhance the efficiency of applied fertilizers, thus reducing the cost of inputs, decreasing the rate of nutrient losses, and enhancing the crop yields. Moreover, using efficient genotypes would have positive effects on environment due to reduced application of chemicals in agriculture (Rengel, 2001).

The Zn-efficient phenotype is a complex trait and the genotypic variations for this trait have been associated with different mechanisms operating within the plant and in the soil-rhizosphere. Ability of plants to extract and accumulate nutrients from sub-optimal growth medium, and utilize them effectively for normal plant growth and high yield is an important adaptive mechanism. Zn hyper-accumulating chickpea genotypes tolerate zinc deficiency, and grow and yield well at suboptimal Zn condition (Siddiqui *et al.*, 2013). In this perspective screening of elite chickpea genotypes containing a high Zn content and the subsequent cultivation of these genotypes with the best Zn fertilizer dose, giving the highest growth and yield, are sustainable and cost-effective approaches for mitigating the adverse effect of zinc deficiency in agricultural crops. We have observed a high diversity for Zn-accumulation among chickpea genotypes (Siddiqui *et al.*, 2013). It was suggested that Zn-accumulating capacity could be exploited for identifying novel genotypes with high Zn efficiency in chickpea.

Given this, the present study was conducted to assess the impact of Zn fertilization on growth and yield attributes of two chickpea genotypes having diverse genetic background with respect to zinc accumulation. Differential response of these genotypes to Zn supply was investigated and the optimum Zn dose was recognized for a desirable growth and yield.

## Materials and Methods

### Experimental Details

Zinc-fertilization studies were conducted in pots under four zinc regimes (0, 2.5, 5 and 10 mg ZnSO<sub>4</sub> kg<sup>-1</sup> soil,

symbolized as Zn<sub>0</sub>, Zn<sub>2.5</sub>, Zn<sub>5</sub> and Zn<sub>10</sub> respectively) and two *C. arietinum* genotypes exhibiting high (IC269837) and low (IC269867) Zn-accumulating capacity in order to assess their performance on the basis of vegetative growth and grain yield.

Ten healthy uniform-sized seeds of each chickpea genotype were surface-sterilized with 0.1% mercuric chloride for 5 min and then vigorously rinsed with deionized water before germination. These seeds were germinated in the dark in non-contaminated sand moistened with deionized water. After one week, five most vigorous and equally developed seedlings were transferred to 23-cm-diameter soil-filled earthen pots lined with polyethylene bags (to avoid contamination). The physico-chemical properties of the soil used in the experiment are given in Table 1.

### Growth Condition and Treatments

Entire experiment was conducted under naturally illuminated condition with a relative humidity of 70-76%. Zinc at concentrations of 0, 2.5, 5 and 10 mg kg<sup>-1</sup> was added to the soil as ZnSO<sub>4</sub>·7H<sub>2</sub>O and thoroughly mixed. The recommended basal doses of N, P, K and S were mixed thoroughly in the soil in order to get 25 kg N, 20 kg P, 30 kg K and 20 kg S ha<sup>-1</sup>, using urea, single super phosphate, muriate of potash (KCl) and gypsum as source respectively. Plants were watered every alternate day with double deionized water (DDW). Watering schedule was adjusted throughout the experimental duration to avert leaching. The treatments were arranged in a randomized design with three replicates and five plants per pot. Plant growth was visually assessed every 5 weeks until harvest. The necessary after-care operations, such as thinning, hand weeding, inter cultivation and plant protection measures, were carried out when required to maintain good and healthy seed crop. The crop was sprayed with Dithane M-45 @ 2.5 mg L<sup>-1</sup> at 35, 45, 75 days after sowing (DAS) to control infestation of *Ascochyta* blight and *Fusarium* wilt. Endosulfan 35 EC @ 1.5 mL L<sup>-1</sup> was sprayed at 35, 50, 70 and 90 DAS for control of pod borer and other sucking pests.

### Plant Harvesting and Determination of Growth and Yield Parameters

At pre-flowering stage (40 DAS), the first sampling was done and four plants were maintained in each pot up to 60 DAS. At flowering stage (60 DAS), the second sampling was done and three healthy plants of uniform size were left growing in each pot until the third sampling at post-flowering stage (90 DAS). Data on plant growth including biomass {dry weights of shoot and root (g per plant), plant height (cm), nodule count (total number of nodules/root system), branch and leaf counts (total number per plant), and leaf area index (LAI)} were recorded from each sample.

