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Variation in growth, physiology and yield of four chickpea cultivars exposed to cadmium chloride

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Abstract

Cadmium is a highly toxic metallic pollutant which adversely affects plant growth. A green house experiment was conducted to study the variation in growth, yield and proline content of four chickpea (*Cicer arietinum* L.) cultivars namely ICC1069, ICC12422, ICC7589 and ICC4969 at two plant growth stages (30 and 60 days after sowing), treated with 0, 25, 50 and 100 mg Cd kg^{-1} soil. Plant growth, plant fresh weight, plant biomass, leaf area, total photosynthetic area, carbonic anhydrase activity, yield and proline content exhibited a dose- dependent response to Cd on four cultivars of *Cicer arietinum* L. The shoot and root length showed a reduction of 10.02, 10.63, 12.97, 7.93 and 4.95, 6.09, 7.85, 9.23% in all the four cultivars respectively, whereas shoot and root dry weight showed a reduction of 18.82, 27.61, 11.27, 44.59 and 10.63, 4.89, 3, 11.94% in all the cultivars respectively at 50 mg Cd kg^{-1} soil at 30 days of growth stage. It was a general observation from the results that all the parameters of plants were reduced in a concentration-duration dependent manner. However, the proline content of leaf is increased with the increase in Cd concentration. It showed an increase of 15.66, 17.5, 18.42 and 23.61% at 100 mg Cd kg^{-1} soil at 30 days of growth stage. Maximal significant reductions in the growth characteristics were observed with 100 mg Cd kg^{-1} soil in all the cultivars in both the samplings. Among cultivars, ICC1069 proved tolerant and showed lesser decrease in the growth characteristics, whereas ICC4969 proved as non-tolerant and showed maximum decrease in the growth characteristics.

Key words

Cadmium, Chickpea cultivars, Growth variations

Introduction

Increased pollution of soils due to continuous use of heavy metal contaminated industrial effluents is critical to crop production globally and a great environmental threat. These metals accumulate in soils and plants in excess and enter food chain (Kashem and Singh, 1999; Stolt *et al.,* 2006; Jamali *et al.,* 2007).

Most abundant metals in the environment with unknown metabolic functions include arsenic, mercury, cadmium, lead and uranium are toxic to plants. Among these, cadmium (Cd) is relatively more noxious soil pollutant and its excessive discharge as a byproduct from industries has worsened the situation (Mengel *et al.,* 2001). It is readily absorbed by the roots and is translocated in different parts of the shoot (Benavides *et al.,* 2005). Reduction in growth and biomass yield with increased levels of Cd arises because of altered physiological phenomenon (Demirevska-Kepova *et al.,* 2006). It causes leaf rotting and chlorosis of foliar parts, hampers leaf water status and photosynthesis (Chugh and Sawhney, 1999; Perfus-Barbeoch *et al.,* 2002), affects ATPase activity of plasmalemma (Fodor *et al.,* 1995; Astolfi *et al.,* 2005), changes lipid composition by enhanced production of reactive oxygen species (Demirevska-Kepova *et al.,* 2006) or decreased activities of antioxidants in a number of plant species (Agrawal and Sharma, 2006; Pal *et al.,* 2006). The ability of plant genotypes to detoxify Cd can differ between and within plant species which play a significant role in the expression of high tolerance in phytotoxicity (Mobin and Khan, 2007).

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell by various substances, for example, organic acids, amino acids, phytochelatins, and metallothioneins; (iv) accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes; (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Cho *et al.,* 2003).

Indeed, heavy metals are known to involve a breakdown of cells membrane lipid due to increased accumulation of reactive oxygen species (ROS) mediated-oxidative stress (Rellan- Alvarez *et al.,* 2006). ROS such as superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Cakmak, 2000), may lead to unspecific oxidation of proteins and membrane lipids, (Cho and Seo, 2005). Unlike redox-active metals, like Cu, Fe, etc., Cd is unable to induce the production of ROS through a Fenton-like reaction. It can induce oxidative stress indirectly by causing a disturbance in chloroplasts (Baszynski *et al.,* 1980). Thus, Cd causes degradation of chlorophyll and carotenoids as well as inhibition of their biosynthesis (Bazzaz *et al.,* 1992), which can result in disturbances in electron transport rates of PS I and PS II leading to the generation of oxygen free radicals (Asada, 1999). Moreover, Cd is known to induce a transient loss of antioxidative capacity, perhaps accompanied by a stimulation or inhibition of oxidant-producing enzymes, resulting in the generation of ROS (Sandalio *et al.,* 2001; Smeets *et al.,* 2005).

