

# Effect of cadmium on photosynthesis, nutrition and growth of mungbean

A. Wahid, A. Ghani, F. Javed

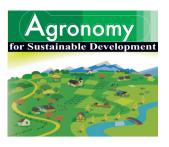
### ▶ To cite this version:

A. Wahid, A. Ghani, F. Javed. Effect of cadmium on photosynthesis, nutrition and growth of mungbean. Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 2008, 28 (2), pp.273-280. hal-00886452

## HAL Id: hal-00886452 https://hal.archives-ouvertes.fr/hal-00886452

Submitted on 1 Jan 2008

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés. Agron. Sustain. Dev. 28 (2008) 273–280 © INRA, EDP Sciences, 2008 DOI: 10.1051/agro:2008010 Available online at: www.agronomy-journal.org



**Original article** 

## Effect of cadmium on photosynthesis, nutrition and growth of mungbean

A. WAHID\*, A. GHANI, F. JAVED

Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan

(Accepted 11 February 2008)

Abstract – Contamination of soils with cadmium is a major threat to ecosystems. Root uptake of cadmium from contaminated soils induces physiological changes such as a decrease in plant growth. Plant species and varieties show differential physiological mechanisms of cadmium tolerance. Here, we studied the effect of cadmium chloride on leaf chlorosis, gas exchange attributes and some essential nutrients in the shoots of selected tolerant and sensitive mungbean (*Vigna radiata*) varieties at the seedling, vegetative and reproductive growth stages. Our results show that elevated concentrations of cadmium led to accumulation of cadmium in the shoot and roots, intervein chlorosis of leaves and loss of pigments. Tolerant mungbean showed steady-state contents of potassium, magnesium, manganese and iron, and photosynthetic pigments at all growth stages. A decrease in the net photosynthesis, increase in substomatal  $CO_2$  level and a decline in the ratio of net photosynthesis and substomatal  $CO_2$  level revealed that cadmium prevented  $CO_2$  fixation by Rubisco. Correlations of shoot cadmium concentration and chlorosis with its nutrient and pigment content, although negative in both varieties, were closer in the tolerant but absent, or weaker if present, in the sensitive mungbean. Most nutrients had close association with the content of photosynthetic pigments of tolerant mungbean, which indicated their involvement in maintaining steady levels of pigments. This finding indicates the involvement of nutrients and pigments in cadmium tolerance. In conclusion, cadmium tolerance in mungbean was attributed to low cadmium uptake and its accumulation in the shoot, leaf chlorosis, improved pigment, nutrient levels and carboxylation efficiency of Rubisco throughout the mungbean phenology. Based on these findings the tolerant mungbean can be grown in moderately cadmium-contaminated soils.

cadmium toxicity / chlorosis / CO2 assimilation / mungbean / nutrients / pigments

#### 1. INTRODUCTION

Contamination of soils with heavy metals has become a serious threat worldwide, because they accumulate in soils and plants in excessive amounts and enter the food chain (Helal et al., 1998; Jamali et al., 2007). Among the many heavy metals, cadmium is the most devastating soil pollutant due to its excessive discharge as a byproduct from industries. It is a nonessential and potentially toxic metal, decreasing dry matter and seed yields (Mediouni et al., 2006). Being highly mobile within phloem, cadmium is translocated into different plant parts (Epstein and Bloom, 2005). Reduction in growth and yield with increased cadmium levels in growth media arises due to leaf rolling, chlorosis of the leaf and stem (Wahid and Ghani, 2008) and a diminished gas exchange rate (Vassilev et al., 2005). Other adverse effects include decreased ATPase activity of the plasmalemma fraction in maize (Astolfi et al., 2005), changed lipid composition by enhanced production of reactive oxygen species and free radicals (Balakhnina et al., 2005), and decreased activities of antioxidants (Agarwal and Sharma, 2006).

Photosynthetic systems are highly damaged by increased cadmium levels. Loss of chlorophyll or its biosynthesis and carbon fixation by Rubisco are of special consideration in crop production. Applied cadmium inhibits chlorophyll biosynthesis, enhances its degradation at the heme level, hampers photochemical and carboxylation reactions of photosynthesis and disrupts the chloroplast metabolism (Vassilev et al., 2005; Ekmekci et al., 2008). In addition, stomatal conductance and its index, transpiration and net photosynthetic rate are influenced by cadmium toxicity (Bindhu and Bera, 2001). Such effects are also assigned to the cadmium-induced membrane damage and activities of antioxidants in pea and sunflower (Hernandez and Cooke, 1997; Azevedo et al., 2005).

