

Comparison of tolerance of *Brassica juncea* and *Vigna radiata* to cadmium

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Abstract

The effect of different cadmium concentrations (6 - 120 μ M) on Hill reaction activity (HRA) of isolated chloroplasts, contents of chlorophylls (Chls) and carotenoids (Cars), and Cd uptake and accumulation in plant organs of Indian mustard (*Brassica juncea* L. cv. Vitasso) and mung bean [*Vigna radiata* (L.) Wilczek] were determined. The Cd stress inhibited photochemical activity of isolated chloroplasts of both species and in both tested developmental stages. On the basis of EC₅₀ values, the mung bean showed a higher sensitivity to Cd treatment than Indian mustard. The higher sensitivity of both species was determined in the earlier than in the older developmental stage. The leaves of Cd-treated plants possessed lower contents of Chls and Cars in both species and the negative effect increased with Cd concentration. A difference between species was also found in Cd uptake and accumulation. In both species, Cd was accumulated more in roots than in shoots, with higher accumulation in Indian mustard than in mung bean.

Additional key words: carotenoids, Cd uptake and accumulation, chlorophylls, developmental stage, Hill reaction, Indian mustard, mung bean.

Introduction

Contamination of soils with heavy metals is a serious worldwide problem both for human health and agriculture. Cadmium belongs to the most dangerous environmental pollutants and has toxic and mutagenic effects on both plants and animals. This metal is readily taken up by plants and translocated to aerial organs, which facilitate its entry into the food chain (Rauser and Meuwly 1995). The toxic effect of Cd on plants has been extensively reviewed (*e.g.* Sanitá di Toppi and Gabbriellini 1999, Siedlecka and Krupa 1999). Cadmium as a stress factor acts on plants and causes many responses at different levels. Toxic Cd-application not only inhibits growth (Breckle 1991, Lunáčeková *et al.* 2003, Dong *et al.* 2005), but also brings about changes in various physiological and biochemical characteristics (Van Assche and Clijsters 1990, Vassilev *et al.* 1997, Dražić *et al.* 2006, Scebba *et al.* 2006). Cadmium affects the water balance (Kirkham 1978, Barceló *et al.* 1989), photosynthesis (Baszynski *et al.* 1980, Krupa *et al.* 1993, Masarovičová *et al.* 1999), photosynthetic electron transport around photosystems PS 1 and PS 2 (Tukendorf

and Baszynski 1991, Siedlecka and Baszynski 1993, Skorzynska-Polit and Baszynski 1995, Vassilev *et al.* 2004), and ultrastructure of chloroplasts (Barceló *et al.* 1988). One of the most important reasons for Cd toxicity is, according to Siedlecka and Krupa (1999), an interaction between Cd and Fe. Cd-induced Fe deficiency leads to serious disturbances of the photosynthetic apparatus such as inhibition of chlorophyll (Chl) synthesis, decrease in the pool of Fe-containing electron carriers (cytochromes and ferredoxin), and disorganization of chloroplast structure.

Over 200 terrestrial species are endemic to metalliferous soils and can tolerate and accumulate high amounts of heavy metals such as Cd, Zn, Cu, and Ni in their organs (Baker *et al.* 1989, Kumar *et al.* 1995, Hajiboland 2005). Species from the genus *Brassica* are good toxic metal accumulators, allocating large amounts of Cd, Pb, and Ni into the shoots (Salt *et al.* 1995, 1996, Jiang *et al.* 2000, Panwar *et al.* 2002, Ghosh and Singh 2005). Mung beans are one of the major grain legume crops in the poorer countries of Asia (Lawn and Ahn

Received 10 April 2006, accepted 17 December 2006.

Abbreviations: Cars - carotenoids; Chls - chlorophyll (*a* + *b*); DCPIP - 2,6-dichlorophenol-indophenol; d.m. - dry mass; EC₅₀ - effective concentration of Cd inducing 50 % DCPIP photoreduction; HRA - Hill reaction activity; PAR - photosynthetic active radiation; PS 2 - photosystem 2.

