Effect of phosphorus on plant growth and nutrient accumulation in a high and a low zinc accumulating chickpea genotypes

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Abstract

The present study compares the effects of phosphorus (P) on growth and bioaccumulation of a high-zinc-accumulating genotype (HZnG) and a low-zinc-accumulating genotype (LZnG) of chickpea (*Cicer arietinum* L.). A green house experiment was conducted in clay-pots filled with sand, at constant zinc (Zn) level with application of varying doses (0, 13.5 and 27 mg kg^{-1} of sand) of P, under naturally illuminated condition. Biomass production in shoot and root, and the Zn and P accumulation in the shoot were measured 90 days after sowing (DAS). P inputs affected all these parameters significantly in both the genotypes. High supply of P had inhibitory effect on most of these traits. An inverse relationship between the Zn and P accumulations in shoot was noticed at the highest dose (27 mg kg^{-1} of sand) of the applied P. HZnG did better than LZnG with all levels of P supply except for P concentration and content. Phosphorus application at the rate of 13.5 mg kg^{-1} of sand was optimum for both the genotypes tested. It has emerged from the study that HZnG chickpea genotype is suitable for zinc-deficient soils and may help in resisting low P availability in the soil.

Key words: *Cicer arietinum*; crop production; nutrition balance; P supply, Zinc content

1. Introduction

Chickpea (*Cicer arietinum* L.), rich in protein and vital mineral nutrients, is an important component of diet in developing countries and has a role in overcoming problems related to nutritional insecurity of poor people. It also contributes significantly to soil fertility through biological nitrogen fixation. On the medicinal side, its seed is used as a tonic, stimulant and aphrodisiac, and also in the ailments related to the liver and spleen disorders (Zia-ul-Haq *et al.*, 2007). Chickpea has a higher dietary fibre content, is the most hypocholesteremic agent among food legumes, and is effective in controlling the cardiovascular diseases and type 2 diabetes (Jukanti *et al.*, 2012; O'Neil *et al.*, 2014). However, its productivity remains low in India and, therefore, it is an import item here. Chickpea requirement in India is projected to be around 10.22 million tonnes by the year 2030, which needs a 4% increase in the annual growth rate (IIPR, 2011). Moreover, the current average global yield of chickpea is 0.9 t/ha, much lower than its estimated potential of 6 t/ha^{-1} under optimum cultivation conditions (FAO, 2012).

Plant nutrients are essential components for improving the quality and quantity of plant products. Non-availability of nutrients is a major constraint of crop productivity. Imbalanced use of plant nutrients markedly affects the crop yield (Ali *et al.*, 2008). Nutrient uptake by crops from the soil solution is affected by the level of nutrients in the soil (Materon and Ryan, 1995). Low availability of mineral nutrients in agricultural soils is a significant cause of yield losses in chickpea (Ahlawat *et al.*, 2007).

Adequate supply of phosphorus (P), an essential plant macronutrient, is a requirement for the optimum performance of

crops (Ryan *et al.*, 2012). Availability of soil P is critical for growth and development of chickpea, and a poor P availability limits its productivity. Phosphorus deficiency is a critical nutrient-deficiency problem in the Indian soils and may cause up to 29-45% yield losses in chickpea (Ahlawat *et al.*, 2007).

Zinc deficiency in agricultural soils is also a wide-spread constraint for chickpea production in India (Ahlawat *et al.*, 2007; Singh, 2008). P and Zn facilitate the availability of each other for crop plants (Ryan *et al.*, 2012). As an essential micronutrient, zinc (Zn) is also equally important for a balanced nutrition. It is a vital element in maintaining the normal physiology, and its deficiency affects multiple functions in the human body. Application of fertilizer seems to be a simple way for duly correcting and improving the soil fertility and plant nutritional status. However, crop genotypes differ in their response to P and Zn application with respect to the uptake and utilization efficiency of these elements (Khan *et al.*, 1998, 2000; Zhu *et al.*, 2001a; Srinivasarao *et al.*, 2007; Ibrikci *et al.*, 2009). Since phosphorus and zinc interact both in plants and soils, they affect the availability and utilization of each other. Furthermore, chickpea genotypes vary widely in their Zn-accumulation capacity and sensitivity to soil Zn deficiency (Siddiqui *et al.*, 2013). Given this, a cautious selection and cultivation of chickpea genotypes that are tolerant to P and Zn deficiencies, coupled with a balanced use of fertilizers, can be the best strategy in the low-input sustainable agriculture systems, especially in the developing countries.