Plants were carefully uprooted from each pot with the root system intact. The roots were washed in running tap water and nodules were detached carefully with forceps and number of nodules per plant was counted. The plants were then rinsed in three lots of deionized water and blotted gently. Roots and shoots were separated and dried in oven at 70°C for 48 h. At constant weight, the dried plant parts were cooled in ambient conditions for two hours before estimating the biomass (g per plant) by weighing the dried material. LAI was determined using the leaf area meter (LICOR model 3100) and calculated as leaf area per plant (cm<sup>2</sup>)/ground area per plant (cm<sup>2</sup>).

All the yield-related parameters were determined at harvesting stage (120 DAS). Pods were shelled manually and seeds separated and weighed. Number of pods per plant, number of seeds per pod, number of seeds per plant and the weight of 100 seeds were recorded as per the ISTA (International Seed testing Association) procedure (Anonymous, 1999).

### Statistical Analysis

Analysis of variance (ANOVA) was performed for the measured variables by using the Graph Pad Prism software program (GraphPad Prism ver. 5, San Diego, California, USA). Tukey test was used to detect the statistical significance of differences ( $P < 0.05$ ) between means.

## Results

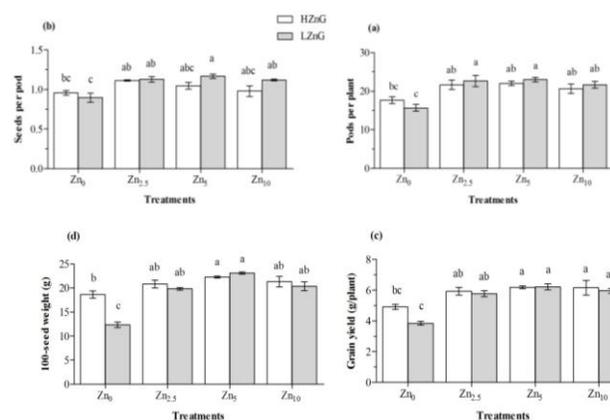
### Growth Characteristics

As to the genotypic difference, the high-zinc-accumulating genotype (HZnG) produced greater amounts of dry matter than the low-zinc-accumulating genotype (LZnG) at all developmental stages, the difference being non-significant at pre-flowering stage. The effect of Zn application was significant for plant biomass production in chickpea genotypes. All the treatments caused a significant increase in dry matter over the control (Zn<sub>0</sub>) at all growth stages of both the genotypes studied, the highest increase being associated with Zn<sub>5</sub> (Table 2). The biomass recorded with 5 mg Zn kg<sup>-1</sup> of soil was significantly greater in comparison to other treatments. However, in response to zinc application, LZnG did better than HZnG at all the three growth stages, showing a highly significant effect ( $P < 0.001$ ) for dry matter production at flowering and post-flowering stages; the HZnG could exhibit only a non-significant variation ( $P > 0.05$ ) from the control.

The height of the plant increased till the post-flowering stage, with a significant difference between the genotypes during all growth stages (Table 2). Irrespective of the genotype, zinc fertilization increased the plant height. In HZnG, the Zn-fertilization effect was significant at the flowering ( $P < 0.01$ ) and post-flowering ( $P < 0.001$ ) stages only, but in LZnG it was significant throughout, in comparison with the control.

**Table 1:** Some physico-chemical properties of the soil used in pot-culture experiment

Soil characteristics	Values
Texture	Loamy Sand (83.6% sand, 6.8% silt and 9.6% clay)
pH	7.1
Conductivity E.C.	0.23 ds m <sup>-1</sup>
Organic C	0.28%
Dry bulk density	1.39 Mg m <sup>-3</sup>
Available N	128 kg ha <sup>-1</sup>
Available P	14 kg ha <sup>-1</sup>
Available K	158 kg ha <sup>-1</sup>
DTPA-extractable zinc	0.78 mg kg <sup>-1</sup>



**Fig. 1:** Effect of Zn fertilization on (a) number of pods per plant, (b) number of seeds per pod, (c) grain yield (g/plant) and (d) 100-seed weight (g) in two chickpea genotypes (the highest and the lowest zinc accumulators). Means followed by different letters are significantly different at  $P < 0.05$

The two genotypes differed significantly ( $P < 0.01$ ) with respect to number of branches per plant at all growth stages studied (Table 2). The maximum number of branches appeared at the post-flowering stage. The effect of zinc application for number of branches was not significant ( $P > 0.05$ ) between the two genotypes.

