

Natural variation in the promoter of *OsHMA3* contributes to differential grain cadmium accumulation between *Indica* and *Japonica* rice

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SUPPORTING INFORMATION

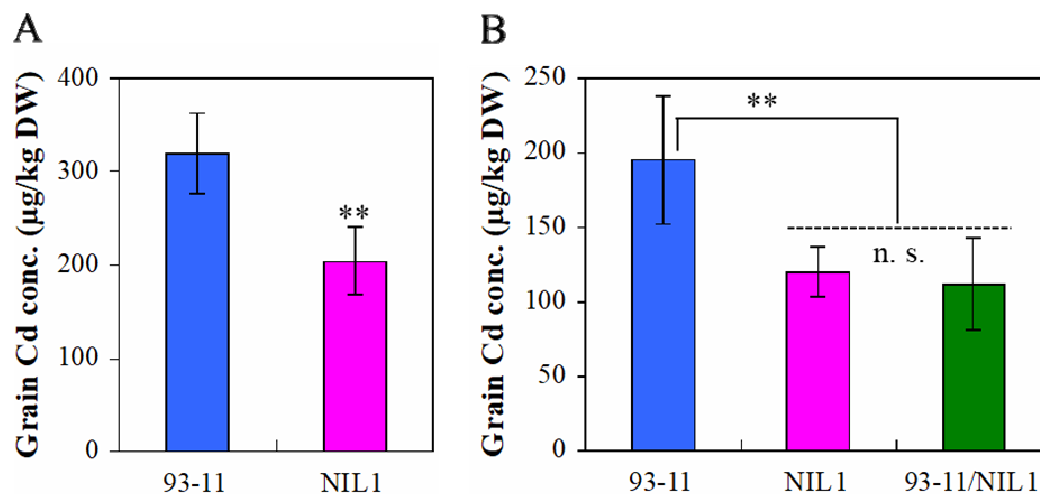


Figure S1. Comparison of grain Cd concentrations in 93-11, NIL1 and 93-11/NIL1 F1 plants

(A) Grain Cd concentrations of 93-11 and NIL1 grown in pots with soils. (B) F1 allelic test. Plants of 93-11, NIL1 and 93-11/NIL1 F1 were grown in a paddy field. Data are means $\pm$ SD (n = 6). ** indicates a significance level at 1%, and n. s. means not significant (unpaired two-tailed *t* test).

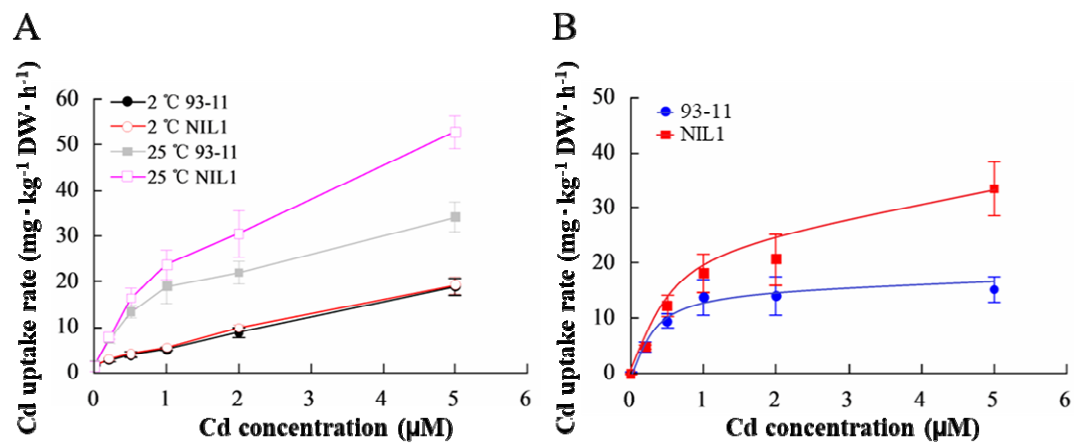


Figure S2. Cd uptake kinetics analysis in intact roots of 93-11 and NIL1

Plants of 93-11 and NIL1 were exposed to 0, 0.2, 0.5, 1, 2 or 5 μM Cd for 20 min at 25°C or 2°C. Absolute (A) and net (B) Cd uptake rate was calculated. Michaelis-Menten curves were generated for net Cd uptake rate. Data are means ± SD (n = 4).

Table S1. QTLs for grain Cd concentration identified from the RIL population of 93-11/PA64s

QTL ^a	Chr.	Marker interval	LOD ^b	PVE (%)	Add. ^c
<i>qGCC3</i>	3	SNP3-114 ~ 3-133	3.15	11.2	19.74
<i>qGCC5</i>	5	SNP5-121 ~ 5-142	3.74	13.3	-22.53
<i>qGCC7</i>	7	SNP7-33 ~ 7-55	3.02	10.9	16.18

Note: ^a Detected QTLs are shown with the italic abbreviation of the trait and the chromosome number. ^b Threshold values of logarithm of odds (LOD)>2.5 are shown. ^c Additive effect (Add.) of the 93-11 allele.

