Introduction

Cadmium (Cd) is nonessential heavy metal toxic to humans, animals and plants at very low concentrations. Most of the Cd in crop plants comes from soils because of animal manures, phosphate fertilizers and sewage sludge (Adriano 2001). Relation between Cd content in soils and in plants has been extensively studied since Cd is relatively mobile and available to plants posing risk to the food chain (Chaney et al. 2000). Some studies demonstrated that Cd in soils does not necessarily correlate with Cd in maize and wheat shoot or grain (Lavado et al 2007). On the other hand, there is substantial genotypic variation in Cd accumulation in leaf and grain in maize (Zhang and Song 2008), suggesting that genetic factors determine differences in Cd accumulation. In durum wheat, a single gene controlling grain Cd concentration with low Cd being dominant has been found (Clarke et al 1997). There are no published similar studies in maize although both Cd accumulation in leaf and grain could be of interest, because maize is used for silage and grain production. Our objectives were to analyze variation for Cd concentration in leaves of a maize mapping population and to detect and determine the effects of QTL associated with the Cd concentration.

Materials and methods

Two temperate inbred lines B84 and Os6-2 which had significantly different ionomic profile according to our previous studies (Brkic et al. 2003) were crossed in order to develop F_4 mapping population. Material development took place in the nursery of the Agricultural institute Osijek and in a winter nursery in Chile in 2003 and 2004. The 294 F_4 families along with six checks, which included the parents as two entries each, and the subsequent F_1 generation as two entries (total of 300 entries), were grown as field trials in Osijek, Croatia (N 45°30', E 18°40') in 2007 and 2008. Soil was eutric cambisol (FAO/ISRIC/ISSS, 1998), the soil type of moderate fertility showing no mineral deficiency (Table 1). The total Cd concentration of 0.38 mg kg^{-1}in soil was lower than limits allowed for agricultural soils.

Table 1. Chemical properties of soil prior to setting up a trial (n=3)

Depth	pH		AL[1]		Organic matter	$CaCO_3$	Cd
(cm)	H_2O	KCl	P_2O_5	K_2O	(%)	(%)	mg kg^{-1}
0-30	7.6	6.96	24.4	29.18	2.5	1.66	0.426
30-60	8.23	7.65	8.8	21.3	1.6	6.24	0.362

[1] AL- extraction method according to Egner et al. 1960

The experiments were conducted in two replications as a 30×10 alpha (0,1) design (Patterson and Williams, 1976) planted at the end of April. Usual crop management practice for maize was applied. Fertilizers were given according to usual requirements for high yielding maize taking into account the soil properties and the previous cropping. The single-row plots were 6 m long with 0.75 m spacing between rows. The ear-leaf at the beginning of the silking stage was taken for chemical analysis (approximately 10 leaves in the mean sample) from each plot. Leaves of four F_4 lines were not available. All leaf samples were dried and ground until 97% of the sample could pass through a 1 mm screen.

Cd concentrations in the maize leaves of 290 F_4 lines were determined by inductively coupled plasma (ICP) technique after microwave digestion. Leaf samples were digested in 65% nitric acid (HNO_3) + 30% hydrogen-peroxide (H_2O_2) (Zarcinas et al. 1987) using the Milestone MLS 1200 microwave. Analyses were performed with a Jobin-Yvon Ultrace 238 ICP-OES spectrometer. After verification of instrument performance (drift, interferences, background

correction), concentrations were determined by linear regression method using blank, standard solutions and internal standards. Plant analysis was conducted in the laboratory of the Research Institute for Soil Science and Agricultural Chemistry (RISSAC) Budapest, Hungary. Cadmium concentrations are expressed on dry matter basis.