Acquisition of essential nutrients in requisite amounts is important for optimal plant growth, since they are either structural or functional components of cells. High cadmium levels perturb the plant mineral nutrition, and a negative correlation has been found for the uptake and distribution of essential

<sup>\*</sup> Corresponding author: drawahid2001@yahoo.com

macro- and micronutrients in various plant parts (Kim et al., 2003; Shukla et al., 2003; Adhikari et al., 2006). Root membrane transporters involved in the uptake of potassium, magnesium and manganese are the first targets of cadmium (Mengel et al., 2001). Being mobile within the plant, cadmium may alter the nutritional status of various plant parts, which may be of greater physiological significance for photosynthesizing leaves (Baryla et al., 2001; Shukla et al., 2003; Wahid et al., 2007).

Mungbean is sensitive to cadmium stress. In screening trials, we noted significant varietal differences in this crop for cadmium tolerance, which led to the selection of cadmiumtolerant (NM-98) and sensitive (NM-28) varieties (Wahid and Ghani, 2008). From the above findings we hypothesized that cadmium tolerance in mungbean might be consistent with lowered chlorosis and maintenance of enhanced  $CO_2$  and nutrient assimilation capacity of the leaves. In view of this prediction, the present study was undertaken to compare the selected tolerant and sensitive mungbean varieties for changes in leaf chlorosis, gas exchange attributes, photosynthetic pigments, some essential nutrients and their interrelationships at three phenological stages.

#### 2. MATERIALS AND METHODS

#### 2.1. Experimental details

Experiments were conducted during February-June, in the 2004 and 2005 sowing seasons, to determine changes in leaf chlorosis, growth, gas exchange, photosynthetic pigments and nutrient content of tolerant and sensitive mungbean [Vigna radiata L. (Wilczek)] varieties. Seeds were sown in plastic pots (5 per pot) containing 10 kg of soil. Thinning was carried out three days after emergence and two uniform seedlings were maintained per pot. The experimental design was completely randomized with four replications. Plants were grown up to predetermined growth stages (seedling, vegetative and reproductive: 15, 40 and 60 days after emergence, respectively) before applying 0 (no cadmium), 3, 6, 9 and 12 mg cadmium per kg soil using cadmium chloride. The cadmium levels used in this study were selected after conducting preliminary experiments under a range of cadmium levels in view of its availability under slightly alkaline pH of the soil, as described elsewhere (Wahid and Ghani, 2008). The physicochemical characteristics of the soil were: texture, sandy loam; saturation, 28-30%; pH, 8; electrical conductivity of soil extract, 2.4 dS/m; organic matter, 1.15%; total nitrogen, 0.52%; and other nutrients (mg/kg) total phosphorus, 6.6; potassium, 160; and calcium, 10.3. Plants were irrigated with tap water (electrical conductivity 0.4 dS/m, sodium adsorption ratio 8, and cadmium nil). Soil moisture was kept at optimum level at all the crop growth periods. Half-strength nutrient solution (Epstein and Bloom, 2005) was applied fortnightly to fulfill nutritional needs. Determinations were made 15 days after the treatment applied at each stage.

## **2.2.** Leaf chlorosis, plant growth leaf gas exchange and pigment determinations

To determine the comparative effects of cadmium stress at all growth stages, intervein chlorosed area was estimated, as described elsewhere (Wahid and Ghani, 2008). Leaf area was determined on a leaf area meter (Model Li-3000, Licor, Licoln, USA). To determine their dry weight, the shoots were cut from roots. The root mass was collected after carefully washing off the adhering soil and briefly blotting the roots dry. Both the parts were put in paper envelopes and kept in an oven at 70 °C for a week. Net photosynthetic and substomatal CO<sub>2</sub> concentrations were determined on the second and third fully expanded leaves using an infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The set of conditions for these determinations was: air flow per unit leaf 335–340 mmol/m<sup>2</sup>/s, atmospheric pressure 98.6–99.8 kPa, photosynthetically active radiation on leaf surface 900–1100  $\mu$ mol/m<sup>2</sup>/s, CO<sub>2</sub> concentration ~357  $\mu$ mol/mol and ambient temperature was different at each stage. The net photosynthesis-to-substomatal-CO<sub>2</sub> ratio, separating the limitations to CO<sub>2</sub> uptake by stomata and its assimilation by Rubisco, was computed.

For the estimation of chlorophylls and carotenoids, the fresh material was extracted in 80% acetone in black-colored bottles, filtered, absorbance of the extract determined immediately using a spectrophotometer and quantities determined as described by Gitelson et al. (2001). Specific absorption coefficients of chlorophyll and carotenoid were used (Lichtenthaler, 1987).

