Variation in the coding region of the heavy metal ATPase *BrHMA3* controls natural variation in cadmium accumulation in widely eaten *Brassica rapa* vegetables

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Supplementary Figures S1–S10 and Tables S1-S4

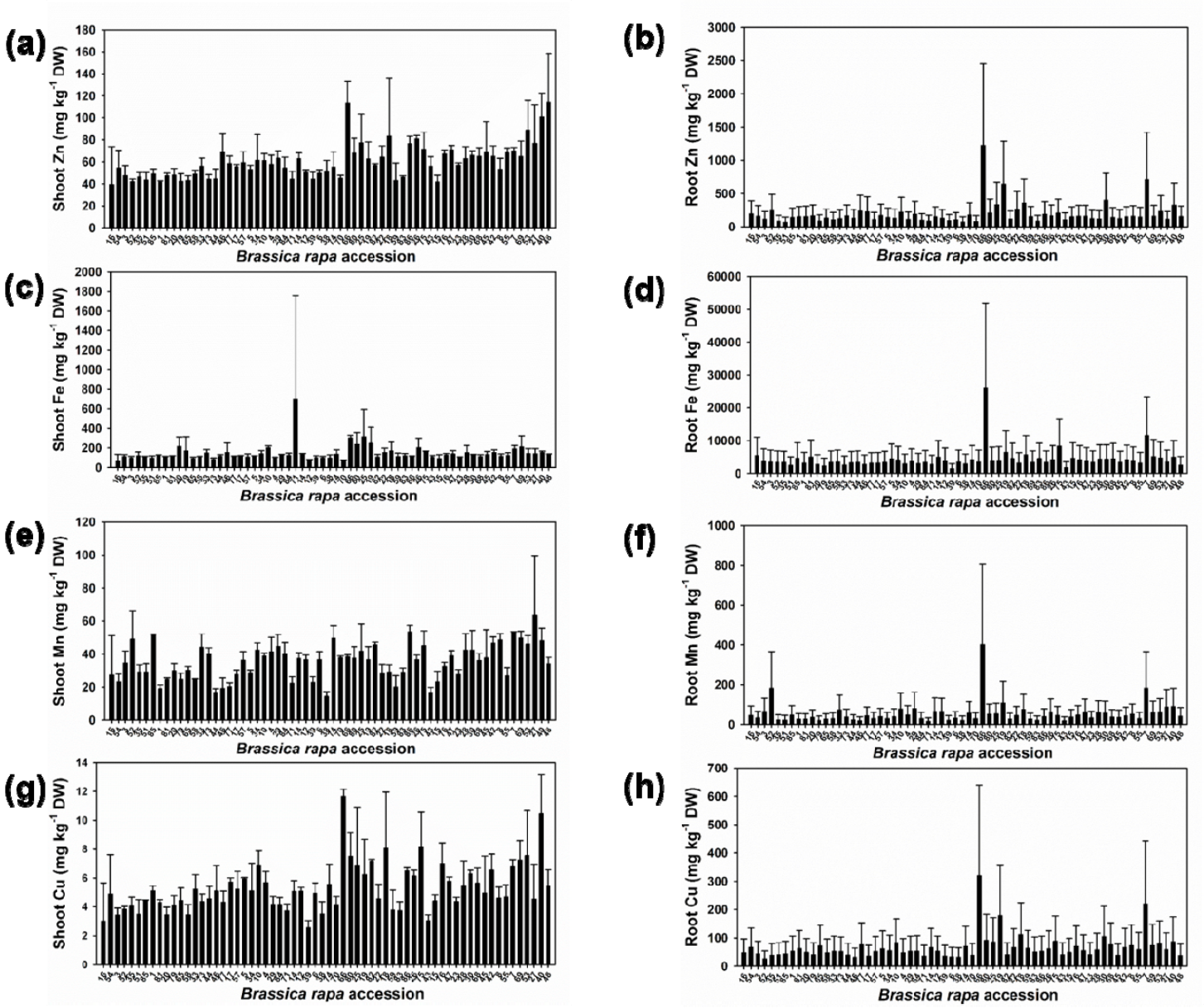


Fig. S1. Concentrations of Zn (a, b), Fe (c, d), Mn (e, f) and Cu (g, h) in the shoots (a, c, e, g) and roots (b, d, f, h) of 64 *Brassica rapa* accessions grown in hydroponic culture. Data are the means ± SD (n = 3).