Genotyping was made by TraitGenetics GmbH (D-06466 Gatersleben) according to the standard protocols. Total genomic DNA was extracted from bulks of ten plants per F_4 line. Following the extraction of the DNA, whole genome amplification (WGA) was performed for the SNP (single nucleotide polymorphism) analyses in order to obtain DNA of equal quality for all samples. For the SSR analyses, the original DNA was used. The SNP markers were analyzed using the SNPlex technology performed on an ABI 3730xl DNA sequencer. Three multiplexes of 48/47/47 SNP markers were derived from a proprietary SNP marker set that has been generated at TraitGenetics GmbH. 56 of the 142 tested SNPs (39%) were polymorphic between the parents of the mapping population and were mapped. SSR Fragment analysis was performed on capillary DNA sequencers (ABI 3100) using dye-labelled primers. 65 of the 69 pre-screened SSR markers were successfully mapped. For the mapping procedure, the data of both marker systems were combined and mapped using the JoinMap® 3.0 program (Van Ooijen and Voorrips, 2001). The map was constructed using Haldane's mapping function and 121 molecular markers (56 SNP and 65 SSR). Subsequently, all 290 F_4 lines were genotyped with 121 polymophic SNP and SSR markers evenly distributed across the chromosomes. The markers mapped predominantly on the expected position similar to the MaizeGDB maps.

Composite interval mapping (CIM) of QTL was performed by PLABQTL computer program (Utz and Melchinger 1996) following the regression approach (Haley and Knott 1992) extended by using cofactors. Cofactors for CIM were selected automatically by the program and added to the regression model with F to enter = 3.5. QTL included in the multiple regression models were limited to those detected with LOD threshold equivalent to an α = 0.05 genome-wide error rate (Cassady et al 2001).The critical LOD score was 3.89 estimated by Bonferroni chi-square approximation. The proportion of phenotypic variance explained by the QTL in the model with adjustment for the number of terms in the multiple regression model, the adjusted R^2 (R^2_{adj}) was calculated as described by Hospital et al. (1997).

Results and discussion

Combined analysis of variance across two environments (data not shown) revealed that the Cd accumulation was significantly affected by the genotype. Zhang and Song (2008) found also different Cd accumulation in root, leaf, stem and grain among three maize genotypes in maturing stage. The concentration of Cd in leaves of the B84 x Os6-2 F_4 progeny varied from 0.1 to 1.7 mg kg^{-1} (Fig. 1). These amounts were below critical concentrations of 5-10 Cd mg kg^{-1} for plants suggested by Sauerbeck et al. (1982). However, substantial genotypic variation in our population indicates that several genotypes are able to accumulate considerable amounts of Cd into leaf. Wang et al. (2007) found that some maize genotypes can be considered Cd hyperaccumulators when exposed to 10^{-4} M Cd solution. These genotypes had a dense root system and a large biomass, so morphology can be an indicator for ability to accumulate Cd. This could be very important in treating Cd-contaminated soils by growing hyperaccumulating maize genotypes since maize plants produce much larger biomass than other common crops.

