African Journal of Biotechnology Vol. 11(8), pp. 1886-1891, 26 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2849
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effect of cadmium on soybean (*Glycine max* L) growth and nitrogen fixation

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Accepted 13 December, 2011

To study the effect of cadmium (Cd) on soybean growth and nitrogen (N_2) fixation, an experiment was performed in sand culture using Hoagland nutrient solution. At the time of sowing, different cadmium level that is 0, 4, 8 and 16 mg kg^{-1} sand was created using Cd $(NO_3)_2$. Soybean shoots and root lengths shoot and root biomass, nodule density and Cd uptake was recorded on 2, 4, 6, 8, 10, and 12 weeks after the emergence. To calculate the relative abundance of ureide and % P_{fix} (proportion of plant N derived from N_2-fixation), xylem sap was collected and analyzed for ureide, nitrate and amino-N at pod fill stage. The application of Cd adversely affected soybean growth, nodulation and N_2 fixation as a function of time and increase in Cd concentration. Maximum reduction in the root and shoot length was found with higher Cd level that is 16 mg kg^{-1} sand after 10 weeks of the growth. Similarly, nodulation and the proportion of plant N (% P_{fix}) derived from N_2 fixation decreased sharply as Cd concentrations increased during the whole growth stages and the maximum reduction was observed in the Cd level of 16 mg kg^{-1} sand followed by 8 and 4 mg kg^{-1} sand, respectively. Cadmium uptake increased with the highest Cd application after each bi-weekly harvest of growth.

Keywords: Cd, growth, nodulation, N_2 fixation, soybean.

INTRODUCTION

Nitrogen (N) requirement of leguminous plants is largely met through a symbiotic relationship with N-fixing bacteria of the genus *Rhizobium, Bradyrhizobium, Mesorhizobium, Allorhizobium, Azorhizobium* and *Sinorhizobium*. This symbiotic association of legumes and bacteria contributes to soil fertility (Skeffington and Bradshaw, 1980). N_2-fixed by soybean ranges from 0 to 450 kg ha^{-1} and the proportion of plant N derived from fixation process (% P_{fix}) vary from 0 to 95% as affected by several genetic traits of the plant, and the soil factors (Keyser and Li, 1992). Elevated levels of heavy metals in soil can severely inhibit N_2-fixation by legume (González et al., 2001). Symbiotic N_2-fixation and nodulation is sensitive to heavy metal (Porter and Sheridan, 1981; Obbard and Jones, 1993; Balestrasse et al., 2001) and (Cd) is considered to be the most toxic element decreasing soil biological activity and plant metabolism even at a low concentration (Sanità and Gabbrielli, 1999).

Presence of Cd in growth media reduces nodule formation and impairs nodule functioning in pea (Hernández et al., 1995), soybean (Huang et al., 1974), alfalfa (Porter and Sheridan, 1981), and bean (Vigue et al., 1981), however N_2-fixation is stimulated to some extent at low level of Cd, and decreases sharply with further increase of Cd concentrations where ultra structure of the root nodule in which the active N_2-fixing and the N_2-fixing cells occur, were reduced (Chen et al., 2003).

Cadmium toxicity is 2 to 20 times greater than any other heavy metals (Kabata-Pendias and Pendias, 2001), and plants accumulate Cd in the roots as a first barrier to restrict its transport to the shoot (Das et al., 1997). In white lupins, more than 80% of total Cd was found in the roots (Römer et al., 2000; Zornoza et al., 2002), where Cd is mainly bound to the cell walls (Zornoza et al., 2002), cadmium uptake through root depends on many soil factors for example pH, redox potential, cation exchange capacity, organic matter content and presence of other metals (Greger, 1999). Soybean is an important economic crop worldwide (Graham and Vance, 2003),

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Table 1. Composition of Hoagland's nutrient solution.

