Supplementary Information

Table S1. Classification of reads mapping to genome and gene body.

Class	A1		K2		K3		K4		Input	
	#	%	#	%	#	%	#	%	#	%
Total Reads [a]	8,616,236		6,955,318		8,280,281		8,563,377		2,300,388	
Total Mapped Reads [a]	6,990,394	81.13	5,584,187	80.29	6,664,691	80.49	6,897,965	80.55	651,339	28.31
Total Mapped Uniquely Reads [a]	1,402,490	20.06	1,109,755	19.87	1,347,598	20.22	1,401,130	20.31	148,306	22.77
5' UTR	4080	0.29	3179	0.29	3984	0.3	4579	0.33	2106	1.42
3' UTR	3061	0.22	2950	0.27	3310	0.25	3387	0.24	2031	1.37
CDS	24,574	1.75	19,585	1.76	24,862	1.84	26,569	1.9	14,508	9.78
Intron	100,356	7.16	75,273	6.78	95,188	7.06	101,794	7.27	13,085	8.82
Promoter (≤1 k)	39,508	2.82	33,178	2.99	38,693	2.87	43,355	3.09	5208	3.51
Promoter (1–2 k)	33,594	2.4	27,626	2.49	32,725	2.43	34,587	2.47	3623	2.44
Promoter (2–3 k)	27,983	2	22,871	2.06	27,082	2.01	28,493	2.03	2988	2.01
Downstream (≤1 k)	24,898	1.78	21,291	1.92	24,591	1.82	26,598	1.9	3553	2.4
Downstream (1–2 k)	25,808	1.84	21,162	1.91	25,066	1.86	26,267	1.87	2862	1.93
Downstream (2–3 k)	21,322	1.52	17,006	1.53	20,305	1.51	21,108	1.51	2273	1.53
InterGenic	1,097,306	78.24	865,634	78	1,051,792	78.05	1,084,393	77.39	96,069	64.78
In CpG islands [b]	998,018	71.16	738,499	66.55	927,881	68.85	894,970	63.81	69,497	46.86

[a] Reads were mapped by Bowtie (version 0.12.8) with the default parameter; [b] CpG Islands were predicted by Takai's CpG island searcher using GCC = 55 OE = 0.65 LENGTH = 500.

Table S2. CpG islands (CpGI) identified in maize genome.

Class	Maize	
	#	%
Total Length of Genome (bp)	2,066,432,718	
Number of CpG Islands [a]	356,833	
Total Length of CpG Islands (bp)	621,019,160	30.05%
Mean Length of CpG Islands (bp)	1,740.36	
Median Length of CpG Islands (bp)	920	
Max Length of CpG Islands (bp)	50,011	

[a] CpG Islands were predicted by Takai's CpG island searcher using GCC = 55 OE = 0.65 LENGTH = 500.

Table S3. Distribution of DNA Methylation in CGIs.

Class	Total	
	#	%
Number of CpG Islands	356,833	
Number of CpG Islands Expressed	223,321	
Expressed In A1	161,777	
Expressed In K2	153,445	
Expressed In K3	162,476	
Expressed In K4	164,296	
In Exon	11,208	
In Intron	6843	
In Promoter	25,666	
In Downstream	14,431	
In InterGenic	170,929	

Table S4. GO annotation of differential methylated genes.

GO Term	Ontology	Description	Number in Input List		Number in BG/Ref	*p*-Value	FDR
GO:0050896	P	response to stimulus	759	2.26%	3421	1.00×10^{-7}	0.00048
GO:0065007	P	biological regulation	1310	3.89%	6272	2.00×10^{-7}	0.00048
GO:0006950	P	response to stress	677	2.01%	3059	7.20×10^{-7}	0.0012
GO:0008152	P	metabolic process	3880	11.53%	20,231	1.00×10^{-6}	0.0012
GO:0010556	P	regulation of macromolecule biosynthetic process	723	2.15%	3393	2.20×10^{-5}	0.0096
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	717	2.13%	3370	2.80×10^{-5}	0.0096
GO:0031326	P	regulation of cellular biosynthetic process	723	2.15%	3393	2.20×10^{-5}	0.0096
GO:0009889	P	regulation of biosynthetic process	723	2.15%	3393	2.20×10^{-5}	0.0096
GO:0050794	P	regulation of cellular process	893	2.65%	4274	2.40×10^{-5}	0.0096
GO:0051171	P	regulation of nitrogen compound metabolic process	717	2.13%	3370	2.80×10^{-5}	0.0096

Table S4. *Cont*.

