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Varietal Differences for Cadmium-induced Seedling Mortality and Foliar-toxicity Symptoms in Mungbean (*Vigna radiata*)

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ABSTRACT

Cadmium (Cd) is a highly toxic soil contaminant affecting crops growth particularly at early stages. Objective of this study was to find varietal differences in mungbean [*Vigna radiata* (L.) Wilczek] for growth and seedling survival under the increased Cd-levels. With substantial varietal difference, applied Cd enhanced the seedling mortality more notably at higher levels. Leaves of all the varieties showed increased signs of chlorosis and necrosis, stunting of shoot and reducing its dry matter and leaf area per pot. Drawing parallels between various symptomatic and growth attributes revealed that Cd-sensitivity of mungbean in survival and growth of plants was closely related to enhanced chlorosis and necrosis of leaves, reduced number and area of leaves per pot. Albeit varietal differences, applied Cd not only enhanced the seedling mortality but also reduced the photosynthetic area by causing chlorosis and necrosis of leaves thus reducing the biomass per pot. In crux, selection of mungbean based on foliar toxicity and seedling survival criteria are imperative to accomplish requisite crop stand and exploitation of marginally Cd-affected soils.

Key Words: Cd-toxicity; Correlations; Nutrients; Seedling survival; *Vigna radiata*

INTRODUCTION

Increased pollution of soils due to continuous use of heavy metal contaminated industrial effluents is critical to crop production globally. The heavy metals greatly accumulate in the soils and plants to show toxicity (Kashem & Singh, 1999; Lone *et al*., 2003). Most abundant metals in the environment with unknown metabolic functions include arsenic, mercury, cadmium, lead and uranium, and are therefore toxic to plants. Of these, cadmium is relatively more damaging due to its excessive discharge as a byproduct from industries (Mengel *et al*., 2001). Cadmium is readily absorbed by the roots and translocated to shoots (Benavides *et al*., 2005). Stunting of growth in terms of elongation and dry matter yield with increased Cd-levels in growth media occurs due to enhanced leaf rolling and chlorosis of foliar parts and hampered leaf water status and photosynthesis (Chugh & Sawhney, 1999; Perfus-Barbeoch *et al*., 2002). Studies further suggest that Cd affects ATPase activity of plasmalemma fraction in wheat and sunflower roots (Fodor *et al*., 1995; Astolfi *et al*., 2005), changes lipid composition by enhanced production of reactive oxygen species (Demirevska-Kepova *et al*., 2006) or decreases activities of antioxidants in a number of plant species (Agarwal & Sharma, 2006; Pal *et al*., 2006).

Available reports show marked differences for Cd-tolerance in various plant species including wheat (Zhang *et al*., 2002), cotton (Wu *et al*., 2004), pea (Metwally *et al*., 2005) and rice (Wu *et al*., 2006). This is because Cd is readily bioavailable during initial hours of exposure (Martin & Kaplan, 1998), and its hyper-accumulation causes the leaf chlorosis (Baryla *et al*., 2001; Smeets *et al*., 2005; Wang & Zhou, 2006). Such symptoms on various plant parts directly determine the severity of stress, and therefore may be useful in the diagnosing stress effects and adopting appropriate strategies to increase stress tolerance (Ahmad *et al*., 2005).

Despite scattered information exists (Rout *et al*., 1999), varietal differences in mungbean for Cd-tolerance merits thorough investigation with specific focus on foliar toxicity symptoms and seedling survival. We surmise that symptoms of Cd-toxicity and seedling survival have close association with each other and determine the final plant stand. In view of this hypothesis, this study reports association of Cd-induced foliar chlorosis, necrosis and seedling mortality in some elite varieties of mungbean, which is an important source of human diet, feed and fodder for animals and green manure (Saleemi, 1998).