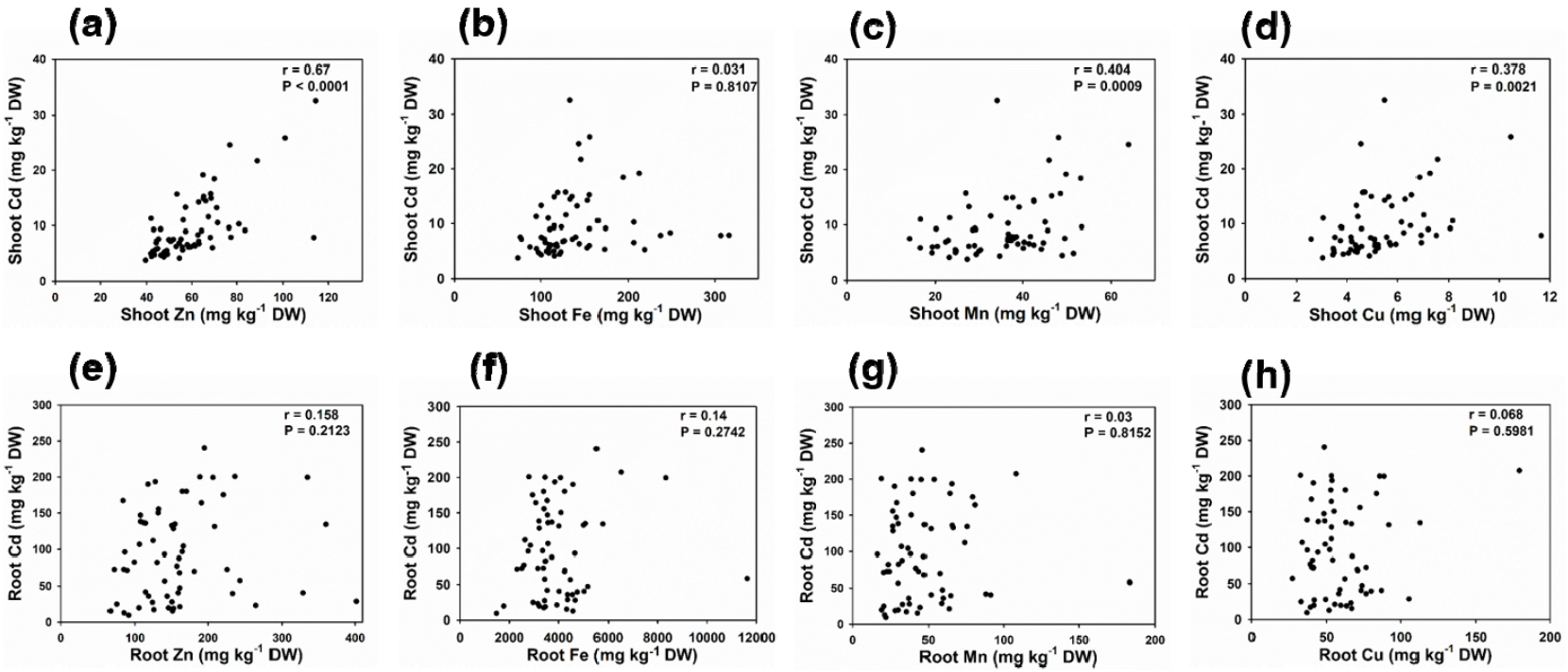


Fig. S2. Relationships between the concentration of Cd with those of Zn (a, e), Fe (b, f), Mn (c, g) and Cu (d, h) in the shoots (a – d) and roots (e – h) of 64 *Brassica rapa* accessions grown in hydroponic culture.

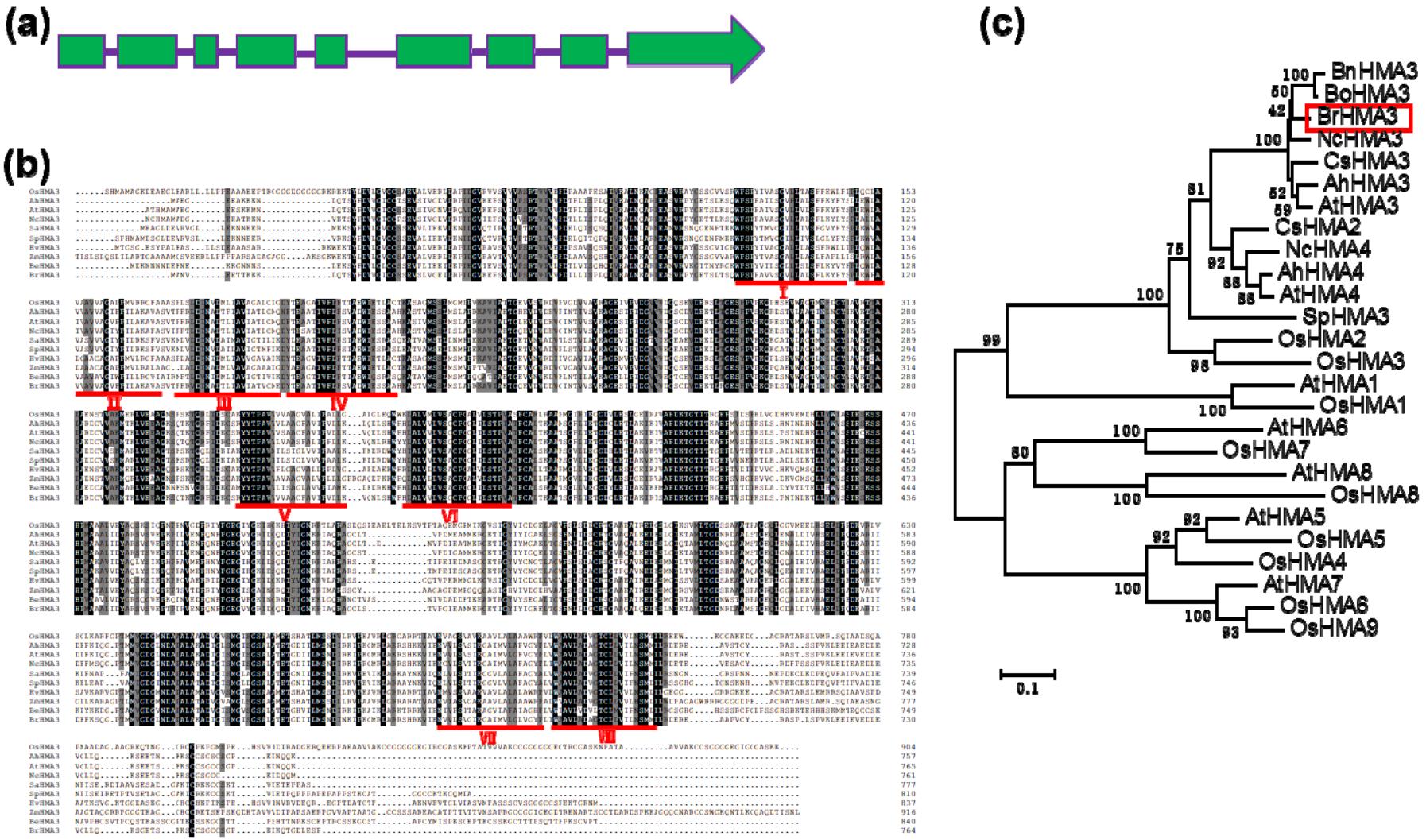


Fig. S3. *BrHMA3* gene in *Brassica rapa.* Schematic gene structure (a), sequence alignment of OsHMA3, AtHMA3, AhHMA3, NcHMA3, SaHMA3, SpHMA3, HvHMA3, ZmHMA3, BcHAM3, BrHMA3, (b), and phylogenetic analysis of P_{1B}-ATPases (c). Transmembrane domains (underlined in b) were predicted by TOPCONS.

