防御素调控植物镉积累和耐受机制

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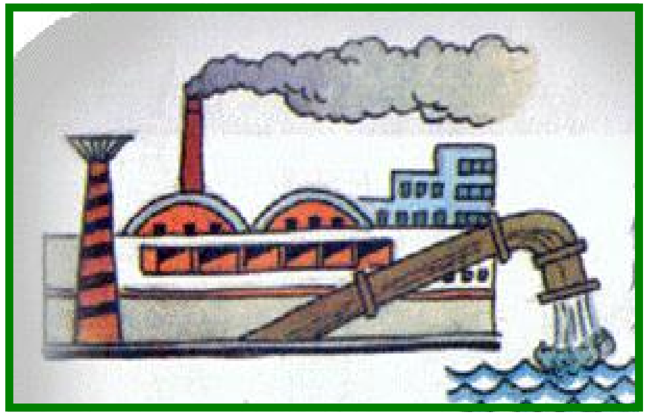
重金属污染危害人类健康



Itai-Itai Disease

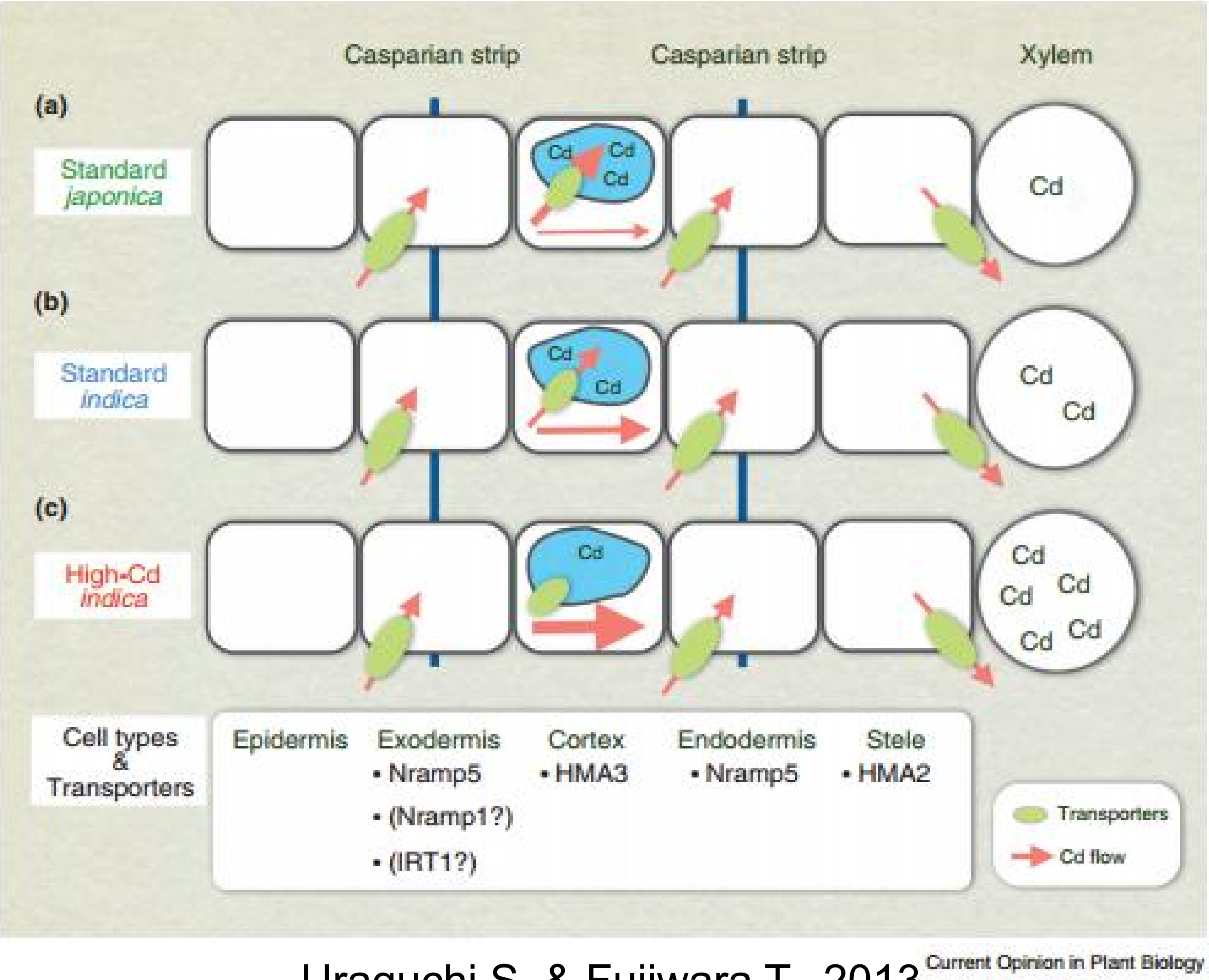
中国土壤重金属Cd污染严重

metal/metalloid	class I (natural background)	class II pH <6.5	class II pH 6.5–7.5	class II pH >7.5	class III pH >6.5	% exceeding the limit
Cd	0.2	0.3	0.3	0.6	1.0	7.0
As						
paddy	15	30	25	20	30	2.7[a]
upland	15	40	30	25	40	
Hg	0.15	0.3	0.5	1.0	1.5	1.6
Cu						
farmland	35	50	100	100	400	2.1[a]
orchard	-	150	200	200	400	
Pb	35	250	300	350	500	1.5
Cr						
paddy	90	250	300	350	400	1.1[a]
upland	90	150	200	250	300	
Zn	100	200	250	300	500	0.9
Ni	40	40	50	60	200	4.8

[a]The percentage exceedance is for all soil types.