#### 2.3. Elemental analysis

Dried ground powder (0.5 g) of shoot and root was digested in a mixture of concentrated nitric acid and perchloric acid (3:1 ratio) on a heating block for about 1 h by gradually raising the temperature to 250 °C, filtered and the volume made up to 50 mL. The analysis of potassium was carried out using a flame photometer (Sherwood Model 410, Cambridge). Cadmium, magnesium, manganese and iron were estimated using an atomic absorption spectrophotometer (Perkin Elmer, Model AAnalyst 3000, Norwalk, Connecticut) as per the instructions of the manufacturer.

#### 2.4. Statistical analysis

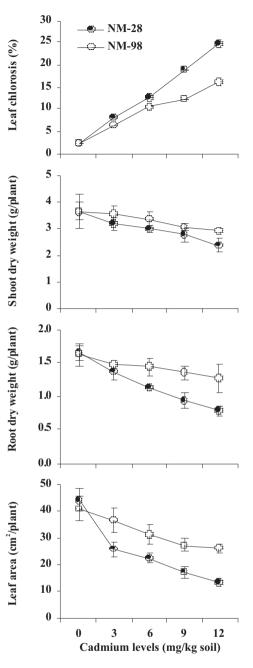
In the absence of any remarkable differences in various growth and analytical characters for both the years, the data were averaged to perform analysis of variance and determine significant differences among varieties, cadmium treatments and their interactions using COSTAT software (COHORT software, 2003, Monterey, California). Correlations were established for shoot cadmium and leaf chlorosis with some gas exchange parameters, pigment and nutrient contents and those of pigments with nutrient contents separately for both the varieties at the seedling, vegetative and reproductive growth stages in order to find the mechanism of cadmium tolerance in mungbean.

#### 3. RESULTS AND DISCUSSION

## 3.1. Plant growth, chlorosis and cadmium concentration

The purpose of this research was to find the physiological basis of cadmium tolerance in the selected tolerant and sensitive mungbean varieties. Adverse effects of cadmium are evident as toxicity symptoms such as chlorosis and necrosis, and diminished growth and yield (Dalla Vecchia et al., 2005). This study revealed significant differences in the chlorosis, dry matter yield of shoot and root, leaf area per plant and cadmium accumulation in the shoot and root at three growth stages (Tab. I). As presented in Figure 1, both the varieties manifested remarkable differences for mesophyll chlorosis with a rise in root zone cadmium levels, although the extent of chlorosis at the seedling, vegetative and reproductive stages was greater in sensitive (eleven-, ten- and nine-fold, respectively) than in tolerant mungbean (seven-, seven- and six-fold, respectively). Greater leaf chlorosis, an important manifestation of cadmium toxicity (Dalla Vecchia et al., 2005; Wahid and Ghani, 2008), mainly on the young leaves of sensitive mungbean, was observed as overall leaf yellowing and tangible chlorosed areas among the veins.

Plant performance under stressful conditions is appraised in terms of reduction in growth, yield and available area for photosynthesis (Bindhu and Bera, 2001; Mediouni et al., 2006). In this study, applied cadmium diminished shoot and root dry weight and leaf area of both the varieties, although this diminution was greater in the sensitive mungbean (Fig. 1). Compared with control, the decline in shoot dry weight at the seedling, vegetative and reproductive stages was 41, 35 and 46% for sensitive and 19, 20 and 31% for tolerant mungbean, while for root dry weight this decline was 60, 52 and 45% for sensitive and 22, 23 and 32% for tolerant mungbean. Likewise, the decline in leaf area per plant at the respective growth stages was 50, 36 and 40% in tolerant mungbean, but 67, 70 and 52% in sensitive mungbean. Increased levels of cadmium are toxic to many plants and the signs of toxicity are evident in the aboveground parts as increased chlorosis, and decreased leaf area, biomass and seed yield in various plant species (Baryla et al., 2001; Wahid and Ghani, 2008). Well-marked difference in the tolerant and sensitive mungbean for shoot and root cadmium accumulation was evident (Tab. I; Fig. 2). The cadmium accumulation at the above-mentioned growth stages was substantially greater for shoots (26, 25 and 20%, respectively) and roots (23, 29 and 28%) of sensitive mungbean as compared with tolerant mungbean (15, 16 and 15% for shoots and 18, 21 and 22% for roots). Negative relationships of leaf chlorosis with shoot dry weight and leaf area (Tab. II) revealed that greater accumulation of cadmium in the leaves of sensitive mungbean as a toxicity response curtailed the available area for photosynthesis, production of photoassimilates and their partitioning for dry matter yield.



**Figure 1.** Leaf chlorosis, shoot and root dry weight, and leaf area of mungbean varieties differing in cadmium tolerance. These data pertain to the plants treated with increased cadmium levels at the vegetative stage and harvested after 15 days. Vertical bars are standard deviations of means.

