[Manuscript draft]

Soybean seed cadmium concentration: Utilizing a QTL affecting cadmium accumulation

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Introduction

Cadmium is one of the most toxic heavy metals showing various adverse effects on human health upon chronic intake. In the human body, cadmium has a long half-time of 10-30 years and is accumulating in the kidney causing renal damages apart from effects on bone health and increased cancer risk (Järup and Åkesson, 2009). Agricultural soils may be contaminated through atmospheric deposition, application of cadmium-containing fertilizers or sewage sludge. As plants contain higher amounts of cadmium than meat or dairy products, plant based food is the major source of cadmium intake. Concentrations of cadmium are particularly high in several mushroom species (e.g. Al Sayegh Petkovšek and Pokorny, 2013) and in oil crops (Järup and Åkesson, 2009). Moreover, consumption of soyfood products has been described as a major source of cadmium intake in non-smoking subjects (Adams et al., 2011). […]

Materials and Methods

Plant materials

The soybean genotypes examined in the present study are listed in Table 1. They include cultivars and breeding lines of the early maturity groups 000 - 0 as well as different specialty germplasm (USDA genebank accessions) utilized for developing food-grade types of soybean. All genotypes were initially screened for their configuration at the SacK149 microsatellite marker locus associated with either a low or a high cadmium accumulation phenotype (Jegadeesan et al., 2010). Subsequently, two high and to low cadmium accumulation genotypes were selected for a pot experiment on seed accumulation of cadmium. In addition, another set of ten genotypes was selected for determining the cadmium content in the harvest from three previously grown field experiments.

DNA extraction and PCR marker analysis

DNA was extracted from 3-6 root tips of 1 cm length, taken from 3 days old seedlings grown on filter paper. The root tips were stored in microtiter plates at -20°C until processing. For DNA extraction the root tips were thawed out for 10 min at room temperature and 100 µl 0.25 m NaOH were added. After incubation for 3 min in boiling water, the samples were immediately grinded with 2 mm glass beads using a swing mill for 3 min. Then 300 µl 0.1 m Tris HCl (pH 8) were added and after centrifugation for 2 min at 1000 G the supernatant was diluted 1:20 with distilled water and stored at -20°C until further processing.

The total volume of the PCR mixture was 10 µl, and contained 3.3 µl DNA, 1x GoTaq® Green Master Mix (Promega), 0.25 pmoles forward primer with an M13 tail added to its 5´ end (5´-CCCAGTCACGACGTTG-3´), 2.5 pmoles reverse primer, and 2.25 pmoles fluorescent labeled M13 tail (FAM, HEX), synthesized by MWG (Ebensburg, Germany). A 2-step PCR was performed as follows: initial denaturation at 95°C for 2 min, followed by seven cycles of 45 s at 94°C, 45 s at 68°C (with each cycle the annealing temperature decreases 2°C), and of 60 s at

72°C. Products were subsequently amplified in the second step for 30 cycles at 94°C for 45 s, 50°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 5 min. The PCR products were separated using 12% polyacrylamide gels, 1x TBE buffer in a C.B.S. electrophoresis chamber (C.B.S. Scientific Inc., Del Mar, CA, USA). Electrophoresis conditions were set at constant 400 V and 10°C for 2 h. Gels were scanned by Typhoon (GE Healthcare, Uppsala, Sweden) in fluorescent mode.

Pot experiment

Field experiments

From the harvest samples of three previously grown experiments, six low and four high cadmium accumulating genotypes were selected for seed quality analysis. The experiments had been grown across three macro-environments in the east of Austria, i.e. Gleisdorf (Styria) in 2005, Watzelsdorf (Lower Austria) in 2005 and Gross Enzersdorf near Vienna (Lower Austria) in 2011. Each experiment had been planted in single row plots of 2.5 m plot length and 50 cm row spacing in randomized complete block designs. Prior to sowing, seeds were inoculated with Nodular G (Serbios, Badia Polesine, Italy) containing soybean specific rhizobia (*Bradyrhizobium japonicum* [Kirchner] Jordan) for promotion of nodule formation and di-nitrogen fixation. Additional nitrogen fertilizer was not applied in any of the experiments.

Analysis of seed quality characters

Seed oil, protein and sucrose content of samples were determined by near-infrared reflectance spectroscopy (NIRS). Samples of 10 g of air-dried seed were ground with a Cyclotec 1093 mill (Foss Tecator, Höganäs, Sweden) equipped with a 2-mm sieve. NIRS spectra were collected in two replications per sample using a Bruker Matrix-I Fourier transform NIRS instrument (Bruker, Ettlingen, Germany) with a RT-PbS detector. Seed oil, protein and sucrose content were predicted in g kg^{-1} on a dry matter basis using calibrations described elsewhere (Sato et al., 2012; Vollmann et al., 2011).

Statistical analysis

Individual plot data were subject to analysis of variance considering the Sack149 SSR marker alleles, genotypes, cadmium treatments or macro-environments as fixed effects; *F*-tests were used to determine significance of particular effects in the respective ANOVA models using the general linear model (GLM) procedure of the SAS statistical software package (SAS, 1988). Effect means were estimated using the LSMEANS procedure of the SAS program.