Table S2. Haplotypes of the seven nucleotide sequence in *OsHMA3* promoter

Haplotype	SNP Position							Number of varieties	Percentage (%)
	-557	-558	-576	-597	-611	-620	-683		
Type1 Var. 93-11	T	T	C	C	C	C	C	1,552	61.2
Type2 Var. PA64s	A	A	T	A	T	T	A	668	26.4
Type3	A	A	C	A	T	T	A	169	6.7
Type4	T	T	C	C	C	T	C	32	1.3
Type5	T	T	C	A	T	T	A	29	1.1
Type6	T	T	C	C	T	C	C	24	0.9
Type7	T	T	C	C	T	T	C	24	0.9
Type8	T	T	C	C	C	C	A	14	0.6
Type9	T	T	T	C	C	C	C	12	0.5
Type10	A	A	C	C	C	C	C	6	0.2
Type11	T	T	C	C	C	T	A	4	0.1

Table S3. Detailed information of selected varieties used for expression and Cd accumulation analysis

Uniform name	Accession name	Subspecies	Haplotype
No.1	93-11	<i>Indica</i>	93-11 type
No.2	CX225	<i>Indica</i>	93-11 type
No.3	B040	<i>Indica</i>	93-11 type
No.4	CX349	<i>Indica</i>	93-11 type
No.5	CX150	<i>Indica</i>	93-11 type
No.6	CX221	<i>Indica</i>	93-11 type
No.7	PA64s	<i>Indica</i>	PA64s type
No.8	IRIS_313-8162	<i>Indica</i>	PA64s type
No.9	IRIS_313-9572	<i>Indica</i>	PA64s type
No.10	IRIS_313-11441	<i>Indica</i>	PA64s type
No.11	IRIS_313-8147	<i>Indica</i>	PA64s type
No.12	IRIS_313-8185	<i>Indica</i>	PA64s type
No.13	IRIS_313-7719	<i>Japonica</i>	93-11 type
No.14	IRIS_313-8069	<i>Japonica</i>	93-11 type
No.15	IRIS_313-8161	<i>Japonica</i>	93-11 type
No.16	IRIS_313-7664	<i>Japonica</i>	93-11 type
No.17	IRIS_313-9187	<i>Japonica</i>	93-11 type
No.18	IRIS_313-10272	<i>Japonica</i>	93-11 type
No.19	B004	<i>Japonica</i>	PA64s type
No.20	IRIS_313-8200	<i>Japonica</i>	PA64s type
No.21	IRIS_313-8023	<i>Japonica</i>	PA64s type
No.22	CX534	<i>Japonica</i>	PA64s type
No.23	B046	<i>Japonica</i>	PA64s type
No.24	IRIS_313-7902	<i>Japonica</i>	PA64s type