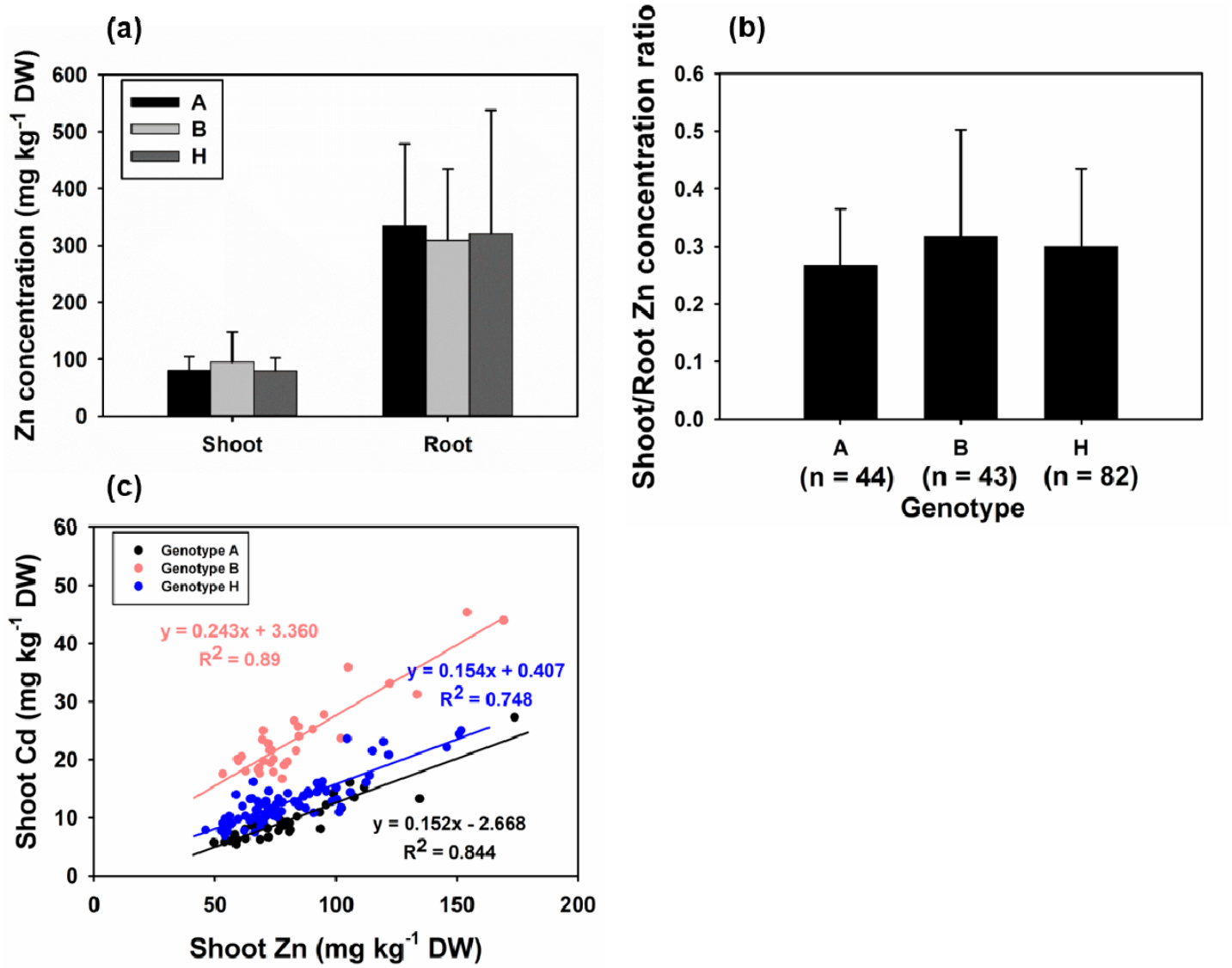
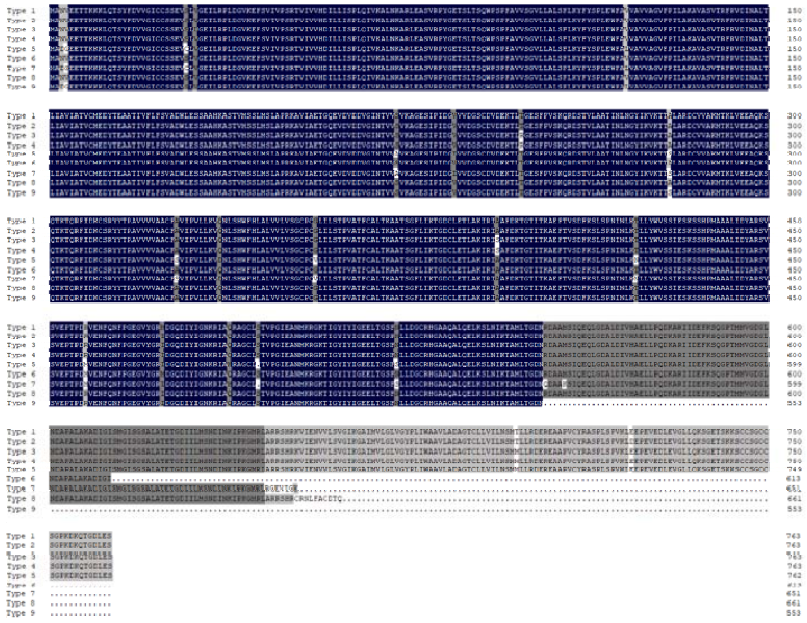


Fig. S4. The concentrations of Zn in the shoots and roots (a), the ratio of shoot/root Zn concentrations (b), and the relationship between the concentrations of Cd and Zn in the shoots (c)of 151 F_2 plants grouped according to *BrHMA3* genotypes. A, B, and H represent *BrHMA3* genotypes of GJCGB, DQMY939 and heterozygous. Data are the means ± SD (n = 43 - 82). DW, dry weight.

(a)



(b)

	Haplotypes	Frequency
Low-Cd	Type1	3
	Type2	2
	Type3	8
	Type4	2
	Type5	27
High-Cd	Type6	4
	Type7	1
	Type8	11
	Type9	6

Fig S5. Amino acid sequence alignment of nine protein haplotypes of BrHMA3 among the 64 *B. rapa* accessions (a) and the frequency distribution of each haplotype (b). Sequences were aligned using the DNAMAN programme.