A similar trend of variation was observed for number of leaves (Table 3). The minimum leaf area index (LAI) in both genotypes occurred in control (Zn<sub>0</sub>) population at all stages of plant growth. However, the effect of Zn application on LAI was significant ( $P < 0.001$ ) at all growth stages (Table 3). The LAI increased gradually from pre-flowering to flowering stages; but a decline was recorded at the post-flowering stage. By comparison, HZnG had a greater LAI than LZnG at all stages of plant growth and the treatment Zn<sub>5</sub> was most effective in both the genotypes (Table 3).

The number of root nodules increased with plant age, being the maximum at the post-flowering stage (90 DAS). The genotypic difference for the number of nodules per plant was significant at all the three growth stages (Table 3).

**Table 2:** Effect of Zn fertilization on biomass (dry matter) production (g/plant), plant height (cm) and number of branches per plant among two chickpea genotypes differing in Zn-accumulation capacity (highest and lowest zinc accumulators) at different growth stages of plants

	Pre-flowering stage				Flowering stage				Post-flowering stage			
	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>
Plant biomass												
HZnG	2.28 <sup>ab</sup>	3.31 <sup>ab</sup>	3.45 <sup>ab</sup>	3.39 <sup>ab</sup>	6.25 <sup>a</sup>	7.02 <sup>a</sup>	7.24 <sup>a</sup>	7.08 <sup>a</sup>	11.92 <sup>ab</sup>	12.65 <sup>ab</sup>	13.08 <sup>ab</sup>	12.87 <sup>ab</sup>
LZnG	1.96 <sup>b</sup>	2.88 <sup>ab</sup>	3.58 <sup>a</sup>	3.33 <sup>ab</sup>	4.76 <sup>b</sup>	6.37 <sup>a</sup>	7.28 <sup>a</sup>	6.69 <sup>a</sup>	9.09 <sup>c</sup>	11.27 <sup>b</sup>	13.19 <sup>a</sup>	12.55 <sup>ab</sup>
ANOVA	G	Ns				*				**		
	T	**				***				***		
	G × T	Ns				Ns				*		
Plant height												
HZnG	25.43 <sup>abc</sup>	27.03 <sup>ab</sup>	27.53 <sup>a</sup>	26.37 <sup>ab</sup>	33.17 <sup>a</sup>	35.53 <sup>a</sup>	36.43 <sup>a</sup>	36.1 <sup>a</sup>	46.63 <sup>a</sup>	48.24 <sup>a</sup>	49.2 <sup>a</sup>	49.13 <sup>a</sup>
LZnG	17.3 <sup>d</sup>	18.67 <sup>cd</sup>	20.53 <sup>abcd</sup>	20.07 <sup>bcd</sup>	24.2 <sup>b</sup>	29.3 <sup>ab</sup>	34.63 <sup>a</sup>	34.37 <sup>a</sup>	32.9 <sup>c</sup>	40.03 <sup>b</sup>	46.9 <sup>a</sup>	46.43 <sup>a</sup>
ANOVA	G	*				*				**		
	T	Ns				**				***		
	G × T	Ns				Ns				***		
No. of branches												
HZnG	15.33 <sup>a</sup>	15.33 <sup>a</sup>	15.67 <sup>a</sup>	15.33 <sup>a</sup>	24.33 <sup>ab</sup>	25 <sup>a</sup>	25.33 <sup>a</sup>	25.33 <sup>a</sup>	35.33 <sup>a</sup>	36.7 <sup>a</sup>	37.33 <sup>a</sup>	37 <sup>a</sup>
LZnG	10.67 <sup>b</sup>	12.33 <sup>ab</sup>	14 <sup>ab</sup>	14 <sup>ab</sup>	19 <sup>b</sup>	20 <sup>ab</sup>	20.67 <sup>ab</sup>	20.33 <sup>ab</sup>	28.67 <sup>a</sup>	31.32 <sup>a</sup>	32.67 <sup>a</sup>	32 <sup>a</sup>
ANOVA	G	**				**				**		
	T	Ns				Ns				Ns		
	G × T	Ns				Ns				Ns		

G = Genotype; T = Treatment. Values are the means of three replicates. Means marked with the same letter are not significantly different at  $P < 0.05$ . Ns = not significant; \*, \*\*, \*\*\* are significant at  $P < 0.05$ , 0.01 and 0.001 respectively

**Table 3:** Effect of Zn fertilization on the number of leaves (no./plant), leaf area index (LAI) and number of nodules (no./plant) in two chickpea genotypes (highest and lowest zinc accumulators) at different growth stages of plants

