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Variable dynamics of cadmium uptake and allocation in four soybean cultivars

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Abstract

Cadmium is a serious environmental pollutant and its uptake by plant represents a serious health risk. Uptake, accumulation as well as sensitivity of soybean plants to metals have been shown to vary with genotype, while the dynamics of this uptake has rarely been studied. Here we studied the uptake and accumulation of Cd^{2+} ions in different parts of soybean plants of four cultivars Moravians, Gallec, Kent and Cardiff. The plants at early developmental stage were immersed in Hoagland nutrient solution in the presence or absence of 50 $mg.L^{-1}$ and the isotope of $^{109}Cd^{2+}$ to monitor its accumulation continuously at 24 h intervals for 10 days. Our results showed that the uptake rate varied among the cultivars, being the highest in roots of the cv. Moravians and the lowest in the cv. Gallec. We also observed a non-even distribution of radioactivity within the entire plants of individual cultivars. The most of Cd^{2+} isotope was translocated into primary leaves and leaves in the cvs. Kent and Moravians; on the contrary, relatively less in the cvs. Cardiff and Gallec. The results were fitted with genetic potential, growth as well as defense parameters such as proline accumulation. Combining uptake dynamics and biochemical data are indicative for different tolerance strategies of soybeans.

Introduction

Contamination of soils represents a serious concern of sustainable environment but also food production. Heavy metals, released to the environment from natural sources (e.g. volcanic activity, weathering of rocks) but in great manner also due to various anthropogenic activities (mining, industry etc.), endanger plants but also animals including humans. Therefore, metal contamination of farmland has widely been discussed due to its potential risk for food safety (Zhao *et al.* 2017).

Cadmium toxicity represents one of the most severely discussed safety issue in many European countries. For example in Slovakia, an average

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concentration of 1.24 mg Cd per kg of soil was reported (Granec and Šurina 1999; Six and Smolders 2014) comparing to 0.4 mg Cd.kg^{-1} worldwide. The exposed organisms are primarily plants, which have to face metal-induced membrane disruption and electrolyte leaking, disruption of water balance, oxidative stress, inhibition of enzymes and others, leading to impaired plant processes and finally reduced yields. The workable strategies to address Cd toxicity in plants include selection of genotypes that either demonstrate low uptake or are hyperaccumulators (Jha and Bohra 2016). For several crop species including rice, wheat and soybean have already been identified genetic markers that allow for selection of genotypes with low genetic potential to accumulate cadmium (Jegadeesan *et al.* 2010). In addition to many transporters and channels on the plasma membrane (Van Kerkhove *et al.* 2010), the metal uptake largely depends on the composition and properties of cell walls since they affect metal binding (immobilizing) and filtering capacity, restricting metal entrance to the cells (Parrotta *et al.* 2015).
The metal uptake and its distribution in plants has widely been studied, however, reports on dynamics of these processes are rather rare. This work was devoted to studying the dynamics of Cd uptake in four soybean cultivars, and to reveal its distribution within the plants. Our findings show that the uptake of Cd by soybean roots is variable among the cultivars and might significantly contribute to the ability of soybeans to cope metal toxicity.

Experimental

Plant material and experimental conditions

Soybean (*Glycine max* L.) seeds of four cultivars Moravians, Cardiff, Gallec and Kent were obtained from Matex, s.r.o (Veľké Kapušany, Slovakia). Seeds were sterilized with 0.5 % (w/v) sodium hypochlorite for 5 minutes and germinated on wet filter paper in Petri dishes. After 2 days, uniforomly germinated seeds were placed into 5 mL vial tubes with ¼ Hoagland solution with presence or absence of 50 mg.mL^{-1} Cd^{2+} in the form of $CdCl_2$. The seedlings were incubated in dark at 23 °C for 48 hours. At daily time intervals the roots were subjected to analyses. At the end of experiment (10 days) the tolerance indexes (TI) were determined for each cultivar as ratio of dry weight of control to metal-exposed tissue x 100. All determinations were performed in triplicate. After verification of normal distribution and variance homogeneity the data were analysed by t-test.

Determining the genetic potential for Cd accumulation

The simple sequence repeat (SSR) genetic markers amplifying the *Cda1* locus for low Cd accumulation capacity (Jegadeesan *et al.* 2010) were used. DNA was isolated according to Békésiová *et al.* (1999). The PCR conditions were described in Socha *et al.* (2015). Reproducible presence/absence of amplicons in at least two of the 3 marker primers SatK147, SatK 149 and SatK 150 (Jegadeesan *et al*. 2010) was taken for evaluation of accumulation potential.

