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REDUCTION OF CADMIUM UPTAKE IN CROP PLANTS: A CASE STUDY FROM SOYBEAN

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ABSTRACT

Cadmium is a toxic heavy metal in agricultural soils which is readily taken up by crop plants thus posing a food and feed safety risk. Genetic variation in seed cadmium accumulation has been described in soybean which enables plant breeding to select genotypes for low cadmium content using genetic markers. Therefore, marker analysis as well as pot and field experiments have been carried out to validate a simple sequence repeat (SSR) marker in early maturity genotypes suitable for soybean production in Central Europe. All soybean genotypes were classified into either high or low cadmium accumulating genotypes. Marker results were clearly verified in a pot experiment with different cadmium levels of soil and in soybean harvest samples from three field locations in Austria with a seed cadmium concentration varying between 0.03 and 0.16 mg kg^{-1}. In general, seed cadmium content was reduced by about 50% in low accumulating genotypes as compared to high accumulating ones. Significant variation has also been found for zinc and manganese content of soybean seed, and zinc was positively correlated with cadmium content. Moreover, genetic variation in cadmium accumulation within both the high and low cadmium uptake groups suggests the action of additional genetic mechanisms which could be utilized by selecting lines from appropriate crosses towards a further reduction in cadmium uptake or translocation to seeds.

Key words: soybean, heavy metal accumulation, cadmium, plant breeding, marker assisted selection

INTRODUCTION

While agricultural soils may be contaminated with toxic heavy metals for either natural reasons or anthropogenically (*i.e.* through application of agro-chemicals, waste disposal, industrial activities, atmospheric deposition or mining), the major risk of their presence is in entering the food chain thereby posing a most serious threat to human health (Oves *et al*., 2012). In addition, heavy metal contamination has various negative effects on the agro-ecosystem such as toxicity to microbial communities and crop plants as well as reducing the overall soil fertility. In particular, cadmium (Cd) is considered as one of the most toxic heavy metals causing adverse health effects to the human body upon chronic intake (Järup and Åkesson, 2009). Concentrations of Cd are generally higher in plant based foods as compared to most foods of animal origin; oilseeds, durum wheat, soybean or vegetables may contain critical amounts of Cd due to their heavy metal uptake properties (Adams *et al*., 2011; Järup and Åkesson, 2009; Greger and Löfstedt, 2004). In soybean, toxic concentrations of cadmium affect plant growth, photosynthesis and other processes (Li *et al*., 2012; Xue *et al*., 2014). For assuring food safety, the European Commission has set maximum levels of Cd and other contaminants in different food products; for soybean, a threshold level of 0.20 mg kg^{-1} Cd has been established (European Commission, 2006).

Soybean (*Glycine max* [L.] Merr.) is a high protein legume crop containing about 40% of protein in the seed (Vollmann *et al*., 2000). While most soybean is utilized for the production of animal feedstuff at present, there is a growing interest in soybean uses for direct food production. Subsequently, soy-food consumption has increased due to a variety of soy-food products newly introduced and health benefits associated with bioactive ingredients of soybean such as isoflavones, dietary fibres and others (Chen *et al*., 2012; L'Hocine and Boye, 2007). Although large amounts of soybean and soybean–meal are imported to Europe at present, the domestic soybean production area has increased from 1.1 mio ha in 2000 to over 3.2 mio ha in 2013 (FAOSTAT, 2015), particularly in south-east and central European regions such as the Danube basin. The expansion of the soybean area to new growing regions with partly unknown status of heavy metal contamination has raised concerns about food and feed safety, as soybean may accumulate significant amounts of heavy metals such as cadmium. For non-smoking individuals consuming soybean products such as tofu on a regular basis, soy-foods could be the major source of cadmium intake (Adams *et al*., 2011). Therefore, soybean sources with low cadmium contamination of the harvest product are important for ensuring soy-food safety; consequently, soil quality, agronomic practices and appropriate soybean cultivar selection appear as key factors for achieving that goal. Genetic differences between soybean genotypes in cadmium uptake and seed accumulation were found by Arao *et al*. (2003) which – for the first time – suggested the feasibility of plant breeding approaches to reduce cadmium contents of the harvest products. Subsequently, a major gene locus controlling cadmium uptake by the soybean root (later designated *Cda1*) was identified in quantitative trait locus (QTL) mapping approaches (Benitez *et al*., 2010; Jegadeesan *et al*., 2010). The QTL for cadmium accumulation was then validated with early maturity soybean genotypes utilized in Central European soybean breeding and production (Vollmann *et al*., 2015).