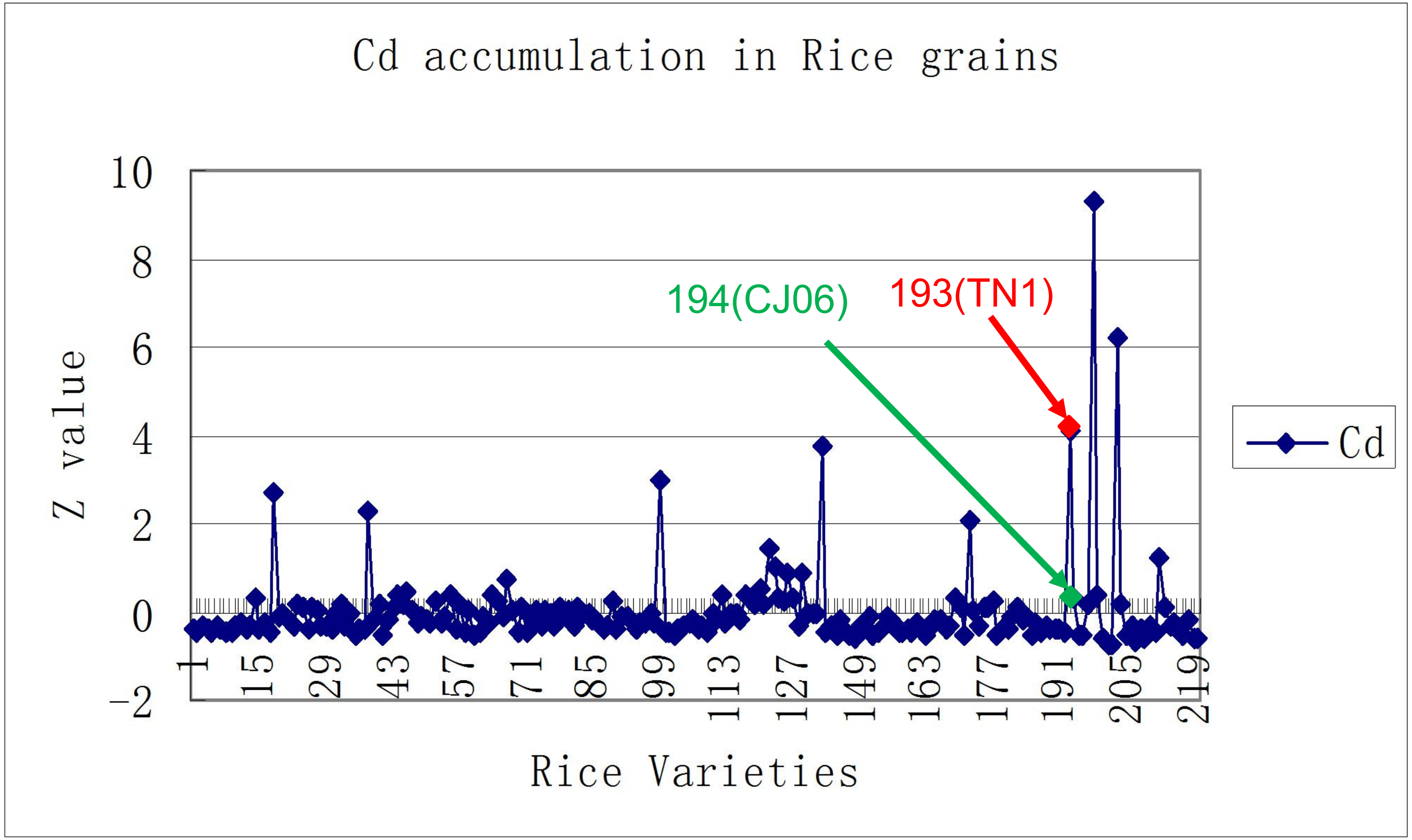
Zhao F J et al., 2015

籼稻地上部Cd积累高于粳稻的机理

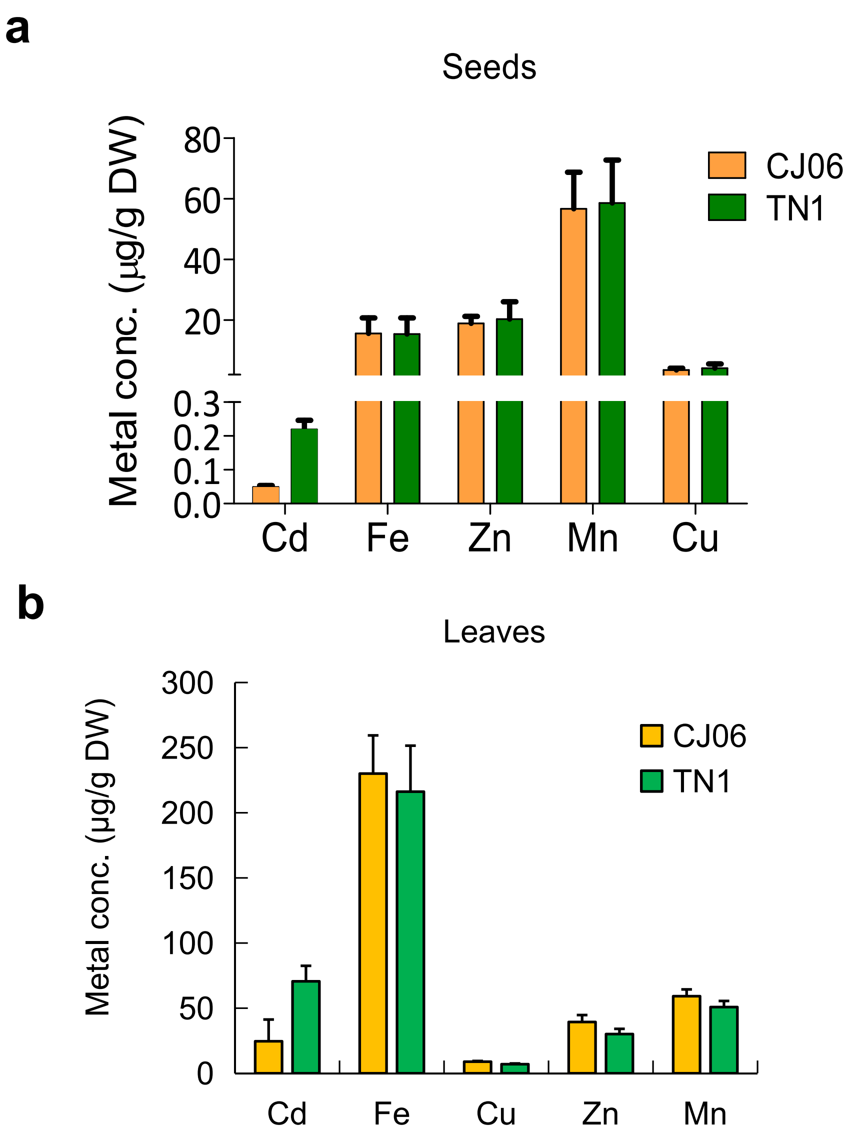


Uraguchi S. & Fujiwara T., 2013

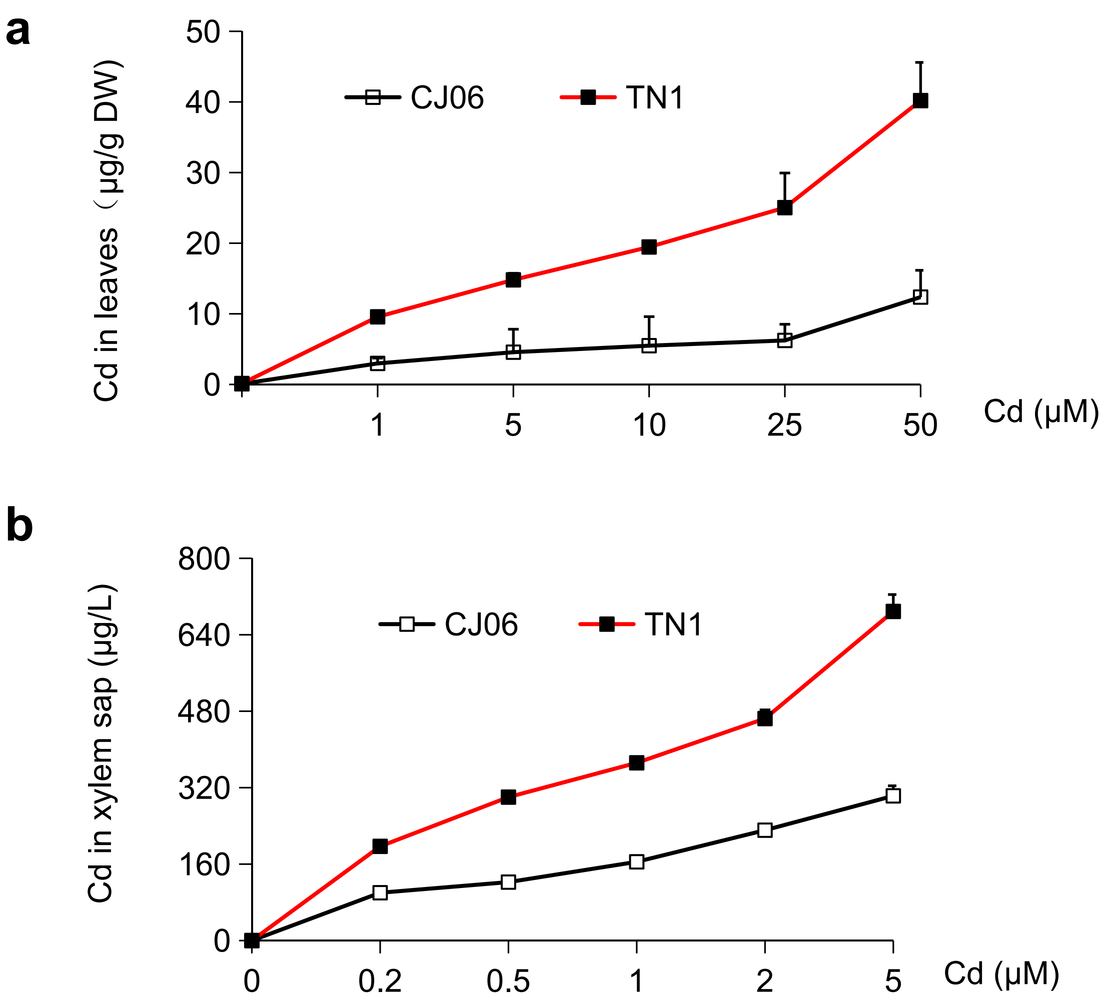
中国核心水稻种质籽粒中Cd积累变异筛选



TN1籽粒和地上部Cd含量显著高于CJ06