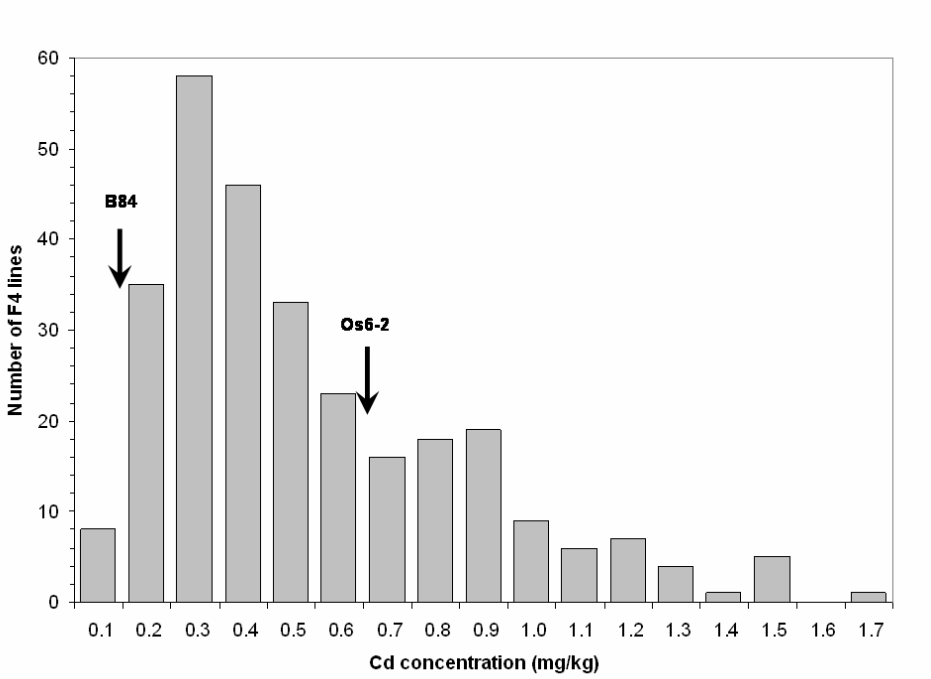

Fig. 1 Frequency distribution for cadmium concentrations (dry matter basis) in leaf of 290 F_4 lines averaged over two environments. Concentrations of two parents B84 and Os6-2 are indicated

The distribution fits the hypothesis of single gene inheritance with the allele for low accumulation being dominant. However, transgressive segregation (particularly phenotypes more extreme than those of the high-accumulating parent Os6-2) indicates that some minor genes also affect Cd accumulation in the population. Similar results were presented for grain Cd accumulation in oat by Tanhuanpää et al. (2007), and in durum wheat by Clarke et al. (1997).

Parental lines differed significantly from each other for Cd concentration (Table 2), while mean of mapping population was closer to the high-accumulating parent Os6-2. Regression model including only one locus detected in the QTL analysis explained 49.8% of the phenotypic (R^2_{adj}) variation.

Table 2. Means of parental lines and mapping population with ± standard error and adjusted percentages of phenotypic variance (R^2_{adj}) explained by detected quantitative trait locus for cadmium concentration in maize leaves

Parameter	mg kg^{-1}
Parent line means (mg kg^{-1})	
B84	0.14
Os6-2	0.67
Significance of difference	**
Mapping population	
Mean (mg kg^{-1})	0.51 ±0.02
R^2_{adj} (%)	49.8

** Significant at $\alpha = 0.01$

One major quantitative trait locus affecting the accumulation of Cd in maize leaves was detected in the population on chromosome 2 (Table 3). The LOD score for the QTL of 32.5

indicates very high probability that this QTL strongly affected amounts of Cd in leaves. Both additive and dominant effects were highly significant suggesting that dominance is important in inheritance for Cd accumulation, corroborating herewith the findings in oat by Tanhuanpää et al. (2007) and in durum wheat by Clarke et al. (1997).

Table 3. Chromosome number (bin), SSR marker associated with Cd accumulation, position of the LOD peak with 1 LOD support interval, partial phenotypic variance (R^2) and effects (additive and dominant) for cadmium concentration in maize leaf

Chrom.	SSR	Position	Supp.int.	LOD	Part. R^2	Effect	
no.-bin	marker	(cM)	(cM)		(%)	add	dom
2-06	bnlg1831	36	34-38	32.5	40.3	-0.35**	-0.13**

** Significant at $\alpha = 0.01$

The SSR marker bnlg1831 (MaizeGDB) can be used in future breeding programs to select low Cd accumulators in maize. This can be increasingly important as high-quality phosphate rock is consumed. On the other hand, selection for high Cd (hyper)accumulators in maize can be of interest in treating Cd- contaminated soils. It can be concluded that our findings could aid rapid development of maize genotypes with increased/decreased Cd accumulation in leaves by direct manipulation of the detected gene.

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