

Identification of QTL and candidate genes for Pb accumulation in maize at maturity stage

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Abstract

Lead (Pb) has become one of the most abundant heavy metal pollutants of the environment. Understanding the genetic basis for the underlying mechanism of tolerance for lead responses in maize (*Zea mays* L) may provide important insights for protecting the environment. Quantitative trait loci (QTL) for Pb accumulation in maize were identified using 207 IBM10 DH at maturity stage. The IBM10 DH and parents were planted in pots which were filled up with soil which was stressed with a Pb(NO₃)₂ solution (500 mg kg⁻¹). Pb concentrations in roots, stems, leaves, and kernels were measured. There was a wide distribution of Pb concentration among the mentioned four tissues and all the DH lines ($P < 0.01$). Pb concentration of kernels displayed significant positive relationships with stems and leaves ($P < 0.01$). The present study has demonstrated that the trend of Pb concentration in different tissues of maize were roots > leaves > stems > kernels. The Pb concentration of kernels was higher significantly correlated with stems. A total of 20 QTLs at logarithm of odds (LOD) ratio ≥ 3 were detected for Pb concentration in different tissues, including roots, stems, leaves, and kernels. Furthermore, RNA-seq data and qRT-PCR analysis led to the identification of two Pb-responsive genes from those QTLs, GRMZM2G137161 and GRMZM2G132995, which were located on chromosome 2 and 6 respectively. The two genes were dramatically up-regulated during Pb stress.

Keywords: maize, Pb concentration, quantitative trait loci (QTL), candidate gene

Introduction

Soil contamination with toxic heavy metals has gained considerable attention in the recent era. Moreover, it seems that this situation will not be mitigated in the near future, especially in developing countries (Valko et al, 2005; Wang and Björn, 2014). Among different kinds of potential toxic metals, Lead (Pb, II) is one of the most common and important pollutants in environment (Tangahu et al, 2011; Watanabe, 1997). Pb is a typical non-essential element to the human body, and excessive intake of the heavy metal can damage the nervous, skeletal, enzymatic, endocrine, and immune systems (Needleman, 2004; Patra et al, 2004). A review of heavy metal contamination in urban soils of China showed that the concentration range of Pb was from 28.6 to 470.19 mg kg⁻¹ (Wei and Yang, 2010). It is generally agreed that soil Pb concentration over 100 mg kg⁻¹ are considered to be excessive (Kabata-Pendias, 2004). The toxic symptoms of Pb in plant are not very specific. Many researches basically involved retardation of plant growth. The inhibitory effects may be due to interference with enzymes essential for normal metabolism and development, photosynthetic processes, water and mineral nutrients absorption, changes in cell ultrastructure and

so on (Assche and Clijsters, 1990; Fodor et al, 1996; Stefanov et al, 1995). For its toxic to plant, animal, and human being, Pb has recently received much attention as a major chemical pollutant of environment. The absorption and transport of Pb by crops are of great concern, especially its accumulation in the edible part. Eventually, Pb poses a serious threat to the health of animals and human beings through entering into the food chain (Li et al, 2014).

In many countries where most maize products are not directly used as human food, they are mostly used as feedstuff for livestock and poultry. Maize may represent the first product in the biological chain. Its quality is important because of the potential for greater translocation and accumulation for heavy metal, especially exposed to Pb (Ali et al, 2013; Liu et al, 2003). So it is very important to understand the differences among maize cultivars and genotypes in Pb uptake and translocation. In maize, different varieties exhibit a wide range of Pb accumulation, especially different tissues (Bi et al, 2009; Brennan and Shelley, 1999). In the present study, line B73 was observed to be more tolerant to heavy metal stress than line Mo17 (Baxter et al, 2013; Lung'aho et al, 2011; Zdunić et al, 2014). However, comparing to other heavy metals and metalloids (Dong et al, 2011; Fu et al, 2014), there

are fewer researches on the accumulation and distribution of Pb in maize. Meanwhile, the genetic basis for Pb accumulation and distribution remains unclear.

The goal of this study was to reveal the mechanism of Pb accumulation and distribution in different maize tissues, to detect QTL associated with Pb tolerance, finally, to identify candidate genes in the QTL intervals based on transcriptional study by RNA-seq.

