

# **A major gene for leaf cadmium accumulation in maize (*Zea mays* L.)**

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# Cadmium – Importance

- 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
- Cd is relatively mobile and available to plants posing risk to the food chain

# Cadmium – Genetics

- Genetic factors determine differences in Cd accumulation in crops
- A single gene controlling grain Cd in durum wheat (Clarke et al, 1997, Crop sci)
- No published studies in maize leaf and grain

# Objectives

- to analyze variation for Cd concentration in leaves of a maize mapping population
- to detect and determine the effects of QTL (quantitative trait loci) associated with the Cd concentration

# Materials and methods

- Two temperate inbred lines B84 and Os6-2 crossed to develop F<sub>4</sub> mapping population
- Genetic material developed at Agricultural institute Osijek in 2003 and 2004
- The 294 F<sub>4</sub> families plus six checks (total of 300 entries) grown in Croatia, Europe in 2007 and 2008
- Eutric cambisol (FAO) - the total Cd concentration of 0.38 mg kg<sup>-1</sup> lower than limits allowed for agricultural soils

## Chemical properties of soil prior to setting up a trial (n=3)

Depth (cm)	pH		AL-method		Org. matter (%)	CaCO <sub>3</sub> (%)	Cd mg kg <sup>-1</sup>
	H <sub>2</sub> O	KCl	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O			
0-30	7.60	6.96	24.4	29.2	2.5	1.66	0.426
30-60	8.23	7.65	8.8	21.3	1.6	6.24	0.362

# Materials and Methods

- Cd concentrations determined by inductively coupled plasma (ICP) technique by Jobin-Yvon Ultrace 238 ICP-OES spectrometer
- The ear-leaf at the silking taken and dried from each plot for chemical analysis (approximately 10 leaves per plot)
- Plant analysis was conducted in the laboratory of the Research Institute for Soil Science and Agricultural Chemistry (RISSAC) Budapest, Hungary. Cd concentrations are expressed on dry matter basis

# Materials and Methods

- genotyping made by TraitGenetics GmbH
- SNP (single nucleotide polymorphism) analyses
- SSR (simple sequence repeats) analyses
- For the mapping procedure, the data of both marker systems combined and mapped: 121 molecular markers (56 SNP and 65 SSR).
- all 290 F4 lines were genotyped with 121 polymorphic SNP and SSR markers evenly distributed across the chromosomes