#### 3.2. Leaf gas exchange and photosynthetic pigments

Photosynthetic systems in plants are specific targets of cadmium damage (Balakhnina et al., 2005; Wahid et al., 2007). Measurements of the changes in net photosynthesis, substomatal  $CO_2$  level and the ratio of net photosynthesis-tosubstomatal  $CO_2$  level revealed significant differences in varieties, cadmium treatments and their interaction at the seedling,

#### A. Wahid et al.

**Table I.** Statistical analysis of variance sources (two mungbean varieties and five cadmium levels including control) applied to the mungbean plants at the seedling, vegetative and reproductive growth stages and harvested 15 days after cadmium treatment application.

Character	Seedling stage		Vegetative stage			Reproductive stage			
	Varieties	Cd levels	$V \times L$	Varieties	Cd levels	$V \times L$	Varieties (V)	Cd levels (L)	V×L
	(V)	(L)		(V)	(L)				
Shoot dry weight	**	**	*	**	**	NS	**	**	NS
Root dry weight	**	**	**	**	**	**	**	**	**s
Leaf area per plant	**	**	*	**	**	**	**	**	NS
Shoot cadmium	**	**	**	**	**	**	**	**	*
Root cadmium	**	**	**	**	**	**	**	**	**
Net photosynthesis	**	**	**	**	**	**	**	**	NS
Transpiration rate	NS	**	NS	NS	**	NS	NS	*	NS
Stomatal conductance	NS	**	NS	NS	*	NS	NS	**	NS
Substomatal CO <sub>2</sub> level	**	*	NS	**	*	NS	**	*	NS
Net photosynthesis-to-	*	**	*	*	*	*	*	**	NS
substomatal-CO2 ratio									
Chlorophyll content	**	**	**	**	**	**	**	**	**
Carotenoid content	**	**	**	**	**	**	**	**	**
Chlorophyll-to-	**	*	NS	**	NS	NS	**	**	**
carotenoid ratio									
Potassium content	**	*	NS	**	**	NS	**	**	NS
Magnesium content	**	**	**	**	**	NS	*	**	**
Manganese content	*	**	NS	**	**	NS	*	**	NS
Iron content	**	**	NS	**	**	NS	*	**	NS

Significant at \*\* P < 0.01; \* P < 0.05 and ns, non-significant.

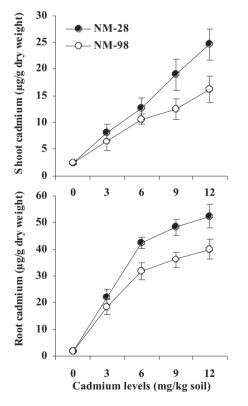


Figure 2. Concentrations of cadmium in the shoot and root of mungbean varieties differing in cadmium tolerance. These data pertain to the plants treated with increased cadmium levels at the vegetative stage and harvested after 15 days. Vertical bars are standard deviations of means.

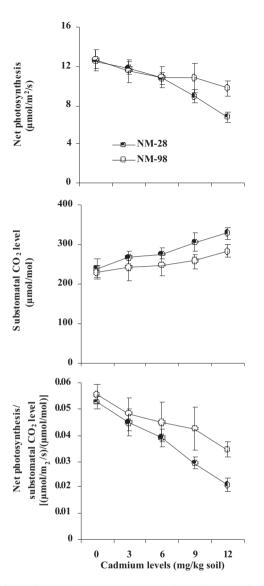
vegetative and reproductive growth stages (Tab. I). Cadmium stress produced a steeper and greater decline in the net rate of photosynthesis of sensitive mungbean (60, 46 and 48%, respectively) than the tolerant mungbean (38, 23 and 39%) at the studied growth stages (Fig. 3). Although applied cadmium increased substomatal CO<sub>2</sub> in both varieties (Fig. 3), nevertheless tolerant mungbean displayed a lower substomatal CO<sub>2</sub> level (34, 23 and 25%) than the sensitive mungbean (40, 37 and 23%) at the seedling, vegetative and maturity stages, respectively. Decline in the net rate of the photosynthesis-tosubstomatal-CO<sub>2</sub> ratio, representing limitation to CO<sub>2</sub> fixation by Rubisco, was greater in the sensitive than the tolerant mungbean (Fig. 3) at all growth periods (72, 61 and 58% for sensitive and 54, 38 and 51% for tolerant mungbean). Overall, these findings suggest that applied cadmium had a discernible effect on the net rate of photosynthesis and substomatal CO<sub>2</sub> concentration of leaves; the former decreased and the latter was enhanced (Fig. 3). This revealed that the effect of cadmium toxicity on CO<sub>2</sub> assimilation was mainly due to the nonstomatal component of photosynthesis, which is contrary to earlier findings for bean (Poschernirieder et al., 1989) and pea (Balakhnina et al., 2005). The elevated substomatal CO<sub>2</sub> concentration and decreased net photosynthesis-to-substomatal-CO<sub>2</sub> ratio revealed that cadmium minimized the carboxylation efficiency of Rubisco as a toxicity response, albeit a sufficient amount of intracellular CO2 was available. The presence of negative correlations of net photosynthesis and the net photosynthesis-to-substomatal-CO2 ratio and positive ones of substomatal CO<sub>2</sub> level with shoot cadmium and leaf chlorosis at the three growth stages of mungbean (Tab. II) further substantiated these effects.