Stock solution composition		stock solution used (mL^{-}L)	Element	Final concentration of element (mM)
KNO_3	(1.00 M)	6.0	N	16
$Ca(NO_3)_2 . 4H_2O$	(1.00 M)	4.0	K	6
$NH_4H_2PO_4$	(1.00 M)	2.0	Ca P	4 2
$MgSO_4 . 7H_2O$	(1.00 M)	1.0	S Mg	1 1
KCl	(50 mM)	1.0	Cl	5×10^{-2}
H_3BO_3	(25 mM)	1.0	B	2.5×10^{-2}
$MnSO_4 . 7H_2O$	(2 mM)	1.0	Mn	2×10^{-3}
$ZnSO_4 . 7H_2O$	(2 mM)	1.0	Zn	2×10^{-3}
$CuSO_4 . 5H_2O$	(0.5 mM)	1.0	Cu	5×10^{-4}
H_2MoO_4 (85% MoO_3)	(0.5 mM)	1.0	Mo	5×10^{-4}
Fe-EDTA	(20 mM)	1.0	Fe	2×10^{-2}

and about 3650 ha are under soybean in Pakistan cultivated for edible oils (Jalaluddin, 2005) where production yield of secondary contribution to soil fertility through N_2-fixation have economic implications. The objectives of this study were to determine Cd effect on growth, nodulation and N_2-fixation in soybean and to quantify the Cd uptake by soybean.

MATERIALS AND METHODS

Soybean was grown in sand medium containing graded level of Cd and irrigated with Hoagland solution (Table 1). Plant growth parameters, N_2 fixation and tissue of Cd concentration were recorded. The sieved sand was washed in 0.1 N HCl solution and rinsed with distilled water. 5 kg sand was filled in plastic pots with piezometer to monitor water level. Cd levels 0, 4, 8, and 16 mg kg^{-1} sand was developed in 18 pots (four replicates) using $Cd(NO_3)_2.4H_2O$ salt. Four uniform weight soybean (cv.NARC-3) seeds surface sterilized by 0.1% $HgCl_2$ solution for 2 min followed by thorough rinsing with distilled water, and inoculated with rhizobia after soaking in 48% sugar solution were planted in 72 pots. Plant shoot, root length, number of nodule, shoot and root biomass were recorded on 14, 28, 42, 56, 70 and 84 days after sowing (DAS). The shoot and root were dried in oven at 65°C for 48 h and analyzed for Cd content (Isaac and Johnson, 1975). Cd uptake was calculated from concentration (µg g^{-1}) and shoot dry weight (g).

N_2 fixation assay by xylem solute technique

At pod fill stage, xylem sap from soybean shoots was collected in vacutainers and analyzed for ureide, nitrate and amino-N (Peoples et al., 1989). The remained sap stored at -15°C until analyses. Relative abundance of ureide (RUN %) was [4 × ureide / (4× ureide + nitrate + amino-N)] ×100, proportion of plant N derived from N_2-fixation (% P_{fix}) was 1.6 (%RUN-15.9). Legume N kg ha^{-1} was (legume dry matter kg ha^{-1}) × (%N) and amount of N_2 fixed kg ha^{-1} = %P_{fix} × Crop N kg ha^{-1} × 1.5*. *This factor was used to include an estimate for contribution by below ground N (Peoples et al., 1989). The data for various characteristics was subjected to analysis of variance using two factor factorial designs with level of Cd as main plot and stages/time as sub plot. Treatment means was compared by LSD for statistical difference at 5% error (Steel et al., 1997). Multivariate analyses of variance (MANOVA) were also employed (Dimiter and Phillip, 2005). The hypothesis of significant week's × Cd level interaction for various treatments was tested by multivariate Test statistics WILKS LAMBDA P0.01.

RESULTS

Plant growth

Soybean shoots and root growth reduced with increase in Cd and the difference increased with time. The difference in plant shoots and roots length in control and the highest Cd level at 14 days after sowing (DAS) was insignificant. The measured root length at 28th day with 16 mg Cd kg^{-1} sand was significantly low than the control treatment, and the difference increased with time (Figure 1a). Similarly, shoot length at 28 DAS with 16 mg Cd kg^{-1} sand reduced as compared to the plant grown in the control treatment and the difference increased with time (Figure 1b).

Maximum shoot and root biomass of soybean plants was obtained in the control treatment and the minimum at Cd 16 mg kg^{-1} sand. Significant reduction in the shoot and root biomass was observed in all the six bi-weekly harvest of soybean growth with increasing Cd concentration. After 28 DAS of soybean growth, the shoot and root biomass reduced by 12 and 31%, respectively in the Cd level of 16 mg kg^{-1} sand as compared to the control treatment. Similar decreasing trend of shoot and root biomass was observed at all stages of soybean growth cycle. Biomass decreased gradually as the concentration of Cd in the sand media increased to 4 to 16 ppm. Similarly, this indicate that the shoot and root biomass reduced with the application of Cd in higher concentration treatment and the reduction increases with time and even some plant conceive death due to high