MATERIALS AND METHODS

Experimental details. Mungbean ([*Vigna radiata* (L.) Wilczek] seeds for the present study were obtained from Nuclear Institute for Agriculture and Biology, Faisalabad, and sown in plastic pots (10 per pot) containing 10-kg soil. Physico-chemical characteristics of soil were: texture sandy loam, saturation 29%, pH 8.1, ECe 2.2 dS m^{-1} and OM 1.15%. After thinning at fourth day of emergence, eight seedlings of uniform size were maintained per pot. The experimental design was completely randomized with four replications. Ten-day old seedlings were applied with increased levels of Cd (1, 2.5, 5, 10 & 15 mg kg^{-1} soi) using $CdCl_2.2.5H_2O$ as soil dressing. Plants were irrigated with subsoil water whenever needed to keep soil moisture up to optimum level. Half strength nutrient solution was applied twice to meet the plant nutrient requirement.

Visual symptoms and growth determinations. All determinations were made 15 day after the Cd treatments were applied. Number of surviving plants was counted to determine the seedling survival. Chlorosis and necrosis of leaves were quantified and expressed as percentage of total area. Number of green photosynthetic leaves was counted on each plant. Leaf area per plant was determined of intact plant by taking the image of leaf on a paper and area determined subsequently on a leaf area meter. The shoots were excised and immediately determined for fresh mass. The shoot samples were put in paper envelops, kept in an oven at 70^{o}C for a week and determined for their dry mass. Data for these parameters was expressed on per pot basis.

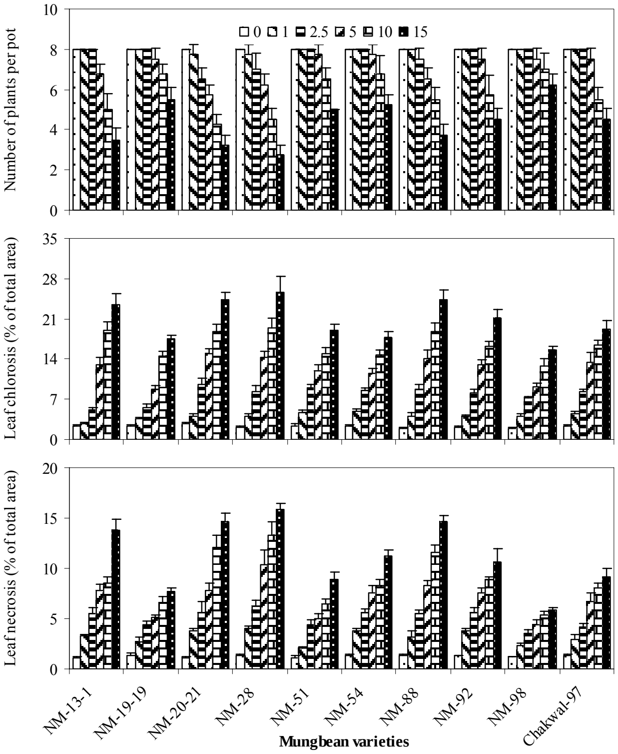
Statistical analysis. Analysis of variance was performed using COSTAT computer package (COHORT software, 2003, Monterey, California) and to find differences among mungbean varieties, Cd treatments and their interactions. Trend lines and correlations coefficients were drawn of the degrees of leaf chlorosis and necrosis with shoot dry matter.

RESULTS

Seedling mortality and visual symptoms of Cd-toxicity. Data revealed significant ($P \leq 0.01$) differences among varieties, Cd-levels and their interactions for seedling survival and symptoms of leaf chlorosis and necrosis. Higher Cd-levels were more damaging to seedling survival than the lower ones. Among the varieties, NM-28 exhibited the lowest seedling survival, while it was the greatest in NM-98 followed by NM-19-19 (Fig. 1). Scattered signs of chlorosis and necrosis on the leaf lamina, between the veins and marginal chlorosis on leaves were well evident, although leaf chlorosis was with greater frequency than necrosis. In control leaves, there was nominal natural chlorosis or necrosis. However, at 15 mg kg^{-1} $CdCl_2$, the chlorosis and necrosis were well evident. Younger leaves were relatively more affected than the older ones; the latter showed enhanced senescence (data not presented). NM-98 and NM-19-19 showed the lowest whereas NM-28 indicated the greatest foliar chlorosis and necrosis (Fig. 1).