Based on that previous study (Vollmann *et al*., 2015), the goal of the present research was to extend the findings on Cd to other heavy metals taken up by soybean, and to re-analyse the results on Cd with respect to variation within different Cd uptake groups. This could contribute to selection of appropriate crossing parents for the development of segregating soybean populations and lines with a further reduction in cadmium uptake, and subsequently to detection and mapping of the respective genes. This would be an important contribution to food safety, because cadmium has a rather long biological half-time of 10–30 years in the human body (Järup and Åkesson, 2009). Moreover, threshold levels for cadmium content of soybean and other plant products might be lowered in the future because of new toxicological evidence and risk assessment.

MATERIALS AND METHODS

Soybean cultivars and breeding lines of maturity groups 000 to 0 were used in the present study both for field experiments and a pot experiment. Using DNA marker analysis, soybean genotypes were classified into either high or low cadmium accumulation groups according to a simple sequence repeat (SSR) marker linked to a gene locus controlling cadmium uptake at the root level (*Cda1* gene). The marker (SacK149) had previously been identified through a QTL analysis as described by Jegadeesan *et al*. (2010).

A pot experiment was carried out in a vegetation hall at Mendel University in Brno (Czech Republic) in Mitscherlich pots containing 6 kg of soil each. Three different cadmium treatments were applied using cadmium acetate during watering of plants (Cd level 1: natural Cd concentration of soil; Cd levels 2 and 3: 0.3 and 0.9 mg of additional Cd kg^{-1} soil, respectively). Field experiments were carried out at three Austrian locations, i.e. Gross Enzersdorf (Lower Austria, near Vienna), Watzelsdorf (northern Lower Austria) and Gleisdorf (Styria). From each experiment, seed samples were harvested and subject to analysis of oil and protein content as well as cadmium (Cd), zinc (Zn) and manganese (Mn) content. Full details of experimental designs of the pot and field experiments, analysis of major seed compositional quality characters, DNA extraction, PCR marker analysis and statistical processing of results has been described in Vollmann *et al*. (2015). In addition, effect means were calculated by the LSMEANS procedure of the SAS statistical software package (SAS 1988), and TUKEY tests were utilized as a multiple comparison procedure for differentiating genotype means in Cd content.

RESULTS AND DISCUSSION

All genotypes could clearly be classified either as high or low Cd accumulators by SSR analysis, as shown in Figure 1. Similar to Jegadeesan *et al*. (2010) and Benitez *et al*. (2010) using mainly Canadian or Japanese germplasm, respectively, no other alleles than the two ones visible in Figure 1 were detected. Based on the SSR results, particular genotypes were selected for subsequent pot and field experiments on heavy metal accumulation.

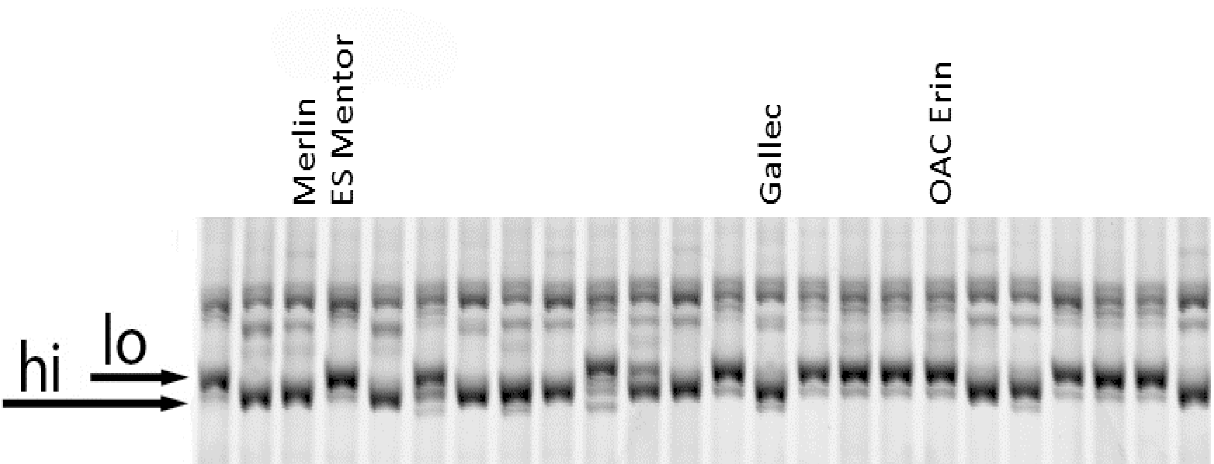

*Figure 1. Differentiation between soybean genotypes at the SacK149 SSR marker locus with two different alleles linked to either high (hi) or low (lo) seed cadmium accumulation (*Cda1 *gene). Lanes for the genotypes used in the pot experiment (Figure 2) are indicated.*

In the pot experiment, both the *Cda1* gene and the soil level of Cd had highly significant effects ($P < 0.0001$) on the soybean seed Cd content. The reaction of different genotypes on increased soil Cd content is shown in Figure 2. A linear increase in seed Cd content has been found for the high Cd accumulating genotypes, and seed Cd content was higher than the food safety threshold level of 0.20 mg kg^{-1} from soil level 2 on. In contrast, the increase in seed Cd was less pronounced for the low Cd accumulating genotypes which demonstrates the effectiveness of the *Cda1* locus.