Acknowledgments: The research was supported by the grant of Comenius University in Bratislava (No. 217/2005) and by the Grant Agency VEGA (No. 1/3489/06). We are also grateful to Mgr. Andrej Pavlovič for his technical assistance.

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1985). For their high nutritive value, they are important agricultural crops as a food source of proteins, calcium, phosphorus, and certain vitamins, and some cultivars possess excellent aroma (Gupta 1983, Betal *et al.* 2004). On the other hand, the seedlings of mung bean can possibly be used as accumulators of arsenic (Van den Broeck *et al.* 1998), lead (Rout *et al.* 2001, Singh *et al.* 2003), and chromium (Samantary 2002).

Materials and methods

Seeds of Indian mustard (*Brassica juncea* L. cv. Vitasso) and mung bean [*Vigna radiata* (L.) Wilczek] were grown in thermostat on wet cellulose wadding at relative air humidity of 80 % and temperature of 25 ± 1 °C for three (*V. radiata*) or four days (*B. juncea*). Then the seedlings were transferred into the plastic containers filled with 2 dm³ of Hoagland solution and cultivated in growth chamber under relative air humidity 60 - 70 %, day/night temperature $25/20 \pm 1$ °C, and 12-h photoperiod with irradiance of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. The solutions were continuously aerated with an aquarium air pump. In the growth stage of primary true leaves in both species (stage 1) and four assimilating leaves in *B. juncea* or the first trifoliate leaves in *V. radiata* (stage 2) the seedlings were transferred to the Hoagland solution with Cd concentrations of 6 - 120 μM [$\text{Cd}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$] (Sigma-Aldrich, Munich, Germany), or only to the Hoagland solution (control variant). The response of plants to the Cd effect was evaluated 5 d after metal application. Twenty plants washed in distilled water were used for growth and physiological analyses in three repetitions.

Hill reaction activity (HRA) was determined according to Henselová *et al.* (2004). The reaction mixture contained 9 cm³ of sodium salt of 2,6-dichlorophenol-indophenol (DCPIP) as redox-system with concentrations of 77.8 - 88.9 $\mu\text{mol cm}^{-3}$, and 1 cm³ of

The aim of the present study was to compare tolerance of Indian mustard and mung bean at different developmental stages to cadmium treatment. We evaluated dry mass of roots and shoots, Hill reaction activity (HRA) of isolated chloroplasts, and contents of photosynthetic pigments with respect to uptake and accumulation of cadmium in individual plant organs.

chloroplasts suspension with resulting Chl content of 73.5 in control plants and from 31.8 to 58.8 $\mu\text{g cm}^{-3}$ in treated plants of mung bean, of 66.4 in control plants and from 32.1 to 48.5 $\mu\text{g cm}^{-3}$ in treated plants of Indian mustard, respectively. After irradiating the mixture for 3 min by $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, the DCPIP photo-reduction of the isolated chloroplasts was measured by following the decreasing concentration of the redox-system at 630 nm using the spectrophotometer Jenway-6400 (London, UK). The EC₅₀ (effective concentration) values were expressed as the logarithms of Cd concentration inducing 50 % DCPIP photoreduction.

Photosynthetic pigments were extracted with the 80 % (v/v) acetone and their concentrations were determined spectrophotometrically (Chl *a* at 663.2, Chl *b* at 646.8, and Cars at 470.0 nm) according to Lichtenthaler (1987).

For determination of Cd content dried powdered samples of roots and shoots were digested with HNO₃, HF, and H₃BO₃ and flame atomic absorption spectrometer (Perkin Elmer 1100, Waltham, USA) was used.

The data represent means of three separate experiments with 20 plants per treatment. The data were analyzed using ANOVA, comparisons between the means values were made by LSD test (least significant difference), and standard error (SE) was calculated.