Materials and Methods

Plant materials and growth conditions

An IBMSyn10 DH population including 207 inbred lines was used in the study. IBMSyn10DH (abbrev, IBM10DH) is a B73 × Mo17 doubled haploid (DH) population obtained after 10 generations of intermat- ing (Hussain et al, 2007).

In 2013, a pot experiment was carried out in a greenhouse at Duoying Farm of Sichuan Agricultural University. Soil samples were taken from Ya'an city, Si Chuan province of China. The Pb concentration in soil was 18.45 mg kg⁻¹, which was under the average background concentration of soil heavy metals (GB15618-1995). Soil was put into some plastic pots and each pot with 15 kg soil, with a diameter of 22 cm and a depth of 28 cm. The seeds of maize were sowed in the pot and fully saturated. Two plants per pot were kept at 3-leaf stage. At this time point, the soil was stressed with a Pb(NO₃)₂ solution (500 mg kg⁻¹). Each genotype was 3 replicates. The whole experiment was under conventional management (water and fertilizer).

Analysis of Pb concentration

At maturity stage, 6 plants, including roots, were harvested. Oven-dried plant tissues (roots, leaves, stems and kernels) were digested with HNO₃-HClO₄ (ratio 4:1) and heated by electric hot plate. The concentration of Pb in roots, leaves, stems and kernels was measured twice by an atomic fluorescence spectrometry (SHIMADZUAA-6600, Japan).

Data analysis and QTL mapping

The linkage map of the IBM10DH which has been previously established was used in QTL analysis (Jansen et al, 2015; Liu et al, 2015). The genetic linkage map was reconstructed using the R statistical software package onemap (Margarido et al, 2007). The linkage map included 6268 bin markers which spanned a map distance of 4,554.31 cM, with an average bin marker distance of 0.73 cM.

For QTL detection, the composite interval map-

ping (CIM; Zmap model 6) of QTL Cartographer version 1.17 (Basten et al, 2005) was used to detect QTLs for the Pb concentration in four measured tissues of maize. Logarithm of odds (LOD) scores were calculated at 1.0 cM intervals. Only QTLs with LOD score ≥ 3.0 were reported here.

Bioinformatic filtering for candidate genes

The QTLs which can explain over 10% for the phenotypic variation and co-localized on same genomic regions were used to search candidate genes. All genes of the QTL confidence intervals were downloaded from MaizeGDB (<http://www.maizegdb.org>). The Singular Enrichment Analysis (SEA) of agriGO was used to GO analysis (Du et al, 2010).

Based on our previous RNA-sequence data using a non-hyperaccumulator genotype (line 9782) at four developmental stages (0, 12 h, 24 h, and 48 h) under the Pb (Pb(NO₃)₂, 1 mol l⁻¹) stress (Gao et al, 2015), genes expressed in specific phase were determine. Eventually, candidate genes were identified by quantitative real-time PCR (qRT-PCR). Primer5 software was used to design the corresponding primers, which were listed in attachment (Supplementary Table 1).

RNA isolation and confirmation of candidate genes by performing qRT-PCR

Total RNA was extracted from roots of two replications of the parental inbreds B73 and Mo17 that were collected for 12 h, 24 h, 48 h, and 72 h, treated with 1,000 mg l⁻¹ of Pb(NO₃)₂ solution using TRIzol reagent (Invitrogen, USA). Afterwards, qRT-PCR was implemented using the SYBR premix Ex Taq kit (TaKaRa, Japan) on an ABI 7500 Real-Time System, as follows: 94°C for 2 min; 95°C for 5 s, 60°C for 30 s, 72°C for 30 s, 72°C for 3 min, 30 cycles. 18S rRNA was set as the endogenous control in this study. The relative quantitative method (2^{-ΔΔCt}) was used to calculate the fold change in the expression level of gene (Schefe et al, 2006).

Results

Phenotypic variation and correlation analysis of Pb concentration in the four tissues

In terms of Pb concentration in the four tissues (Table 1), the parent Mo17 had a lower Pb concentration (34.28 mg kg⁻¹) than parent B73 (53.81 mg kg⁻¹) in the roots. However, for leaves and kernels, the Pb concentration in B73 (17.81 and 0.24 mg kg⁻¹) was lower than those in Mo17 (33.62 and 0.68 mg kg⁻¹). The data demonstrated that the Pb distribution in

Table 1 - Statistical analysis of Pb concentration of the parents and IBM10DH population in different tissues of maize.