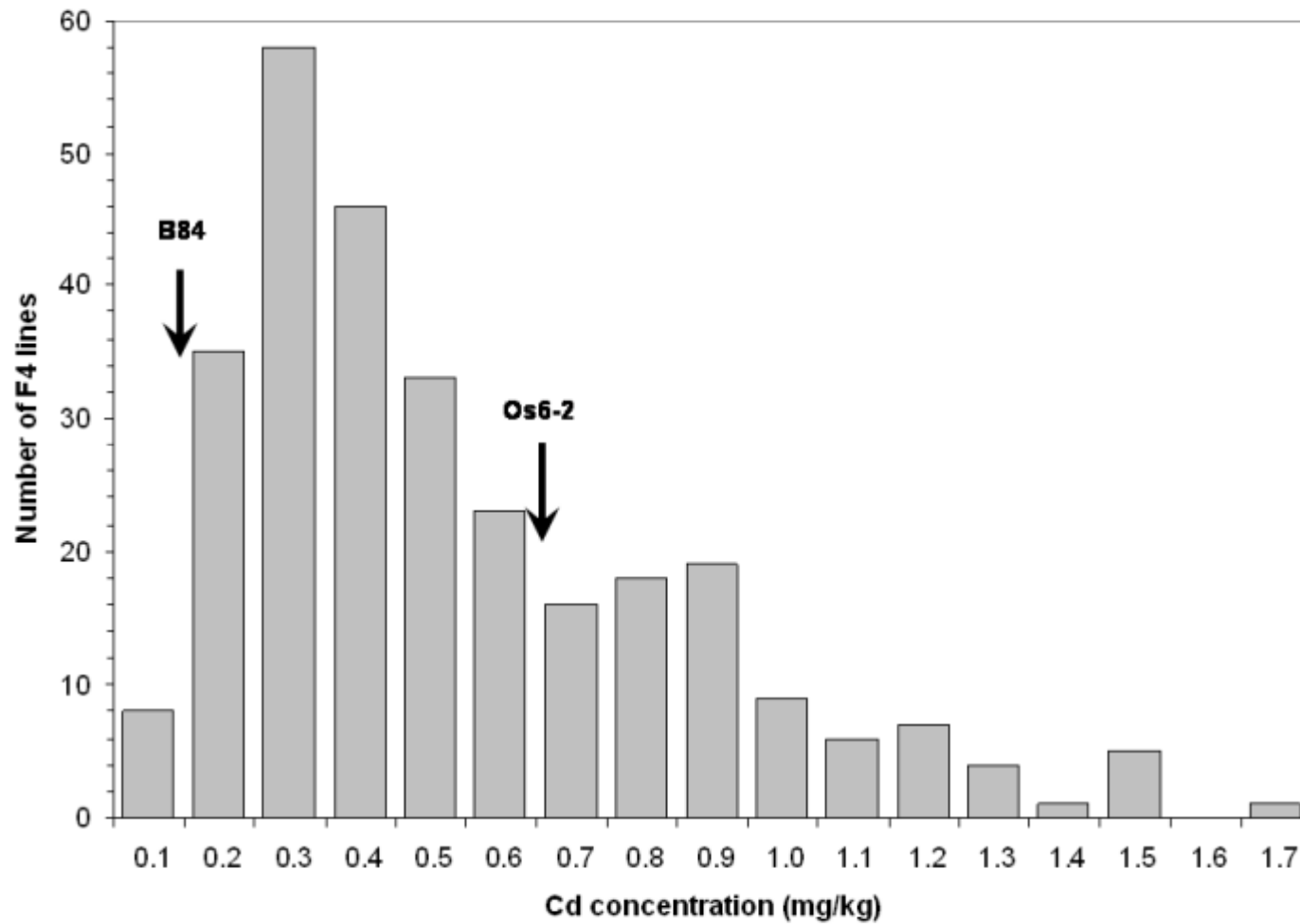
# Materials and Methods

- Composite interval mapping (CIM) of QTL performed by PLABQTL computer program (Utz and Melchinger 1996)
- The critical LOD score = 3.89 (Bonferroni chi-square approximation)
- Phenotypic variance explained by the adjusted  $R^2$  (Hospital et al. 1997)

# Results

- Combined analysis of variance across two environments revealed that the Cd accumulation was significantly affected by the genotype
- The concentration of Cd in leaves of the B84 x Os6-2 F<sub>4</sub> progeny varied from 0.1 to 1.7 mg kg<sup>-1</sup> (below critical concentrations of 5-10 Cd mg kg<sup>-1</sup> for plants)
- substantial genotypic variation in our population indicates that several genotypes are able to accumulate considerable amounts of Cd into leaf

# Results



# Results

- The distribution fits the hypothesis of single gene inheritance with the allele for low accumulation being dominant.
- Parental lines differed significantly from each other for Cd concentration, 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.

Means of parental lines and mapping population with  $\pm$  standard error and adjusted percentages of phenotypic variance ( $R^2$  adj) explained by detected quantitative trait locus for cadmium concentration in maize leaves