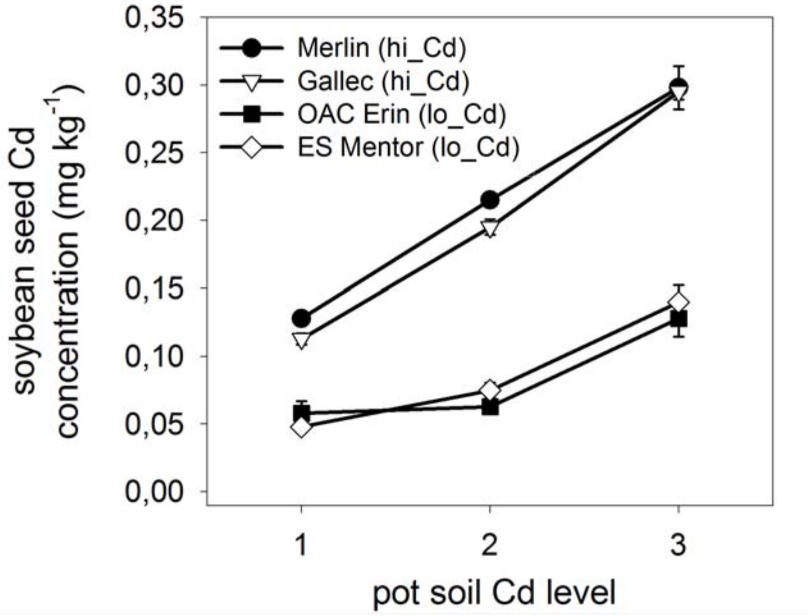

Figure 2. Cadmium content (mg kg^{-1} dry matter) of soybean seed from cultivars carrying either the marker allele linked to high (cvs. Merlin, Gallec) or low (cvs. OAC Erin, ES Mentor) cadmium accumulation as determined from plants grown in pots with three different cadmium levels. Bars are indicating the standard errors of means.

The joint analysis of field experiments from three Austrian locations revealed soybean seed cadmium levels from 0.03 to 0.16 mg kg^{-1} of seed dry matter. Highest seed cadmium contents were found in samples from the locations with the lowest soil pH. As Cd is fixed at higher soil pH levels, agronomic measures such as liming might promote precipitation or immobilization of heavy metals as suggested by Simon *et al*. (2010) for heavy metal contamination of soil. Similarly, the relationship between soil pH and Cd uptake has also been reported in other food crops such as wheat grown in different field locations across Austria (Spiegel *et al*., 2009). The results of a combined analysis of variance for seed compositional characteristics (i.e. seed protein and oil content, Cd, Zn and Mn content) is given in Table 1. Environmental effects were highly significant for all traits analysed. The Cd accumulation marker locus had a highly significant effect on Cd, Zn and Mn content suggesting similar uptake channels for these heavy metals. As a consequence, the positive relationship between Cd and Zn content is also revealed in a scatter plot given in Figure 3. Moreover, the Cd marker locus was also significantly associated with oil content (Table 1) which might be due to genetic linkage at the soybean linkage group K, were genes affecting oil content have been located (Mansur *et al*., 1993).

Table 1. ANOVA significance levels (F-test) of the Cd marker locus and other model effects on soybean seed characteristics (protein and oil content, Cd, Zn and Mn content) from the combined analysis of three experiments at Austrian locations (3 macro-environments, 10 genotypes, 2 replications)

Source of variation	**Df**	**Protein content**	**Oil content**	**Cd content**	**Zn content**	**Mn content**
Replication (env)	3	n.s.	n.s.	0.0134	0.4013	0.2792
Environment	2	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
Cd marker	1	n.s.	<0.0001	<0.0001	0.0179	<0.0001
Genotype (Cd marker)	8	<0.0001	0.0001	<0.0001	<0.0001	0.0001
Env × Cd marker	2	n.s.	n.s.	<0.0001	0.7673	0.0290
Env × geno (Cd marker)	16	0.0032	n.s.	0.0025	0.0022	0.0095