Plant growth attributes. Shoot length and its dry mass, and number and area of leaves per pot indicated significant ($P \leq 0.01$) differences among varieties and Cd-levels with an interaction ($P \leq 0.01$) of these factors for shoot dry mass and leaf area per pot, but no interaction was evident for shoot length and number of leaves. Under control condition, maximum shoot length was produced by NM-88 followed by Chakwal-97, while a lowest one by NM-13-1 (Fig. 1). However, under Cd-stress NM-98 and Chakwal 97 exhibited the maximum shoot length while it was the minimum in NM-20-21 and NM-28. Under control condition no marked varietal difference was noted for shoot dry mass. At 15 mg kg^{-1} Cd level this attribute was reduced in all the varieties, being the lowest in NM-13-1, NM-20-21 and NM-28, but highest on NM-98 (Fig. 2). The number of leaves per pot did not differ much under control, but was reduced in all the varieties, although substantial vartietal difference was evident. At highest Cd-level, maximum leaf number was evident in NM-54 and NM-98, whilst the minimum in NM-20-21 and NM-88 (Fig. 2). Leaf area per pot was the most affected attribute among all growth parameters. Under control, NM-51 followed by NM-28 manifested greatest leaf area per pot. Under Cd-stress, NM-51 followed by NM-98 indicated better leaf area per pot whilst it was the lowest in NM-28 (Fig. 2).

Fig. 1. Changes in plant survival, leaf chlorosis and leaf necrosis in mungbean varieties under increased Cd-levels at three growth stages. Legends are levels of $CdCl_2$ kg^{-1} soil.



Correlations. Parallels were drawn of plant survival and shoot dry mass with foliar toxicity symptoms and plant growth attributes separately at 0 and highest (15 mg kg^{-1}) Cd level (Table I). None of these relationships were evident under no-Cd applied. However, at the highest Cd-level, seedling survival was positively related to shoot dry mass per pot. Furthermore, leaf chlorosis and necrosis were negatively related to plant survival and its dry mass, while positively to number and area of leaves per pot (Table I).

DISCUSSION

This study showed that despite substantial varietal differences, increased Cd-levels were detrimental to mungbean as evident from gradual reductions in the plant survival, increased symptoms of foliar toxicity (Fig. 1) and reductions in shoot elongation and its dry mass, as well as

Fig. 2. Changes in some growth attributes of mungbean varieties exposed to increased Cd levels. Legends are $CdCl_2$ kg^{-1} soil.

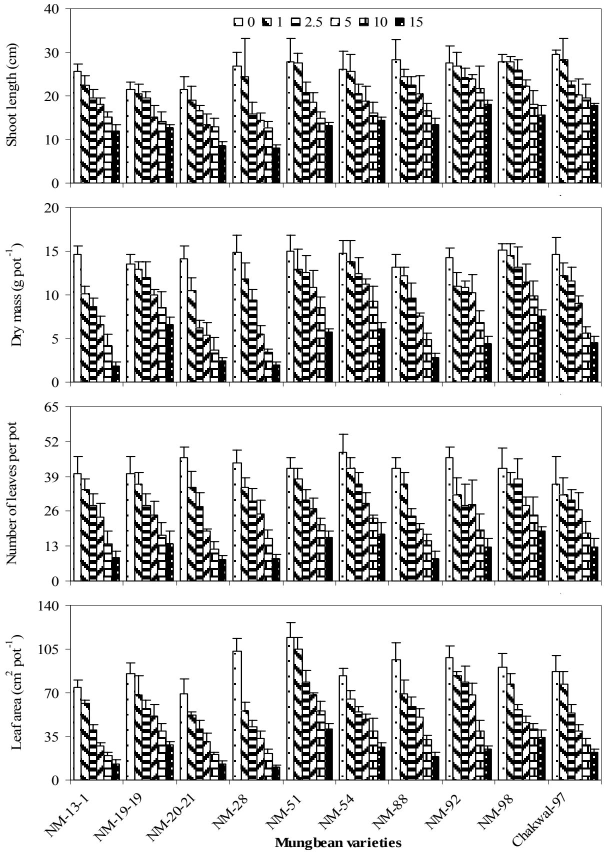


Table I. Correlation coefficient (r) of plant survival and shoot dry weight with Cd-induced chlorosis and necrosis and number and area of leaves under 0 and 15 mg $CdCl_2$ kg^{-1} soil