Trait	Parent B73±SD	Mo17±SD	IBM10DH Average ± SD	CV%	Skewness	Kurtosis	Range	F
RPC	53.81±1.74**	34.28±1.92	48.18±7.29	14.81	-0.25	1.25	21.80-69.60	8.11**
SPC	12.71±0.79*	9.36±0.69	15.31±5.59	35.82	0.55	0.22	2.50-33.40	9.35**
LPC	17.81±0.29	33.62±1.66**	25.87±8.29	30.09	0.16	-0.29	5.05-41.15	16.12**
KPC	0.24±0.08	0.68±0.15**	0.47±0.25	67.28	0.87	1.07	0.04-1.37	17.42**

SD - standard deviation; CV - coefficient of variation; ** significant at P < 0.01

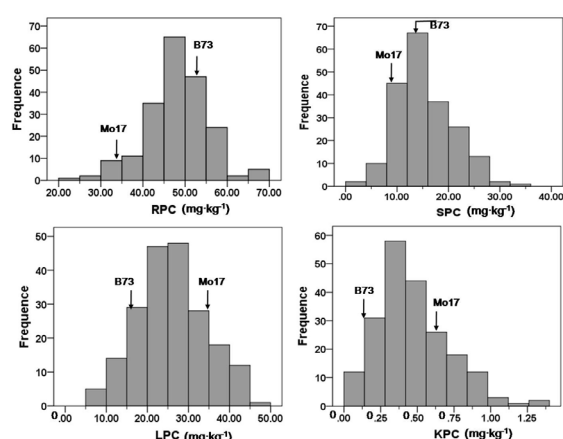


Figure 1 - The frequency distribution of four traits in IBM10 DH derived from B73 × Mo17. The trait values for B73 and Mo17 are indicated by arrows. LPC - Pb concentration in the leaves, RPC - Pb concentration in the leaves, SPC - Pb concentration in the stems, KPC - Pb concentration in the kernels.

the four tissues were significantly different between the two parents. Among the IBM10DH families, the 207 distributions of the Pb concentration of each tissue were approximately normal, and there were wide variations in four tissue (Figure 1). It also suggested that the IBM10DH was suitable for QTL analysis.

Analysis of variance (ANOVA) was used to test the statistical significance of the differences in the four tissues. According to variance analysis, the Pb concentration for the four measured tissues (kernels, stems, leaves, and kernels) in the IBM10DH exhibited significant variations at $P < 0.01$ level (Table 1). Among different tissues, the roots had the highest Pb concentration (48.18 mg kg^{-1}), followed by leaves (25.87 mg kg^{-1}) and stems (15.31 mg kg^{-1}), with kernels showing the lowest Pb concentration (0.47 mg kg^{-1}).

For the Pb concentration in four measured tissues, The Pb concentration in kernels was highly correlated with Pb concentration in stems in the IBM10 DH population grown on Pb-polluted pot soil ($r = 0.69$; Table 2). The low correlation coefficient ($r = 0.15$) between LPC and RPC was observed. However, there were no significant relationships among RPC, SPC, and KPC ($P > 0.05$). It indicated that Pb concentration in kernels maybe simultaneously increased with Pb concentration of stems.

QTL analysis for Pb concentration in the four tis-

Table 2 - Correlation coefficients among four measured tissues in the IBM10DH population.

	RPC	SPC	LPC	KPC
RPC		0.01	0.15*	-0.01
SPC	0.01		0.25**	0.69**
LPC	0.15*	0.25**		0.31**
KPC	-0.01	0.69**		0.31**

*, ** significant at $P < 0.05$ and 0.01 , respectively.

sues of maize

Twenty different QTLs were identified for Pb concentration in the four tissues with LOD scores ranging from 3.06 to 9.77 and located on maize chromosomes except 8 (Table 3, Figure 2).