Results

Increasing contents of Cd in nutrient solutions significantly decreased the dry mass yield of roots and shoots in both crops, and both developmental stages (Table 1). In mung bean the dry mass of roots decreased with increasing Cd-concentration by 7.7 - 62.0 % at stage 1, and by 5.9 - 33.3 % at stage 2. The dry mass of shoots in mung bean decreased by 5.6 - 64.2 % at stage 1 and by 4.2 - 50.0 % at stage 2. In Indian mustard the dry mass of roots was reduced by 14.9 - 55.0 % at stage 1 and by 6.8 - 47.2 % at stage 2. Dry mass of shoots decreased by 3.8 - 49.1 % at stage 1 and by 2.5 - 47.0 % at stage 2. Cd induced also morphological changes, especially drying of older leaves, chlorosis and necrosis of young leaves, reduction in length of lateral roots, and browning of roots. Furthermore, at the highest Cd concentrations of 60 and 120 μM we noted fading of plants, mainly in mung bean.

The 12 - 60 μM Cd in nutrient solutions reduced the photochemical activity of isolated chloroplasts at both developmental stages (Fig. 1). HRA was more negatively influenced at stage 1 than at stage 2 in both species. The effective concentration (EC₅₀) was achieved in *B. juncea* at 38.1 at stage 1 and 55.9 μM Cd at stage 2. In *V. radiata* the EC₅₀ values were 18.8 and 26.8 μM Cd, respectively (Fig. 1). The lower EC₅₀ values for mung bean at both developmental stages indicated that *V. radiata* was more sensitive to Cd than *B. juncea*.

A significant decrease in contents of Chls and Cars was established under the influence of Cd at both growth stages. This effect was dependent on Cd concentration in nutrient solution (Table 1). At stage 1 a lower Chl content was found in control plants of Indian mustard than of mung bean. At stage 2 Chl content was higher in Indian

Table 1. Effect of cadmium on the root and shoot dry mass [mg plant^{-1}], chlorophyll (Chl) and carotenoid (Car) contents [$\text{g kg}^{-1}(\text{d.m.})$], and the photochemical activity of isolated chloroplasts [% of DCPIP photoreduction] of Indian mustard and mung bean seedlings 5 d after treatment with different Cd concentrations. Means \pm SE of three independent experiments with 20 plants per treatment. Data followed by the same letter are not significantly different at 5 % level according to LSD test.

