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Quantitative trait loci for metal accumulation in maize leaf

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Abstract

Maize, as a major crop, has been investigated for decades for metal accumulation, but not in the context of leaf ionome to identify putative genetic factors participating in the control of metal accumulation. Our objectives were to analyze variation for copper (Cu), iron (Fe), potassium (K), manganese (Mn), magnesium (Mg), and strontium (Sr) concentrations in leaves of a maize mapping population, and to detect and determine the effects of quantitative trait loci (QTL) associated with the metal concentrations. Ear-leaf samples at the beginning of the silking stage was taken for elemental analysis (ICP-OES) of 290 F4 lines of a biparental population (B84×Os6-2) grown in field trials in Croatia. The population and parents differed significantly in Cu, Fe, K, Mg, Mn, and Sr concentrations. The population was mapped using sets of 56 SNP and 65 SSR polymorphic markers. Eleven significant QTLs were detected for all six metal concentrations. Of them, QTLs for Cu, Fe, and Mg were colocalized on chromosome 5 in the region of *ys1* gene. Significant dominant effect of these QTLs supports the involvement of *ys1* in accumulations of these metals. Some QTLs had no obvious candidate genes offering the possibility of identifying unknown genes that affect metal accumulation.

Keywords: leaf, metals, QTLs, *Zea mays* L

Introduction

Metals, along with nucleic acids, proteins, and metabolites, are involved in almost every process in an organism. The functional genomics of all ions including all metals, called ionomics (Lahner et al, 2003), has been established recently, enabling measurement of concentrations of many metals simultaneously, which requires the application of high-throughput elemental analysis technologies. Accordingly, the leaf ionome of a plant represents its mineral and trace element content (Salt et al, 2008), and it is dependent on many factors including soil properties, multiple physiological processes and genotypic traits. When alterations of these factors occur, transport of metal ions from the soil solution to the shoot (leaf) could affect the shoot ionome (Baxter et al, 2008). Compared to other "-omics", ionomics is generally at the inception, since the majority of genes and gene networks in ionome regulation are still unknown.

Two prospective biotechnological applications that arise from the ionomic studies in plants are biofortification and phytoremediation. The goal of biofortification is to increase density of beneficiary metals (micronutrients) in the edible portions of staple crops by breeding; particularly staple crops, by plant breeding; whereas the goal of phytoremediation is (hyper) accumulation of toxic metals into plants to clean up contaminated soils (Zhao and McGrath, 2009). These goals, although very different, are actually the two sides of the same coin (Guerinot and Salt, 2001), since relations among beneficiary and toxic metals could be tight.

Maize (*Zea mays* L), as a major crop has been investigated for decades for metal accumulation, particularly iron (Fe) and zinc (Zn) accumulation in grain for biofortification purposes (Ortiz-Monasterio et al, 2007 for a review); but concentrations of copper (Cu), potassium (K), manganese (Mn), magnesium (Mg), calcium (Ca) have also been considered (Menkir, 2008). Moreover, from McHargue (1925) to present date, it was addressed the significance of the occurrence of many metals in both grain and silage maize. Compared to all other metals, data pertaining to Sr in maize is very limited. As an alkaline earth metal, Sr is the second abundant element in the earth's crust after Ca, and is a well known pollutant, especially ^{90}Sr isotope. Seregin and Kozhevnikova (2004) found in the xylem cell walls, in the vascular bundles of coleoptile, mesocotyl, and leaves of maize, an evidence for high Sr mobility, whose transport was similar to Ca. In contrast to toxic cadmium (Cd), stable Sr might be beneficiary for humans in some cases (Reginster et al, 2005).

To our knowledge, there is no published study of leaf ionome in maize as a multivariable system to detect the plant's physiological status and to identify putative genetic factors participating in the control of

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metal accumulation. Our objectives were to analyze variation for Cu, Fe, K, Mg, Mn, and Sr concentrations in leaves of a maize mapping population, and to detect and determine the effects of QTL associated with the metal concentrations.

Materials and Methods

Two temperate inbred lines B84 and Os6-2, which had significantly different ionomic profile according to our previous studies (Brkić et al, 2003), were crossed in order to develop a mapping population. The line B84 is well known BSSS line, while OS6-2 is related to the line C103 of Lancaster origin. Liu et al, (2003) gave detailed background of B84 and C103 and their relation. Development of the biparental population B84xOs6-2 was described by Šimić et al, (2009b) in detail.