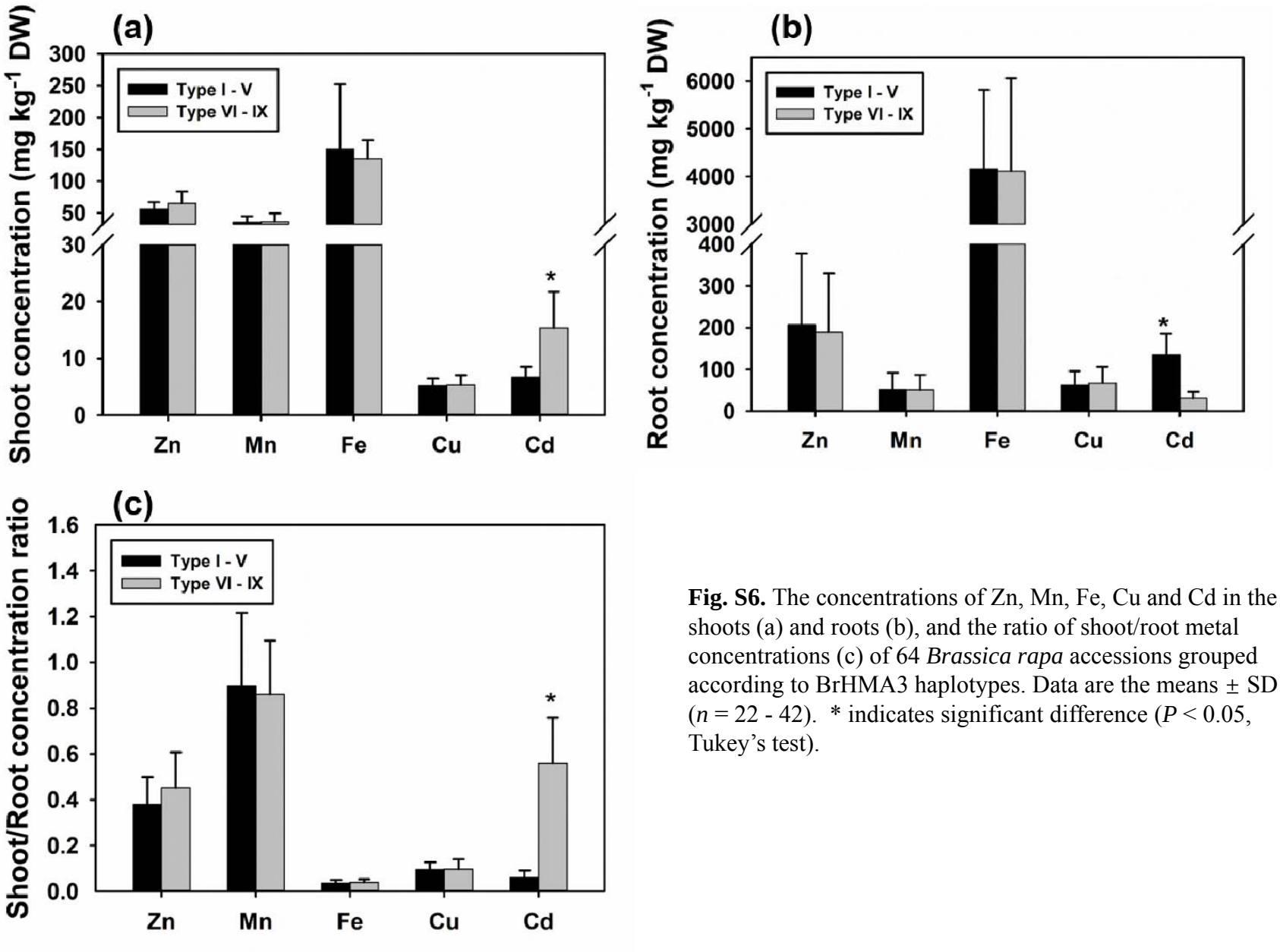


Fig. S6. The concentrations of Zn, Mn, Fe, Cu and Cd in the shoots (a) and roots (b), and the ratio of shoot/root metal concentrations (c) of 64 *Brassica rapa* accessions grouped according to BrHMA3 haplotypes. Data are the means ± SD (n = 22 - 42). * indicates significant difference ($P < 0.05$, Tukey's test).