	Pre-flowering stage				Flowering stage				Post-flowering stage			
	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>	Zn <sub>0</sub>	Zn <sub>2.5</sub>	Zn <sub>5</sub>	Zn <sub>10</sub>
No. of leaves												
HZnG	37 <sup>a</sup>	36.67 <sup>ab</sup>	38 <sup>a</sup>	37.33 <sup>a</sup>	49 <sup>abc</sup>	49.33 <sup>ab</sup>	49.67 <sup>a</sup>	49.33 <sup>ab</sup>	89.67 <sup>a</sup>	90.67 <sup>a</sup>	90.33 <sup>a</sup>	89.33 <sup>a</sup>
LZnG	24 <sup>c</sup>	24.33 <sup>c</sup>	26.67 <sup>abc</sup>	25 <sup>bc</sup>	42.33 <sup>c</sup>	42.67 <sup>bc</sup>	43 <sup>abc</sup>	42.67 <sup>bc</sup>	79.67 <sup>b</sup>	85.67 <sup>ab</sup>	86.67 <sup>ab</sup>	85 <sup>ab</sup>
ANOVA	G	**				**				*		
	T	Ns				Ns				Ns		
	G × T	Ns				Ns				Ns		
Leaf area index												
HZnG	0.97 <sup>c</sup>	1.36 <sup>b</sup>	1.56 <sup>ab</sup>	1.54 <sup>ab</sup>	1.75 <sup>a</sup>	1.99 <sup>a</sup>	2.03 <sup>a</sup>	2.01 <sup>a</sup>	1.43 <sup>ab</sup>	1.58 <sup>a</sup>	1.63 <sup>a</sup>	1.6 <sup>a</sup>
LZnG	0.66 <sup>d</sup>	1.35 <sup>b</sup>	1.7 <sup>a</sup>	1.69 <sup>a</sup>	1.4 <sup>b</sup>	1.92 <sup>a</sup>	2.02 <sup>a</sup>	1.98 <sup>a</sup>	1.25 <sup>b</sup>	1.43 <sup>ab</sup>	1.46 <sup>ab</sup>	1.44 <sup>ab</sup>
ANOVA	G	Ns				Ns				Ns		
	T	***				***				***		
	G × T	***				**				Ns		
No. of nodules												
HZnG	10 <sup>ab</sup>	12 <sup>a</sup>	12.33 <sup>a</sup>	11.67 <sup>ab</sup>	18.67 <sup>ab</sup>	20.33 <sup>a</sup>	21 <sup>a</sup>	20.67 <sup>a</sup>	25.67 <sup>a</sup>	27 <sup>a</sup>	27.7 <sup>a</sup>	27.33 <sup>a</sup>
LZnG	7.67 <sup>b</sup>	10.67 <sup>ab</sup>	11.33 <sup>ab</sup>	11 <sup>ab</sup>	15.67 <sup>b</sup>	19 <sup>ab</sup>	20.33 <sup>a</sup>	20 <sup>a</sup>	20.67 <sup>a</sup>	23 <sup>a</sup>	24.33 <sup>a</sup>	24 <sup>a</sup>
ANOVA	G	*				**				**		
	T	*				*				Ns		
	G × T	Ns				Ns				Ns		

G = Genotype; T = Treatment. Data are means of three replicates. Means marked with the same letter, are not significantly different at  $P < 0.05$ . Ns = not significant; \*, \*\*, \*\*\* are significant at  $P < 0.05$ , 0.01 and 0.001 respectively

Zinc treatment affected the nodule number significantly at pre-flowering and flowering stages, and non-significantly at post-flowering stage. On the whole, treatment Zn<sub>5</sub> was superior to other treatments; however, the difference between Zn<sub>5</sub> and Zn<sub>10</sub> was only marginal.

### Yield and Yield Attributes

The effect of zinc application, genotype and their interaction

was conspicuous on the yield attributes of the crop, viz. pods per plant, seed count per pod, 100-seed weight, and total seed yield.

The HZnG produced more pods than LZnG in the untreated (Zn<sub>0</sub>) population. Zinc fertilization improved the pod number per plant, significantly ( $P < 0.001$ ) in LZnG and non-significantly ( $P > 0.05$ ) in HZnG (Fig. 1a). Effect of genotype-treatment interaction (G × Zn) was non-significant ( $P > 0.05$ ).