The 294 F4 families of the population along with six checks, which included the parents as two entries each, and the subsequent F1 generation as double entries (total of 300 entries), were grown as field trials in Osijek, Croatia (45°30'N, 18°40'E) in 2007 and 2008. Details about the trials were given by Sorić et al, (2009). Briefly, 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. Soil was eutric cambisol, the soil type of moderate fertility with no metal imbalances. Chemical properties of the soil prior to setting up the trial were presented by Soric et al, (2009). Fertilizers were given according to usual requirements for high yielding maize, taking into account the soil characteristics and the previous cropping. No additional fertilizers with micronutrients were applied. 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 F4 lines were not available. After drying and grinding until 97% of the leaf powders could pass through a 1 mm screen, samples of the 290 F4 lines were digested in 65% nitric acid (HNO_3) and 30% hydrogen-peroxide (H_2O_2) (Zarcinas et al, 1987), using the Milestone MLS 1200 microwave, and the concentrations of Cu, Fe, K, Mg, Mn, Sr and Zn determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES). Plant analyses were conducted in the laboratory of the Research Institute for Soil Science and Agricultural Chemistry (RISSAC) Budapest, Hungary (Sorić et al, 2009). After verification of instrument performance (drift; interferences, background correction), elemental concentrations in samples were determined by linear regression method using blank, standard solutions and internal standards of RISSAC. Finally, the metal concentrations were expressed on leaf dry matter basis.

290 F4 lines of the population were genotyped using sets of SNP (single nucleotide polymorphisms) and SSR (simple sequence repeats) molecular markers. All steps of the DNA analysis were conducted by TraitGenetics GmbH, Germany, according to the standard protocols (Šimić et al, 2009a). In total, 142 SNP markers (three multiplexes of 48/47/47 markers) were analyzed. They were derived from a proprietary SNP marker set that has been generated at TraitGenetics, identified through amplicon resequencing method, and validated through the analysis of many maize lines at TraitGenetics (Ganal et al, 2009). SNPlex analysis was performed on an ABI 3730xl DNA sequencer, whereby internal and external standards were used for size determination. 65 of the 69 prescreened SSR markers were successfully mapped. The four remaining markers were either not functional/not useful or not polymorphic between the parents of the mapping population. Status of marker data, linkage map, percentages of homozygosity, and genome of the Parent 1 (B84) were presented by Šimić et al (2009a). For the mapping procedure, the data of both marker systems were combined and mapped using Haldane's mapping function and 121 molecular markers (56 SNP and 65 SSR) (Šimić et al, 2009a). SNP markers were denoted in this study with "Z". Data about used SSR markers are available via online database MaizeGDB (Andorf et al, 2010).

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 a log of the odds (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). Zinc was excluded from further assessment, since just one nonsignificant QTL was detected (LOD=3.30). Minor nonsignificant QTLs with LOD scores between 3.25 and 3.89 were detected also for Fe (one QTL), Mg (two QTLs), and Sr (four QTLs). They were not included in further analyses either.

Results

Leaf metal concentrations in 290 F4 lines varied considerably: 7.06-23.90 mg/kg for Cu, 101.3-239.0 mg/kg for Fe, 17887-33593 mg/kg for K, 710-3620 mg/kg for Mg, 48.46-166.59 mg/kg for Mn, and 3.59-10.78 mg/kg for Sr (Figure 1). The pattern of the frequency distribution in F4 lines revealed a continuous variation for all metal concentrations. Although Shapiro-Wilk test detected that data for all traits are not normally distributed, the variation in all metals suggest that metal accumulation are controlled by several QTLs.