For the Pb concentration in roots, 6 QTLs were identified on chromosomes 1, 2, 3, 9, 10, which explained 4.71% - 11.66% of the phenotypic variation. qRPC3, which was located on chromosome 3, had a 11.66% phenotypic variation with LOD score 6.91, with a direct raise 2.42 mg kg^{-1} Pb concentration of the roots. For the Pb concentration in the stems, 5 QTLs were identified on chromosome 2, 4, 7, and 10 with the LOD score 3.19 to 5.29, which explained 5.11% to 8.25% of the phenotypic variation. For Pb concentration in the leaves, 6 QTLs were identified with the LOD scores 3.08 - 8.45, which explained 4.82% to 14.28% of the phenotypic variation. Of the 6 QTLs, qLPC6 had a high phenotypic variation (14.28%), with a direct decrease of 2.30 mg kg^{-1} Pb concentration in the leaves. For Pb concentration in the kernels, three QTLs were identified on chromosomes 1, 2, and 5, respectively, which explained 8.39% to 18.88% of the phenotypic variation. Of the 3 QTLs, qKPC2 and qKPC5 had a high contribution (10.02 and 18.88%) to the variance in Pb concentration of kernels, with a direct decrease 0.12 mg kg^{-1} and 0.17 mg kg^{-1} Pb concentration in the kernels.

Out of the 20 QTLs detected for the Pb concentration in different tissues, the widest and narrowest QTL confidence interval were qKPC2 (13.91 cM) and qLPC10 (2.37 cM), respectively. In addition, QTLs for LPC, SPC, and KPC, which colocalized small genomic regions on chromosome 2, had a physical interval of just 4 Mb with relatively high LOD scores (Table 3). The additive effect of other major QTLs, which were located on chromosome 3, 6, and 5, were negative values (contributed by B73).

These results verified that Pb uptake from soil to root and translocation from root to shoot were possibly controlled by two separate genetic mechanisms.

Filtering and identifying genes of QTL intervals

With a relatively high mapping resolution, the QTLs which could explain over 10% for the phenotypic variation and co-localized on same genomic regions were used to search candidate genes. For the RPC QTLs, qRPC3 was located in the region that ranged from 7.05 to 8.25 Mb on chromosome 3 (Table 3). For the LPC QTLs, qLPC6 was mapped to the region that ranged from 136.67 to 142.72 Mb on chromosome 6. For the KPC QTLs, qKPC2, which overlapped with the position of the qLPC and qSPC, was located in the region that ranged from 41.05 to 45.85 Mb on chromosome 2. In addition, qKPC5 was located the 4.75 Mb to 5.85 Mb on chromosome 5.

Based on the current annotation of the B73 RefGen_v2 genome sequence, a total number of 321 genes underlying the four detected QTLs were identified (Supplementary Table 2). Of these genes, 95

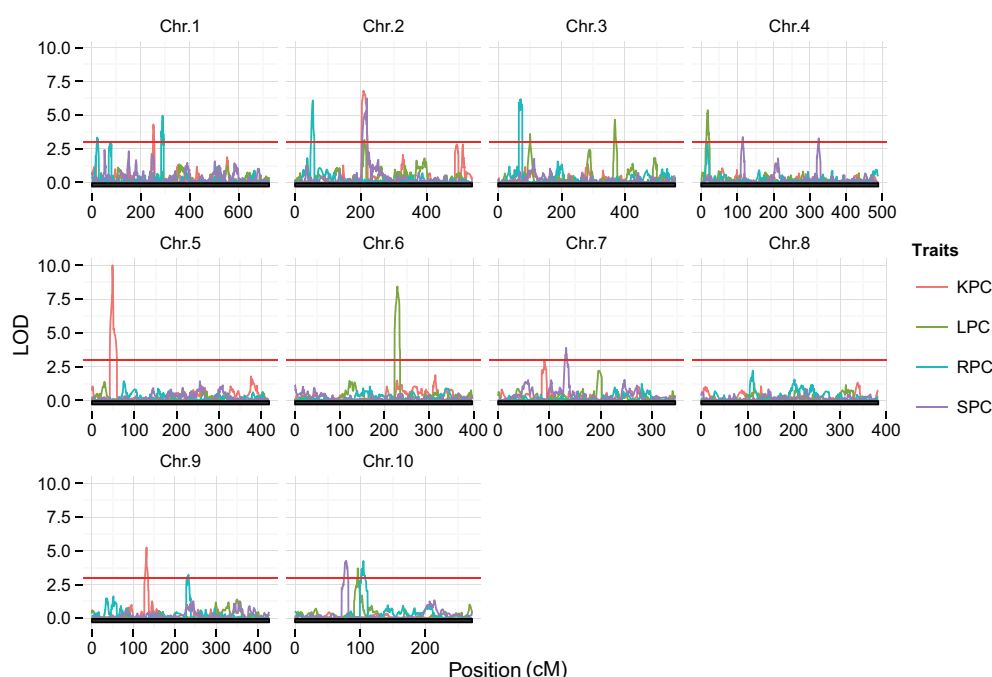


Figure 2 - The LOD profiles of QTLs detected for Pb concentration in maize four tissues.

genes were catalogued as “unknown protein function” (Supplementary Table 3). 160 potentially coding genes were associated to at least a single GO term (Figure 3). The results revealed that these genes were encompassed diverse functional categories such as DNA binding, catalytic, electron carrier and so on.