Results

Marker analysis
Pot experiment
Field experiments

Table 1:
Scores for the SacK149 SSR marker locus alleles for either high (hi) or low (lo) seed cadmium accumulation in 48 soybean genotypes

no.	name	Cd score	no.	name	Cd score
1	Merlin	hi	25	GH9X-3	lo
2	Sigalia	hi	26	Sayamusume	hi
3	Daccor	lo	27	PI 592923 (USDA)	hi
4	Aligator	hi	28	GH8X-10	lo
5	Petrina	lo	29	Sapporomidori	hi
6	Lissabon	hi	30	PI 200508 (USDA)	lo
7	Sultana	hi	31	GI1X-1	hi
8	Cordoba	hi	32	Sigalia	hi
9	Malaga	hi	33	G1635-2011	hi
10	GH13X-1-4	lo	34	G471	lo
11	Apache	lo	35	PI 591539 (USDA)	hi
12	Primus	hi	36	Vanessa	hi
13	ES Mentor	lo	37	Flavia	lo
14	Gallec	hi	38	G470	hi
15	Christine	lo	39	GF2X-9-1-7	lo
16	Essor	hi	40	GH13X-4	lo
17	Minsoy	hi	41	GK5X-3-8	lo
18	Toyopro	hi	42	GF4X-21-5-2	lo
19	G1070-2011	hi	43	Proto	hi
20	Lotus	hi	44	PI 243545 (USDA)	hi
21	Kent	hi	45	PI 567476 (USDA)	lo
22	OAC Erin	lo	46	Josefine	lo
23	Toyopro	hi	47	G-M6X-89	lo
24	PI 511866 (USDA)	hi	48	AC Proteus	hi

Table 2:
Significance levels (*F*-test) of the Cd marker allele and other ANOVA model effects on soybean seed characteristics (3 macro-environments, 10 genotypes, 2 replications)

Source of variation	df	1000 seed weight	protein content	oil content	sucrose content	Cd content
replication (env)	3	n.s.	n.s.	n.s.	n.s.	0.0134
environment	2	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
Cd marker allele	1	n.s.	n.s.	<0.0001	n.s.	<0.0001
genotype (Cd marker)	8	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
env × Cd marker	2	n.s.	n.s.	n.s.	n.s.	<0.0001
env × geno (Cd marker)	16	<0.0001	0.0032	n.s.	n.s.	0.0025

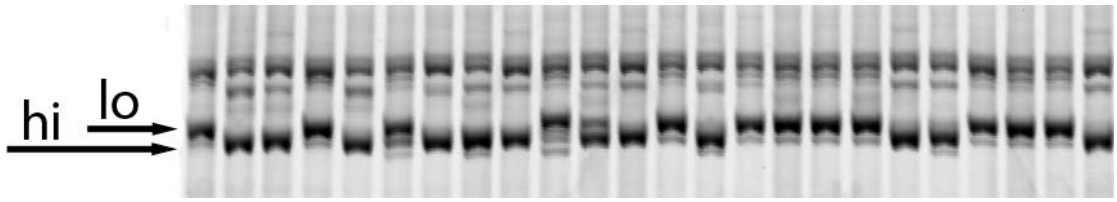

Fig. 1:
Differentiation between soybean genotypes at the SacK149 SSR marker locus with alleles for either high (hi) or low (lo) seed cadmium accumulation