Table S4. The list of primers used in this study

Primer name	Primer sequence (5' to 3')	Purpose
L4683F	AACCTCGAATTCCTTGGGAG	InDel marker for fine mapping <i>qGCC7</i>
L4683R	TTCGACTCCAAGGAGTGCTC	
L6446F	GCAATTGGCGGATATTTTCACG	InDel marker for fine mapping <i>qGCC7</i>
L6446R	GCAATTGGCGGATATTTTCACG	
L6952F	GGGAGCCAAAACAGTAGCAG	InDel marker for fine mapping <i>qGCC7</i>
L6952R	GCTGTGCCCACTAAGTTAGC	
L7150F	GAGCAGCTAATTGCTTGTA	InDel marker for fine mapping <i>qGCC7</i>
L7150R	AGGTAAATTGAGTGTCAGTAGCA	
L7260F	AGAAGAACCTCCCTCCTCCT	InDel marker for fine mapping <i>qGCC7</i>
L7260R	CCAAGGGTAAGAAGAGGCCA	
L7337F	TTAGAAGGTGATTAGCAAACATCAA	InDel marker for fine mapping <i>qGCC7</i>
L7337R	ACAAATATGCAGAACCCCATGT	
L7351F	CAAGACGTTTTGGGCTCATG	InDel marker for fine mapping <i>qGCC7</i>
L7351R	TTGGGTTTCTCGGATCTGGT	
L7395F	ACGAGGAGAAGCAGAGCG	InDel marker for fine mapping <i>qGCC7</i>
L7395R	GTGCACCTGTCCCTCGTG	
L7420F	TCTATGTATTTTGATCTCCGGGG	InDel marker for fine mapping <i>qGCC7</i>
L7420R	AAAATAACTTTGAGCGTACCGTG	
L7434F	ATTATTCTTTTGCCTAAGTGTGTGG	InDel marker for fine mapping <i>qGCC7</i>
L7434R	TTGAGCAACGCCAATTATTCTATTT	
L7547F	GCTACCCCTGGAGTCCAC	InDel marker for fine mapping <i>qGCC7</i>
L7547R	TCCAATAGTAAATGGCCAGTGTG	
L7653F	ACCCCTTACGCCATTCAAGA	InDel marker for fine mapping <i>qGCC7</i>
L7653R	CTTTCTTGCTTGACGTGGCT	
L7847F	AACCGAGCTGTCATCTTTCA	InDel marker for fine mapping <i>qGCC7</i>
L7847R	TCTCTCGCAATGAAAGCCAA	
L8155F	GCGATACCAGGACTCTAAAGC	InDel marker for fine mapping <i>qGCC7</i>
L8155R	GGTCGCCCGGTTTGTTAAC	
L8451F	TCCCATATCTAGACAAGGG	InDel marker for fine mapping <i>qGCC7</i>
L8451R	TAACATCACACGACAGTAC	
L9312F	AGGGTCCTCGTCTTCTTTGA	InDel marker for fine mapping <i>qGCC7</i>
L9312R	AGTCCTATCCAGATTGCCCTG	
RM14795F	GCGCGATAAACGTTTGAGAGAAGG	SSR marker for genotyping allele of <i>qGCC3</i> locus
RM14795R	AAATCAGTCGCGGTTGCTGTCC	
RM16676F	TGCATAACACCCAAATGCCTTC	SSR marker for genotyping allele of <i>qGCC3</i> locus
RM16676R	TATGTTACAATGTTACGGTCC	
RM18384F	TACGTACCAACAGGCGGAGG	SSR marker for genotyping allele of <i>qGCC5</i>
RM18384R	TTCTTGGTGCTATCTTCGTGCTC	
RM430F	AAACAACGACGTCCCTGATC	SSR marker for genotyping allele of <i>qGCC5</i>
RM430R	GTGCCTCCGTGGTTATGAAC	

<i>qOsHMA3F</i>	TCCATCCAACCAAACCGAAA	Real time RT-PCR of <i>OsHMA3</i>
<i>qOsHMA3R</i>	TGCCAATGTCCTTCTGTCCCA	
<i>qHistone H3F</i>	GGTCAACTTGTTGATTCCCTCT	Real time RT-PCR of <i>Histone H3</i>
<i>qHistone H3R</i>	AACCGCAAAATCCAAAGAACG	
<i>qOsHMA3:CCGF</i>	AGGTGCGGCGCGTCCAAGAATC	Real time RT-PCR of <i>OsHMA3</i> in Changchungu variety
<i>qOsHMA3:CCGR</i>	CAACATTTGGCAACAACGGCCG	
<i>OsHMA3-1F</i>	acgaattcgagctcggtaccGCAGATTGCGAATAGTTTATC	Complementation test of <i>OsHMA3</i>
<i>OsHMA3-1R</i>	ttgcatgcctgcagtcgacTAATTAATAGATAACTTGCCATGC	
<i>OsHMA3-2F</i>	ggtaccggggatcctctagaATGGCCGGAAGGATGAG	Amplification of <i>OsHMA3</i> CDS for <i>pOsHMA3:OsHMA3</i> fusion construct
<i>OsHMA3-2R</i>	tcacctttcacttcaccg	
<i>OsHMA3-3F</i>	agggtgtgcgcaagacg	Amplification of <i>OsHMA3</i> downstream sequence for <i>pOsHMA3:OsHMA3</i> fusion construct
<i>OsHMA3-3R</i>	aacgacggccagtgccagcttTAATTAATAGATAACTTGCCATGC	
<i>OsHMA3-4F</i>	atgaccatgattacgaattcTGACTAGTGGCAGCTGTAGA	Amplification of <i>OsHMA3</i> promoter sequence for <i>pOsHMA3:OsHMA3</i> fusion construct
<i>OsHMA3-4R</i>	cttcgcctcatcctttccggccat	
<i>OsHMA3-5F</i>	ggccagtccaagcttTAAGATGACTAGTGGCAGCT	Amplification of <i>OsHMA3</i> promoter sequence for <i>proOsHMA3:LUC</i> fusion construct
<i>OsHMA3-5R</i>	tatgctgcttaaagttcatgag	
<i>OsHMA3-6F</i>	ttgggtgtaaagttgtgcatc	Amplification of <i>OsHMA3</i> promoter sequence for <i>proOsHMA3:LUC</i> fusion construct
<i>OsHMA3-6R</i>	agggtcttcagatctTCAAGGTACGTCTTCTCCG	