Therefore, selection of plant genotypes with high ability to tolerate Cd toxicity is a reasonable approach to counteract the adverse effect of Cd in crop growth and yield. Chickpea (*Cicer arietinum* L.) is an important pulse crop of India which supplies 70 to 90 % of the world's chickpea. It contains 18.20% protein, 62% carbohydrate, 4% fat and is a rich source of Ca, Fe and niacin. It can grow in low rainfall and poor soils (Wealth of India, 2001).

In the present investigation, four varieties of chickpea (*Cicer arietinum* L.) were treated with different concentration of Cd to evaluate their relative tolerance to Cd toxicity on the basis of growth, yield and proline content.

Materials and Methods

The seeds of different cultivars of chickpea (*Cicer arietinum* L.) were obtained from National Seed Corporation Ltd., New Delhi. Seed were sterilized with 0.1% sodium hypochlorite solution for 10 min and rinsed with double distilled water. Seeds of all four cultivars namely ICC1069, ICC12422, ICC7589 and ICC4969 were sown in earthen pots of 10 inches filled with a mixture of soil and compost (3:1) proportion. Soil properties such as texture, porosity, degree of saturation, density and water content were determined by the method of Moodie *et al.* (1959). The pH and EC of soil extract, organic matter, total nitrogen (N) and phosphorus (P) were estimated by the method of Black (1965). The pots were kept under natural photoperiod and 3 days after emergence, the seedlings were thinned to five per pot. The plant was treated with different concentrations of $CdCl_2$ solutions (25, 50 and 100 mg kg^{-1} soil) and pot receiving no Cd served as control. Plants were watered with distilled water as when required.

Growth in terms of shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, leaf area and total photosynthetic area were determined at 30 and 60 days after treatment. Among biochemical characteristics, total chlorophyll content, carotenoid content, carbonic anhydrase activity and proline content were measured twice at 30 and 60 days after treatment. Number of seeds per plant and weight of 100 seeds were taken as yield characteristics. Shoot length was measured on meter scale. Leaf area was measured by a leaf area meter (LA-211, Systronics, India). Dry weight was recorded by drying the plants in an oven at 80^o C till constant weight. The total photosynthetic area was obtained by multiplying leaf area and leaf number per plant. Total chlorophyll content and carotenoid content were measured by the method of MacKinney (1941). Carbonic anhydrase activity was measured by the method described by Dwivedi and Randhava (1974) whereas proline content was estimated following the method of Bates *et al.* (1973).

Analysis of variance was performed using SPSS computer package (SPSS ver. 17 inc.) to find significant differences among chickpea varieties. Cadmium treatments and their interactions, trend lines and correlation coefficients were drawn of the degrees of leaf area and total photosynthetic area with root dry matter.

Results and Discussion

Data revealed significant ($P \leq 0.01$) differences among varieties, Cd–levels and their interaction for biomass, carotenoid content, total chlorophyll content, proline content, carbonic anhydrase activity and yield. Among the varieties, ICC4969 exhibited lowest reduction in plant length (root and shoot length), while it was highest in ICC1069 followed by ICC12422 at 30 and 60 DAS (Table 1). The order of performance of varieties in terms of biomass was ICC1069>ICC 12422>ICC7589>ICC4969. Growth reductions of Cd treated chickpea plants has earlier been reported due to higher accumulation of Cd and reduction in the availability of other nutrients resulting in disturbed metabolism. (Ouzounidou *et al.,* 1997; Wu and Zhang, 2002; Wu *et al.,* 2003; Khan *et al.,* 2007; Ahmad *et al.,* 2009) The reduction in shoot dry weight by Cd toxicity in wheat cultivars could be the direct consequence of the inhibition of photosynthesis (Khan *et al.,* 2006).