Since application of fertilizer P is critical for improving chickpea yield, and since genotypic variations exist for Zn-accumulation capacity and tolerance to zinc deficiency, it is necessary to evaluate the effect of P fertilizers on Zn bioavailability to chickpea. Although phosphorus-zinc interactions have been widely investigated in plants (Das *et al.*, 2005; Das, 2015), this aspect remains little explored. We, therefore, made an attempt to investigate whether the genotypic background of this crop has some impact on the availability of P, and eventually on the crop performance. Keeping

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the above in view, a pot experiment was designed to investigate the effect of P supply on plant growth, and on the uptake and concentration of P and Zn in two chickpea genotypes that differ in their Zn-accumulation capacity.

2. Materials and Methods

2.1 Plant material and growth conditions

A phosphorus-fertilization study was conducted in sand-filled pots having a constant level of zinc and varying amounts of phosphorus (0, 13.5 and 27.0 mg P kg^{-1} of sand, symbolized as P_0, $P_{13.5}$ and P_{27}, respectively) with two chickpea (*Cicer arietinum* L.) genotypes IC269837 and IC269867, having a high and a low Zn-accumulating capacity, respectively (Siddiqui *et al.*, 2013), under naturally illuminated green house condition at the Jamia Hamdard Campus, New Delhi.

Ten uniform-sized healthy seeds were surface-sterilized with 0.1% mercuric chloride for 5 min, rinsed vigorously with deionized water and then germinated in the dark on non-contaminated sand moistened with deionized water. After one week, 5 uniformly germinated seedlings were transferred to clay-pots filled with acid-washed sand. Before potting, recommended basal doses of N, K, S, and Zn were mixed thoroughly in the sand in order to get 25 kg N, 30 kg K, 20 kg S and 10 kg Zn ha^{-1}. Urea, muriate of potash (KCl), gypsum and zinc sulphate ($ZnSO_4$) were used as the source for the four nutrients, respectively. Phosphorus in the form of single super phosphate was added to the sand at concentrations of 0, 13.5 and 27.0 mg kg^{-1} and mixed thoroughly. The experimental design was block randomized with three replicates and three treatments. The average day/night temperatures were 33/20±2 °C, with a relative humidity of 60-70%. The crop was given protective irrigation throughout the experiment, using double deionized water (DDW), depending upon the water requirement. Plants were maintained up to 90 days after sowing (DAS), and all measures were taken to ensure a healthy plant growth.

Plants were uprooted carefully, rinsed several times with deionized water and blotted gently. Roots and shoots were separated and oven-dried at 70ºC for 48 h, before estimating the biomass (in g per plant) by weighing the dried material. For estimation of Zn and P concentrations, dried root and shoot samples were ground to fine powder, digested in a mixture of concentrated nitric and perchloric acid (4:1 ratio) at high temperature (up to 200 ºC), and then diluted using deionized water. Zn concentration was analyzed on atomic absorption spectrophotometer (AAS ZEEnit 65, Germany), whereas P concentration was determined by the vanadomolybdate yellow colour method (Jackson, 1973), in acid-digested extract solution. The concentration of Zn and P in plant samples was expressed as mg kg^{-1} DW and g kg^{-1} DW, respectively. The total contents of Zn and P were determined by multiplying shoot dry matter (g per plant) with the concentration of each of the elements separately, and expressed in μg per plant and mg per plant, respectively.

2.2 Statistical analysis

Analysis of variance (ANOVA) was performed using the Graph Pad Prism software (GraphPad Prism ver. 5, San Diego California USA). The data were presented as mean ± standard error (n = 3). Treatment means were compared, using the Duncan's multiple range test (DMRT) and taking p <0.05 as significant.