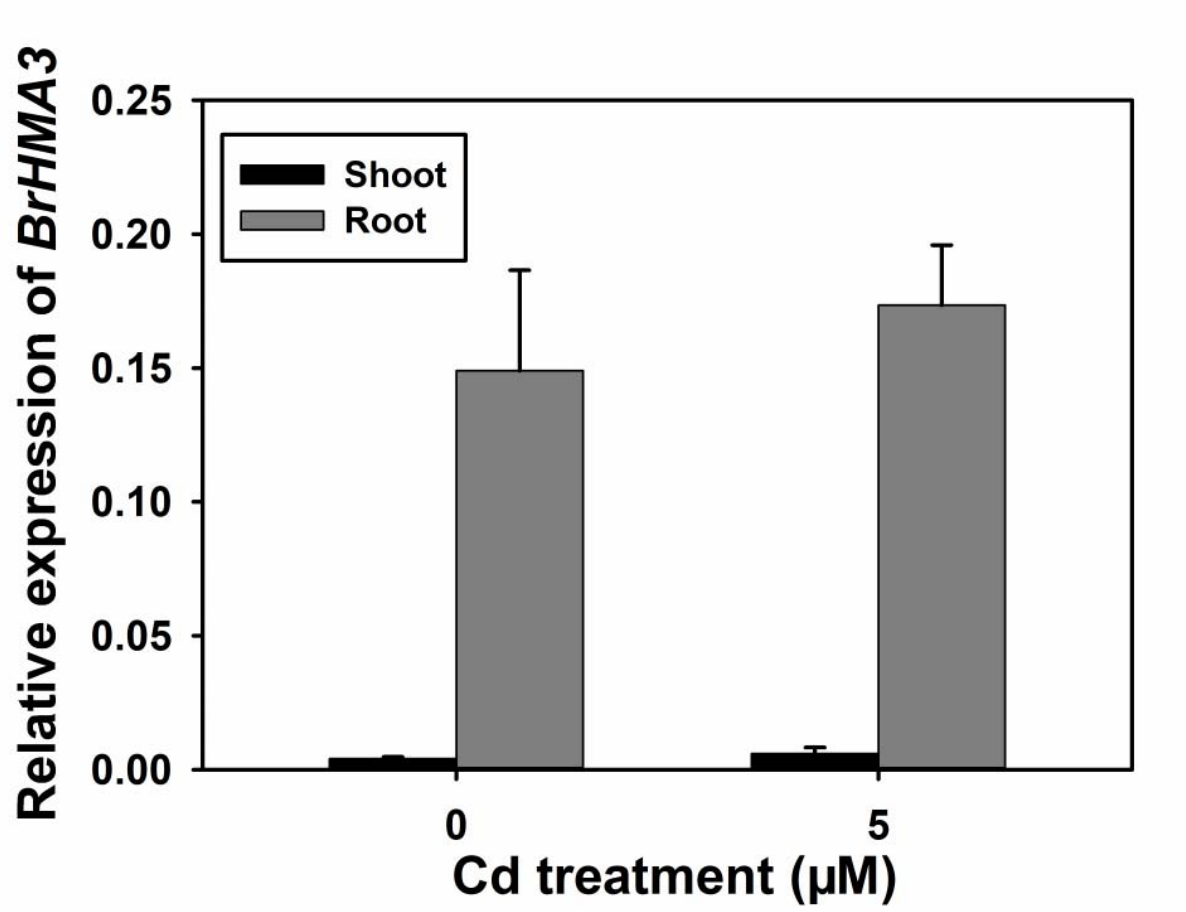


Fig. S7. The expression pattern of *BrHMA3* in the roots and shoots of *Brassica rapa* (cv. Chiifu) grown in hydroponic culture with or without 5 μM Cd for 3 d. Expression of *BrHMA3* was calculated as $2^{-\Delta CT}$ relative to *Bra005178*. Data are the means ± SD (n = 3).

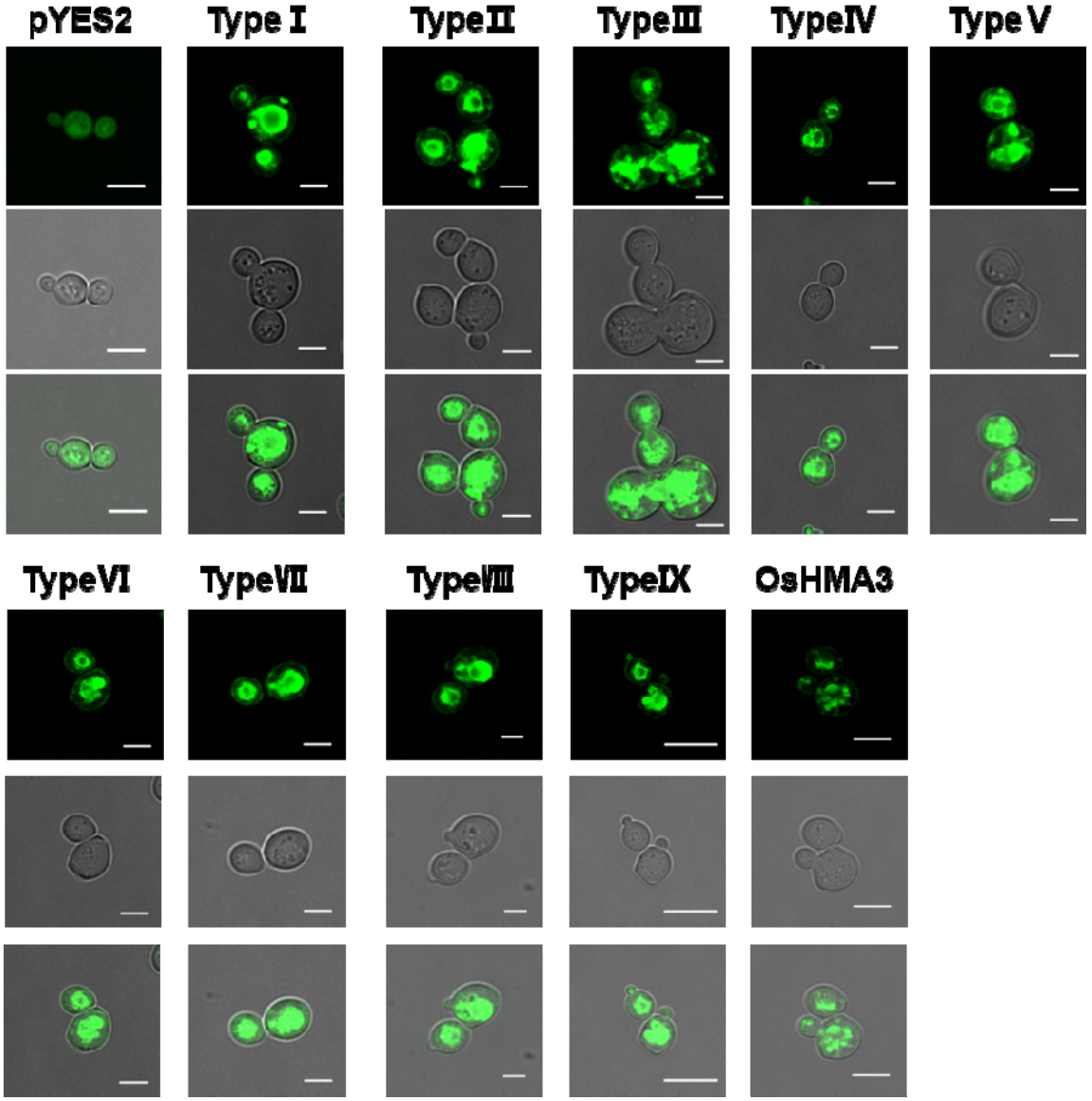


Fig. S8. Intracellular localization of GFP-BrHMA3 and GFP-OsHMA3 expressed in yeast strain INVSc1. Bar = 2 μm.