Content of proline

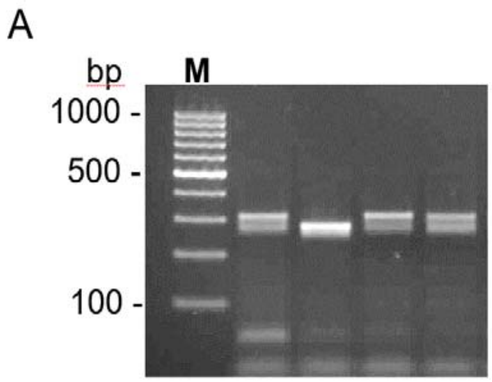
Content of proline in plant tissues upon homogenization with liquid nitrogen was determined spectrophotometrically as described by Bates *et al.* (1973).

Radiometric analysis

Determinations of radioisotope ^{109}Cd in liquid samples of culture medium (expressed as Bq/mL) or in solid samples of washed plant biomass (expressed as Bq/g tissue) was implemented by scintillation gammaspectrometry with well type NaI(Tl) crystal, using a scintillation gammaspectrometer, type 76BP76/3 (Scionix, The Netherland). Calibration of the instrument and calculation of radioactivity were realized using a library of analyzed radionuclides and the program ScintiVision-32 (Ortec, USA).

Results

The genetic potential for accumulation of cadmium was determined for the tested varieties. The data showed that the *Cda1* locus was present in only the variety Gallec, which therefore we assigned as of low Cd accumulation potential (Fig. 1). The tolerance indexes for growth in presence of Cd showed highest values for the cultivars Kent and Gallec, while the variety Moravians was the most sensitive one (Table 1). To estimate the stressed nature of tissues we measured the levels of proline as typical multifunctional metabolite accumulating



B

Cultivar	Marker/Primer *SatK 147*	*SatK 149*	*SatK 150*	Accumulation type
Cardiff	+	nd.	nd.	high
Gallec	+	+	nd.	low
Kent	nd.	nd.	nd.	high
Moravians	+	nd.	nd.	high

Fig. 1. PCR-detection of the locus *Cda1* for low Cd accumulation capacity. Three primers including SatK150 (A) were used, and reproducible presence of corresponding amplicons for at least two of them conditioned the assignment of a genotype as a low metal-accumulating type (B).

Table 1. Relative values of individual parameters in soybeans exposed to 5 mg/L of cadmium.

Variety	Genetic potential to accumulate Cd	Tolerance index (%)	Proline	SD
Moravians	high	62	1.1780*	0.5434
Cardiff	high	75	1.2243	1.5104
Gallec	low	78	1.7488**	4.5375
Kent	high	80	1.3125***	5.2231

Statistically significant differences are marked * at $P \leq 0.05$ and *** $P \leq$ at 0.001.

in plants under metal stress. Significant elevation was observed in each cultivar, except for the variety Cardiff (Table 1). With radiological analyses, we studied the uptake, accumulation and allocation of cadmium in different parts of soybeans. The plants were immersed in Hoagland nutrient solution in the presence or in the absence of the defined amount of the isotope $^{109}Cd^{2+}$. First, its depletion from solution by roots was measured continuously at 24 h intervals for 10 days. Our results showed that the uptake rate greatly differs among cultivars. Fastest and most intense cadmium uptake (removal) from the solution was typical for the cv. Cardiff already after 1 day and Moravians after 2 days (Fig. 2A), in contrast to the cvs. Gallec and Kent. In the latter two varieties the uptake was clearly delayed and ~10 times lower after 2 days than in the cv. Moravians. The difference in the uptake rates from environment (solution) among the varieties Moravians/Cardiff and Gallec/Kent decreased with time, nevertheless still remained more than ~ 2 fold at the end of the experiment (Fig. 2A). The two former cultivars removed up to 85% of cadmium from the solution, while the latter ones only 50 – 60% (Fig. 2).

Part of cadmium taken up by plants from the solution accumulated in the plant tissue. Due to different physiology we again detected differences among tested cultivars. Highest radioactivity representing the amount of cadmium accumulated in the tissue was detected in the cv. Cardiff (Fig. 2B).

The Cd contents in the other three cultivars (including the cv. Moravians) were comparable to each other, but obviously lower comparing to the cv. Cardiff (Fig. 2B). The differences in metal deposition in tissue among the cultivars were not as pronounced as in case of the uptake rate from solution; while up to only 5 fold difference was observable after 2 days, at the end of experiment the amount of cadmium deposited in tissues of all cultivars was in a range of 120 – 170 µg/g of tissue (Fig. 2B). The total amount of Cd accumulated in the plant tissue during 10 days was determined. Highest total amount of cadmium accumulated in the cv. Cardiff, in contrast the lowest amount was detected in the cv. Moravians (Fig. 3A).