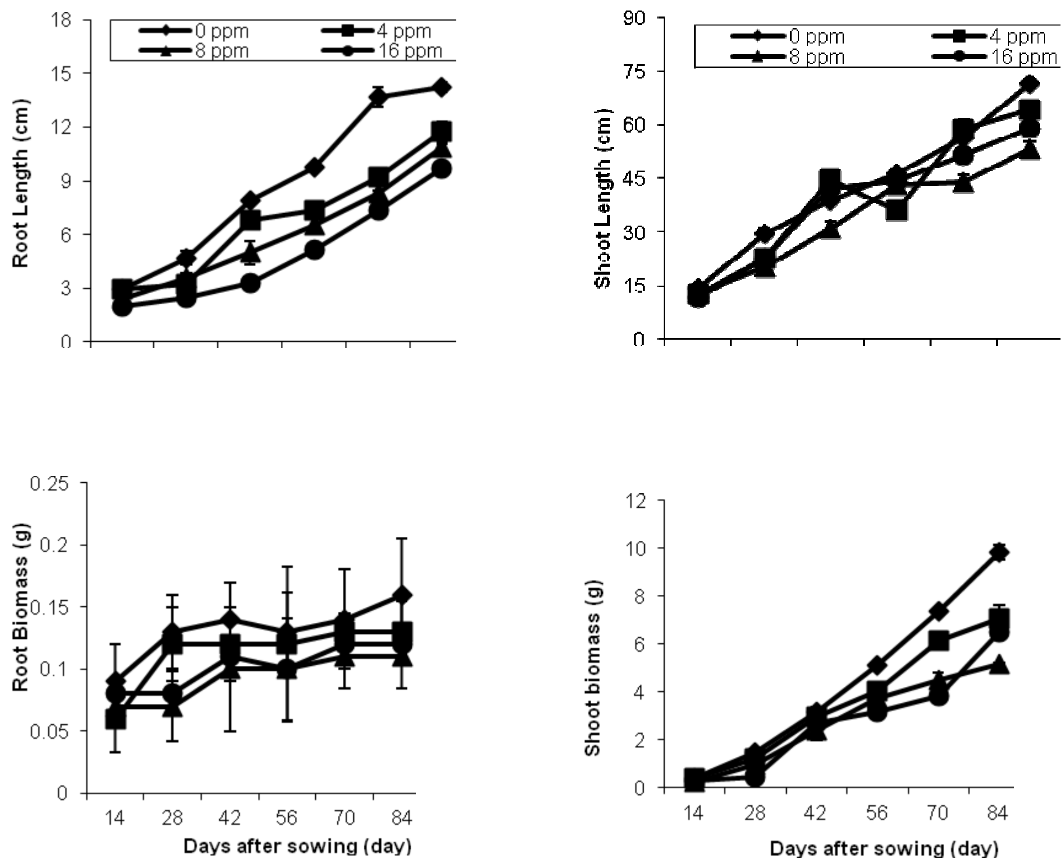

Figure 1. Effect of different levels of cadmium on root length (a), shoot length (b), root biomass (c) and shoot biomass (d) of soybean.

concentration of Cd in sand medium.

Cadmium effect nodulation

The number of root nodules decreases as the Cd concentrations increased. At 28 DAS, similar number of nodules was seen in the control treatment. Similar, trend was observed throughout soybean growth. At the time of 70 DAS, the maximum nodules were counted in the control treatment. As illustrated in (Figure 2a), the maximum number of nodulation was counted in the control treatment and with the increase in Cd concentration the nodules number was significantly decreased.

Nitrogen fixation as affected by Cadmium

The effect of different Cd levels on proportion of nitrogen derived by soybean through nitrogen fixation (P_{fix}) at pod fill stage is shown in Figure 2b. The application of Cd causes reduction in % P_{fix} as Cd concentration increased. The maximum reduction (50%) in % P_{fix} was found in the highest concentration (16 mg kg^{-1}) Cd followed by 41% by the plants grown in the Cd treatment of 8 mg kg^{-1} sand as compared to the control treatment. There was significant difference in the % P_{fix} by the plants grown in the control treatment and in the Cd concentration of 16 mg kg^{-1}.