Because of the abundant genes, we used previous RNA-seq data (Gao et al, 2015) to further filter the genes (Supplementary Table 4). According to the significant difference of the gene expression levels in maize roots at four developmental stages, 6 candidate genes were filtered in the end (Figure 4, Table

4). Three genes were located on chromosome 2 and three genes were located on chromosome 6, which were significantly up-regulated under Pb stress condition and closely related to transport and efflux.

qRT-PCR validated the putative candidate genes

To assess the responses of these 6 putative candidate genes to Pb stress in maize, a quantitative real-time PCR (qRT-PCR) analysis was performed using the two parents B73 and Mo17. As shown in Table 3, the candidate gene GRMZM2G137161 is predicted to encodes transmembrane amino acid transporter protein, which was located Chromo-

Table 3 - QTLs detected for Pb concentration of four tissues in maize.

Trait	QTL	Chr.	Pos(cM) ^a	LOD	R ² (%)	A ^b	Genetic distance (cM) ^c		Physical distance (Mb) ^d	
RPC	qRPC1-1	1	21.34	3.25	5.23	1.71	18.31	22.03	4.45	4.85
	qRPC1-2	1	288.34	4.94	7.56	-1.79	285.02	291.33	94.75	96.42
	qRPC2	2	53.95	5.94	9.65	-2.45	50.74	55.43	7.25	7.92
	qRPC3	3	70.05	6.91	11.66	2.42	65.21	74.23	7.05	8.25
	qRPC9	9	231.05	3.06	4.71	1.61	227.66	233.46	109.15	115.55
	qRPC10	10	103.85	3.45	5.54	1.72	99.72	111.45	104.55	116.00
SPC	qSPC4-1	2	206.78	5.29	8.25	-2.27	198.42	210.54	39.95	43.90
	qSPC4-2	4	114.81	3.36	5.25	-1.27	109.25	116.23	17.95	19.85
	qSPC4-3	4	324.53	3.19	5.11	1.26	320.26	325.25	181.95	182.05
	qSPC7	7	132.88	3.82	5.89	1.76	130.04	138.05	105.05	112.55
	qSPC10	10	78.31	4.16	6.84	1.53	71.54	81.13	14.35	61.15
	qLPC2	2	211.01	3.08	4.82	-1.76	207.83	215.07	42.40	44.35
LPC	qLPC3-1	3	100.91	3.51	5.65	-1.94	98.49	101.16	11.32	12.35
	qLPC3-2	3	368.21	4.55	6.88	-2.11	364.94	370.92	198.45	199.2
	qLPC4	4	18.15	5.23	8.25	2.36	12.02	22.84	2.85	4.05
	qLPC6	6	230.02	8.45	14.28	-2.31	220.79	234.33	136.67	142.72
	qLPC10	10	96.16	3.61	5.62	1.94	95.21	97.58	87.53	92.23
	qKPC1	1	252.72	4.29	8.39	-0.07	248.43	256.31	69.25	75.95
KPC	qKPC2	2	208.06	6.79	12.02	-0.12	203.38	217.29	41.05	45.85
	qKPC5	5	48.43	9.77	18.88	-0.17	42.14	52.85	4.75	5.85

^aPosition of peak with highest LOD in cM. ^bThe additive effect of the QTL. Negative values indicate that the alleles for increasing trait value are contributed by B73, positive values indicate that the allele for increasing trait value are contributed by another parent Mo17. ^cThe confidence interval of QTL position. ^dThe physical distance of the bin makers corresponding to genetic distance.