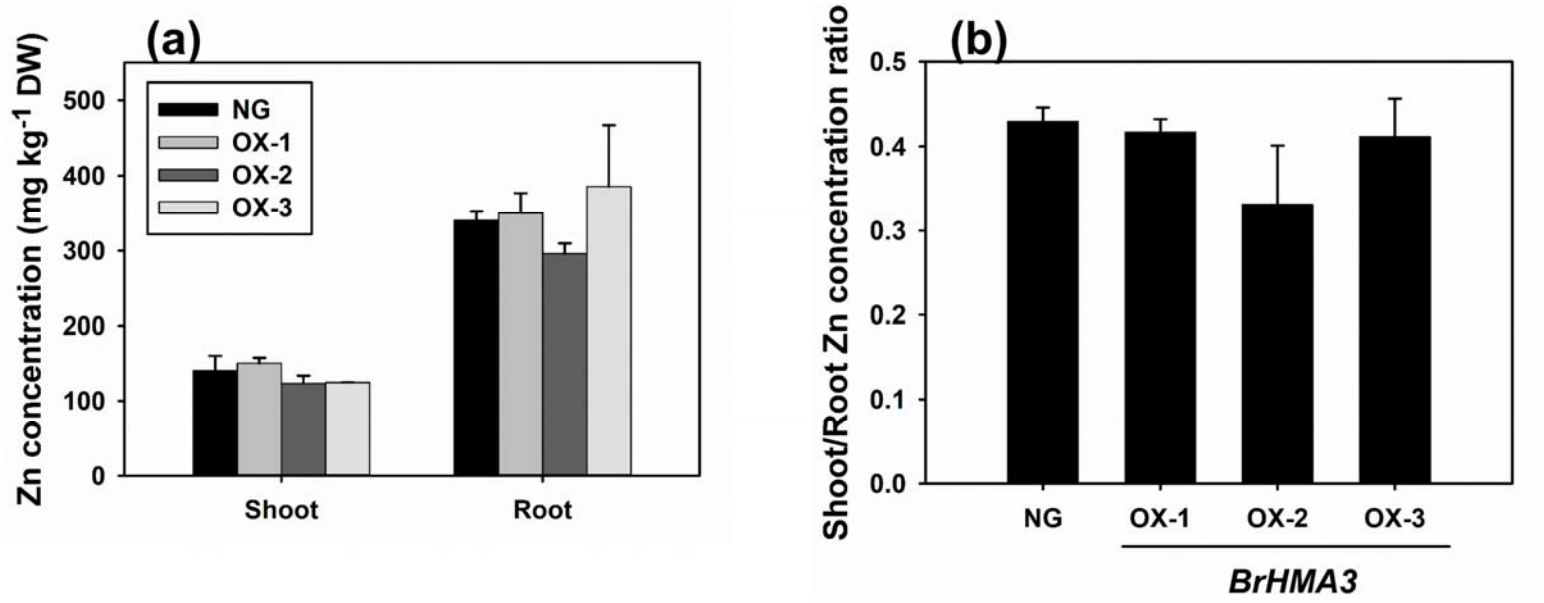


Fig. S9. The concentrations of Zn in the roots and shoots *(a)* and the shoot/root Zn concentration ratio of transgenic rice plants expressing *BrHMA3* (b). NG, non-transgenic segregation line control; OX, overexpressing lines. Data are the means ± SD (n = 3).

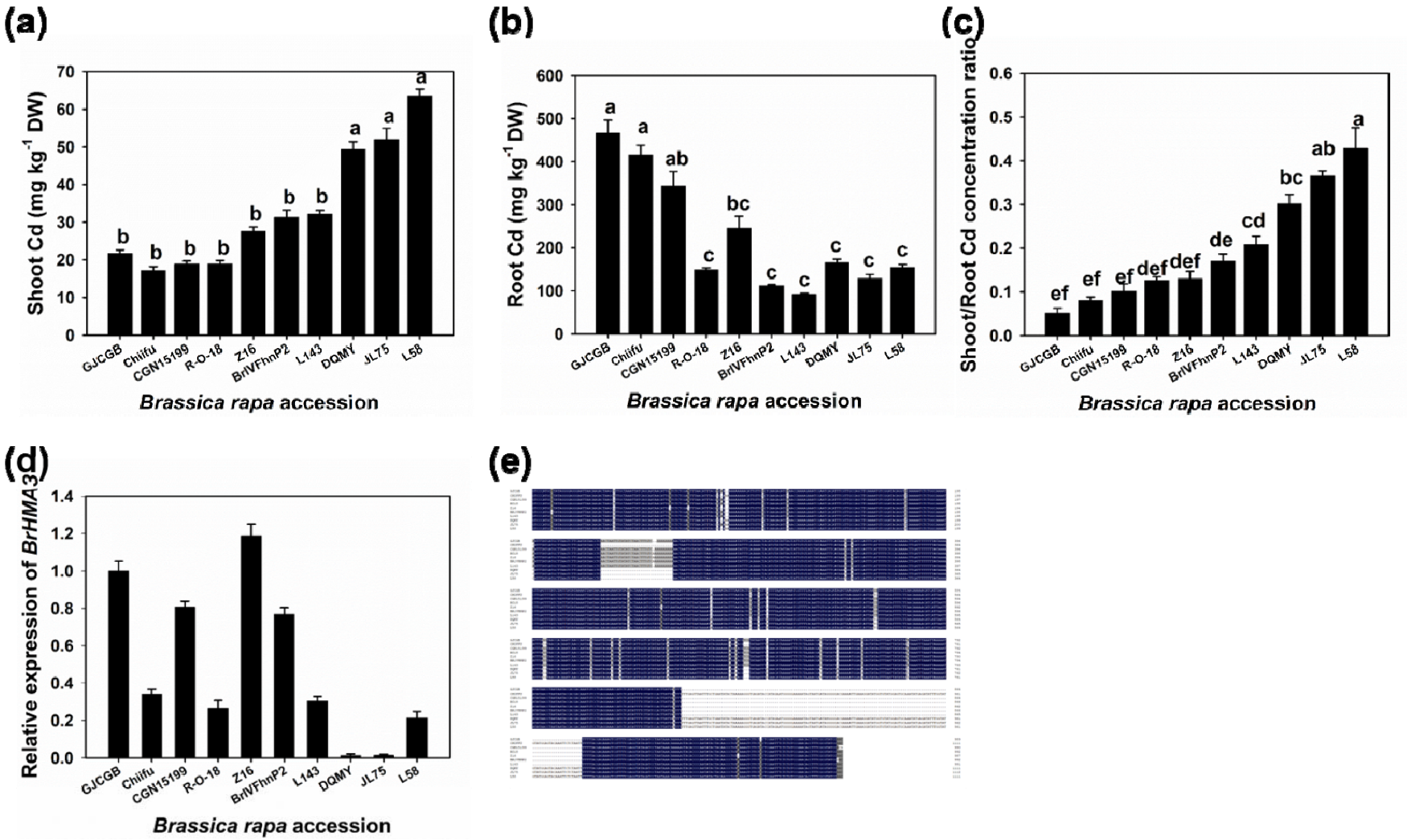


Fig. S10. Cadmium concentrations in the shoots (a) and roots (b), and the ratio of shoot/root Cd concentrations of ten accessions of *Brassica rapa* grown in hydroponic culture with 0.5 μM Cd for 10 d; relative expression of *BrHMA3* in the roots (d) and the alignment of *BrHMA3* promoter sequences in the ten accession of *B. rapa* (e). Data are the means ± SD ($n = 3$). Different letters above the bars indicate significant difference at $P < 0.05$ (Tukey's test).

