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

Roberta Soric, University of Osijek, Glas Slavonije d.d, Croatia

Zdenko Loncaric, Vlado Kovacevic, University of Osijek, Croatia

Ivan Brkic, Domagoj Simic, Agricultural Institute Osijek, Croatia
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 F4 mapping population
- Genetic material developed at Agricultural institute Osijek in 2003 and 2004
- The 294 F₄ 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⁻¹ lower than limits allowed for agricultural soils
Chemical properties of soil prior to setting up a trial (n=3)

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>pH H2O</th>
<th>pH KCl</th>
<th>AL-method P2O5</th>
<th>AL-method K2O</th>
<th>Org. matter (%)</th>
<th>CaCO3 (%)</th>
<th>Cd mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>7.60</td>
<td>6.96</td>
<td>24.4</td>
<td>29.2</td>
<td>2.5</td>
<td>1.66</td>
<td>0.426</td>
</tr>
<tr>
<td>30-60</td>
<td>8.23</td>
<td>7.65</td>
<td>8.8</td>
<td>21.3</td>
<td>1.6</td>
<td>6.24</td>
<td>0.362</td>
</tr>
</tbody>
</table>
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 F₄ progeny varied from 0.1 to 1.7 mg kg⁻¹ (below critical concentrations of 5-10 Cd mg kg⁻¹ 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 (R2 adj) variation.
Means of parental lines and mapping population with ± standard error and adjusted percentages of phenotypic variance (R² adj) explained by detected quantitative trait locus for cadmium concentration in maize leaves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>B84</td>
<td>0.14</td>
</tr>
<tr>
<td>Os6-2</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Significance of difference: **

Mapping population

<table>
<thead>
<tr>
<th>Mean ± standard error</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.51 ±0.02</td>
<td></td>
</tr>
</tbody>
</table>

R² adj (%) 49.8
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.
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!