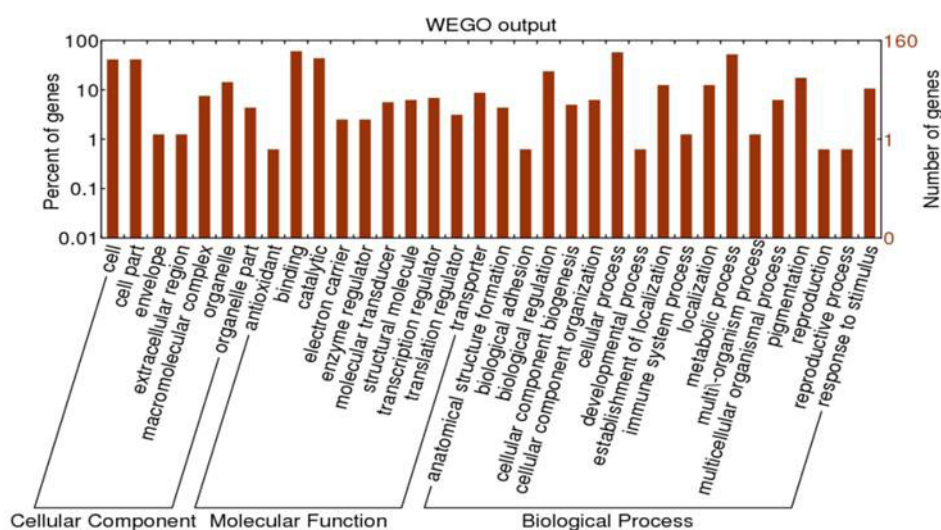


Figure 3 - GO analysis of all genes of four QTL intervals.

some 2. GRMZM2G132995 is one of MATE efflux family protein, which was located on chromosome 6. There were almost similar expression trend between RNA-seq data (Figure 4C) and qRT-PCR data (Figure 5), and dramatically up-regulated during the Pb stress. Comparison of changes in gene expression between control group and Pb 1000 treatment, GRMZM2G137161 expression of B73 significantly increased 1.59 - 14.35 fold ($P < 0.05$) than control group. GRMZM2G132995 expression of B73 was significantly overexpressed 0.75 - 4.62 fold ($P < 0.05$) than control group. However, the expression of the two candidate genes in Mo17 had not significant difference with that of CK. In addition, the expressions of the remaining four genes were not significantly differences between B73 and Mo17 (data no shown).

Discussion

Lead (Pb) has become one of the most abundant heavy metal pollutants of the environment. Plants can easily take up Pb from the soil. And a small fraction is translocated upward to the shoot and leaf, even to kernel in most plant species (Liu et al, 2009; Patra et al, 2004), which has posed critical concern to human health and environmental safety. Therefore, understanding the genetic basis for the underlying mechanism of Pb accumulation in maize may provide important insights for the selection and development of Pb-extreme maize cultivars. One side, Low Pb accumulation lines can be produced to avoid Pb going into food chain. One the other side, Pb-absorbing lines can be produced to reduce the Pb from the soil to protect the environment. Therefore, the distribution and accumulation of Pb in different tissues have received close attention in previous studies.

Genotypic variation of traits related to Pb distribution and accumulation

Pb is absorbed and accumulated in differ-

ent plant tissues (Schreck et al, 2012; Zhang et al, 2012), generally with the highest concentration in the root tissues (Raskin et al, 1997). Based on the results, a transgressive segregation of Pb concentration in maize roots, stems, leaves and kernels was observed, which showed highly significant difference between all lines and the parents.

Following the uptake of metals by roots, xylem loading of metals is suggested as the next important transport process for metal-accumulation in plant shoots (Clemens et al, 2002). In this study, although Pb concentration in the roots of Mo17 was lower than in B73, Pb concentrations in the leaves and kernels were higher in Mo17. This indicated that more rapid and greater root-to-shoot Pb translocation was observed in Mo17. This results also suggested the higher loading ability of Pb to the stem in Mo17, which led to higher Pb accumulation in leaf and kernel.

In general, a very large amount of Pb was retained in roots compared to its concentration in leaves and kernels in maize (Fu et al, 2010; Pallavi and Shanker Rama, 2005), which was similar to other heavy metals (Zhang et al, 2008). We found that the same trend of Pb concentration in different tissues were roots > leaves > stems > kernels. Among 207 IBM10DH, the Pb concentrations of 32 lines in kernels are under the level of National Food Hygiene Standard. Kato reported a significant correlation between kernel and stem Cd concentration in rice (Kato et al, 2010). In this study, the Pb concentration of kernels was higher significantly correlated with stems. However, The Pb concentration of roots was non-significant with the Pb concentration of stems and kernels. The results further confirmed that high Pb accumulation level in kernels of maize may be partially due to the elevated translocation of Pb from stems to maturing kernels.