The soil moisture ranged from 4 to 8% and moisture was maintained in green house condition. Physico-chemical properties of soil showed sandy loam texture, saturation of ~29%; pH 8.2;

Table- 1 : Effect of different concentrations of cadmium on growth of four varieties of chickpea at 30 and 60 days after sowing

		Cd treatments (mg kg^{-1} soil)							
		Shoot length (cm)				Root length (cm)			
DAS	Cultivars	0	25	50	100	0	25	50	100
30	ICC 1069	13.07±0.017	13.12±0.02	11.76±0.046	10.83±0.053	10.7±0.066	10.77±0.092	10.17±0.082	9.75±0.066
	ICC12422	12.80±0.026	12.86±0.03	11.44±0.046	10.59±0.02	9.53±0.026	9.60±0.062	8.95±0.056	8.23±0.082
	ICC7589	10.87±0.02	10.92±0.02	9.46±0.053	8.69±0.046	9.33±0.026	9.39±0.087	8.60±0.1	7.96±0.13
	ICC4969	9.33±0.053	9.37±0.017	8.59±0.046	7.79±0.056	9.10±0.1	9.18±0.026	8.26±0.057	7.10±0.095
60	ICC 1069	28.0±2.66	28.1±0.053	27.73±0.10	26.54±0.075	17.47±0.026	17.54±0.12	17.26±0.01	16.87±0.082
	ICC12422	27.6±0.2	27.69±0.12	26.82±0.085	25.49±0.046	17.07±0.026	17.15±0.026	16.77±0.017	16.15±0.036
	ICC7589	24.07±0.098	24.1±0.026	23.59±0.066	22.73±0.82	11.30±0.2	11.39±0.12	11.10±0.058	10.57±0.02
	ICC4969	20.21±0.115	20.3±0.044	19.67±0.029	18.80±0.104	9.10±0.29	9.18±0.036	8.86±0.036	8.23±0.026
		Dry weight of shoot (g)				Dry weight of root (g)			
30	ICC 1069	0.340±0.026	0.348±0.0026	0.276±0.0053	0.196±0.0036	0.160±0.0044	0.167±0.0017	0.143±0.0062	0.129±0.0046
	ICC12422	0.297±0.011	0.306±0.0036	0.215±0.0044	0.173±0.0082	0.143±0.0072	0.150±0.0044	0.136±0.0017	0.113±0.0053
	ICC7589	0.213±0.0062	0.221±0.0026	0.189±0.004	0.113±0.0026	0.100±0.017	0.110±0.02	0.097±0.013	0.065±0.0098
	ICC4969	0.157±0.002	0.163±0.0053	0.087±0.002	0.035±0.0053	0.067±0.02	0.073±0.0026	0.059±0.0053	0.032±0.0026
60	ICC 1069	1.72±0.036	1.8±0.056	1.59±0.12	1.39±0.046	2.71±0.13	2.78±0.03	2.46±0.036	2.11±0.036
	ICC12422	1.59±0.04	1.61±0.098	1.43±0.062	1.29±0.046	2.61±0.066	2.69±0.10	2.32±0.061	1.96±0.026
	ICC7589	1.39±0.046	1.46±0.026	1.27±0.02	1.09±0.053	2.32±0.089	2.40±0.040	2.17±0.02	1.83±0.053
	ICC4969	1.25±0.044	1.26±0.026	0.95±0.044	0.79±0.046	2.18±0.026	2.29±0.036	1.85±0.053	1.62±0.035

electrical conductivity (EC) 285 µmhos cm^{-1}; soil organic matter, 1.15%; total N, 0.52%; total P, 6.6 mg kg^{-1} and K^+, 160 mg kg^{-1}, respecively

Under control conditions, dry weight of ICC4969 showed maximum reduction on 30 and 60 days. At 100 mg kg^{-1} Cd level, this attribute was reduced in all the varieties, lowest in ICC4969, ICC7589 and ICC12422, and highest in ICC1069 on 30 and 60 days of sampling (Table 1). The leaf area per pot did not differ much under control, but was reduced in all the varieties under Cd stress, although substantial varietal differences were evident. At highest Cd level, maximum leaf area per pot was evident in ICC1069 and ICC12422, whilst minimum in ICC7589 and ICC4969 at both sampling stages (Fig.1).