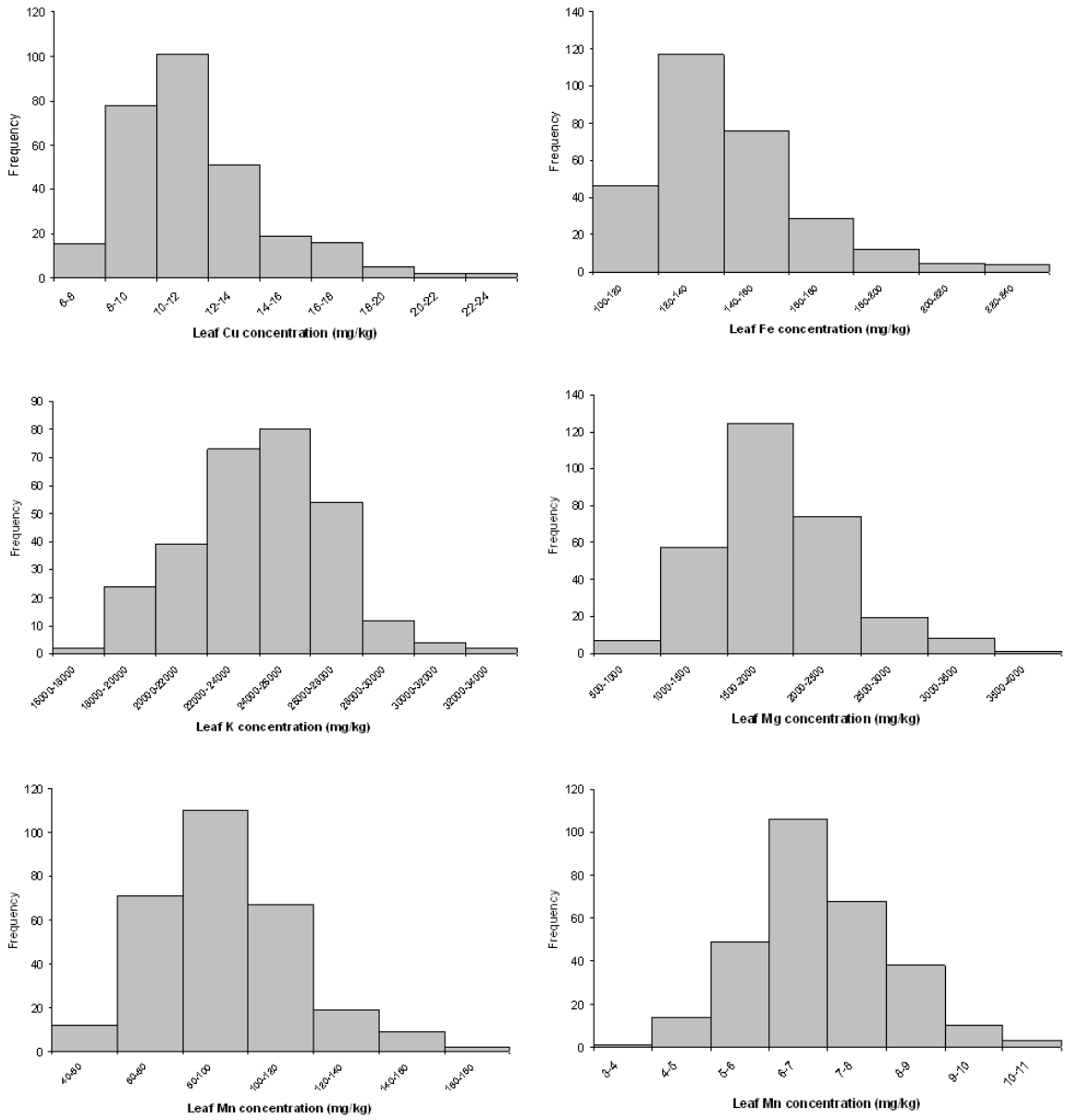

Figure 1 - Frequency distributions for the concentrations of Cu, Fe, K, Mg, Mn and Sr (dry matter basis) in leaves of 290 F4 lines averaged over two environments.

The parental lines B84 and Os6-2 differed significantly for all metal concentrations (Table 1). Among the metals compared in the experiment, the largest differences between the parents were observed for Cd in the accompanying study (Sorić et al, 2009) and Cu concentrations, which were respectively 4.8 and 2.9 times higher in Os6-2 than in B84. Transgressive segregants both below and beyond parents were observed for all metals, except for Cu where the parent B84 had the lowest concentration in the experiment. Three QTLs were detected for leaf Cu and K, two QTLs for Sr, while only one QTL was detected for Fe, Mg and Mn concentrations, respectively. Greatest adjusted percentages of phenotypic variance explained by detected QTLs were for K, Cu and Sr concentrations.