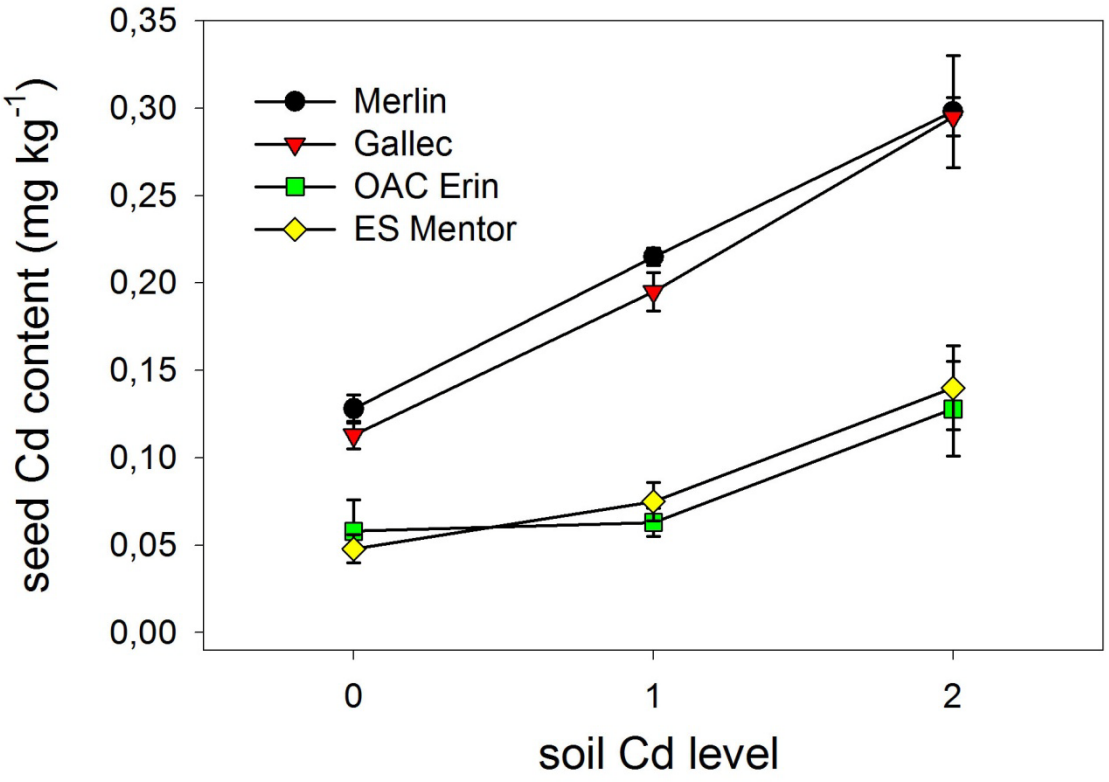

Fig. 2:
Seed cadmium content (mg kg^{-1}) of soybean cultivars carrying either the marker allele for high (Merlin, Gallec) or low (OAC Erin, ES Mentor) Cd accumulation grown in pots at three different cadmium levels

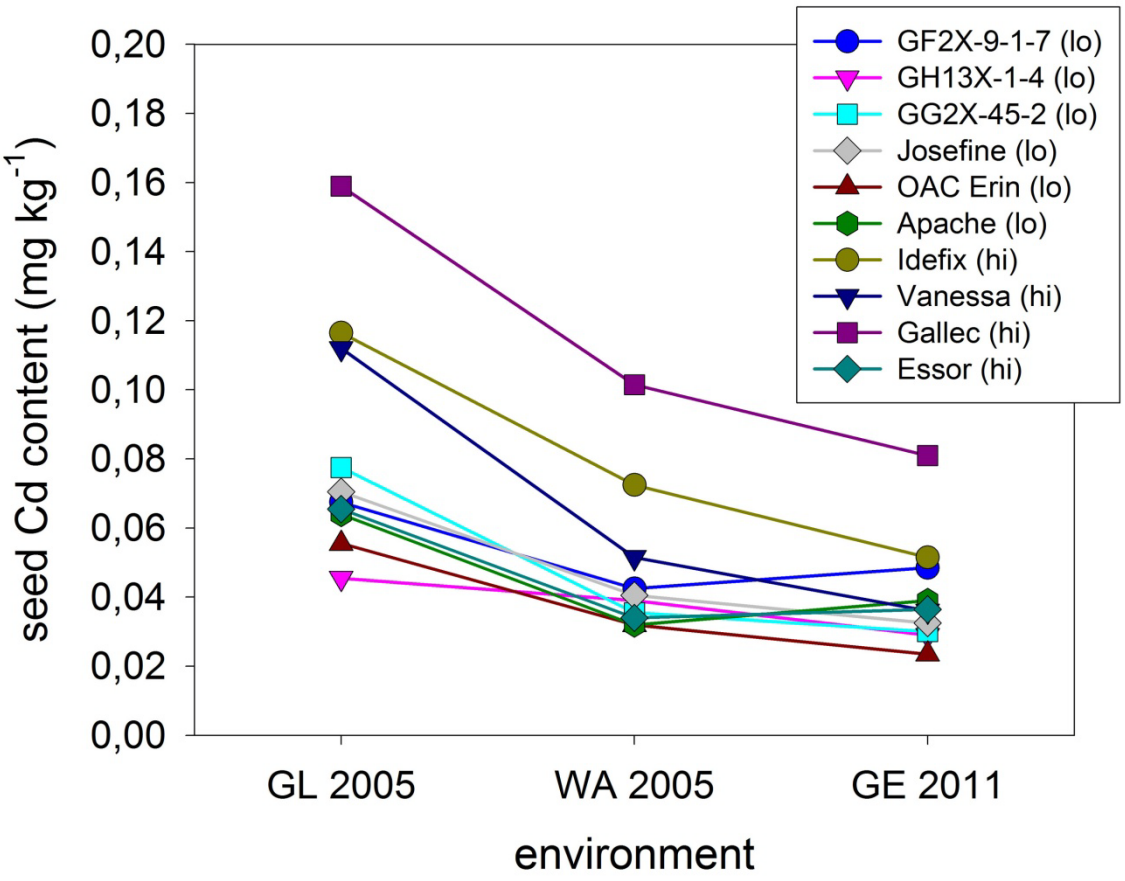

Fig. 3:
Seed cadmium content (mg kg^{-1}) of ten soybean genotypes grown in three different environments of east Austria (GL 2005 = Gleisdorf 2005; WA 2005 = Watzelsdorf 2005; GE 2011 = Gross Enzersdorf near Vienna 2011)

Discussion

Acknowledgments

This research was partly supported by project no. 63p4 of the AKTION Austria – Czech Republic programme.

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