Under Cd stress, ICC1069 followed by ICC12422 indicated better total photosynthetic area per pot whilst it was lowest in ICC4969 at both the sampling (Fig. 2).This study showed that despite substantial varietal differences, increased Cd levels were detrimental to chickpea as evident from gradual reduction in leaf area and total photosynthetic area. This may be attributed due to the senescence and death of older leaves and appearance of injury symptoms on the younger leaves at high Cd stress, thereby reducing leaf area per pot and total photosynthetic area per pot (Ghani and Wahid, 2007).

Total chlorophyll and carotenoid content was found maximum in ICC1069 and ICC12422 and minimum in ICC7589 and ICC4969 at 30 and 60 DAS (Fig. 3 and 4). The decrease in chlorophyll and carotenoid is due to detrimental effect of increased Cd level (Larsen *et al.* 1998). Similarly, decreased synthesis of chlorophyll due to Cd treatment has been reported earlier by several workers. The decreased chlorophyll and carotenoid would ultimately decrease photosynthesis rate (Vassilev *et al.,* 1993; Costa and Morel, 1994; Zhang *et al.,* 2003; Mobin and Khan, 2007).

Under control conditions, maximum carbonic anhydrase activity was shown by ICC1069, and minimum by ICC4969. At 100 mg kg^{-1} Cd level, the activity reduced in all the varieties (Fig. 5) at both the growth stages. This may be the consequence of a general inhibitory effect of Cd on enzyme activities and alteration in their structure. It has been reported by different workers that activity of carbonic anhydrase is decreased under Cd stress (Mobin and Khan, 2007; Khan *et al.,* 2007).

The level of proline in leaves showed an increasing trend at 25 and 50 mg Cd kg^{-1} soil and was found to be maximum at 100 mg Cd kg^{-1} soil in all the varieties at 30 and 60 DAS (Fig. 6). At low Cd stress, the increase in proline accumulation serves as a biomarker of heavy metal stress and this has been proposed as a mechanism of storage of excess nitrogen (Rhodes *et al.,* 1999). Among the four tested heavy metals that induce proline accumulation, Cd was the strongest inducer of proline (Roy *et al.,* 1992; Kastori *et al.,* 1992; Singh and Tiwari, 2003). Proline accumulation is a symptom of injury which does not confer tolerance against metal or other stresses (Lutts *et al.,* 1996). In contrast, suggestions have been made that proline might protect plants from heavy metal toxicity (Smirnoff and Cumbes, 1989; Kavi Kishore *et al.,* 1995).

In yield attributes, weight of 100 seeds as well as number of seeds per plant showed reduction with increase in Cd concentration except at 25 mg Cd kg^{-1} soil in all the varieties at both 30 and 60 days

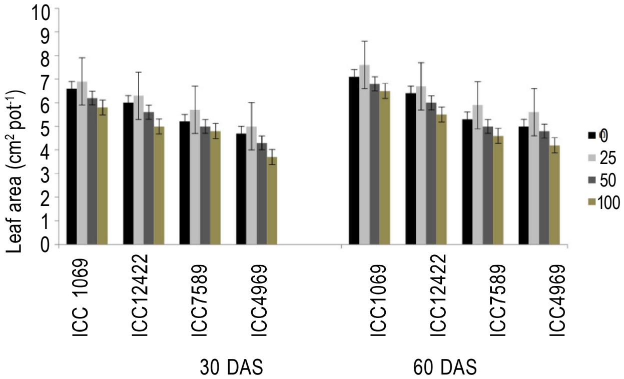

Fig.1: Effect of different concentrations of Cd on leaf area in four varieties of chickpea at 30 and 60 DAS

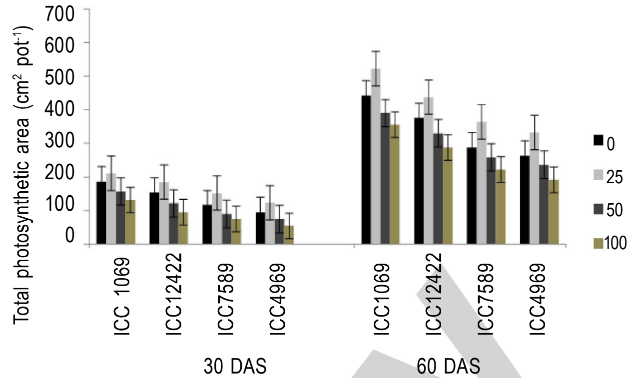

Fig.2: Effect of different concentrations of Cd on Total photosynthetic area in four varieties of chickpea at 30 and 60 DAS