GO Term	Ontology	Description	Number in Input List		Number in BG/Ref	*p*-Value	FDR
GO:0006355	P	regulation of transcription, DNA-dependent	492	1.46%	2208	1.40×10^{-5}	0.0096
GO:0051252	P	regulation of RNA metabolic process	493	1.47%	2211	1.30×10^{-5}	0.0096
GO:0031323	P	regulation of cellular metabolic process	731	2.17%	3439	2.60×10^{-5}	0.0096
GO:0010468	P	regulation of gene expression	727	2.16%	3419	2.60×10^{-5}	0.0096
GO:0045449	P	regulation of transcription	713	2.12%	3352	3.00×10^{-5}	0.0096
GO:0065008	P	regulation of biological quality	474	1.41%	2139	3.20×10^{-5}	0.0096
GO:0032774	P	RNA biosynthetic process	518	1.54%	2364	3.60×10^{-5}	0.01
GO:0006351	P	transcription, DNA-dependent	517	1.54%	2362	4.10×10^{-5}	0.011
GO:0032501	P	multicellular organismal process	441	1.31%	1983	4.60×10^{-5}	0.012
GO:0042592	P	homeostatic process	453	1.35%	2047	5.30×10^{-5}	0.013
GO:0009987	P	cellular process	3519	10.46%	18,476	7.30×10^{-5}	0.017
GO:0006464	P	protein modification process	660	1.96%	3113	8.20×10^{-5}	0.018
GO:0016070	P	RNA metabolic process	660	1.96%	3121	0.0001	0.022
GO:0050789	P	regulation of biological process	926	2.75%	4504	0.00011	0.022
GO:0080090	P	regulation of primary metabolic process	751	2.23%	3595	0.00012	0.022
GO:0060255	P	regulation of macromolecule metabolic process	757	2.25%	3632	0.00014	0.025
GO:0019222	P	regulation of metabolic process	763	2.27%	3667	0.00015	0.027
GO:0043412	P	macromolecule modification	682	2.03%	3252	0.00017	0.029
GO:0043687	P	post-translational protein modification	619	1.84%	2929	0.00018	0.03
GO:0009266	P	response to temperature stimulus	389	1.16%	1765	0.00023	0.034
GO:0001659	P	temperature homeostasis	387	1.15%	1758	0.00026	0.034
GO:0050826	P	response to freezing	387	1.15%	1758	0.00026	0.034
GO:0009409	P	response to cold	387	1.15%	1758	0.00026	0.034
GO:0042309	P	homoiothermy	387	1.15%	1758	0.00026	0.034
GO:0048871	P	multicellular organismal homeostasis	387	1.15%	1758	0.00026	0.034
GO:0009628	P	response to abiotic stimulus	407	1.21%	1856	0.00023	0.034
GO:0044238	P	primary metabolic process	3032	9.01%	15,885	0.00028	0.035
GO:0055114	P	oxidation reduction	580	1.72%	2743	0.00028	0.035
GO:0006468	P	protein amino acid phosphorylation	509	1.51%	2387	0.00035	0.044
GO:0006350	P	transcription	768	2.28%	3730	0.00039	0.047

Table S4. *Cont.*

GO Term	Ontology	Description	Number in Input List		Number in BG/Ref	*p*-Value	FDR
GO:0016740	F	transferase activity	1202	8.51%	5727	3.30×10^{-7}	0.00087
GO:0016758	F	transferase activity, transferring hexosyl groups	156	1.10%	542	5.20×10^{-7}	0.00087
GO:0005506	F	iron ion binding	295	2.09%	1187	9.10×10^{-7}	0.001
GO:0016757	F	transferase activity, transferring glycosyl groups	197	1.40%	747	2.80×10^{-6}	0.0024
GO:0001883	F	purine nucleoside binding	1120	7.93%	5398	5.20×10^{-6}	0.0026
GO:0001882	F	nucleoside binding	1120	7.93%	5400	5.40×10^{-6}	0.0026
GO:0030554	F	adenyl nucleotide binding	1120	7.93%	5398	5.20×10^{-6}	0.0026
GO:0004497	F	monooxygenase activity	151	1.07%	553	8.60×10^{-6}	0.0036
GO:0016773	F	phosphotransferase activity, alcohol group as acceptor	620	4.39%	2858	1.60×10^{-5}	0.0062
GO:0016301	F	kinase activity	647	4.58%	3006	2.30×10^{-5}	0.0079
GO:0005524	F	ATP binding	1056	7.48%	5133	3.10×10^{-5}	0.0096
GO:0032559	F	adenyl ribonucleotide binding	1056	7.48%	5137	3.50×10^{-5}	0.0097
GO:0003824	F	catalytic activity	3368	23.86%	17,617	4.70×10^{-5}	0.012
GO:0003700	F	transcription factor activity	321	2.27%	1390	5.20×10^{-5}	0.012
GO:0020037	F	heme binding	213	1.51%	876	8.40×10^{-5}	0.019
GO:0004674	F	protein serine/threonine kinase activity	489	3.46%	2248	0.00011	0.021
GO:0046906	F	tetrapyrrole binding	213	1.51%	880	0.0001	0.021
GO:0050824	F	water binding	387	2.74%	1758	0.00026	0.046
GO:0050825	F	ice binding	387	2.74%	1758	0.00026	0.046
GO:0031224	C	intrinsic to membrane	481	6.82%	2183	4.50×10^{-5}	0.017
GO:0005623	C	cell	2687	38.09%	13,914	7.10×10^{-5}	0.017
GO:0044464	C	cell part	2687	38.09%	13,914	7.10×10^{-5}	0.017
GO:0044425	C	membrane part	576	8.17%	2684	8.60×10^{-5}	0.017
GO:0016021	C	integral to membrane	476	6.75%	2156	4.20×10^{-5}	0.017
GO:0005576	C	extracellular region	147	2.08%	583	0.00027	0.045