The N_2 fixation of soybean was reduced by Cd concentration and the difference increases with time. The difference in plant N_2 fixation by soybean in the control treatment and highest Cd concentration treatment up to 42 DAS was insignificant. Nitrogen fixation at 84^{th} day with 16 mg Cd 16 mg kg^{-1} sand was significantly low than the control treatment (Figure 2c). Nitrogen fixation by soybean was firstly increased slightly as the Cd concentration increased, but decreased significantly once the Cd concentration was above 8 mg kg^{-1} sand.

Cadmium uptake by Plant

The Cd concentrations in shoot increased with increasing Cd concentration and with time; however the Cd concentration in the shoot of soybean plants increased with the highest Cd application after each bi-weekly

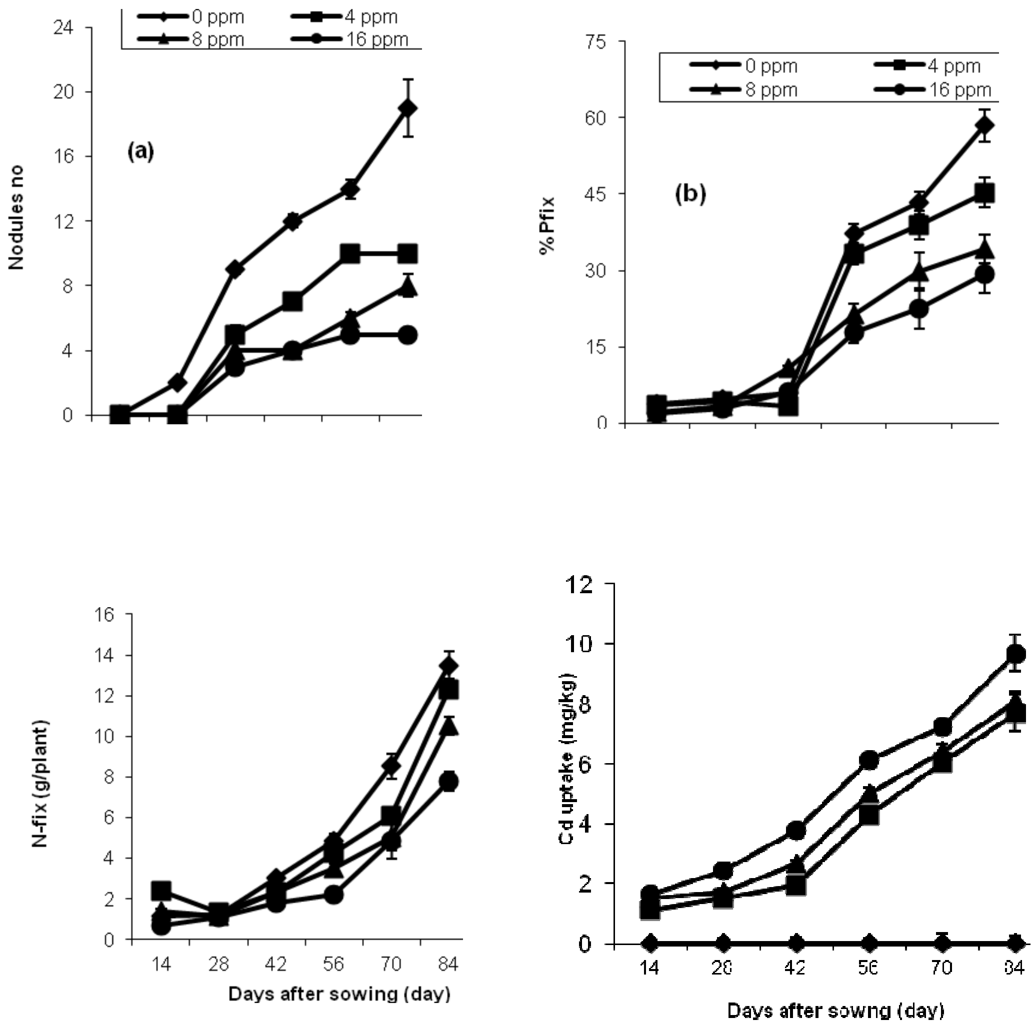

Figure 2. Effect of different levels of cadmium on nodule numbers (a),%Pfix (b), N2-fixed (c) and Cd uptake (d) of soybean.

harvest of growth. At 28 DAS of growth, the maximum Cd was obtained by the plants grown under Cd concentration of 16 mg kg^{-1} sand and the minimum was taken by the plants grown in the control treatment (Figure 2d). Similar, pattern was also found after each harvest of growth.