Mapping Pb tolerance QTLs in maize

By combining genotype data with the value of

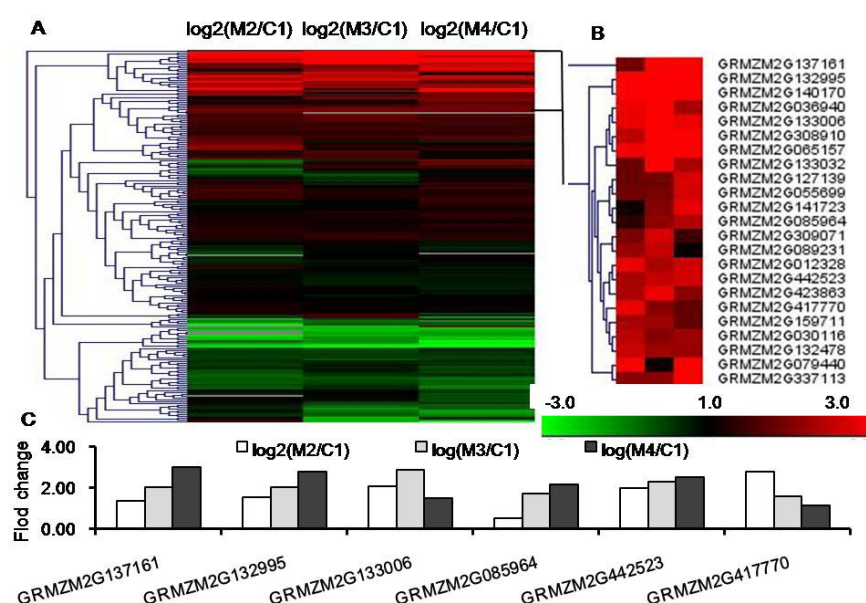


Figure 4 - Mapping information of all expression genes from majority QTLs: (A) Venn diagram of all genes from four RNAseq libraries underlying the 4 QTLs in maize roots at four developmental stages under the Pb stress (CK sample (C1), Pb 1000 treat for 12 h (M2), Pb1000 treat for 24 h (M3), Pb 1000 treat for 48 h (M4). (B) The list of significant up-regulated genes was enlarged. (C) Bar-plot of 6 differential expression genes filtered according to current functional annotation.

Pb concentration in roots, stems, leaves, and kernels at maturity age, we found that three QTLs were co-localized on chromosome 2. This result indicated the insights that these traits might be highly inter-correlated. Some studies have suggested that the main determinant of the Cd concentration in shoot tissues was the ability to translocate Cd from root to shoot through the xylem, rather than Cd uptake by the roots (Tanaka et al, 2008; Uraguchi et al, 2009). Nearly 100% of the Cd in rice kernels is attributable to phloem transport (Tanaka et al, 2007). The QTL detection results indicated that Pb uptake from soil and translocation from root-to-shoot were possibly controlled by two separate genetic mechanisms. In addition, the Pb accumulation could be controlled by multiple genes.

Since the location of markers flanking the loci can be found in the Maize Genome Project genetic map, we found that qKPC2 (qLPC2, qSPC2) and qLPC6 were close to qAl2 and qAl6 (Ninamango-Cárdenas et al, 2003), respectively. Meanwhile, we also detected an additional QTL on chromosome 5 that accounted for 18.88% of the phenotypic variation for KPC. This new QTL has not been reported previously. Different

QTLs may be responsible for different processes involved in accumulation of Pb.

Candidate genes analysis

Major genes for metal accumulation might be associated with ion transport and efflux as detected in Arabidopsis and Rice (Ueno, 2010; Waters and Grusak, 2008), but these genes are mostly still unknown in maize. Because of the multiple QTLs and relatively long list of genes in confidence interval, we used RNA-seq data analyses to further decrease the number of candidate genes and gain insight into the potential roles of the candidate genes. Eventually, through qRT-PCR assess, the expression of the candidate genes (GRMZM2G137161 and GRMZM2G132995) in roots of B73 were significantly higher than Mo17. The one gene, GRMZM2G137161, is predicted to encode a homolog of the vacuolar amino acid transporter protein. GRMZM2G137161, which was localized in the vacuolar is highly overexpressed in roots of B73. Pb could be compounded with amino acids such as histidine and with organic acids such as citric, fumaric, malic acids and phytochelatins (PCs) (Ghnaya et al, 2013; Shahid et al, 2012). Pb might be transported into the vacuole and was stored in the form of Pb-

Table 4 - Candidate genes in four QTL intervals that showed differential expression levels in the RNA-seq data.