In summary, eleven significant QTLs were detected for concentrations of Cu, Fe, K, Mg, Mn and Sr (Table 2). Three of them, QTLs for Cu, Fe, and Mg, are colocalized and associated with the SNP marker Z00831 on chromosome 5, having support interval of just 4 cM and relatively high LOD scores. The respective QTLs for Fe and Mg are the only significant QTLs for these traits. The colocalized QTLs on chromosome 5 had only highly significant dominant effect. There are other two significant QTLs for Cu,

Table 1 - Means of parental lines and mapping population with ± standard error and adjusted percentages of phenotypic variance (R^2_{adj}) explained by detected quantitative trait loci for concentration of six metals in maize leaves.

Parameter	Copper	Iron	Potassium	Magnesium	Manganese	Strontium
Parent line means (mg kg^{-1})						
B84	6.37	105.1	21220	2201.2	67.57	6.93
Os6-2	18.33	180.5	27260	1969.4	97.77	5.71
Significance of difference	**	**	**	**	**	**
Mapping population (mg kg^{-1})	11.58 ± 2.82	141.7 ± 25.0	24147 ± 2768	1862 ± 480	92.95 ± 22.16	6.89 ± 1.28
Number of QTL	3	1	3	1	1	2
R^2_{adj} (%)	21.7	7.5	32.7	9.2	15.2	22.2

** Significant at P = 0.01

with lower LOD scores and significant negative additive effect, indicating that the B84 allele decreases the trait value. The same is true for three significant QTLs for K on chromosomes 2, 6, and 8. The QTL on chromosome 6 flanking the SSR marker umc1887 had the highest LOD score of 7.34 in the study. The only significant QTL on chromosome 3 was for Mn, whereas on chromosome 1 there was just one significant QTL for Sr. QTLs on chromosome 8 for K and Sr are colocalized and flanked with the SSR marker bnlg1131, having support interval of 10 cM.

Phenotypically, the tightest positive correlation was between Cu and Fe, followed by correlations between Fe and Mg, and between Fe and Mn (Table 3). It indicates that Fe leaf concentration may be simultaneously increased together with Cu, Mg and Mn concentrations. Therefore, phenotypic correlations corroborate the QTL analysis that accumulation of Cu, Fe and Mg seem to be connected. In contrast, K is neither correlated nor negatively associated with all other elements, suggesting independent accumulation of K.

Discussion

The amount of metal ions ultimately accumulating in the plant leaf depends on a plethora of processes in multiple tissues: uptake into the roots, translocation (xylem loading/unloading or phloem loading/unloading), chelations, storage, encoding regulatory proteins. Therefore, QTLs for increased leaf metal concentrations may indicate genes that are important for any of these processes. Briefly, putative genes may be categorized by predicted function: transporters, chelators/storage, regulators of transporters, and genes involved in metabolism. In maize, there are some known genes, controlling particularly Fe, K, and Mg concentrations, including genes encoding Fe-sulfur protein (*isp1*, *ris1*, *ris2*), *ferric chelate reductase* (*fcr1*), K channel (*kch1*, *kch2*, *kch3*, *kch4*, *kch5*, *ork1*), and Mg chelatase subunits (*chld1*, *chlh1*, *chlh2*, *oy1*) (MaizeGDB, 2010). These genes are involved either in chelation or regulation of transporters, but none of them seem to be associated with QTLs detected in this study. We realized, according to relative small percentages of explained phenotypic variance, that the majority of metal-related genes had been undetected, remaining outside the QTL support intervals. Majority of genes for metal concentrations might be associated with ion transport as detected in Arabidopsis (Waters and Grusak, 2008), but these genes

Table 2 - Chromosome number - bin, markers associated with position of the LOD peak with 1 LOD support interval (LOD threshold was 3.89 equivalent to an $\alpha = 0.05$ genome-wide error rate), partial phenotypic variance (R^2) and effects (additive and dominant) for concentrations of six metals in ear-leaves of the maize B84xOs6-2 population.

Chromosome number-bin	Marker	Position (cM)	Supp.int. (cM)	LOD	Part. R^2 (%)	Effect additive	Effect dominant
Copper							
2-05	Z00820	32	26-38	4.90	7.5	-1.03*	-1.18*
5-06	Z00831	38	36-40	5.59	8.5	0.26	1.71**
8-05	bnlg1782	22	20-34	4.21	6.5	-1.00**	-0.73
Iron							
5-06	Z00831	38	36-40	4.89	7.5	2.96	21.38**
Potassium							
2-08	Z00823	54	50-58	6.16	9.3	-1,206**	-518
6-03	umc1887	18	14-22	7.34	11.0	-1,558**	-84
8-09	bnlg1131	42	36-46	4.28	6.7	-1,183**	115
Magnesium							
5-06	Z00831	38	36-40	7.31	11.0	175.5	153.3**
Manganese							
3-05	Z01376	22	18-24	5.22	8.0	-10.7**	-6.8*
Strontium							
1-04	Z00876	28	24-32	4.85	7.4	0.52**	0.33*
8-09	bnlg1131	42	36-46	4.02	6.3	0.53**	-0.01