Table S5. The detailed components of the nutrient solution.

Component	Concentration	Component	Concentration
$Ca(NO_3)_2 \cdot 4H_2O$	4 mmol/L	$MnSO_4$	0.1 μmol/L
KNO_3	6 mmol/L	$CuSO_4 \cdot 7H_2O$	0.1 μmol/L
NH_4NO_3	1 mmol/L	$MgSO_4 \cdot 7H_2O$	1umol/L
Fe·EDTA	100 μmol/L	$(NH_4)_6Mo_7O_{24}(4H_2O)$	0.016 μmol/L
H_3BO_3	1 μmol/L	$ZnSO_4 \cdot 7H_2O$	1 μmol/L
KH_2PO_4	1 μmol/L		

Table S6. The primers of candidate methlylation genes association with Pb stress.

Primer Name	Gene ID	Primer Sequence (3'–5')	Function Prediction
Z1-F	GRMZM2G406099	GACAAAGTCAAGCGGAGATG	AP2/ERF transcription factor
Z1-R	GRMZM2G406099	CTACGGCGAAGAAATCCTCT	
Z2-F	GRMZM2G406074	CGTAGATCGTGCTTACCGAA	F-box domain, cyclin-like protein
Z2-R	GRMZM2G406074	TGCTCTTCTTGCAACTGCTT	
Z3-F	GRMZM5G865576	TGAACAACCAAACAATTCCC	Glycoside hydrolase, family 28
Z3-R	GRMZM5G865576	GSAAGGCTGCATCCTCTTAGG	
Z4-F	GRMZM2G145458	CTGCAGCAGTTCATGGAGAG	Glycosyl transferase, family 48
Z4-R	GRMZM2G145458	CTGTATCAGAGCGTGAGCGT	
Z5-F	GRMZM2G062499	GCAGCGATAGGAGAGAGACC	Leucine-rich repeat protein
Z5-R	GRMZM2G062499	CTTCTTGTTGCTCTCGTGGA	
Z6-F	GRMZM2G082487	CGGAGGAGGGATCACTAGC	Tetratricopeptide repeat-containing protein
Z6-R	GRMZM2G082487	GATTCAGGGAGAGCAGCAA	
Z7-F	GRMZM2G048883	CGCTTCCAACCTTCTCTTTC	Zinc finger, C2H2-like protein
Z7-R	GRMZM2G048883	ATGGCTTCAAGAACAAGGCT	
Z8-F	GRMZM2G482657	AACAACCTCCGGGTACTGTC	Zinc finger, RING-type protein
Z8-R	GRMZM2G482657	CCGACGGAAACCATATCTCT	

Figure S1. All and differentially methylated genes identified in maize roots. (**A**) A total of methylated genes identified in the four samples, including 161,777 in A1, 153,445 in K2, 162,476 in K3, and 164,296 in K4, respectively; (**B**) Differentially methylated genes unique or shared among three comparisons of K2 *versus* A1, K3 *versus* A1, and K4 *versus* A1.