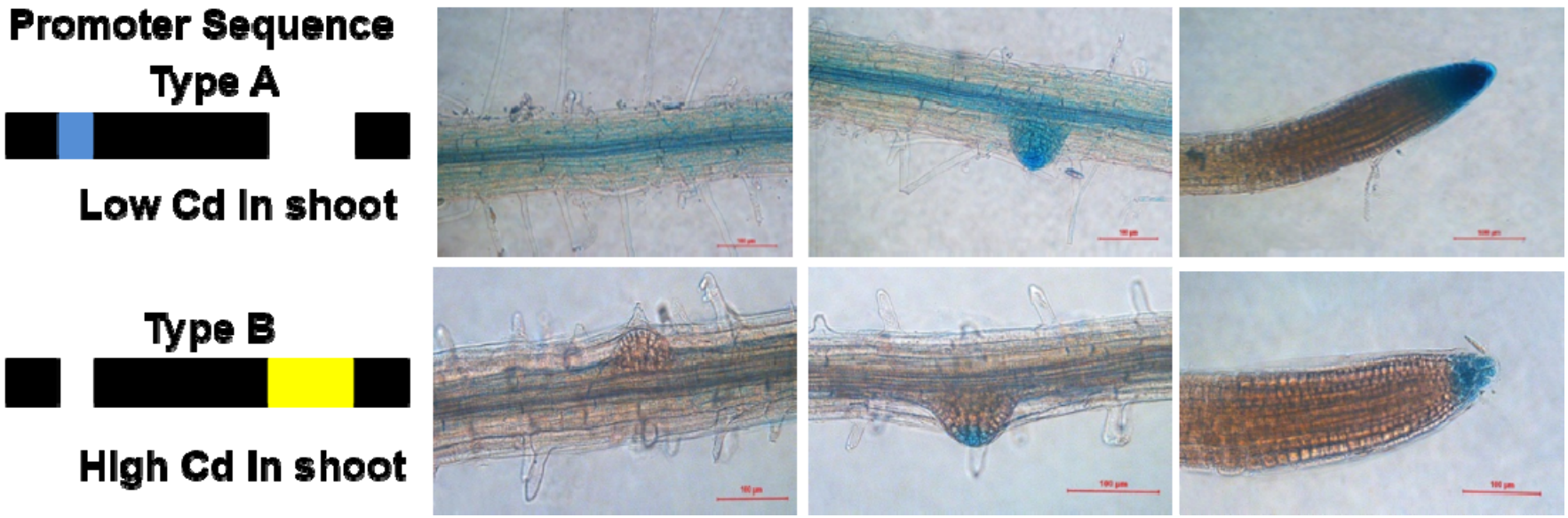


Fig. S11. *BrHMA3* promoter activity**.** GUS staining of transgenic *Arabidopsis thaliana* containing two types of *Pro*^{BrHMA3}*::GUS* constructs. The blue and yellow parts indicate 35 bp deletion and 152 bp insertion. Bar = 100 μm.

Table S1. The code, name, subspecies, and BrHMA3 haplotype of 64 *Brassica rapa* accessions used in the present study