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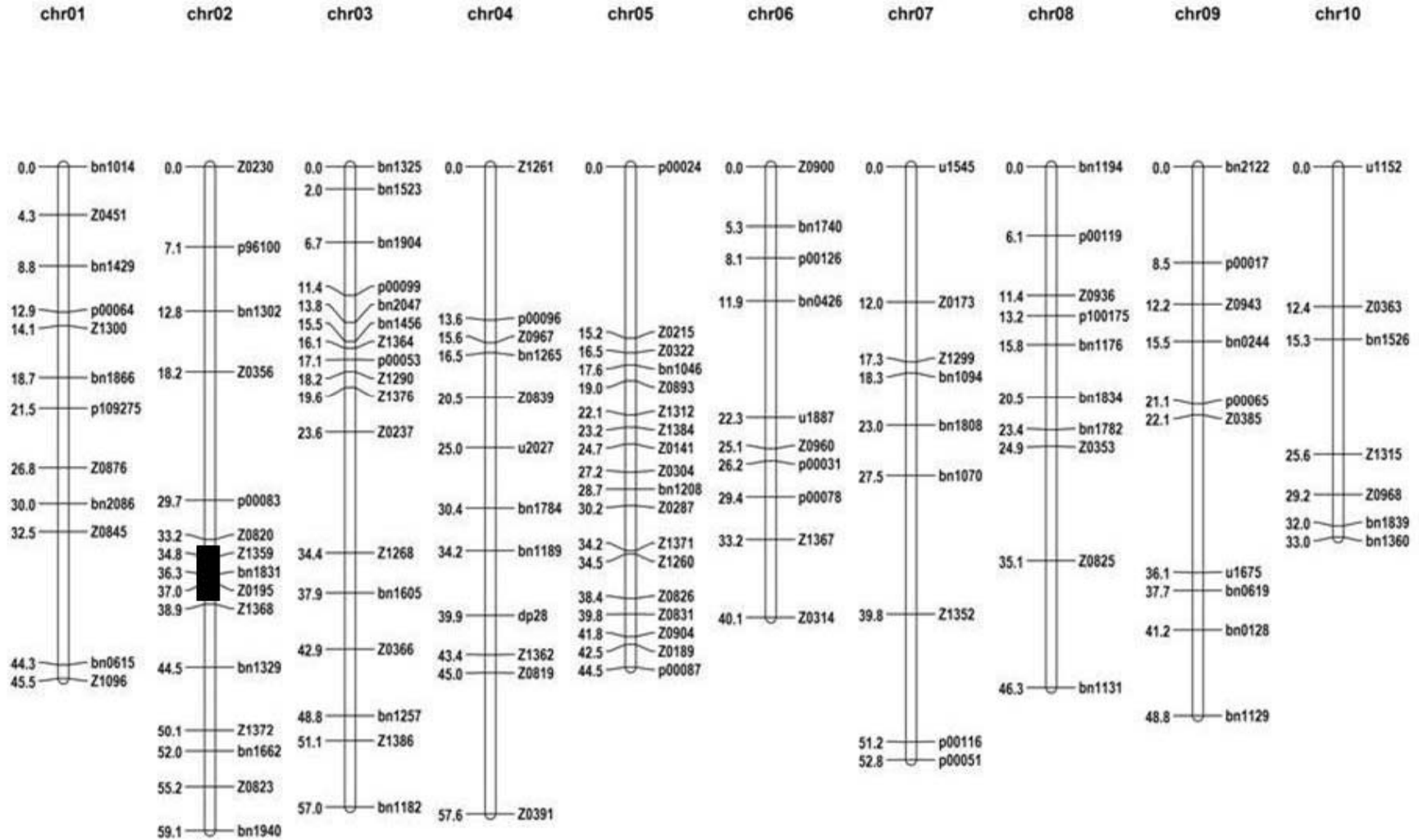
Parameter	mg kg <sup>-1</sup>
B84	0.14
Os6-2	0.67
Significance of difference	**
Mapping population	
Mean $\pm$ standard error	0.51 $\pm$ 0.02
$R^2$ adj (%)	49.8

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# Results

- One major quantitative trait locus affecting the accumulation of Cd in maize leaves was detected in the population on chromosome 2
- 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

# Linkage map



# Conclusions

- The SSR marker bnlg1831 can be used in future breeding programs to select low Cd accumulators in maize.
- Selection for high Cd (hyper)accumulators in maize can be of interest in treating Cd-contaminated soils.
- Our findings could aid rapid development of maize genotypes with increased/decreased Cd accumulation in leaves by direct manipulation of the detected gene.

Thank you for your attention!