3. Results

3.1 Dry matter production

The effect of P application on dry matter production in shoot and root was significant in both the chickpea genotypes (Table 1).

HZnG did better than LZnG with each of the treatments given. Genotypic differences were significant among untreated (control) as well as N-grown plants. Root biomass of both the genotypes increased significantly (p < 0.05) with increase in P supply (Table 1). The increase was maximum, about 55% in HZnG and 61% in LZnG, with $P_{13.5}$ treatment (13.5 mg P kg^{-1} sand), as compared to their respective controls. At the highest level of P (P_{27}), a decline set in and the increase over the control was only about 23% and 31% in HZnG and LZnG, respectively. Shoot biomass also increased significantly (p < 0.01) with increase in P supply in both the genotypes (Table 1). However, HZnG showed a significantly higher shoot biomass than LZnG. The percent increase in shoot biomass was the highest with $P_{13.5}$ treatment in both HZnG (41.71%) and LZnG (67.81%), in comparison to the control. On addition of a high dose of P (P_{27}), shoot biomass was reduced in both the genotypes; it was 23.16% in HZnG and 14.93% in LZnG, compared to $P_{13.5}$ treatment. The effect of P was non-significant (p > 0.05) on the ratio of root and shoot biomass (Table 1). Although the ratio was greater in HZnG than in LZnG, it did not differ significantly between the genotypes (p > 0.05). P treatment improved the shoot biomass more than the root biomass, and therefore, the root:shoot ratio in general decreased with increase in P supply to both the genotypes. The reduction in the ratio was nearly 21% and 18% in HZnG and about 16% and 22% in LZnG, with 13.5 and 27 mg P kg^{-1} sand, respectively, as compared to the control.

3.2 Zn and P accumulation in shoot

Phosphorus application, up to 13.5 mg P kg^{-1} sand, significantly (p < 0.001) increased shoot zinc concentration (Figure 1a) in both HZnG (18.51%) and LZnG (15.13%), but declined by 8.87% in HZnG and 6.44% in LZnG at a high (P_{27}) P level, in comparison to the control. Zn concentration was significantly higher (p < 0.05) in HZnG than in LZnG with each level of applied P. The effect of interaction between genotypes and P levels applied (G × P) was non-significant (p > 0.05).

The effect of P application on shoot Zn content was also significant (p < 0.001) (Figure 1b). The highest increase was recorded at $P_{13.5}$ treatment in both the genotypes, but on further increase in P level the content decreased for both the genotypes. Zinc content was 48.79, 105.78 and 63.80 μg per plant in HZnG, while it was 32.18, 73.36 and 50.83 μg per plant in LZnG, at P_0, $P_{13.5}$ and P_{27} treatments, respectively. HZnG had a significantly higher (72.79 μg per plant) Zn content than LZnG (52.12 μg per plant), irrespective of P dose applied (Figure 1b). The G × P interaction effect was non-significant (p > 0.05) for both the genotypes.

Likewise, phosphorus application significantly increased the shoot P concentra-tions of both the genotypes over their respective controls (p < 0.01) (Figure 2a). Compared to the control, it increased by 61.34% and 142% in HZnG, whereas by 99.24% and 149.24% in LZnG at $P_{13.5}$ and P_{27} treatments, respectively. On the whole, P concentration in LZnG (2.41 g kg^{-1} DW) was significantly (p < 0.05) higher than in HZnG (1.99 g kg^{-1} DW). However, untreated populations of both genotypes did not differ significantly (p < 0.05) for P concentration. The highest concentration was observed at P_{27}, which was relatively greater in LZnG (3.29 g kg^{-1} DW) than in HZnG (2.88 g kg^{-1} DW), showing a significant difference (p < 0.05). The G × P effect was non-significant (p > 0.05) in both the genotypes.