Species	Stages	Parameters	0	6	12	24	60	120 μM Cd
<i>Brassica juncea</i>	1	root dry mass	7.46 \pm 0.69a	6.35 \pm 1.01ab	6.31 \pm 0.83ab	5.92 \pm 0.85ab	4.30 \pm 0.22bc	3.36 \pm 0.25c
		shoot dry mass	55.66 \pm 4.33	53.53 \pm 8.02a	50.42 \pm 8.45ab	49.04 \pm 5.11ab	35.23 \pm 2.29bc	28.34 \pm 0.96c
		Chl (a + b)	14.62 \pm 1.49a	10.89 \pm 0.95b	8.45 \pm 0.52bc	7.15 \pm 0.80c	6.43 \pm 0.54c	5.66 \pm 0.89c
		Car	9.21 \pm 0.54a	8.05 \pm 0.32a	6.22 \pm 0.43b	5.93 \pm 0.21b	5.68 \pm 0.17b	5.21 \pm 0.60b
		HRA	-	88.71 \pm 4.83a	73.26 \pm 5.05b	58.82 \pm 4.70b	36.27 \pm 4.24b	-
	2	root dry mass	24.72 \pm 2.03a	23.04 \pm 1.20a	22.63 \pm 2.15a	20.17 \pm 2.65ab	15.33 \pm 1.38bc	13.06 \pm 1.64c
		shoot dry mass	189.67 \pm 9.04a	184.86 \pm 8.35a	179.35 \pm 8.96a	169.54 \pm 9.89a	127.32 \pm 3.48b	100.44 \pm 9.92c
		Chl (a + b)	25.59 \pm 1.52a	23.47 \pm 2.84ab	19.89 \pm 1.97bc	17.24 \pm 1.17cd	12.53 \pm 1.51de	10.86 \pm 1.40e
		Car	15.27 \pm 0.60a	13.68 \pm 0.53ab	12.60 \pm 0.84bc	10.92 \pm 0.56cd	9.74 \pm 0.77d	7.01 \pm 0.70e
		HRA	-	98.11 \pm 4.55a	82.68 \pm 5.25b	69.37 \pm 5.60b	42.64 \pm 4.76b	-
	1	root dry mass	2.71 \pm 0.16a	2.50 \pm 0.27a	1.80 \pm 0.14b	1.25 \pm 0.24bc	1.11 \pm 0.14c	1.03 \pm 0.24c
		shoot dry mass	12.79 \pm 0.96a	12.08 \pm 1.42a	10.54 \pm 0.95ab	8.75 \pm 0.98bc	5.92 \pm 0.95cd	4.58 \pm 0.90d
		Chl (a + b)	20.93 \pm 1.66a	14.67 \pm 1.45b	12.61 \pm 1.51b	10.25 \pm 0.91bc	7.05 \pm 2.02cd	3.63 \pm 1.54d
		Car	4.16 \pm 0.14a	3.53 \pm 0.34a	3.41 \pm 0.45a	2.35 \pm 0.21b	2.09 \pm 0.16b	1.95 \pm 0.13b
		HRA	-	81.10 \pm 5.02a	64.75 \pm 4.16b	38.78 \pm 4.20b	16.27 \pm 3.66b	-
	2	root dry mass	27.35 \pm 4.54a	25.75 \pm 3.83a	20.29 \pm 3.28a	19.41 \pm 2.61a	19.12 \pm 1.23a	18.24 \pm 1.36b
		shoot dry mass	108.33 \pm 8.13a	103.73 \pm 9.22a	93.53 \pm 4.50ab	77.24 \pm 6.71bc	61.18 \pm 5.40cd	54.14 \pm 4.68d
		Chl (a + b)	16.25 \pm 1.45a	14.80 \pm 1.59ab	11.28 \pm 1.47bc	8.70 \pm 1.49cd	5.91 \pm 1.12de	2.98 \pm 1.05e
		Car	7.80 \pm 0.76a	6.75 \pm 0.46a	5.33 \pm 0.34b	4.31 \pm 0.41bc	3.04 \pm 0.27cd	1.80 \pm 0.27d
		HRA	-	87.30 \pm 3.75a	66.15 \pm 3.96a	56.80 \pm 4.20b	20.45 \pm 4.23b	-

Table 2. Cadmium accumulation [$\text{mg kg}^{-1}(\text{d.m.})$] in roots and shoots of Indian mustard and mung bean 5 d after Cd treatment of the seedlings.

Cd [μM]	<i>Brassica juncea</i>				<i>Vigna radiata</i>			
	stage 1 root	shoot	stage 2 root	shoot	stage 1 root	shoot	stage 2 root	shoot
0	3.8	2.5	4.4	0.7	4.2	1.6	5.7	2.2
12	1396.0	243.4	1412.0	151.3	316.2	51.1	309.0	3.4
60	2745.2	1148.0	4858.0	449.4	2128.0	310.4	1498.0	6.2
120	7408.0	1562.3	10146.0	892.0	3972.0	703.6	3534.0	28.9

mustard than in mung bean (Table 1). More considerable Cd effects appeared at stage 2 (Table 1).

In both plant species Cd accumulation in roots and shoots as well as effects of Cd toxicity on growth correlated with increasing Cd concentrations in the nutrient solution (Table 2). The roots of both species accumulated higher amounts of Cd than the shoots. The amount of Cd in roots of Indian mustard at 120 μM

concentration was 5 and 11 and in mung bean 6 and 122 times higher than in the shoots, respectively. Indian mustard was more tolerant to Cd and had higher accumulating ability than mung bean and the content of Cd correlated with dry mass of plant organs (Tables 1 and 2). Roots of Indian mustard accumulated approximately 4.5 times more Cd than roots of mung bean at 12 μM Cd concentration at both developmental stages (Table 2).