DISCUSSION

An increase in Cd concentration significantly inhibited the root and shoot growth in soybean plant except at the early stages. Cadmium is strongly phytotoxic and growths inhibit or even cause plant death. Cadmium alters various physiological processes affecting growth, inhibition of enzymes, and altered stomatal action (Barcelo and Poschenrieder, 1990; Das et al., 1997). Cadmium is retained in the roots and only small amounts are transported to the shoots (Cataldo et al., 1983). The inhibitory effect of Cd on root elongation is mediated through altered cell growth. Root and shoot elongation per day was 1.15 mm day^{-1} and 7.04 mm day^{-1} at 16 mg Cd kg^{-1} sand, respectively which was less to root and shoot elongation in control treatment 1.69 mm day^{-1} 8.51 mm day^{-1}, respectively. Cadmium also inhibits nutrients uptake by plants (Obata et al., 1996). Higher Cd concentrations caused reduction in the plant biomass. Higher doses of heavy metal can affect physiology; reduce plant growth and dry biomass yield (Grifferty and Barrington, 2000; Nwosu et al., 1995). Cadmium is strongly phytotoxic and causes growth inhibition and even plant death. Cadmium alters in various physiological processes including growth, inhibits enzymes, and stomatal action (Barcelo and Poschenrieder, 1990; Das et al., 1997). Mean plant biomass of soybean decreased as the concentrations increased from 4 to 8 to 16 ppm of Cd as noted on 84 DAS, (Figure 1c and d).

The results also imply that cadmium stress has significant deleterious effects on root nodulation, especially at the level of 8 and 16 mg Cd kg^{-1} sand. Cadmium has an adverse effect on legume nodule metabolism even at low concentration. The presence of Cd inhibits nitrogenase activity and photosynthesis, affecting the number of nodules and shoot, root, leaf and nodule biomass (Neumann and Werner, 2000; Balestrasse et al., 2003, 2005a) and inducing nodule senescence (Balestrasse et al., 2004). Cadmium is also toxic to the microsymbiont (Ibekwe and Angle, 1996). Cadmium induced oxidative stress produces a decrease in carbohydrate and soluble protein at the nodule, leghemoglobin depletion, inhibition of antioxidant enzyme activity and an increase in lipid peroxidation and thiols (Carpena et al., 2003; Balestrasse et al., 2001, 2003, 2004, 2005a, b). Nodule ultrastructure is also altered by the presence of Cd (Carpena et al., 2003; Balestrasse et al., 2004). The negative effect of environmental stresses on N_2-fixation includes plant infection by Rhizobia; effects nodule growth and function (González et al., 2001). The plant N derived from N_2-fixation was significantly reduced by the application of heavy metal in higher amounts (Broos et al., 2004). Rother et al., (1983) while examining nodulation and N_2-fixation by white clover growing on mine spoils with up to 216 mg Cd kg^{-1}, 20 g Zn kg^{-1} and 30 g Pb kg^{-} observed slight increase as growth progressed from the early flowering stage to the podding stage. The deleterious effect of Cd was also observed on the nodulation and N_2-fixation with a much lower level of Cd added. The number of rhizobium present in the soils was greatly reduced in most contaminated treatments (Obbard et al., 1993). The results imply that low level Cd contamination might stimulate N_2 fixation by root nodules to some extent, whereas severe contamination of Cd would result in the significant inhibition of N_2 fixation (Chen et al., 2003). Among heavy metals Cd is considered dangerous due to its high mobility at small concentration and effects plants germination (Barcelo and Poschenrieder, 1992; Jarvis et al., 1976). Once Cd has penetrated the root it can travel to the xylem by an apoplastic and/or symplastic pathway (Sanita di Toppi and Gabbrielli, 1999). Cadmium in plant is typically complexed by organic acids and phytochelatins (Cataldo et al., 1983; Przemeck and Haase, 1991; Senden et al., 1992; Salt et al., 1995).

Conclusion

Growth, nodulation and N_2 fixation of soybean was adversely affected with increased in Cd concentration and the difference increases with each bi-weekly harvest from germination till maturity. Results also showed increased Cd uptake and higher uptake were observed with higher Cd level. The lower Cd levels of this experiment may reflect realistic towards natural soil environment. These results suggests further time course studies on the mechanisms of metal toxicity and recovery by rhizobia to assess accurately the role that Cd toxicity plays on growth and N_2 fixation of legumes.

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