**Table II.** Interrelationships (correlation coefficient, r) established between leaf chlorosis, some photosynthetic parameters, pigment and some essential nutrient contents of cadmium-sensitive and tolerant mungbean varieties at three growth stages.

X variable	Y variable	Seeding		Vegetative	Vegetative		Reproductive	
		Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	
		mungbean	mungbean	mungbean	mungbean	mungbean	mungbean	
Shoot cadmium	Leaf chlorosis	0.986**	0.982**	0.944*	0.958**	0.977**	0.992**	
	Net photosynthesis	-0.909*	-0.974**	-0.985**	-0.955*	-0.985**	-0.955*	
	Substomatal CO <sub>2</sub> level	0.871ns	0.877ns	0.871ns	0.774ns	0.871ns	0.774ns	
	Net photosynthesis-to-	-0.941*	-0.940*	-0.936*	0.986**	-0.981**	0.929*	
	substomatal CO2 ratio							
	Chlorophylls	-0.918*	-0.987**	-0.982 **	-0.976**	-0.982**	-0.976**	
	Carotenoids	-0.945*	-0.991**	-0.896*	-0.933*	-0.896*	-0.933**	
	Chlorophyll-to-	-0.589ns	-0.837ns	-0.922*	-0.968**	-0.922*	-0.968**	
	carotenoid ratio							
	Potassium	-0.671ns	-0.972**	-0.773ns	-0.979**	-0.773ns	-0.979**	
	Magnesium	-0.707ns	-0.895*	-0.765ns	-0.943*	-0.765ns	-0.943**	
	Manganese	-0.716ns	-0.987**	-0.749ns	-0.988 **	-0.749ns	-0.988**	
	Iron	-0.711ns	-0.960**	-0.784ns	-0.978**	-0.784ns	-0.978**	
Leaf chlorosis	Net photosynthesis	-0.962**	-0.917*	-0.984**	-0.984**	-0.997**	-0.972**	
	Substomatal CO <sub>2</sub> level	0.938*	0.999**	0.982**	0.947*	0.937*	0.802ns	
	Net photosynthesis-to-	-0.981**	-0.988**	0.999**	-0.942*	0.999**	0.949**	
	substomatal CO <sub>2</sub> ratio							
	Chlorophylls	-0.966**	-0.988**	-0.947*	-0.987**	-0.986**	-0.987**	
	Carotenoids	-0.973**	-0.985**	-0.924*	-0.977**	-0.934*	-0.960**	
	Chlorophyll-to-	-0.713ns	-0.878*	-0.770ns	-0.981**	-0.910*	-0.975**	
	carotenoid ratio							
	Potassium	-0.749ns	-0.992**	-0.934*	-0.959**	-0.860ns	-0.994**	
	Magnesium	-0.730ns	-0.948**	-0.939*	-0.961**	-0.875ns	-0.976**	
	Manganese	-0.777ns	-0.962**	-0.920*	-0.970**	-0.853ns	-0.971**	
	Iron	-0.744ns	-0.968**	-0.940*	-0.988**	-0.883*	-0.987**	
Chlorophylls	Potassium	0.883*	0.993**	0.881*	0.926*	0.878*	0.994**	
	Magnesium	0.819ns	0.951*	0.847ns	0.922*	0.842ns	0.950*	
	Manganese	0.868*	0.985**	0.782ns	0.938*	0.798ns	0.948*	
	Iron	0.842ns	0.966**	0.855ns	0.993**	0.843ns	0.976**	
Carotenoids	Potassium	0.868ns	0.976**	0.827ns	0.898*	0.962**	0.956*	
	Magnesium	0.852ns	0.933*	0.795ns	0.919*	0.833ns	0.882*	
	Manganese	0.869ns	0.973**	0.724ns	0.907*	0.735ns	0.894*	
	Iron	0.867ns	0.940*	0.820ns	0.982**	0.796ns	0.967**	
	·							
Chlorophyll-to- carotenoid ratio	Potassium	0.778ns	0.929*	0.614ns	0.977**	0.621ns	0.979**	
	Magnesium	0.524ns	0.914*	0.768ns	0.915*	0.786ns	0.923*	
	Manganese	0.747ns	0.889*	0.739ns	0.988**	0.842ns	0.951**	
	Iron	0.576ns	0.924*	0.723ns	0.987**	0.843ns	0.952**	

Significant at \*\*  $P \le 0.01$ , \*  $P \le 0.05$  and ns  $P \ge 0.05$ .