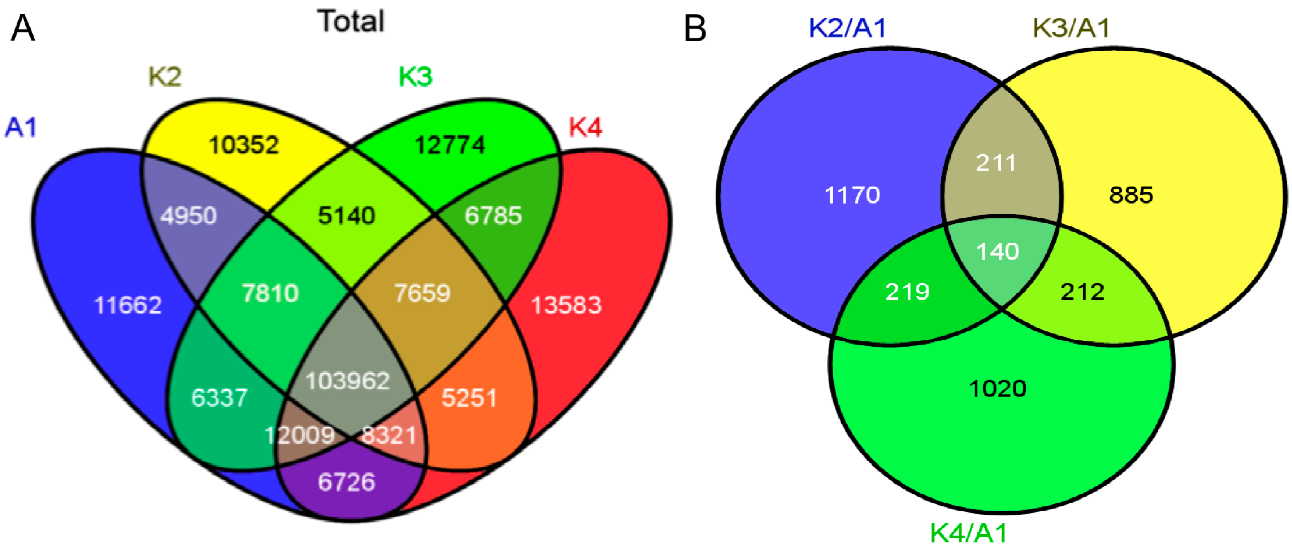

Figure S2. The photos of the seedlings of maize treated with 3 mM of Pb.

Figure S3. Dynamic changes of SOD, POD enzyme activity in maize roots and leaves under Pb stress. (**A**) Dynamic changes of SOD activity; (**B**) Dynamic changes of POD activity.

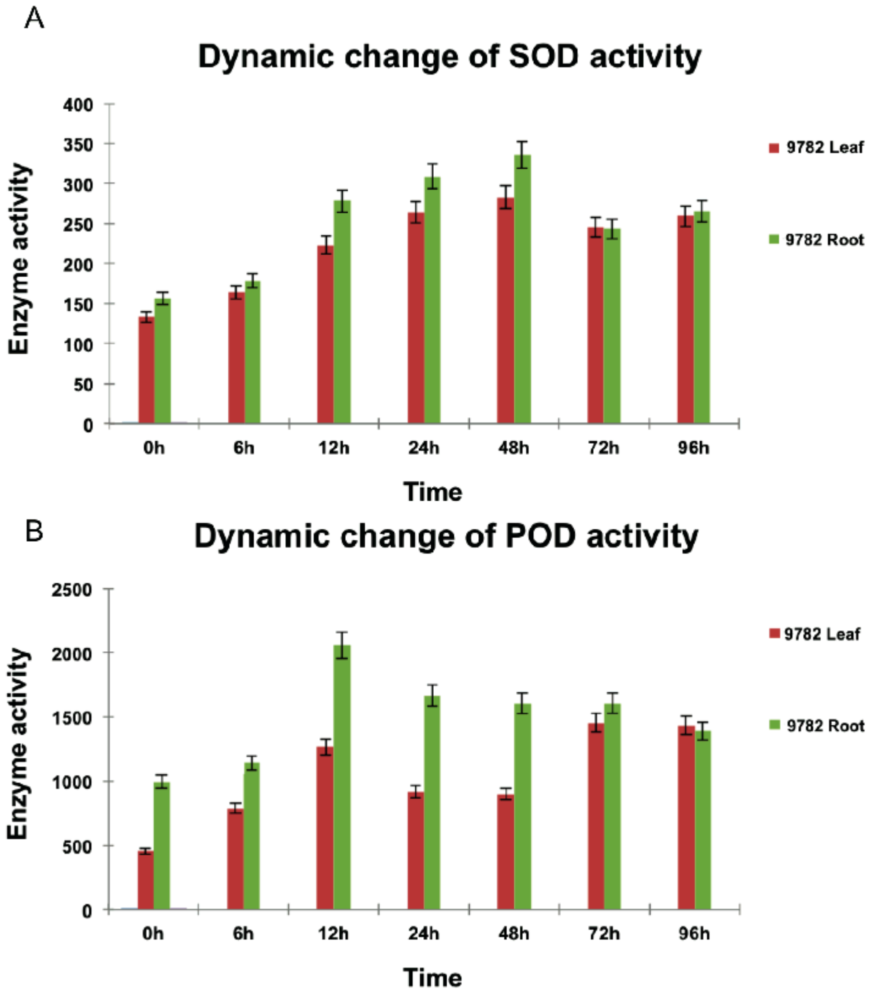

Figure S4. Average reads density in gene body of the maize genome. (**A**) Distance to TSS (−5K/+5K) used to qualify the average reads density in gene body; (**B**) Relative distance of gene body (5'-3') used to qualify the average reads density in gene body.