X-variable	Y-variable	0	15
Number of plants survived	Leaf chlorosis (% of total area)	-0.220ns	-0.978**
	Leaf necrosis (% of total area)	0.409ns	-0.941**
	Shoot dry weight (g pot^{-1})	-0.300ns	0.981**
	Number of leaves per pot	0.060ns	0.939**
	Leaf area (cm2 pot^{-1})	-0.445ns	0.867**
Shoot dry weight (g pot^{-1})	Leaf chlorosis (% of total area)	0.062ns	-0.969**
	Leaf necrosis (% of total area)	-0.379ns	-0.928**
	Number of leaves per pot	0.101ns	0.952**
	Leaf area per pot (g pot^{-1})	0.123ns	0.881**

Significant at ** $P \leq 0.01$ and ns non-significant

number and area of leaves per pot (Fig. 2). This indicated that higher levels of applied Cd accelerated the senescence and death of older and producing injury symptoms on the younger leaves, thereby reducing leaf area per pot. Visual symptoms of Cd-damage as noted here are reported in certain other plant species e.g., radish (Khan & Frankland, 1983), pea (Hernandez & Cooke, 1997) and of leaf chlorosis in oilseed rape (Baryla *et al.*, 2001; Carrier *et al.*, 2003) and rice (Adhikari *et al.*, 2006) grown in Cd-contaminated soils. These effects appeared to result from an antagonistic effect of Cd accumulation on the levels of essential nutrients in leaves (Epstein & Bloom, 2005; Adhikari *et al.*, 2006). The symptoms of Cd-damage noted in this study were similar to the deficiency of essential nutrients including potassium, magnesium, manganese and iron (Epstein & Bloom, 2005), which is important in view of the fact that all these elements are either structurally or functionally related to chlorophyll synthesis and its activity. Since the occurrence of such toxicity signs results directly in the loss of chlorophyll, the reduction in growth is a likely reason for the crippled nutritional status of leaves. Nevertheless, this aspect certainly merits further investigations.

To substantiate the validity of the above findings, the parallels were drawn between plant survival, foliar toxicity symptoms and growth attributes (Table I). Existence of positive correlations of plant survival with shoot elongation, its dry mass and number and area of green leaves, while negative ones of leaf chlorosis and necrosis revealed that plant growth was crippled primarily due to reduced photosynthetic area (Carrier *et al.*, 2003; 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 genetic variability for its tolerance, effects of Cd-damage on the seedling survival and subsequent growth are complex, which appeared to mainly involve the deficiencies of certain essential nutrients and reduced photosynthetic area for dry matter yield. Nevertheless, findings of this study carry great implications for accruing better mungbean stand in marginally Cd-contaminated soils.

REFERENCES

Adhikari, T., E. Tel–Or, Y. Libal and M. Shenker, 2006. Effect of cadmium and iron on rice (*Oryza sativa* L.) plant in chelator–buffered nutrient solution. *J. Plant Nutr*., 29: 1919–40

Agarwal, V. and K. Sharma, 2006. Phytotoxic effects of Cu, Zn, Cd and Pb on in vitro regeneration and concomitant protein changes in Holarrhena antidysenterica. *Biol. Plant*., 50: 307–10

Ahmad, S., A. Wahid, E. Rasul and A. Wahid, 2005. Comparative morphological and physiological responses of green gram genotypes to salinity applied at different growth stages. *Bot. Bull. Acad. Sin*., 46: 135–42

Astolfi, S., S. Zuchi and C. Passera, 2005. Effect of cadmium on H^+–ATPase activity of plasma membrane vesicles isolated from roots of different S–supplied maize (*Zea mays* L.) plants. *Plant Sci*., 169: 361–8

Baryla, A., P. Carrier, F. Franck, C. Coulomb, C. Sahut and M. Havaux, 2001. Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium–polluted soil: causes and consequences for photosynthesis and growth. *Planta*, 212: 696–709