Zinc application significantly ( $P < 0.001$ ) improved

seed number per pod over the control (Fig. 1b). The maximum production of seeds was recorded at Zn<sub>2.5</sub> (HZnG) and Zn<sub>5</sub> (LZnG), the genotypic difference being non-significant. With the increase in soil-zinc levels, genotypic variation was non-significant ( $P > 0.05$ ), and so was with the interaction ( $G \times Zn$ ) effect ( $P > 0.05$ ).

In the control population, HZnG gave a greater yield than LZnG, but the latter was more responsive to soil zinc application. Our results showed a significant increase in grain yield ( $P < 0.001$ ) with the graded zinc dose applied (Fig. 1c). On an average, the highest seed yield, about 42% greater than the control (Zn<sub>0</sub>), was achieved with application of 5 mg Zn kg<sup>-1</sup> soil. No significant difference ( $P > 0.05$ ) was noted between the genotypes, and the same was true for the  $G \times Zn$  effect.

Comparing the genotypes, the 100-seed weight was greater in HZnG than in LZnG. Each genotype responded significantly to zinc application (Fig. 1d). Irrespective of the genotype, 100-seed weight was highest with Zn<sub>5</sub> treatment and lowest with Zn<sub>0</sub> (control). Treatment Zn<sub>5</sub> was the optimum for both the genotypes. Significant genotypic difference ( $P < 0.05$ ) was noticed for this trait. The  $G \times Zn$  effect was also significant.

## Discussion

Zinc is involved in all metabolic and cellular functions in plants and is thus essential for normal plant growth and development. Zinc deficiency as well as zinc fertilization caused a significantly different effect on two chickpea genotypes with reference to growth attributes, as reported earlier by Khan *et al.* (1998). Zinc deficiency causes reduction in dry matter production of many crop plants (Khan *et al.*, 2004; Wang and Jin, 2005; Cakmak, 2008), including the chickpea genotypes. The growth defects of the plants growing on low-zinc supply are attributed largely to the disturbance of auxin metabolism, which may involve decreased IAA synthesis or enhanced IAA degradation by reactive oxygen species (ROS) produced under Zn-deficient conditions (Robson, 1994; Cakmak, 2011). Although low zinc supply suppressed vegetative growth of plants, yet both genotypes of chickpea differed in their sensitivity to zinc deficiency. Under zinc-deficient conditions, high Zn-accumulating genotype (HZnG) maintained better growth, having highest values for all growth attributes, suggesting that it had inherent capacity to tolerate low available zinc. This genotype extracts zinc efficiently from low-zinc growth medium and maintains intracellular Zn level required for various metabolic processes to function normal.

In the present study, zinc applications had a positive effect on plant growth, leading to increase in biomass production, plant height and number of root nodules (Tables 2 and 3). The apparent mechanism for achieving these improvements was increase in leaf area index (LAI), chlorophyll content and relative growth rate (Khan *et al.*, 2008; Zhoori *et al.*, 2009; Chaab *et al.*, 2011). Similar

increase in plant biomass due to Zn application was recorded in chickpea (Pathak *et al.*, 2012), tomato (Ejaz *et al.*, 2011), wheat and rice (Khan *et al.*, 2009), corn, dry bean and soybean (Fageria *et al.*, 2008). Increase in LAI due to Zn supply (Tharanathan and Mahadevamma, 2003) owes the role of Zn in the synthesis of tryptophan and IAA, which increase the leaf area. Zinc also activates certain enzyme systems and has an important role in cell enlargement, resulting in increased plant height.

Root nodulation is directly related to nitrogen economy of leguminous plants and affects their productivity. Poor root nodulation of chickpea plants grown on Zn-deficient soil, as observed in the present investigation, could be related to the reduction in nitrogen fixation (Ahlawat *et al.*, 2007), which further contributes to decrease in crop yield. Zinc fertilization has a significant effect on the number of nodules among chickpea genotypes and many other plant species (Grewal, 2001).