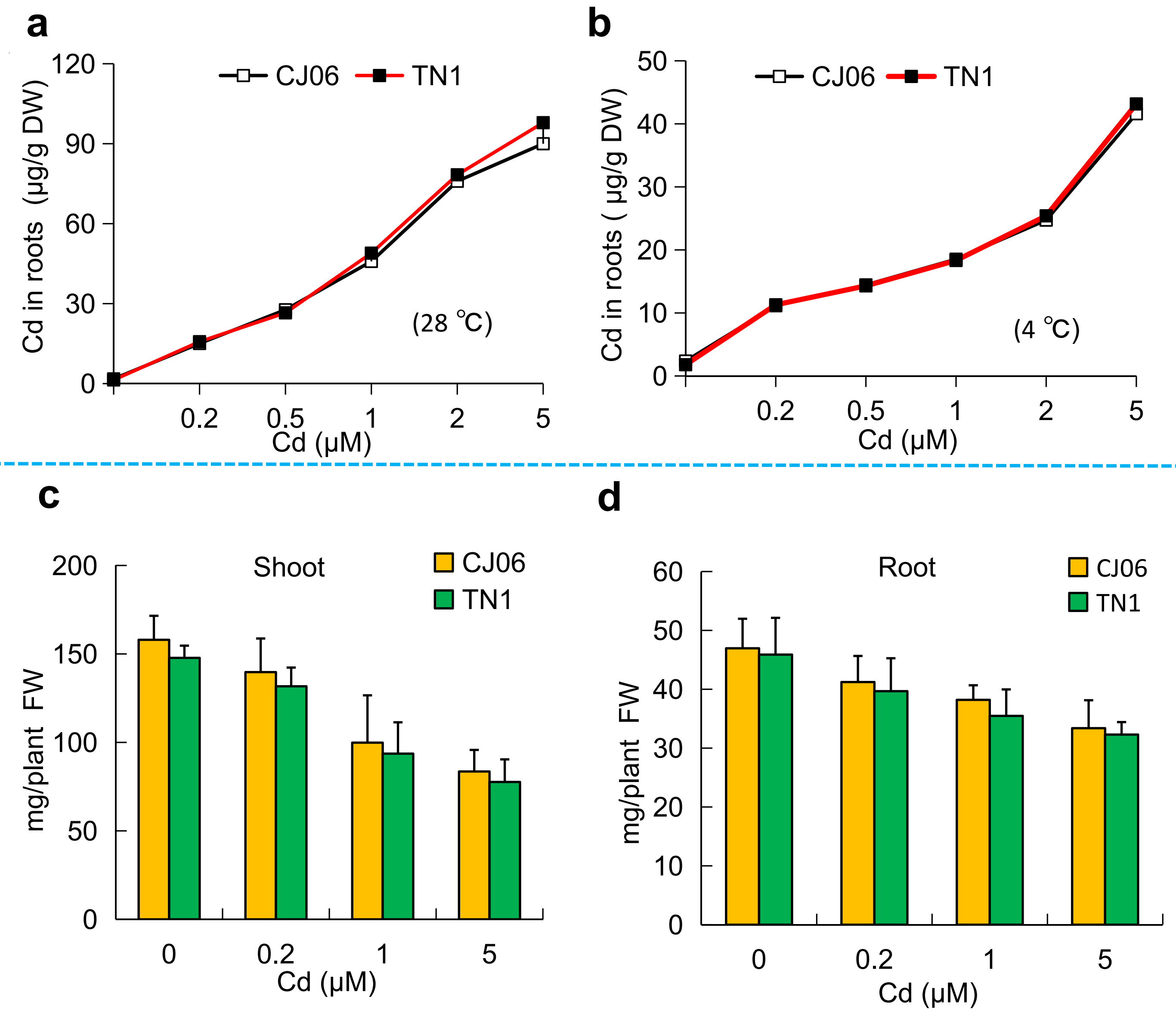
TN1中Cd长途转运显著高于CJ06



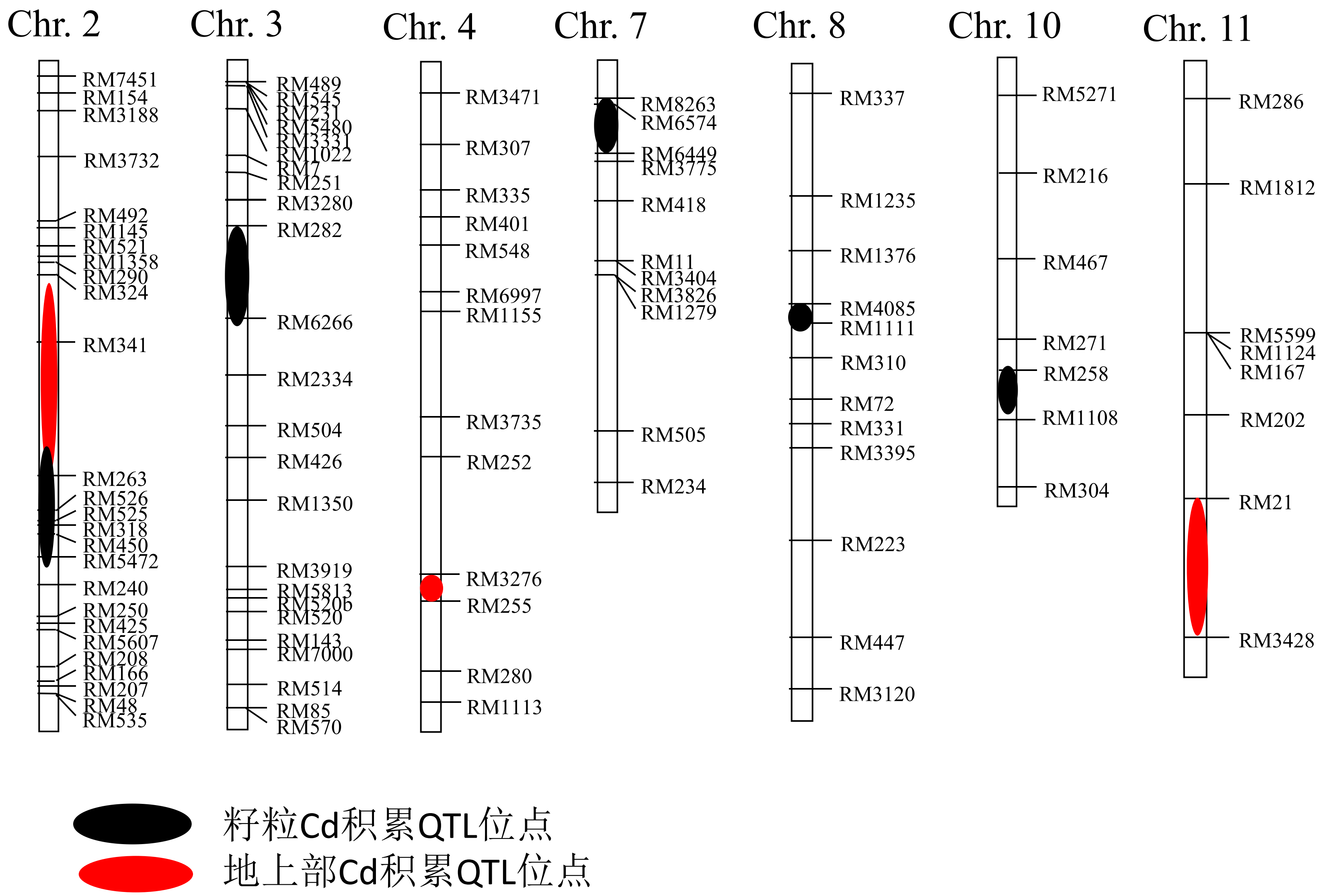
TN1与CJ06对Cd的吸收和耐受性无明显差异

镉吸收

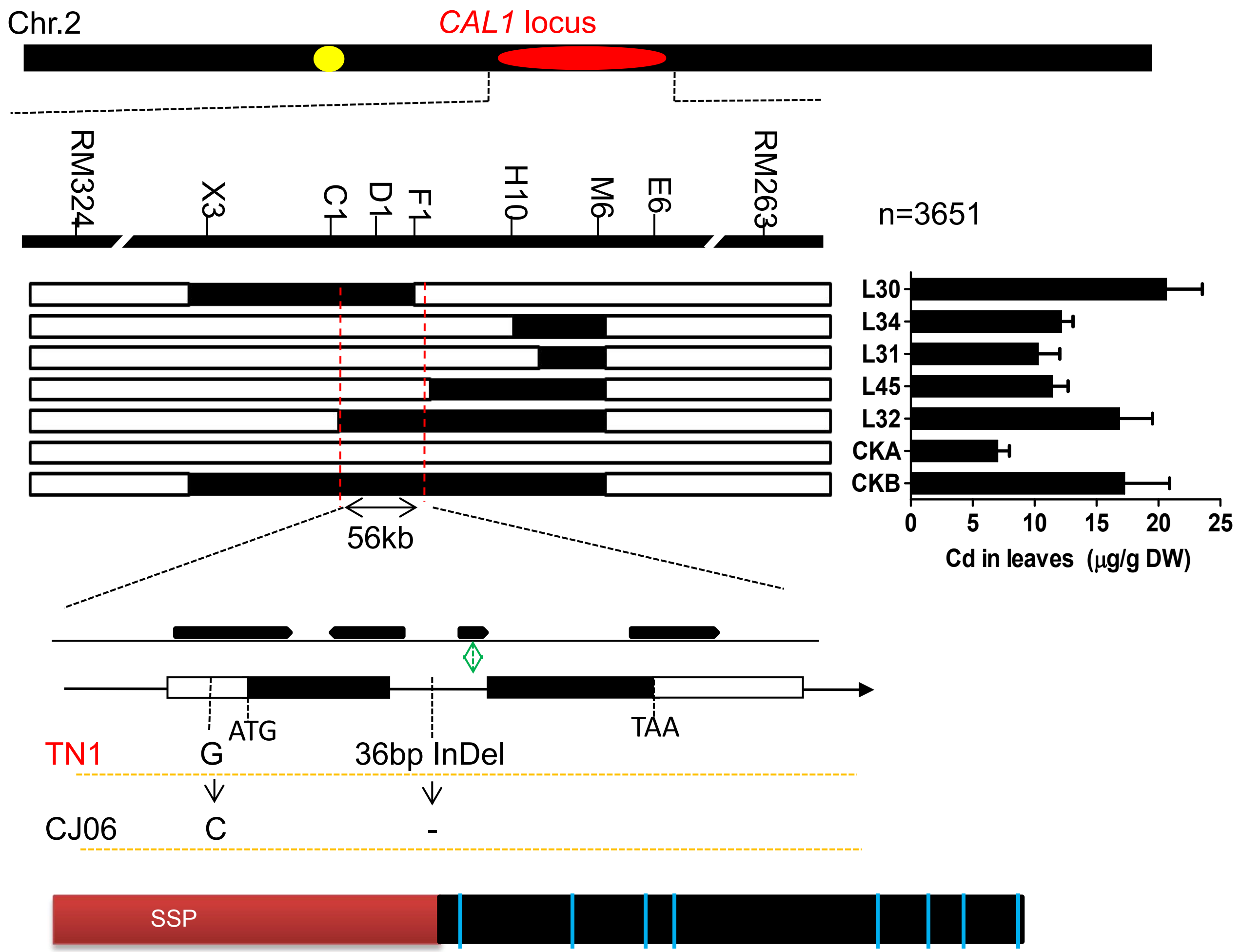
镉耐受



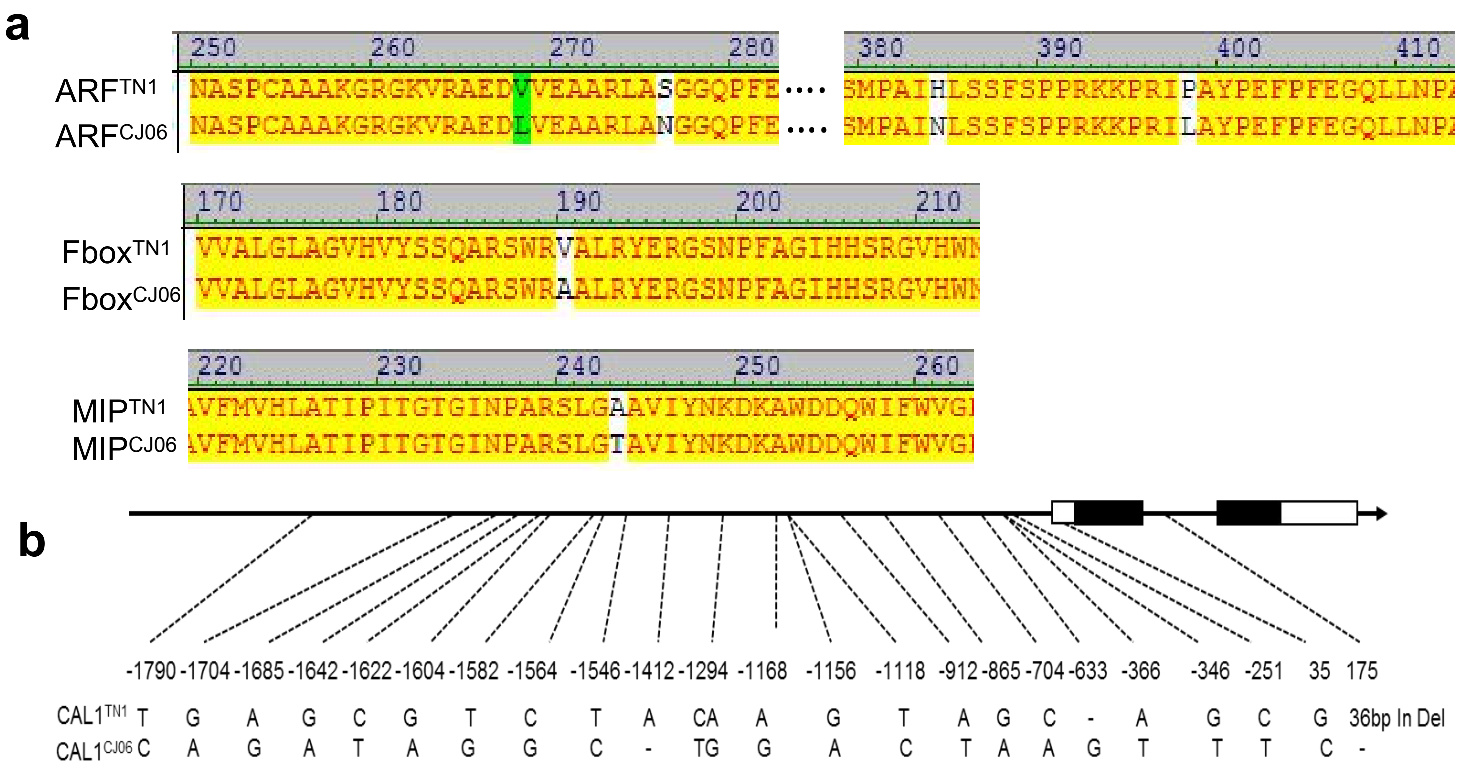
TN1和CJ06来源DH群体定位Cd积累QTL位点



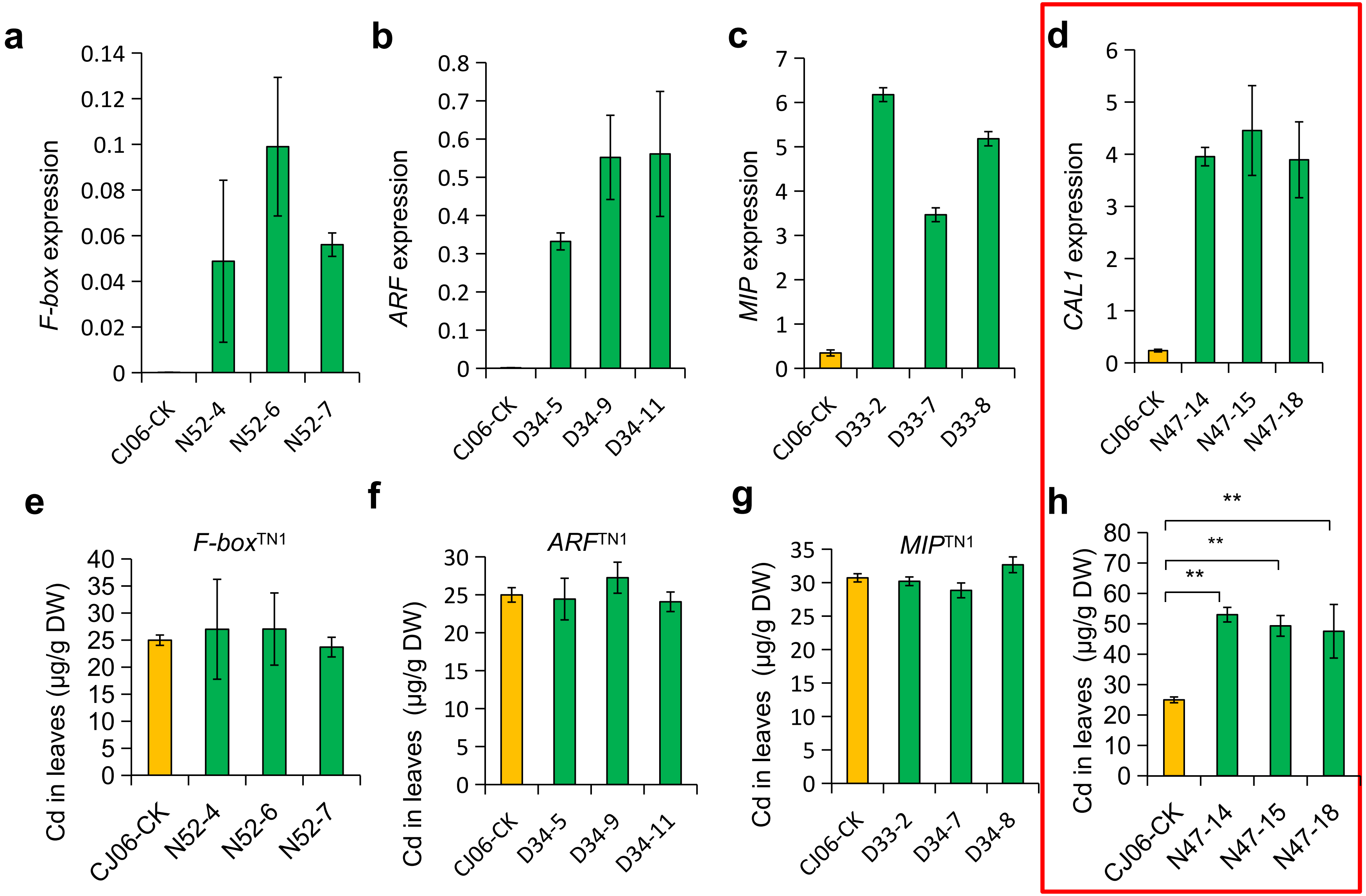