Maintenance of steady levels of photosynthetic pigments is an important manifestation of metal tolerance in plants (Bhattacharjee and Mukherjee, 2003). Chlorophylls and carotenoids are involved principally in light harvesting and a balance in their amounts (given as the chlorophyll-tocarotenoid ratio) is imperative for optimum light energy capture in photosynthesis (Wahid and Ghazanfar, 2006). Leaf chlorophyll concentration revealed significant differences between the varieties and cadmium levels and their interaction at all stages (Tab. I). As depicted in Figure 4 for the vegetative stage, cadmium-treated tolerant mungbean indicated a lesser decline in the concentration of chlorophylls (18, 15 and 24%) than the sensitive mungbean (59, 49 and 61%) at the respective growth stages. Likewise, carotenoid content also decreased (Fig. 4) but indicated significant differences between varieties, cadmium treatments and an interaction of varieties and cadmium treatments at all stages (Tab. I). The decline in carotenoid concentrations was 50, 42 and 39% for sensitive



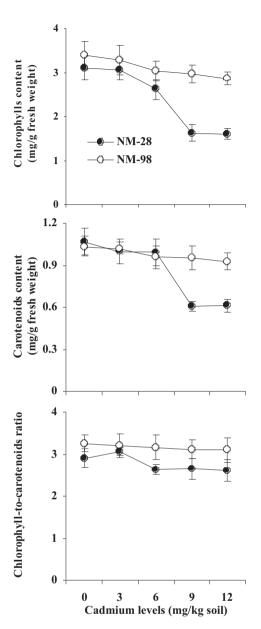


Figure 3. Leaf gas exchange parameters of mungbean varieties differing in Cd tolerance. The measurements were taken at the time of harvesting with an infrared gas analyzer. These data pertain to the plants treated with increased cadmium levels at the vegetative stage and harvested after 15 days. Vertical bars are standard deviations of means.

mungbean and 15, 11 and 27% for tolerant mungbean at the seedling, vegetative and reproductive growth stages. These changes in chlorophyll and carotenoid contents significantly changed the chlorophyll-to-carotenoid ratio in both the varieties with increased cadmium levels (Tab. I; Fig. 4). The decline in this ratio for the sensitive mungbean was 18, 10 and 34%, and it either nominally decreased (3 and 5% at the seedling and vegetative stages) or increased (4% at the reproductive stage) in the tolerant mungbean. The loss of chlorophyll may be due to either an increased activity of the chlorophyll-degrading enzyme chlorophyllase or diminished activity of 5-aminolevulinic acid dehydratase (ALAD), a key

**Figure 4.** Changes in leaf photosynthetic pigments of mungbean varieties differing in Cd tolerance. These data pertain to the plants treated with increased cadmium levels at the vegetative stage and harvested after 15 days. Pigments were extracted from fresh materials preserved at the time of harvest. Vertical bars are standard deviations of means.

enzyme in the chlorophyll biosynthesis pathway (Bhattacharjee and Mukherjee, 2003), or both.

Leaf chlorosis as a result of cadmium toxicity was directly associated with the loss and/or hampered biosynthesis of chlorophyll, as noted in this study for mungbean at various growth stages (Tab. II) and reported previously for pea (Hernandez and Cooke, 1997), brassica (Baryla et al., 2001) and rice (Adhikari et al., 2006). However, tolerant mungbean showed more negative and explicit relationships than sensitive mungbean. More notably, the tolerant, but not the sensitive mungbean, maintained a steady-state Chl-to-Car ratio, which

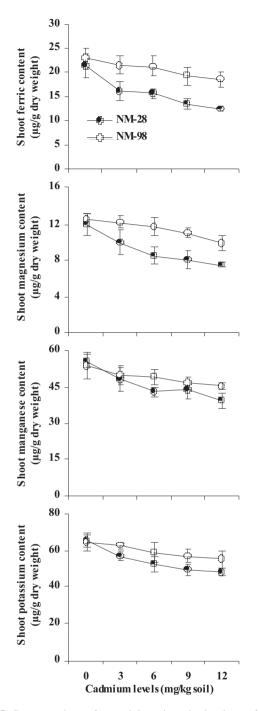


Figure 5. Concentrations of essential nutrients in the shoot of mungbean varieties differing in cadmium tolerance. These data pertain to the plants treated with increased cadmium levels at the vegetative stage and harvested after 15 days. Vertical bars are standard deviations of means.