Plants grown with low zinc supply showed a significant reduction in all yield attributes in both chickpea genotypes. Zinc deficiency induces flower abortion and infertility of pollen and ovules, which result in a low seed setting, leading ultimately to yield reductions (Pathak *et al.*, 2012). There was a significant difference between the two chickpea genotypes for all the yield parameters; HZnG was statistically superior to LZnG for all yield attributes at deficient zinc levels. Differential response to zinc-stress conditions and variability in fruiting potential of the genotypes were the reasons for differences in yield contributing parameters. Application of Zn significantly improved yield and yield attributes of the two chickpea genotypes studied, possibly due to a positive influence of Zn on plant metabolism, auxin biosynthesis and nutrient uptake (Cakmak *et al.*, 1999; Agrawal *et al.*, 2010). Zinc, added at 5 mg kg<sup>-1</sup>, caused a significant increase in the number of pods per plant, seeds per pod, grain weight per plant and 100-seed weight, as compared with the control (no Zn) in both the chickpea genotypes differing in their zinc-accumulation capacity. The positive response of zinc application on all yield-related parameters was due to the fact that Zn improved the reproductive potential of the plants (Pathak *et al.*, 2012).

Increase in zinc supply significantly increased the pod counts per plant in our study. Application of micronutrients, especially zinc sulphate, had a positive effect on formation of stamens and pollens and we can attribute the increase in number of pods per plant to this phenomenon. Since chickpea is a self-pollinating plant, therefore, increased activity of stamen will enhance fertility status of flowers, which may end up with increased number of pods. Similar increase in the number of pods per plant due to Zn application occurred in chickpea (Pathak *et al.*, 2012) and soybean (Banks, 2004). Furthermore, increase in the number of spikes per square meter was reported in wheat due to application of zinc sulphate (Yilmaz *et al.*, 1997).

The present study indicated a poor grain yield in chickpea grown in Zn-deficient soil, as found earlier in green gram (Pandey *et al.*, 2006), and was attributed to a reduced receptivity of stigma, affecting the fertilization and seed development under Zn deficiency. Effect of zinc application on seeds per pod was significant in both the genotypes. However, HZnG maintained more yield in comparison to LZnG at low level of zinc, probably due to their effective Zn-extraction ability from low-zinc growth medium and their enhanced ability to utilize this nutritionally important element at cellular level, as suggested by Cakmak *et al.* (1999). Addition of zinc increased the seed weight per plant in both chickpea genotypes with different zinc-accumulation capacity. However, the yield increased only up to Zn<sub>5</sub>, while Zn<sub>10</sub> treatment caused a decrease in grain yield. Similar increase in chickpea grain yield due to Zn fertilization was reported by Brennan *et al.* (2001) and Pathak *et al.* (2012), which might have a link with increased number of pods per plant, as in other leguminous species (Valenciano *et al.*, 2007). Khan (1998) reported increased grain yield mainly because of increasing pod bearing when Zn was applied in conditions of high moisture availability. Excessive Zn applications may lead to a slight decrease in yield (Tripathi *et al.*, 1997).

A significant improvement in 100-seed weight was obtained with increase in zinc levels in the two genotypes, the maximum occurring at 5 mg kg<sup>-1</sup> zinc application. Similarly, application of zinc increased the 100-kernel weight in common bean (Nadergoli *et al.*, 2011) and 1000-seed weight in chickpea (Valenciano *et al.*, 2009). In the present study, additional Zn supply was better utilized by low Zn-accumulating genotype (LZnG) for yield related parameters.

ZnSO<sub>4</sub> application to soil minimized the Zn-deficiency effect among both genotypes of chickpea; however these genotypes varied in their requirement of zinc fertilizer. For a hyperaccumulating genotype, the 2.5 mg Zn kg<sup>-1</sup> soil dose was sufficient to mitigate the adverse effect of zinc deficiency, but the low zinc-accumulating types required 5 mg Zn kg<sup>-1</sup> soil for similar performance. However, supply of Zn at 5 mg kg<sup>-1</sup> was effective for increasing the overall growth and yield attributes in both the genotypes studied. Although a zinc dose at the rate of 10 mg kg<sup>-1</sup> significantly improved the growth and yield parameters over the control; yet it had no significant differences from the treatment Zn<sub>5</sub>. This suggests that beyond an optimal requirement, Zn application does not improve growth and yield potential of chickpea genotypes. Thus, zinc fertilizer applied at the rate of 5 mg Zn Kg<sup>-1</sup> soil was most effective in the present investigation.

## Conclusion

There exists genotypic variation in the requirement of zinc

fertilizer for chickpea production. The present investigation has shown that chickpea genotypes capable to accumulate more zinc in their tissue require a relatively small exogenous zinc supply. Such genotypes can be effectively used in the modern agriculture in conjunction with zinc fertilizer, especially in areas where spatial and temporal variability hinders zinc availability, and for which the effectiveness of fertilization is low. Considering the large scale Zn-deficiency, genotypic variability could be exploited in plant-breeding programs to produce high yield chickpea genotypes.

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