植物防御素家族*CAL1*为水稻叶片Cd积累QTLs候选基因



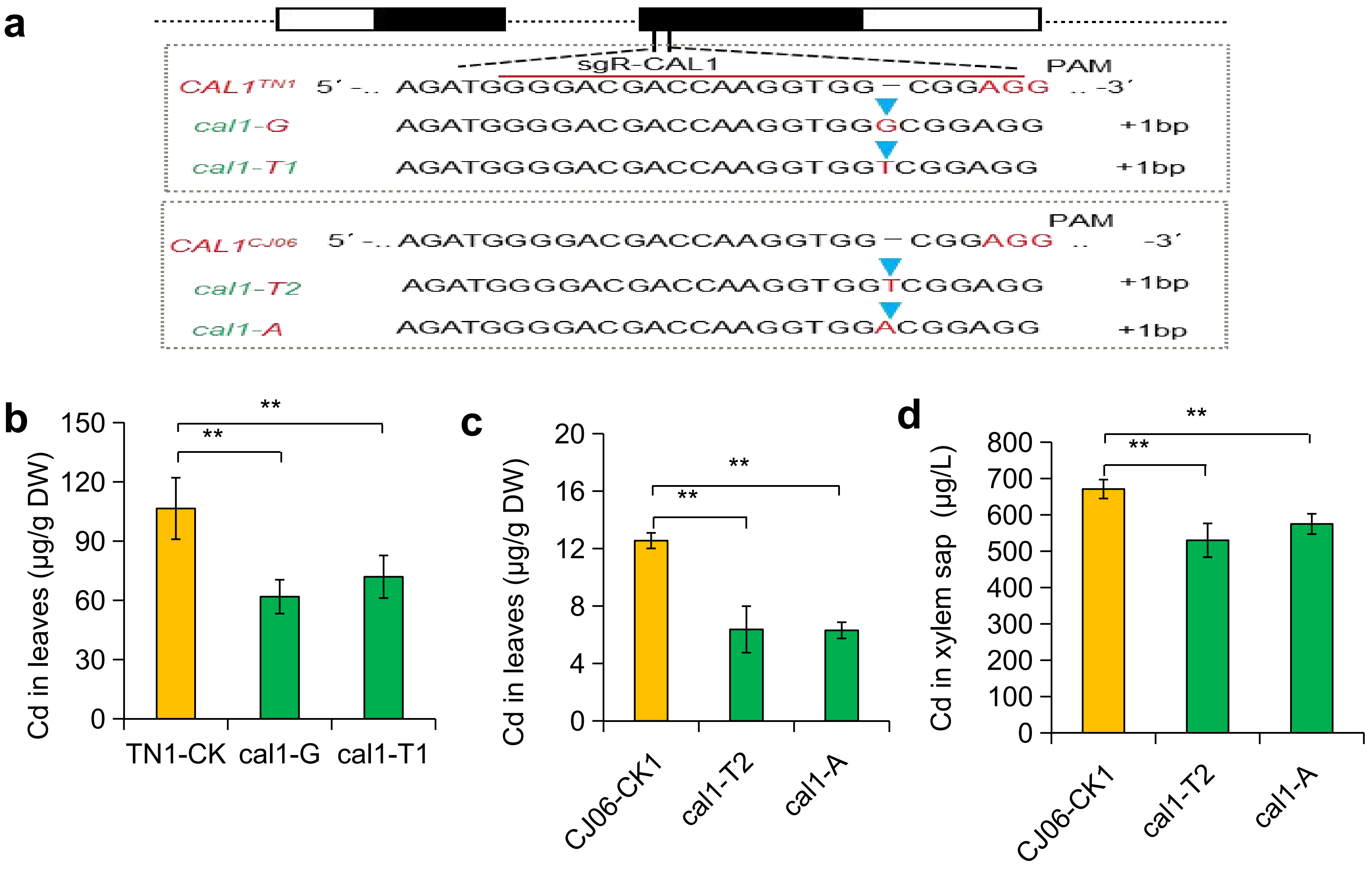
候选基因变异位点确定


a
250 260 270 280
380 390 400 410
ARFTN1 NASPCAAAKGRGKVRAEDVVEAARLASGGQPFE.... SMPAIHLSSFSPPRKKPRIPAYPEFPFEGQLLNP
ARFCJ06 NASPCAAAKGRGKVRAEDLVEAARLANGGQPFE.... SMPAINLSSFSPPRKKPRILAYPEFPFEGQLLNP
170 180 190 200 210
FboxTN1 VVALGLAGVHVYSSQARSWRVALRYERGSNPFAGIHHSRGVHWN
FboxCJ06 VVALGLAGVHVYSSQARSWRAALRYERGSNPFAGIHHSRGVHWN
220 230 240 250 260
MIPTN1 AVFMVHLATIPITGTGINPARSLGAAVIYNKDKAWDDQWIFWVGI
MIPCJ06 AVFMVHLATIPITGTGINPARSLGTAVIYNKDKAWDDQWIFWVGI
b
-1790 -1704 -1685 -1642 -1622 -1604 -1582 -1564 -1546 -1412 -1294 -1168 -1156 -1118 -912 -865 -704 -633 -366 -346 -251 35 175
CAL1TN1 T G A G C G T C T A CA A G T A G C - A G C G 36bp In Del
CAL1CJ06 C A G A T A G G C - TG G A C T A A G T T T C -