was closely associated with the plant dry weight ( $r = P \le 0.01$  for tolerant mungbean at all stages, while  $r = P \le 0.05$  at the seedling and  $P \ge 0.05$  at the vegetative and reproductive stages for sensitive mungbean). In addition to the photochemical function, carotenoids have a specific role in mitigating oxidative damage on the thylakoid membranes produced un-

der environmental stresses (Havaux, 1998; de Pascale et al., 2001; Smeets et al., 2005; Wahid and Ghazanfar, 2006). Therefore, mungbean sensitivity to cadmium stress in this study is also assignable to a decreased chlorophyll-to-carotenoid ratio, which led to a limited light harvesting capacity of leaves.

#### 3.3. Shoot nutrients

Cadmium has been shown to hamper the status of essential macro- and micronutrients in a number of plant species (Shukla et al., 2003; Kim et al., 2003; Ghnaya et al., 2007). In this study, determinations were made for potassium, magnesium, manganese and iron, since their deficiency produces visible signs of chlorosis on leaves (Epstein and Bloom, 2005). Statistical analysis of the data indicated significant differences in the mungbean varieties and cadmium levels for the concentrations of potassium, magnesium, manganese and iron (Tab. I; Fig. 5). With substantial differences, both the mungbeans indicated a decline in the contents of potassium (52, 41 and 38% for sensitive and 38, 18 and 34% for tolerant), magnesium (48, 37 and 33% for sensitive and 16, 21 and 34% for tolerant), manganese (29, 30 and 20% for sensitive and 13, 16 and 16% for tolerant) and iron (30, 26 and 26% for sensitive and 19, 15 and 21% for tolerant) at the seedling, vegetative and reproductive stages. The chlorosis of leaves under adverse conditions quite often results from the deficiency of certain essential nutrients (Mengel et al., 2001). Correlations of the studied nutrients with photosynthetic pigments revealed that except for a positive correlation of potassium with chlorophyll, none of the nutrients were related to the carotenoids or chlorophyllto-carotenoid ratio at any stage in the sensitive mungbean; contrarily, these relationships were closer and positive in tolerant mungbean at all growth stages (Tab. II). This revealed that sensitive mungbean appeared to lack an effective control over the accumulation of cadmium and other nutrients, thereby showing decreased growth. In view of their involvement in the biosynthesis of photosynthetic pigments and the role of light and dark reactions of photosynthesis as structural or functional components, we believe that the accumulation pattern of the nutrients as displayed by tolerant mungbean is imperative for its cadmium tolerance.

#### 4. CONCLUSION

In physiological terms, cadmium tolerance of mungbean was related to improved shoot nutrients and photosynthetic pigments, maintenance of a steady-state chlorophyllto-carotenoid ratio and low intervein (mesophyll) chlorosis. These changes in the tolerant mungbean resulted in greater photosynthetic area and an improved  $CO_2$  assimilation capacity of leaves, which emerged as an important mechanism of cadmium tolerance. Based on these findings, tolerant mungbean can be cultivated in moderately cadmium-contaminated soil for sustained production of this important leguminous crop species.