Benavides, M.P., S.M. Gallego and M.L. Tomaro, 2005. Cadmium toxicity in plants. *Brazilian J. Plant Physiol*., 17: 49–55

Carrier, P., A. Baryla and M. Havaux, 2003. Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long–term growth on cadmium–contaminated soil. *Planta*, 216: 939–50

Chugh, L.K. and S.K. Sawhney, 1999. Photosynthetic activities of Pisum sativium seedlings grown in presence of cadmium. *Plant Physiol. Biochem*., 37: 297–303

Demirevska–Kepova, K., L. Simova–Stoilova, Z. Stoyanova and U. Feller, 2006. Cadmium stress in barley: growth, leaf pigment, and protein composition and detoxification of reactive oxygen species. *J. Plant Nutr.*, 29: 451–68

Epstein, E. and A.J. Bloom, 2005. *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd edition. Sinauer Associates, Massachusetts

Fodor, E., A. Szabo–Nagy and L. Erdei, 1995. The effects of cadmium on the fluidity and H^+–ATPase activity of plasma membrane from sunflower and wheat roots. *J. Plant Physiol*., 147: 87–92

Hernandez, L.E. and D.T. Cooke, 1997. Modification of the roots plasma membrane lipid composition of cadmium treated Pisum sativum. *J. Exp. Bot.*, 48: 1375–81

Kashem, M.A. and B.R. Singh, 1999. Heavy metal contamination of soil and vegetation in the vicinity of industries in Bangladesh. *Water Air Soil Pollut*., 115, 347–61

Khan, D.H. and B. Frankland, 1983. Effects of cadmium and lead on radish plant with particular reference to movement of metals through soil profile and plant. *Plant Soil,* 70: 335–45

Lone, M.I., S. Saleem, T. Mahmood, K. Saifullah and G. Hussain, 2003. Heavy metal contents of vegetables irrigated by sewage/tubewell water. *Int. J. Agri. Biol.*, 5: 533–5

Mengel, K., E.A. Kirkby, H. Kosegarten and T. Appel, 2001. *Principles of Plant Nutrition*, 5th edition. Springer, Heidelberg

Metwally, A., V.I. Safronova, A.A. Bellimov and K.J. Dietz, 2005. Genotypic variation of the response to cadmium toxicity in Pisum sativum L. *J. Exp. Bot*., 56: 167–78

Pal, M., E. Horvath, T. Janda, E. Paldi, and G. Szalai, 2006. Physiological changes and defence mechanisms induce by cadmium stress in maize. *J. Plant Nutr. Soil Sci*., 169: 239–46

Perfus–Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier, 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32: 539–48

Rout, G.R., S. Samantaray and P. Das, 1999. Differential cadmium tolerance of mung bean and rice genotypes in hydroponic culture. *Acta Agri. Scandinavica,* 49: 234–41

Saleemi, M.A., 1998. *Environmental Assessment and Management of Irrigation and Drainage Schame for Sustainable Agriculture Growth*. Environmental Pprotection Agency, Lahore

Smeets, K., A. Cypers, A. Lamrechts, B. Semane, P. Hoet, A.V. Laere and J. Vangronsveld, 2005. Induction of oxidative stress and antioxidative mechanisms in Phaseolus vulgaris after Cd application. *Plant Physiol. Biochem*., 43: 437–44

Wang, M.E. and Q.X. Zhou, 2006. Joint stress of chlorimuron–ethyl and cadmium on wheat Triticum aestivum at biochemical levels. *Environ. Pollut*., 144: 572–80

Wu, F., H. Wu, G. Zhang and D.M.L. Bachir, 2004. Differences in growth and yield in response to cadmium toxicity in cotton genotypes. *J. Plant Nutr. Soil Sci.*, 167: 85–90

Wu, F., J. Dong, G. Jia, S. Zheng and G. Zhang, 2006. Genotypic difference in the responses of seedling growth and Cd toxicity in rice (Oryza sativa L.). *Agri. Sci. China*, 5: 68–76

Zhang, G., M. Fukami and H. Sekimoto, 2002. Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crop Res.*, 77: 93–8

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