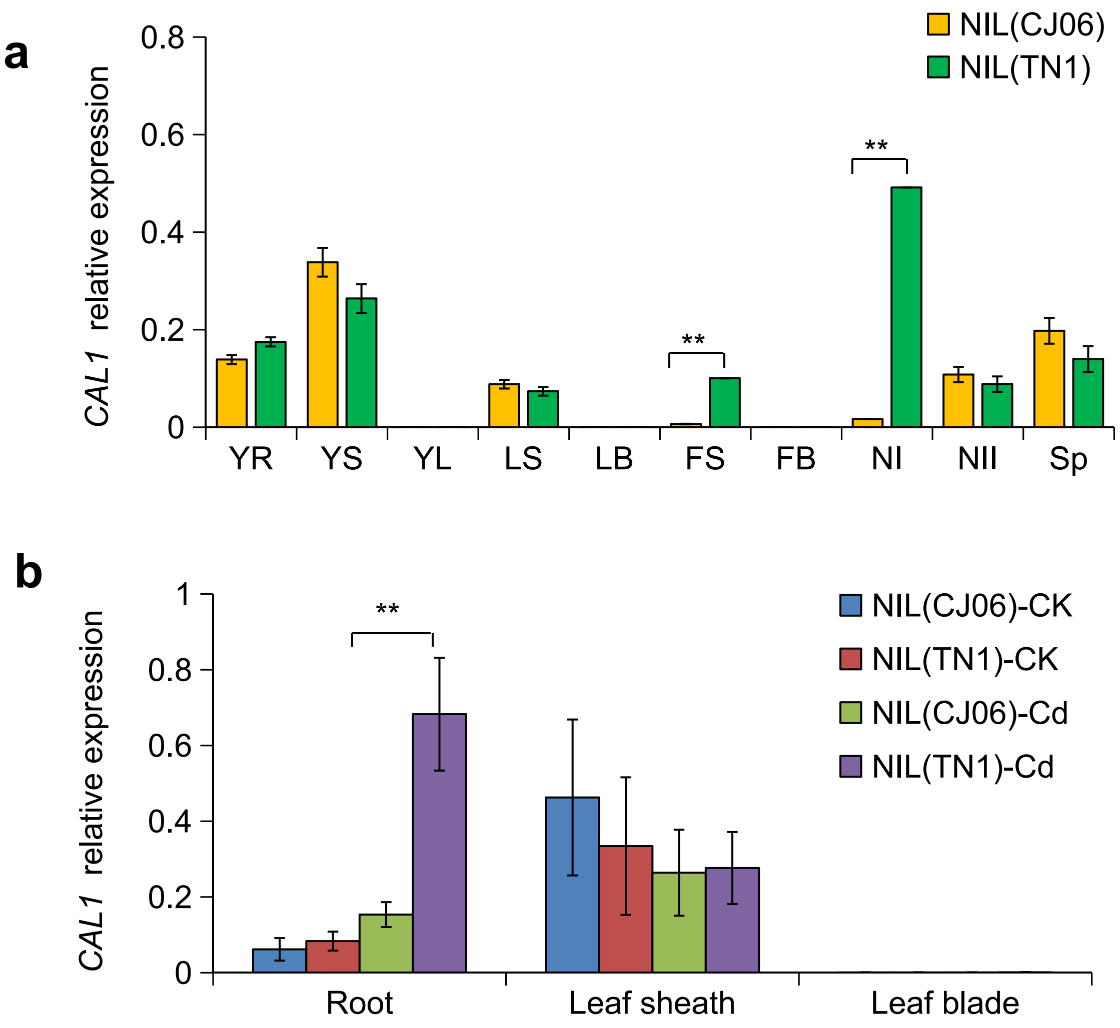
*CAL1*为控制叶片Cd积累的目标基因



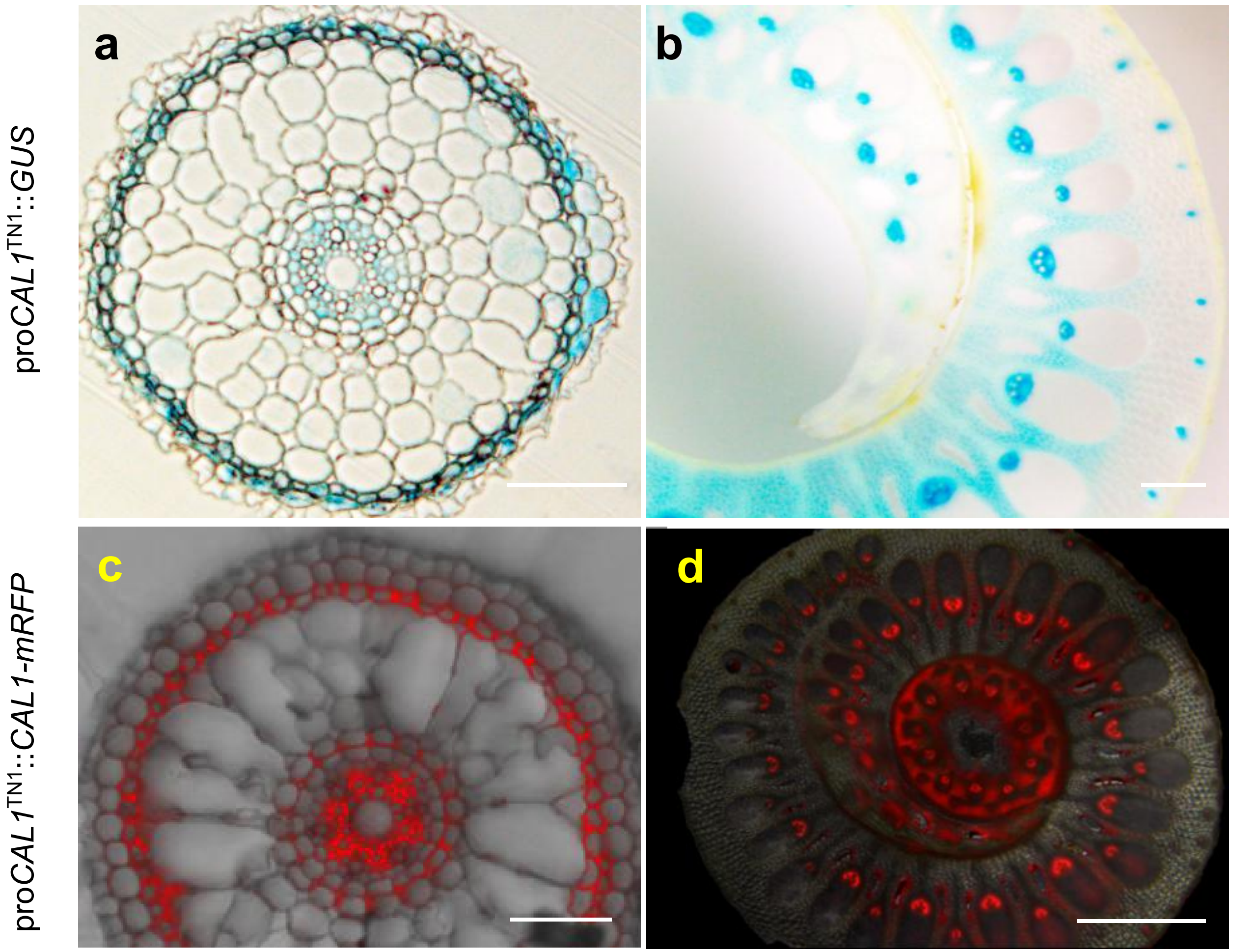
*CAL1*突变降低了水稻叶片和木质部伤流液Cd含量



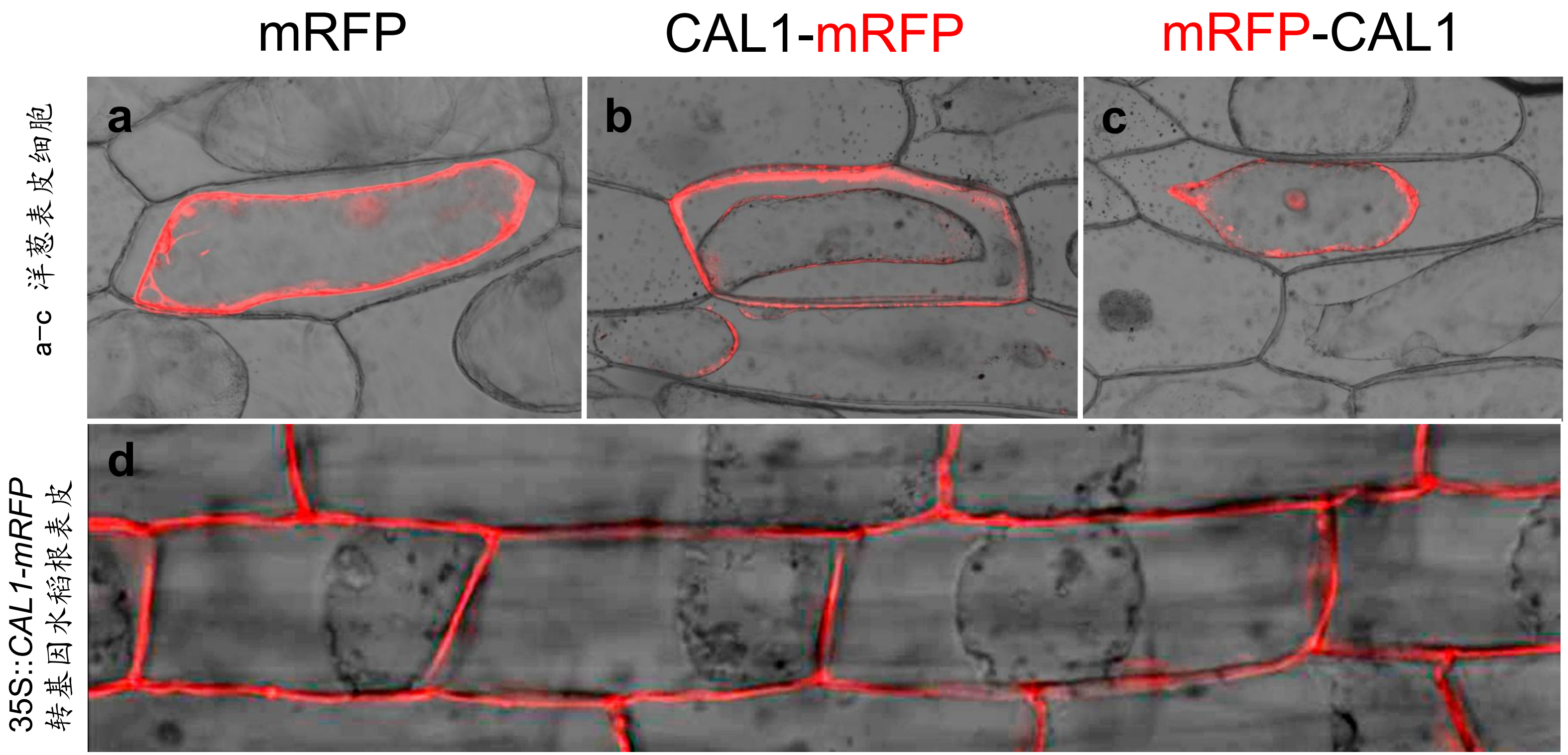
*CAL1*差异表达调控Cd积累



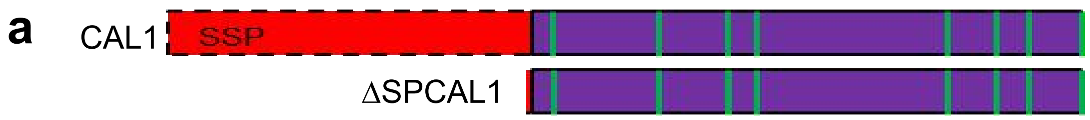