#### REFERENCES

- Adhikari T., Tel-Or E., Libal Y., Shenker M. (2006) Effect of cadmium and iron on rice (*Oryza sativa* L.) plant in chelator-buffered nutrient solution, J. Plant Nutr. 29, 1919–1940.
- Agarwal V., Sharma K. (2006) Phytotoxic effects of Cu, Zn, Cd and Pb on in vitro regeneration and concomitant protein changes in *Holarrhena antidysenterica*, Biol. Plantarum 50, 307–310.
- Astolfi S., Zuchi S., Passera C. (2005) Effect of cadmium on H<sup>+</sup>-ATPase activity of plasma membrane vesicles isolated from roots of different S-supplied maize (Zea mays L.) plants, Plant Sci. 169, 361–368.
- Azevedo H., Glória Pinto C., Santos C. (2005) Cadmium effects in sunflower: membrane permeability and changes in catalase and peroxidase activity in leaves and calluses, J. Plant Nutr. 28, 2233–2241.
- Balakhnina T., Kosobryukhov A., Ivanov A., Kreslavskii V. (2005) The effect of cadmium on CO<sub>2</sub> exchange, variable fluorescence of chlorophyll, and the level of antioxidant enzymes in pea leaves, Russ. J. Plant Physiol. 52, 15–20.
- Baryla A., Carrier P., Franck F., Coulomb C., Sahut C., Havaux M. (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.
- Bhattacharjee S., Mukherjee A.K. (2003) Heavy metals alter photosynthetic pigment profiles as well as activities of chlorophyllase and 5-aminolevulinic acid dehydratase (ALAD) in *Amaranthus lividus* seedlings, J. Environ. Biol. 24, 395–399.
- Bindhu S.J., Bera A.K. (2001) Impact of cadmium toxicity on leaf area, stomatal frequency, stomatal index and pigment content in mungbean seedlings, J. Environ. Biol. 22, 307–309.
- Dalla Vecchia F., La Rocca N., Moro I., De Faveri S., Andreoli C., Rascio N. (2005) Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium, Plant Sci. 168, 329–338.
- de Pascale S., Maggio A., Fogliano V., Ambrosino P., Ritieni A. (2001) Irrigation with saline water improves carotenoids content and antioxidant activity of tomato, J. Hortic. Sci. Biotech. 76, 447–453.
- Ekmekci Y., Tanyolac D., Ayhan B. (2008) Effect of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars, J. Plant Physiol. doi: 10.1016/j.jplph.2007.01.017.
- Epstein E., Bloom A.J. (2005) Mineral Nutrition of Plants: Principles and Perspectives, 2nd ed., Sinauer Associates, Inc. Publishers, Massachusetts.
- Ghnaya T., Slama I., Messedi D., Grignon C., Ghorbel M.H., Abdelly C. (2007) Effects of Cd<sup>2+</sup> on K<sup>+</sup>, Ca<sup>2+</sup> and N uptake in two halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: consequences on growth, Chemosphere 67, 72–79.
- Gitelson A.A., Merzlyak M.N., Chivkunova O.B. (2001) Optical properties and nondestructive estimation of anthocyanin in plant leaves, Photochem. Photobiol. 74, 38–45.

- Havaux M. (1998) Carotenoids as membrane stabilizers in chloroplasts, Trends Plant Sci. 3, 147–151.
- Helal M., Baibagyshew E., Saber S. (1998), Uptake of Cd and Ni by spinach, Spinacea oleracea (L.) from polluted soil under field conditions as affected by salt water irrigation, Agronomie 18, 443–448.
- Hernandez L.E., Cooke D.T. (1997) Modification of the roots plasma membrane lipid composition of cadmium treated *Pisum sativum*, J. Exp. Bot. 48, 1375–1381.
- Jamali M.K., Kazi T.G., Arain M.B., Afridi H.I., Jalbani N., Memon A.R. (2007) Heavy metal contents of vegetables grown in soil, irrigated with mixtures of wastewater and sewage sludge in Pakistan, using Ultrasonic-assisted Pseudo-digestion, J. Agron. Crop Sci. 193, 218–228.
- Kim C.-G., Bell J.N.B., Power S.A. (2003) Effects of soil cadmium on *Pinus sylvestris* L. seedlings, Plant and Soil 257, 443–449.
- Lichtenthaler H.K. (1987) Chlorophyll and carotenoids: pigments of photosynthetic biomembranes, Method. Enzymol. 148, 331–382.
- Mediouni C., Benzari O., Tray B., Ghorbel M.H., Jemal F. (2006) Cadmium and copper toixicity for tomato seedlings, Agron. Sustain. Dev. 26, 227–232.
- Mengel K., Kirkby E.A., Kosegarten H., Appel T. (2001) Principles of Plant Nutrition, 5th ed., Springer, Heidelberg.
- Poschernirieder C., Gunse B., Barcelo J. (1989) Influence of cadmium on water relations, stomatal resistance, and abscisic acid contents in expanding bean leaves, Plant Physiol. 90, 1365–1371.
- Shukla U.C., Singh J., Joshi P.C., Kakkar P. (2003) Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis and macromolecules of wheat seedlings, Biol. Trace Elem. Res. 92, 257–274.
- Smeets K., Cypers A., Lamrechts A., Semane B., Hoet P., Laere A.V., Vangronsveld J. (2005) Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application, Plant Physiol. Biochem. 43, 437–444.
- Vassilev A., Perez-Sanz A., Semane B., Carleer R., Vangronsveld J. (2005) Cadmium accumulation and tolerance of two salix genotypes hydroponically grown in presence of cadmium, J. Plant Nutr. 28, 2159–2177.
- Wahid A., Ghazanfar A. (2006) Possible involvement of some secondary metabolites in salt tolerance of sugarcane, J. Plant Physiol. 163, 723–730.
- Wahid A., Ghani A. (2008) Varietal difference in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation, Ann. Appl. Biol. 152, 59–69.
- Wahid A., Ghani A., Ali I., Ashraf M.Y. (2007) Effects of cadmium on carbon and nitrogen assimilation in shoots of mungbean [Vigna radiata (L.) Wilczek] seedlings, J. Agron. Crop Sci. 193, 357–365.