BrHMA3 Haplotype	Subspecies	Cultivar name	Code
Type I	Brassica chinensis L.	Gaojiaochigengbai(GJCGB)	3
Type I	Brassica pekinensis	DatouqingV02A155(DTQV02A155)	34
Type I	Brassica pekinensis	Chiifu	52
Type II	Brassica chinensis L.	Chiqingcai(CQC)	4
Type II	Brassica pekinensis	V02A0555xiaoshizitou(V02A0555XSZT)	57
Type III	Brassica chinensis L.	Sijixiangcuixiaobaicai(SJXCXBC)	10
Type III	Brassica chinensis L.	Shanghaisiyueman(SHSYM)	14
Type III	Brassica narinosa L. H. Bailey	Zhongbayewutaicai(ZBYWTC)	19
Type III	Brassica pekinensis	Shangdong4(SD4)	26
Type III	Brassica pekinensis	Fushan212(FS212)	54
Type III	Brassica parachinensis L. H. Bailey	Zicaitai(ZCT)	65
Type III	Brassica parachinensis L. H. Bailey	Gailiangshiyuehong(GLSYH)	66
Type III	Brassica parachinensis L. H. Bailey	SynBr05	86
Type IV	Brassica chinensis L.	Huaguanqinggengbaicai(HGQGBC)	17
Type IV	Brassica narinosa L. H. Bailey	Lvlingwutacai(LLWTC)	18
Type V	Brassica chinensis L.	V02B0645Huoqiuquanxinwu(V02B0645HQQXH)	1
Type V	Brassica chinensis L.	Shianyoucai(SAYC)	5
Type V	Brassica chinensis L.	Zhongheqingcai(ZHQC)	6
Type V	Brassica chinensis L.	Material from Holland by Zhaojianjun	12
Type V	Brassica chinensis L.	V02B0591Takucai(V02B0591TKC)	16
Type V	Brassica pekinensis	Z16	20
Type V	Brassica pekinensis	Jianchun(JC)	25
Type V	Brassica pekinensis	424-2-3	29
Type V	Brassica pekinensis	14Hetaowenxiaoqingkou(14HTWXQK)	33
Type V	Brassica pekinensis	Beijingxinsanhao(BJXSH)	35
Type V	Brassica pekinensis	V02A1351Shimabaicai(V02A1351SMBC)	44
Type V	Brassica pekinensis	CGN07201	46
Type V	Brassica pekinensis	Kenshin	51
Type V	Brassica pekinensis	Yunhongzhongjiang(YHZJ)	58
Type V	Brassica campestris L.	Liuye70tianyouqingcaixin(LY70TYQCX)	70
Type V	uncultivated	Chamagudaoyebaicai(CMGDYBC)	71
Type V	Yellow Sarson	R-o-18	73
Type V	Yellow Sarson	L143	74
Type V	Komatsuna	L41	75
Type V	Komatsuna	CGN17281	76
Type V	Brassica juncea var.multisecta L.H.Bailey	Xizhangyeshengbaicai8#(XZYSBC8#)	77
Type V	Brassica rapa L.	CGN15199	79
Type V	Brassica rapa L.	CGN15220	80
Type V	Brassica rapa L.	CGN15201	81
Type V	Brassica rapa L.	CGN06721	82
Type V	Brassica pekinensis	Sijichun (SJC)	84
Type V	Rapid cycling	SynBr02	85
Type VI	Brassica chinensis L.	Shanghaiqing(SHQ)	7
Type VI	Brassica chinensis L.	Suzhouqing(SZQ)	8
Type VI	Brassica pekinensis	Xianfengixiayang(XFXY)	22
Type VI	Brassica pekinensis	Beijing75(BJ75)	30
Type VII	Brassica campestris L.	L58DH	68
Type VIII	Brassica chinensis L.	Jingyanwuyueman(JYWYM)	15
Type VIII	Brassica pekinensis	BrIVFhn P1	23
Type VIII	Brassica pekinensis	Xinfeng90(XF90)	38
Type VIII	Brassica pekinensis	33--3	40
Type VIII	Brassica pekinensis	Huabai2(HB2)	42
Type VIII	Brassica pekinensis	V02A1499Zhulongcai(V02A1499ZLC)	43
Type VIII	Brassica pekinensis	Xinlv85(XL85)	47
Type VIII	Brassica pekinensis	Xiakang40(XK40)	48
Type VIII	Brassica pekinensis	Zhulongcai(ZLC)	53
Type VIII	Brassica pekinensis	Qingbai'er5-4-2(QBE5-4-2)	55
Type VIII	Brassica campestris L.	Dongguanbaishayouqing45tiantiancaixin(DGBSYQ45TTCX)	69
Type IX	Brassica pekinensis	Ji'nandagenV02A0806(JNDGV02A0806)	27
Type IX	Brassica pekinensis	Jinlv75(JL75)	28
Type IX	Brassica pekinensis	L488-3	39
Type IX	Brassica pekinensis	V02A1396Xingchengxiaocuocai(V02A1396XCXCC)	45
Type IX	Brassica pekinensis	Daqingmaye939(DQMY939)	59
Type IX	Brassica rapa L.	CGN06688	83

Table S2. Nine *Brassica rapa* accessions selected for the soil pot experiment.

BrHMA3 Haplotype	Subspecies	Name	Code
Type I	Brassica pekinensis	Chiifu	52
Type II	Brassica pekinensis	V02A0555xiaoshizitou(V02A0555XSZT)	57
Type III	Brassica pekinensis	Shangdong4(SD4)	26
Type IV	Brassica chinensis L.	Huaguanqinggengbaicai(HGQGBC)	17
Type V	Brassica pekinensis	Z16	20
Type VI	Brassica chinensis L.	Shanghaiqing(SHQ)	7
Type VII	Brassica campestris L.	L58DH	68
Type VIII	Brassica pekinensis	BrIVFhn P1	23
Type IX	Brassica pekinensis	Daqingmaye939(DQMY939)	59

Table S3. Primers used in the present study

Purpose	Forward(5' - 3')	Reverse (5' - 3')
BrHMA3 ORF (Genomic DNA)	TCCGAACACCTTTCGCGTAT	GTACTTACCAATTCATACAGATTC
PYES2-BrHMA3	AGGGAATATTAAGCTTGGTACCATGGCCAACGTTGAAGAGACAAC	GGCGGCCGTTACTAGTGGATCCTCAGAAACTCTCTAAGTCCCCTGTTT
BrHMA3-GFP	CTTCGAATTCTGCAGTCGACGGTACCATGGCCAACGTTGAAGAGACA	CTCGCCCTTGCTCACCATCAGGATCCAGAAACTCTCTAAGTCCCCTGTTT
Genetic marker (Promoter)	ATAACCTAATAATACCACGAC	CGTTGGATACGCGAAAGGTGTT
Genetic marker (ORF)	GCAATGCTCACAGGAGATAGC	GCTGTAAAAATGATAACGTTTTGGTTATT
Pro^{BrHMA3}-GUS	TTAATTAAATGGCCAACGTTGAAGAGACAAC	GGCGCGCCTCAGAAACTCTCTAAGTCCCCTGTTT
Arabidopsis Actin	TCGTTGCCCCTCCAGAGA	GGTACTGAGGGAGGCCAAGAT
AtHMA3 promoter	TAAAACGACGGCCAGTGCCAAGCTTACGCTTGAAACAGTTGAGAAAA	GTTGTCTCTTCAACGTTGGCCATTTGAGATCTCGGACTAAATTTCG
Rice actin	GGTCAACTTGTTGATTCCCCTCT	AACCGCAAAATCCAAAGAACG
OsHMA3 qRT-PCR	TCCATCCAACCAAACCCGGAAA	TGCCAATGTCCTTCTGTTCCCA
Brassica rapa Actin	CTCACCGAAGCACCTCTCAA	GACCACTGGCGTAAAGGGAG
BrHMA3 qRT-PCR	TTCTTGCAGATGCAGGGACTT	AGTCCCCTGTTTGCTTGTCC

Table S4. Ten *Brassica rapa* accessions selected for promoter analysis

BrHMA3 Haplotype	Subspecies	Name
Type I	Brassica chinensis L.	Gaojiaochigengbai(GJCGB)
Type V	Brassica pekinensis	Z16
Type V	Yellow Sarson	R-o-18
Type I	Brassica pekinensis	Chiifu
Type V	Yellow Sarson	L143
Type IX	Brassica pekinensis	Jinlv75(JL75)
Type VII	Brassica campestris L.	L58DH
Type IX	Brassica pekinensis	Daqingmaye939(DQMY939)
Type V	Brassica rapa L.	CGN15199
Type I	Brassica pekinensis	BrIVFhn P1