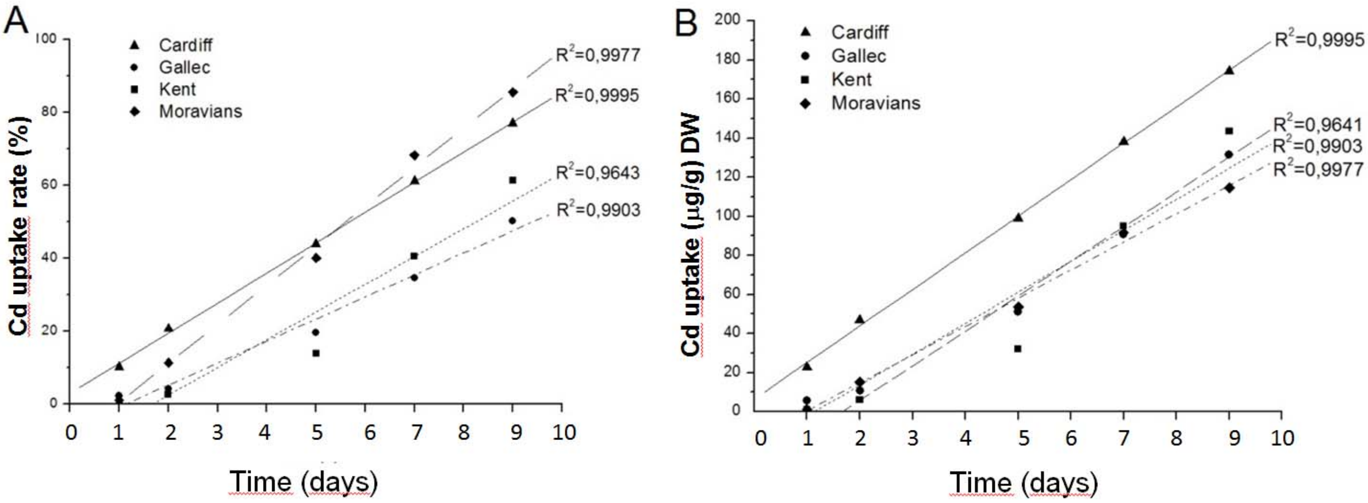


Fig. 2. The dynamics of uptake (depletion) of $^{109}Cd^{2+}$ from the growth solution by the root system of four soybean cultivars (Cardiff, Gallec, Moravians, Kent) after 9 days of culturing in the liquid Hoagland medium. Intake of Cd^{2+} by the tissue is expressed in % of the initial amount of Cd^{2+} solution (A) and in mg/g (dry weight) (B). Data represent the average values obtained from 3 independent experiments.

The metal was distributed within the soybean plants, while the allocation pattern depended on cultivar. The cv. Moravians retained most of metal in roots, in contrast the cv. Kent translocated a considerable amount of Cd^{2+} into aerial parts – mainly to the stem and to primary leaves (Fig. 3B).

Discussion

Sensitivity/tolerance of plants (including soybean) to cadmium results partly from genetic potential. Therefore, uptake of the metal from environment and the ability of plants to alleviate toxicity vary among genotypes/cultivars. Chromosome loci have been identified (Benitez *et al.* 2010; Jegadeesan *et al.* 2010) that can explain to some extent the variability in tolerance to cadmium. In our study, the locus for low cadmium accumulation potential *Cda1* was present in the cultivar Gallec (Fig. 1), and confirmed (radio)analytically (Fig. 3A). In this cultivar, the metal uptake is probably governed mainly by the transporters (and other genes) on the chromosome 9. However, comparable (or even lower) amounts of metal did accumulate in the other tested cultivars, in which the *Cda1* locus was absent. This is in agreement with the fact that the given locus explains ~60% of observed variability for soybean tolerance to cadmium (Jegadeesan *et al.* 2009; Socha *et al.* 2015), and suggests for these cultivars another mechanisms regulating the metal uptake.

The dynamics of cadmium entrance to the root cells (and their compartments) can vary, depending on several factors including pH of soils and metal speciation (Rodriguez-Serrano *et al.* 2009), or metal concentration in the growth media (Zhao *et al.* 2017). At given dose we observed a gradually increasing uptake of Cd with time during the 10 days of experiment. The uptake of metal from solution was very rapid in case of the cvs. Cardiff and Moravians (Fig. 2A), but the metal amount measured in the tissue was correspondingly high only for cv. Cardiff (Fig. 2B). Interestingly, this cultivar appears as relatively tolerant to Cd, while under similar conditions we observed lack of induction of defense components such PR protein synthesis (Bardáčová *et al.* 2016) or proline accumulation (this study). In addition, some part of cadmium was transported to the areal parts of plants (Fig. 3B). We propose that the cv. Cardiff might have an efficient system for detoxification and/or intracellular sequestration of cadmium ions, e.g. high level of phytochelatins, glutathion or organic acids (Schützendübel and Polle 2002).