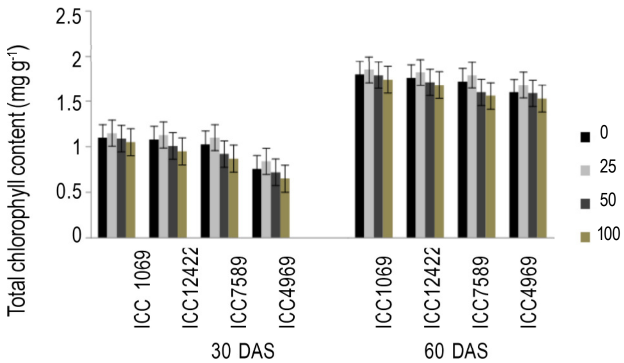

Fig.3: Effect of different concentrations of Cd on Total chlorophyll content in four varieties of chickpea at 30 and 60 DAS

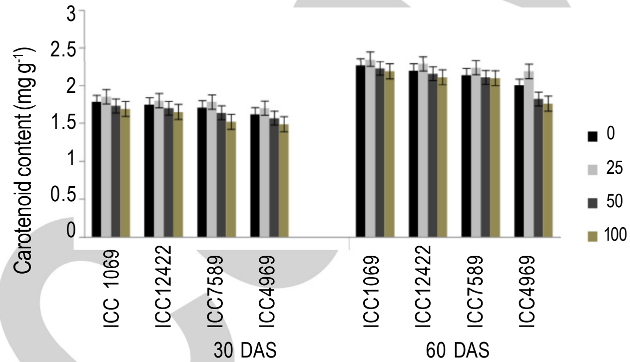

Fig.4: Effect of different concentrations of Cd on carotenoid content in four varieties of chickpea at 30 and 60 DAS

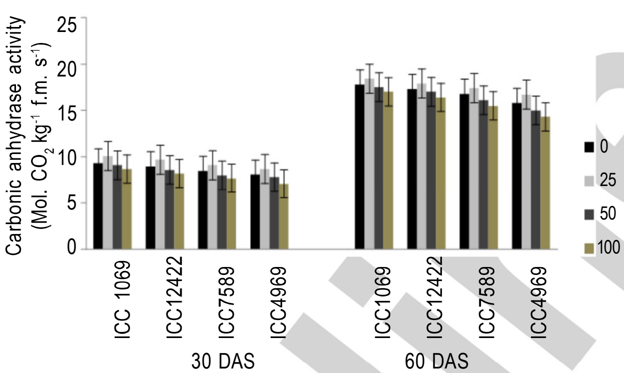

Fig.5: Effect of different concentrations of Cd on carbonic anhydrase activity in four varieties of chickpea at 30 and 60 DAS

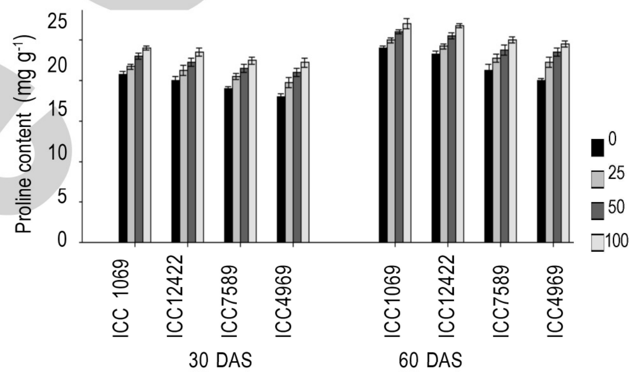

Fig.6: Effect of different concentrations of Cd on proline content in four varieties of chickpea at 30 and 60 DAS