The shoot P content also increased significantly with increase in P application rates (p < 0.01) (Figure 2b). It was 2.35, 7.01 and 7.97 mg per plant in HZnG, while 1.91, 7.60 and 7.93 mg per plant in LZnG with P_0, $P_{13.5}$ and P_{27}, respectively. Although P content was high at the higher dose (P_{27}), it did not differ significantly (p > 0.05) from that at $P_{13.5}$ treatment in both the genotypes. The genotypic difference was non-significant (p > 0.05) in control as well as P-treated plants. The G × P interaction effect was also non-significant (p > 0.05).

4. Discussion

Significant genotypic differences due to P application were apparent in parameters studied in the two chickpea genotypes (Table 1; Figures 1 and 2). Our results demonstrate that an increase in the available soil P was associated with low Zn concentrations in shoots, which agrees with the findings of Gianquinto *et al.* (2000) and Zhu *et al.* (2001b). This P-induced decline in Zn concentration was possibly due to the dilution effect of increased shoot growth than to a reduced Zn uptake by roots (Singh *et al.*, 1988; Gianquinto *et al.*, 2000). However, in the present case, reduced Zn concentrations cannot be explained fully by a dilution effect, because changes in biomass due to treatments were less marked than changes in shoot Zn concentration (Table 1; Figure 1). Zn concentration in HZnG was higher than in LZnG with all the three treatments. This genotypic difference could be because (a) high P uptake may depress Zn uptake by roots, as shown in Figures 1 and 2, where HZnG always had a higher Zn uptake than LZnG under P stress, and (b) high P uptake may involve a high rate of P transport from root to shoot *via* the xylem, which may hinder root-to-shoot Zn translocation, the proportion of shoot Zn was consistently lower in LZnG than in HZnG. Across the genotypes, shoot zinc content increased with increase in P supply from P_0 to $P_{13.5}$ but significantly declined at P_{27} in both the genotypes, which could be due to mutually antagonistic effect of P and Zn (Singh *et al.*, 1988).

Our observations on increase in root and shoot dry matter and shoot P concentration are in agreement with those of Li *et al.* (2003), who observed a significant increase in plant shoot biomass and tissue P concentrations due to increase in P supply in the two cultivars of barely differing in Zn and P efficiencies. Similarly, maize responded well to P supply in growth medium with reference to shoot P concentration (Gill *et al.*, 2004). Zhu *et al.* (2001a) reported a significant increase in P concentrations in both shoots and roots of spring wheat due to P supply irrespective of the genotypes.

These results indicate that in P-deficient medium ($P_{13.5}$), Zn had a positive interaction with P. However, at higher P levels (P_{27}), an antagonistic effect manifested, as observed earlier by Srinivasarao *et al.* (2007); the significantly negative relationship between shoot Zn and P concentrations could be correlated to P translocation from roots to shoot.

5. Conclusion

A balanced use of fertilizer is necessary for efficient uptake, mobilization and utilization of macro as well as micronutrients. An optimum performance of chickpea can be maintained by a balanced application of P, and crop production can be improved by exploiting genotypic variations in tolerance to low availability of soil Zn and P. Genotypes tolerant to deficiency of a particular nutrient may possibly resist nutrition disorders caused by the nonavailability of other nutrients also.

Table 1: Effect of phosphorus (P) application on root dry matter (RDM) and shoot dry matter (SDM) production (expressed in g per plant) in a high (HZnG) and a low (LZnG) zinc accumulating chickpea genotype. Plants were harvested at 90 days after sowing

Treatments	RDM		SDM		Root: shoot ratio	
	HZnG	**LZnG**	**HZnG**	**LZnG**	**HZnG**	**LZnG**
P_0	1.45 ± 0.25[bc]	1.03 ± 0.04[c]	1.99 ± 0.40[bc]	1.46 ± 0.18[c]	0.78 ± 0.15[a]	0.73 ± 0.11[a]
$P_{13.5}$	2.25 ± 0.15[a] (55.17)	1.66 ± 0.35[abc] (61.16)	3.67 ± 0.32[a] (84.42)	2.88 ± 0.26[ab] (97.26)	0.62 ± 0.06[a] (-20.51)	0.61 ± 0.19[a] (-16.44)
P_{27}	1.78 ± 0.19[ab] (22.76)	1.35 ± 0.05[bc] (31.07)	2.82 ± 0.43[ab] (41.71)	2.45 ± 0.31[bc] (67.81)	0.64 ± 0.05[a] (-17.95)	0.57 ± 0.07[a] (-21.92)
ANOVA	RDM		SDM		Root: shoot ratio	
Genotype (G)	**Ns**		**Ns**		**Ns**	
Treatment (T)	*		**		**Ns**	
G × T	**Ns**		**Ns**		**Ns**	