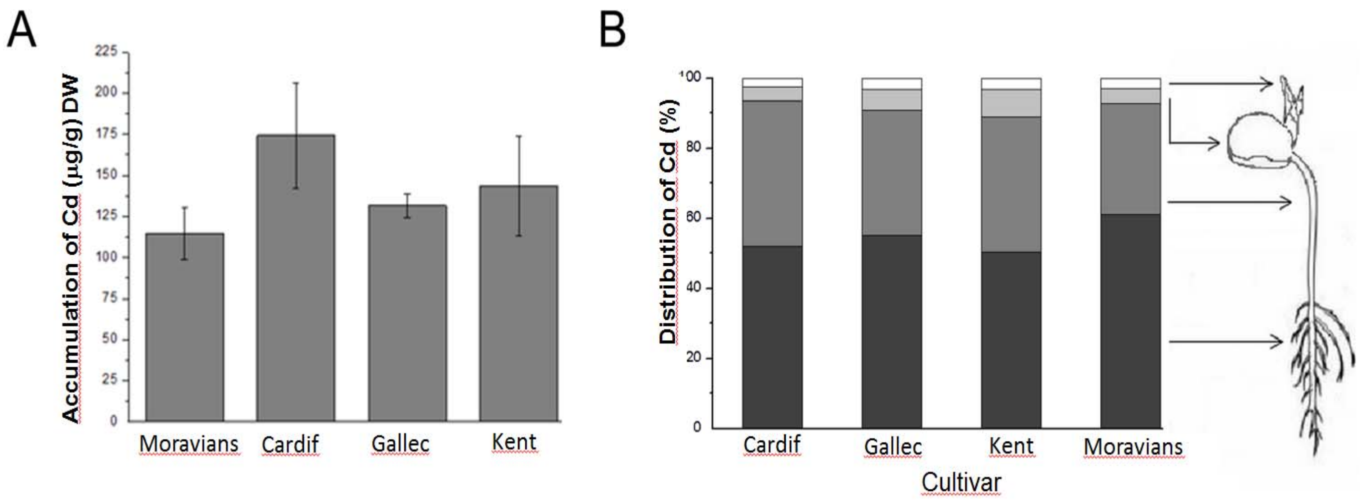


Fig. 3. Cadmium allocation in four soybean cultivars (Cardiff, Gallec, Moravians, Kent). Total amounts of Cd^{2+} deposited in soybean plants were measured (A) and the relative abundances within different plant parts was calculated (B). The data represent average values of three independent experiments from plants after 10 days of culturing in liquid medium with 50 mg/L Cd^{2+} and spiked with $^{109}CdCl_2$ (141.7 kBq/L).

Among the tested soybeans, the cv. Moravians took up Cd^{2+} from solution as rapidly as Cardiff, however, accumulated Cd in the tissue like the cvs. Gallec and Kent (Fig. 2B).Moreover, after 9 days we quantified in this cultivar the lowest amounts of Cd of all tested genotypes. Noteworthy that Gallec is genetically (Socha *et al.* 2015; this study) and also practically (this study) a low-Cd accumulator. Therefore, we conclude that part of Cd ions likely binds to the cell walls on the surface of roots (Cataldo *et al.* 1983; Piršelová *et al.* 2012) but never enters the root. This fits with relatively high tolerance index of this cultivar, too.
The allocation pattern of Cd in soybean plantlets points to even higher complexity of metal transport in plats than expected. Furthermore, metal distribution and allocation within plants reflect to different strategies applied by soybeans to withstand negative impacts, as suggested previously (Arao *et al.* 2003). The cv. Gallec exerted low genetic predisposition for cadmium accumulation, and the both uptake dynamics and real accumulation rate supported the molecular prediction. In contrast, the cv. Moravians with relatively slow but intensive uptake and translocating the metal to shoot revealed the poorest strategy resulting in high sensitivity to cadmium. Dynamics of uptake as well as deposition rate and allocation of metals within a plant might be indicative to defense efficiency and strategy of a plant genotype to cope metal toxicity.

Conclusions

Sensitivity of soybean plants to cadmium can partly be explained by genetic determinants. However, uptake of the metal from environment varies among cultivars. Furthermore, metal distribution and allocation within plants reflect different strategies applied by (soybean) plants to withstand negative impacts. The cv. Gallec exerted low genetic predisposition for cadmium accumulation and the both uptake dynamics and accumulation rate supported the molecular prediction. In contrast, the cv. Moravians with relatively slow but intensive uptake and translocating the metal to shoot revealed the poorest strategy resulting in high sensitivity to cadmium. Dynamics of uptake as well as deposition rate and allocation of metals within a plant might be indicative to defense efficiency of a plant genotype to cope metal toxicity.

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