*significant at $\alpha = 0.05$; ** significant at $\alpha = 0.01$
note: a negative value of additive effect indicate that the B84 allele decreases the trait value

Table 3 - Phenotypic correlations between entry-means for metal concentrations in the maize B84xOs6-2 population. Asterisk indicates significance of correlation coefficients at P = 0.05.

	Cu	Fe	K	Mg	Mn
Fe	0.56*				
K	0.02	0.04			
Mg	0.22*	0.34*	-0.37*		
Mn	0.36	0.25*	-0.03	0.15*	
Sr	-0.23*	-0.04	-0.12*	0.14*	0.28*

are mostly still unknown in maize.

Previously in the same experiment, we demonstrated in our accompanying study (Sorić et al, 2009) that Cd accumulation in maize leaf seems to be controlled by only few genes; perhaps one, two, or three, since we detected one major QTL with the LOD score of 32.5, explaining 49.8% of the phenotypic variation. This is in accordance with the results in other Poaceae species such as rice (Ishikawa et al, 2010) and oat (Tanhuanpää et al, 2007). Accumulation of metals evaluated in this study, though, appear to be controlled by several genes. This could be supported by different frequency distributions for Cd compared to distributions of metals evaluated herein. Moreover, we made classical quantitative genetic analysis by estimating minimum number of effective genes controlling the traits (Castle-Wright formula, an application in maize among others by Šimić and Hallauer, 2001) wherein numerous methodical requirements should be met (Hedrick, 1999), and we found that, for example, Cu accumulation is controlled at least by four genes (data unpublished).

We identified colocalized QTLs for Cu, Fe and Mg accumulation on chromosome 5 in the region where the gene *ys1* is positioned. The gene *ys1* (*yellow stripe 1*) encodes the ferric–phytosiderophore transporter (Curie et al, 2001), but it is also involved in the intracellular transport of other metals such as Zn and Cu (Schaaf et al, 2004). No published studies demonstrated the *ys1* gene is involved in Mg transport. *Ys1* was described by Beadle (1929) as a factor for chlorophyll deficiency which may also indicate Mg deficiency. However, Bell et al (1958) demonstrated that *ys1* mutant was not related to magnesium metabolism. *Ys1* is a well known maize mutant phenotypically expressed by leaf chlorosis controlled by the recessive *ys1* allele. Significant dominant effect of all three colocalized QTLs for increasing Cu, Fe and Mg concentrations also indicate involvement of *ys1* in accumulations of these metals. Further physiological and QTL studies should validate possible relationships existing between the *ys1* gene and Cu and Mg accumulation.

Generally, colocalization of QTLs for multiple elements was observed in Arabidopsis seeds (Vreugdenhil et al, 2004) and rice seedlings (Shimizu et al, 2005) also. Colocalized QTLs for K and Sr concentrations at the end of long arm of chromosome 8 appear to have no obvious candidate genes. It offers the possibility of identifying unknown genes that affect uptake and translocation of K and Sr. QTL analysis for Ca concentration would be reasonable in order to clarify relations among Ca, K, and Sr, and possibly to detect colocalized QTLs for all three metals. Ca had been measured by ICP technique in our experiment, though the results were inconsistent, and repeatability was low.

Our QTL data based on results in only one maize population should be interpreted with caution. Further analyses are needed to test robustness of QTLs across germplasm pools, as stressed by Lee et al, (2009) for C-glycosyl flavone synthesis in maize. However, our preliminary unpublished results for the more marker-saturated IBM population (Lee et al, 2002) indicate that variations for metal accumulation are not necessarily significant, and therefore not of much interest as a source population for ionomic study. Although this population is valid for QTL identification per se because QTLs can be detected in populations derived from two not differing parents, breeders are more interested in inclusion of novel alleles with larger effects in their programs, that is, inclusion of one parent as a donor of high metal accumulation. This would be plausible for the traits controlled by not many genes, such as metal accumulation.

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