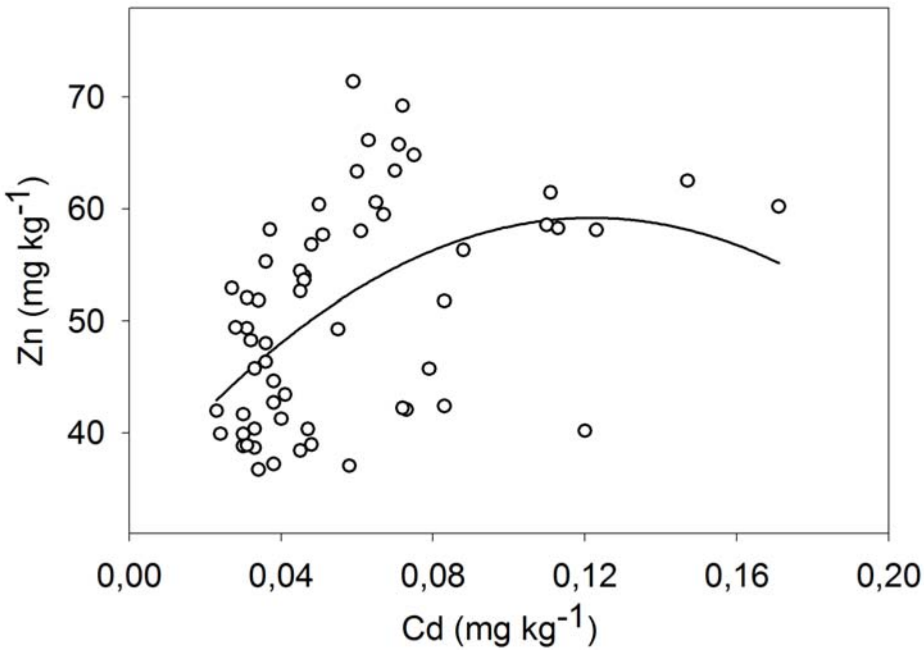

Figure 3. Relationship between cadmium and zinc content (mg kg^{-1} dry matter) of soybean seed in 60 seed samples from three locations in Austria (second order regression, r^2=0.25).

In analysis of variance, highly significant genotype effects nested within each Cd marker class (see Table 1, line "Genotype (Cd marker)") have been detected for Cd and other traits. For seed Cd uptake, this finding is of particular interest as it represents evidence of an additional genetic variation within both the high and low Cd uptake groups which might be due to genes other than the *Cda1* locus. While the soybean *Cda1* locus has been well characterised on a functional level (see Vollmann *et al*. (2015) for an overview and more details), other genetic factors and their influences are unknown at present. However, their effect on seed Cd content is clearly visible and statistically significant as shown in Table 2 for each of the two *Cda1* groups. Thus, in crosses between appropriate genotypes, recombinant lines might be selected with a further reduction in seed Cd content either through reduced uptake or lower re-mobilization rate into the seed, and additional gene loci might be identified by QTL mapping or similar breeding research approaches.

Table 2. Variation in cadmium content (mg kg^{-1}) of soybean genotypes within either the high or the low Cd accumulation group (genotype means across 3 macro-environments and 2 replications each; Tukey-test at P=0.05)

High Cd accumulation group			Low Cd accumulation group		
Genotype	**Cd cont.**	**Tukey test**	**Genotype**	**Cd cont.**	**Tukey test**
Gallec	0.114	a	GF2X-9-1-7	0.053	a
Idefix	0.081	b	Josefine	0.048	a
Vanessa	0.067	b	GG2X-45-2	0.048	a
Essor	0.045	c	Apache	0.045	ab
			GH13X-1-4	0.038	b
			OAC Erin	0.037	b

Breeding for reduced uptake of heavy metals is an important contribution to improving food and feed safety of plant harvest products. Similar to the present case study in soybean, a locus controlling cadmium uptake has also been identified in durum wheat offering comparable options of selection for reduced Cd content in seed (Zimmerl *et al*., 2014). The high relevance of such approaches is clearly underlined by the finding of numerous durum wheat samples from locations in south-western Germany exhibiting cadmium levels higher than the 0.20 mg kg^{-1} food safety threshold value (Zimmerl *et al*., 2014).

CONCLUSIONS

The results of the present study clearly confirm the effectiveness of the soybean *Cda1* locus on controlling and reducing seed Cd accumulation in early maturity soybean germplasm. Furthermore, the results suggest the presence of additional genetic factors in soybean which could be utilized for a further reduction of seed cadmium content.

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