Discussion

Dry mass, HRA, and contents of photosynthetic pigments were influenced by Cd treatment. The effect of Cd depended on its concentration in solution, growth stage of plants and plant species. The response of Indian mustard and mung bean to Cd decreased with age of plants. Mung

bean was more sensitive to Cd treatment than Indian mustard in both developmental stages. In both species roots were more sensitive to Cd than shoots, which corresponds to the higher contents of Cd in roots than in shoots. Similar effects on growth parameters were found

by Lunáčková *et al.* (2003) and Šotníková *et al.* (2003) in fast growing woody plants (*Salix alba*, *Populus robusta*). According to Skorzyńska-Polit *et al.* (1995) and Tukendorf *et al.* (1997) plants in the earliest developmental stage responded to Cd mainly by growth inhibition, while older plants responded by acceleration of the senescence. In comparison to shoots, root growth is especially susceptible in *V. radiata* to metal toxicity; this has been extensively used as a convenient criterion of metal tolerance (Šimonová *et al.* 2004). *V. radiata* seedlings can be used as a bio-indicator for the environmental quality of soils or water, respectively. They grow much faster than *Phaseolus vulgaris* which is the frequently used model plant. The plants of *V. radiata* are reliable and fast bio-indicators also for evaluating water and soil contamination by other heavy metals (e.g. Al, As, Pb, Cr) or herbicides. The growth inhibition as well as pigment contents in the leaves are good parameters to be included in a phytotoxicity index.

Photosystem 2 (PS 2) is inactivated by heavy metals (Siedlecka and Baszynski 1993) such as Cd. This effect is related to disorders in Chl biosynthesis or Chl destruction. The EC₅₀ values determined for both species confirmed a reduction in HRA under the influence of 6 - 60 µM Cd. Similar results with isolated chloroplasts of maize were obtained by Siedlecka and Baszynski (1993) and of barley by Vassilev *et al.* (2004). Heavy metals similarly to herbicides probably inhibited PS 2 electron transport in chloroplast thylakoid membranes and the resistance to them was caused by a specific change in structure of the D1 protein (Devine and Shukla 2000). Moreover, PS 2 reaction centres and PS 2 electron transport are affected by an interaction of Cd impairing enzyme activity and protein structure. The interaction of heavy metals with the functional SH-groups of proteins according Van Assche and Clijsters (1990) is a possible

mechanism of action for heavy metals.

The contents of Chls decreased in both species with increasing Cd concentrations, which corresponded with the results reported by Fargašová (2000) for Cd, Zn, Pb, Cu, and Fe in mustard (*Sinapis alba*) and by Samantary (2002) for Cr in mung bean. However, no significant decrease of Chl and Car contents by Cd was established in woody plants by Lunáčková *et al.* (2003) or in runner beans by Tukendorf *et al.* (1997). The decrease in pigment contents in Cd-treated plant of Indian mustard and mung bean was similar to that found in *Phaseolus vulgaris*. This decrease agrees with ultrastructural disorders as Cd probably induces premature senescence of leaves (e.g. Barceló *et al.* 1988).

In plants, roots are the first organs to come into contact with toxic metal concentrations and they usually accumulate significantly more metal than shoots as shown by our results and by Salt *et al.* (1995), Wójcik and Tukiendorf (1999), and Rout *et al.* (2001). Our results show that *B. juncea* is a more effective crop in removing heavy metals from substrate than *V. radiata*. Plants accumulating more than 100 mg kg⁻¹ of Cd in their shoots are defined as Cd hyperaccumulators (Baker and Brooks 1989). Maximum Cd contents in the shoots of *B. juncea* were several times higher than is the criterion set by these authors. Cd is usually accumulated in plant roots, but the endodermis is the main barrier (Brune *et al.* 1995) for its uptake and translocation. A barrier for Cd transport in mung bean may exist between the root and the hypocotyls; therefore a very little amount of Cd is translocated into the shoots in comparison with roots.

It could be concluded, that *B. juncea*, as a hyperaccumulator of Cd, could be potentially used for remediation of Cd contaminated soils and *V. radiata* as a fodder and food under our environmental and climatic conditions.

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