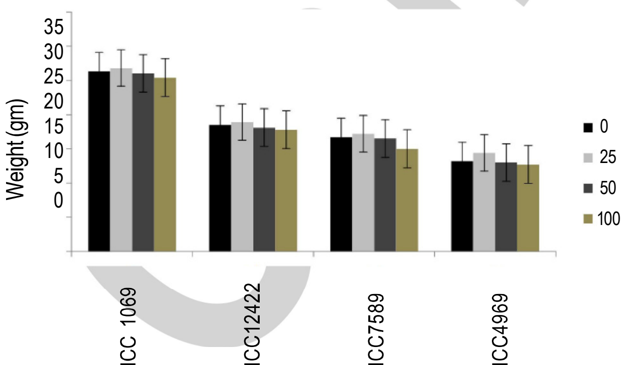

Fig.7: Effect of different concentrations of Cd on weight of 100 seeds (gm) in four varieties of chickpea at harverting stage

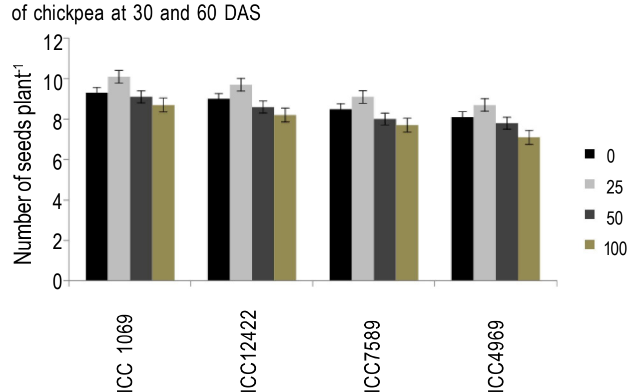

Fig.8: Effect of different concentrations of Cd on number of seeds plant^{-1} in four varieties of chickpea at harvesting stage

Table 2- : Correlation coefficient (r) of shoot and root dry weight with leaf area, total photosynthetic area, total chlorophyll and carotenoid content under 0 and 100 mg Cd kg^{-1} soil at 30 and 60 days after treatment

X-variable	Y-variable	0	100	0	100
		30 DAT		60 DAT	
Shoot dry weight	Leaf area	0.966*	0.951*	0.960*	0.933ns
	Total photosynthetic area	0.995**	0.989*	0.958*	0.943ns
	Total chlorophyll content	0.828ns	0.930ns	0.996**	0.984*
	Carotenoid content	0.994**	0.974*	0.956*	0.926ns
Root dry weight	Leaf area	0.949ns	0.933ns	0.905ns	0.957*
	Total photosynthetic area	0.986*	0.943ns	0.936ns	0.968*
	Total chlorophyll content	0.856ns	0.984*	0.934ns	0.973*
	Carotenoid content	0.991**	0.926ns	0.972*	0.987*

* = P<0.05; ** = P<0.01; ns = not significant

of growth stages (Fig. 7 and 8). The order of performance of cultivars in terms of percent reduction was ICC1069> ICC12422> ICC7589> ICC4969. Panwar *et al.* (1999), Dunbar *et al.* (2003) and Khan *et al.* (2006) also showed that the application of Cd to soil has been found to decrease the yield of crop plants.

Parallels were drawn of shoot dry weight and root dry weight with leaf area, total photosynthetic area and pigment contents of leaf separately at 0 and highest (100 mg kg^{-1}) Cd level at both the sampling stages (Table 2). None of these relationships were evident when no Cd treatment was given. However, at highest Cd level, shoot dry weight and root dry weight were positively related to leaf area, total photosynthetic area and pigment contents at both the sampling stages. Existence of positive correlation of plant biomass with leaf area, total photosynthetic area and pigment contents revealed that plant growth was crippled primarily due to reduced photosynthetic area (Carrier *et al.,* 2003; Adhikari *et al.,* 2006) and reduction in pigment contents (Phetsombat *et al.,* 2006) appeared mainly to be due to the deficiencies of certain essential nutrients as a result of antagonistic effect of Cd accumulation (Epstein and Bloom, 2005; Adhikari *et al.,* 2006).

Therefore, selection of varieties capable of producing greater photosynthetic area under Cd stress may be a lasting solution to the problem in view. In crux, despite substantial variability for its tolerance, effect of Cd damage on plant biomass and subsequent growth are complex. The findings of this study carry great implications for accruing better chickpea stand in marginally Cd-contaminated soils.

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