Values in parentheses indicate percent variation with reference to respective controls. Each value is a mean ± SE of three replicates. The data followed by different letters are significantly different (p < 0.05, Duncan's multiple range test). **Ns** = not significant; *, ** are significant at p < 0.05 and p < 0.01, respectively.
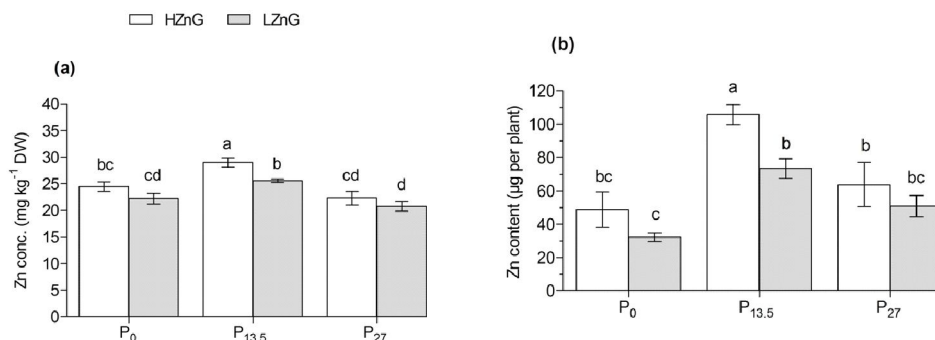

Figure 1: Effect of different levels of phosphorus (P) supply on (a) shoot-zinc concentration (mg kg⁻¹ DW) and (b) shoot-zinc content (μg per plant) in two chickpea genotypes (a high and a low zinc-accumulators). Plants were harvested at 90 DAS. Vertical bars represent ± standard error of means of three replicates. The data followed by different letters are significantly different (p < 0.05, Duncan's multiple range test)
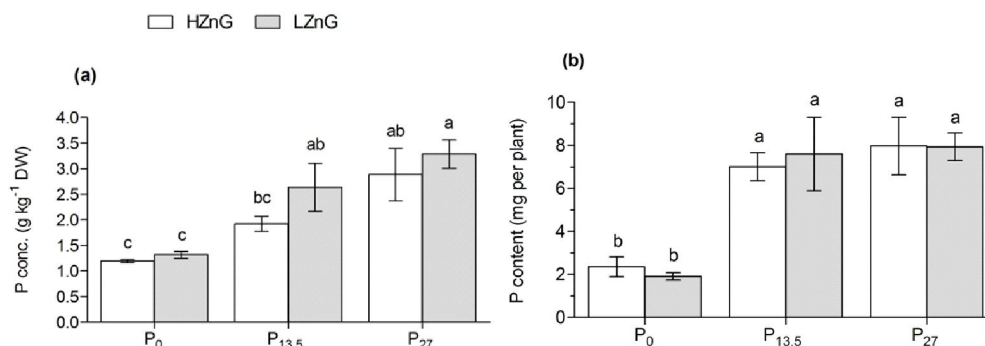
Figure 2: Effect of different levels of phosphorus (P) supply on (a) shoot-phosphorus (P) concentration (g kg^{-1} DW) and (b) shoot-phosphorus content (mg per plant) in a high (HZnG) and a low (LZnG) zinc-accumulating genotype of chickpea as analyzed at 90 DAS. Vertical bars represent ± standard error of means of three replicates. The data followed by different letters are significantly different (p < 0.05, Duncan's multiple range test)

Conflict of interest

We declare that we have no conflict of interest.

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