Gene-ID	Chr	Position	Current annotation
GRMZM2G137161	2	42000195 42002952	transmembrane amino acid transporter protein, putative, expressed
GRMZM2G442523	2	43370373 43375906	transporter family protein, putative, expressed
GRMZM2G417770	2	43943602 43945578	ATP-dependent protease, putative, expressed
GRMZM2G085964	6	138562322 138563604	AP2 domain containing protein, expressed
GRMZM2G132995	6	139591107 139593447	MATE efflux family protein, putative, expressed
GRMZM2G133006	6	139593708 139596321	MATE efflux family protein, putative, expressed

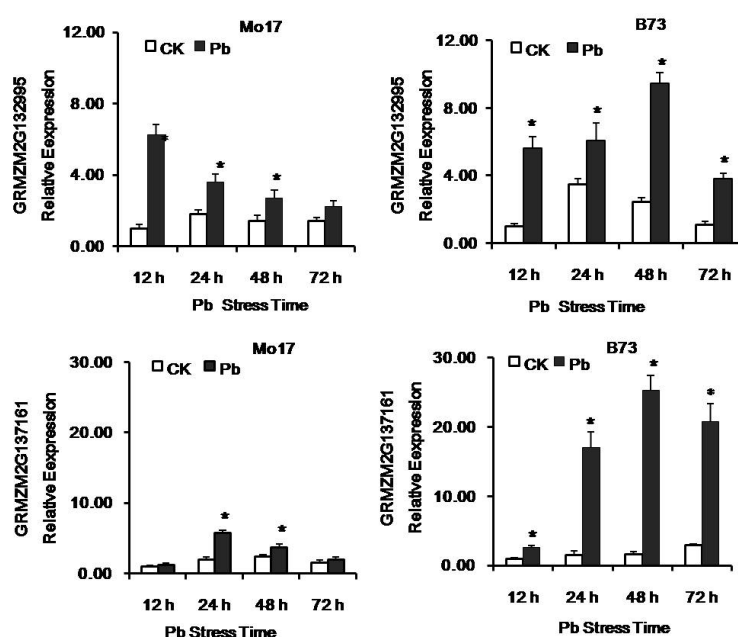


Figure 5 - Relative expression of GRMZM2G137161 and GRMZM2G132995 analysed by qRT-PCR at the different Pb stress times: The standard deviation calculated from two biological replicates. *represents the significant in $P < 0.05$.

chelate complex, thus inducing the damage for cell structure. It suggested that GRMZM2G137161 might play a role in enhancing Pb tolerance and decreasing Pb transport. The other gene, GRMZM2G132995, which was predicted to encode toxin extrusion (MATE) proteins, was also strongly up-regulated in roots of B73, especially at 48 h of Pb treatment. The vesicular monoamine transporter (VMAT) is a transport protein integrated into the membrane of synaptic vesicles of presynaptic neurons. VMATs utilize a proton gradient generated by V-ATPases in vesicle membranes to power monoamine import. It has been previously unraveled that the abundance of V-ATPase subunit A increased with the presence of copper (Morris et al, 2014). Plant MATE protein capable of transporting citrate have recently been identified; some of these act as a transporter responsible for Al-activated root citrate release underlying Al tolerance (Magalhaes et al, 2007). While others are citrate transporters related to other physiological processes such as iron translocation (Yokosho et al, 2009), Cd transport (van de Mortel et al, 2008), and phosphorus efficiency (Ukde-Stone et al, 2003). This suggested that the role of GRMZM2G132995 in Pb absorption via import from intercellular space is to increase Pb accumulation in roots of plant.

Although the two candidate genes were filtered, expression data should be gathered for a larger number of genotypes, and QTLs should be validated in different genetic and environmental backgrounds under field conditions of many years and many places. It is worth examining this region thoroughly in order to confirm that the QTLs resides in this region.

Acknowledgements

The authors thank all those who collaborated in the analysis, interpretation of data and writing the article. This research was supported by a grant from the National Science Foundation of China (CN) (31171567).

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