CAL1主要在木质部薄壁细胞和根的外皮层表达

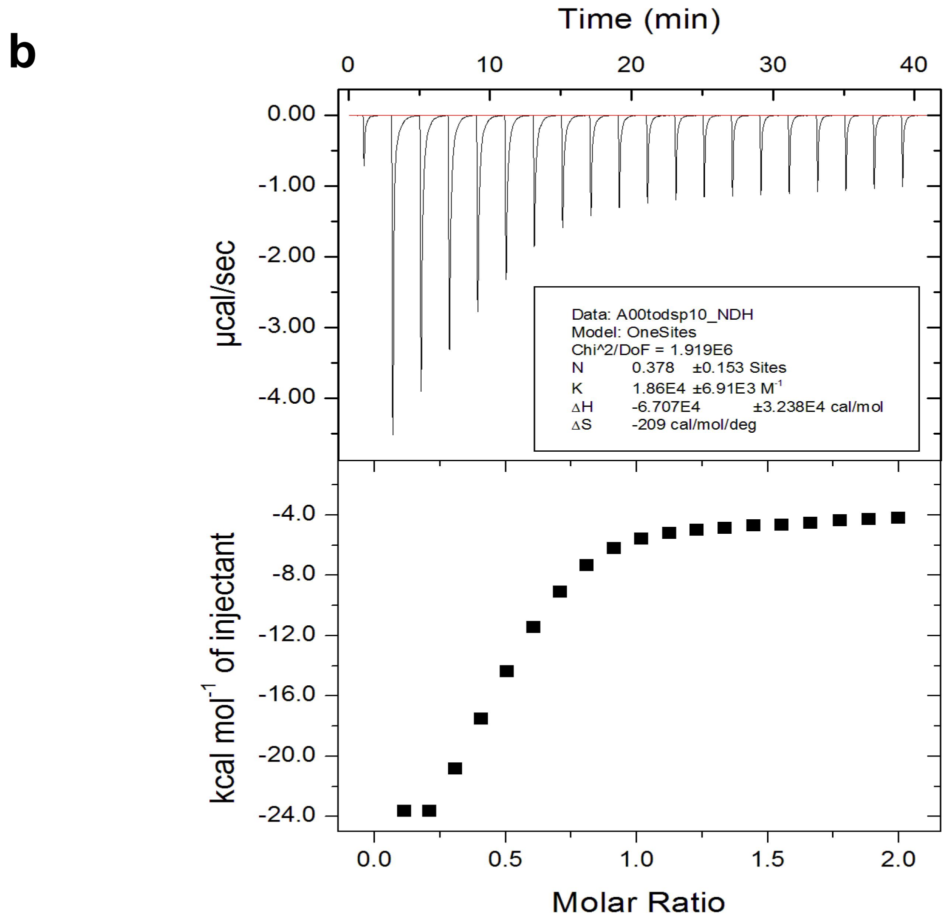


CAL1亚细胞定位于质外体空间



ITC实验确定ΔSPCAL1为低亲和力的Cd结合蛋白



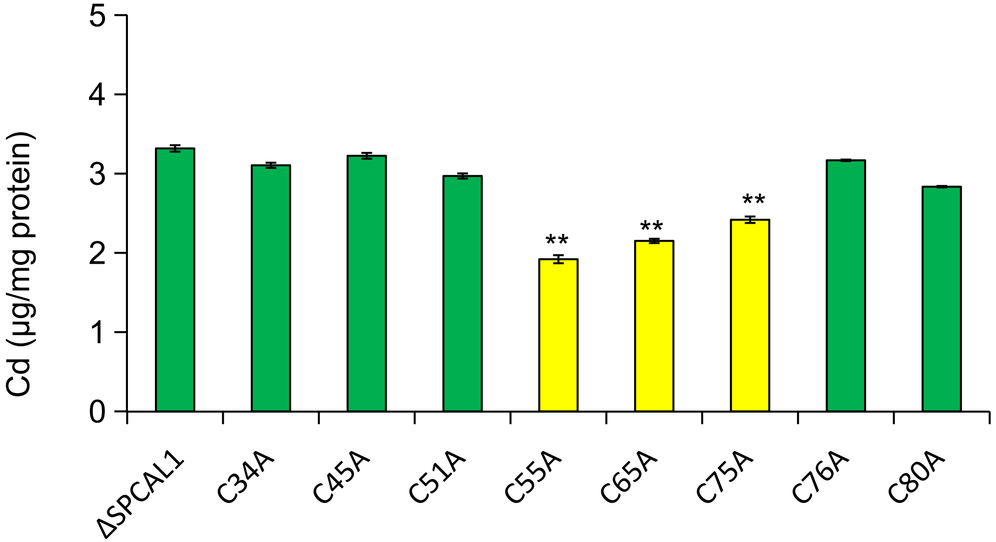


Dissociation constant/KD = 53.7μM

ΔSPCAL1蛋白中三个Cys定点突变降低了其与Cd结合能力

a

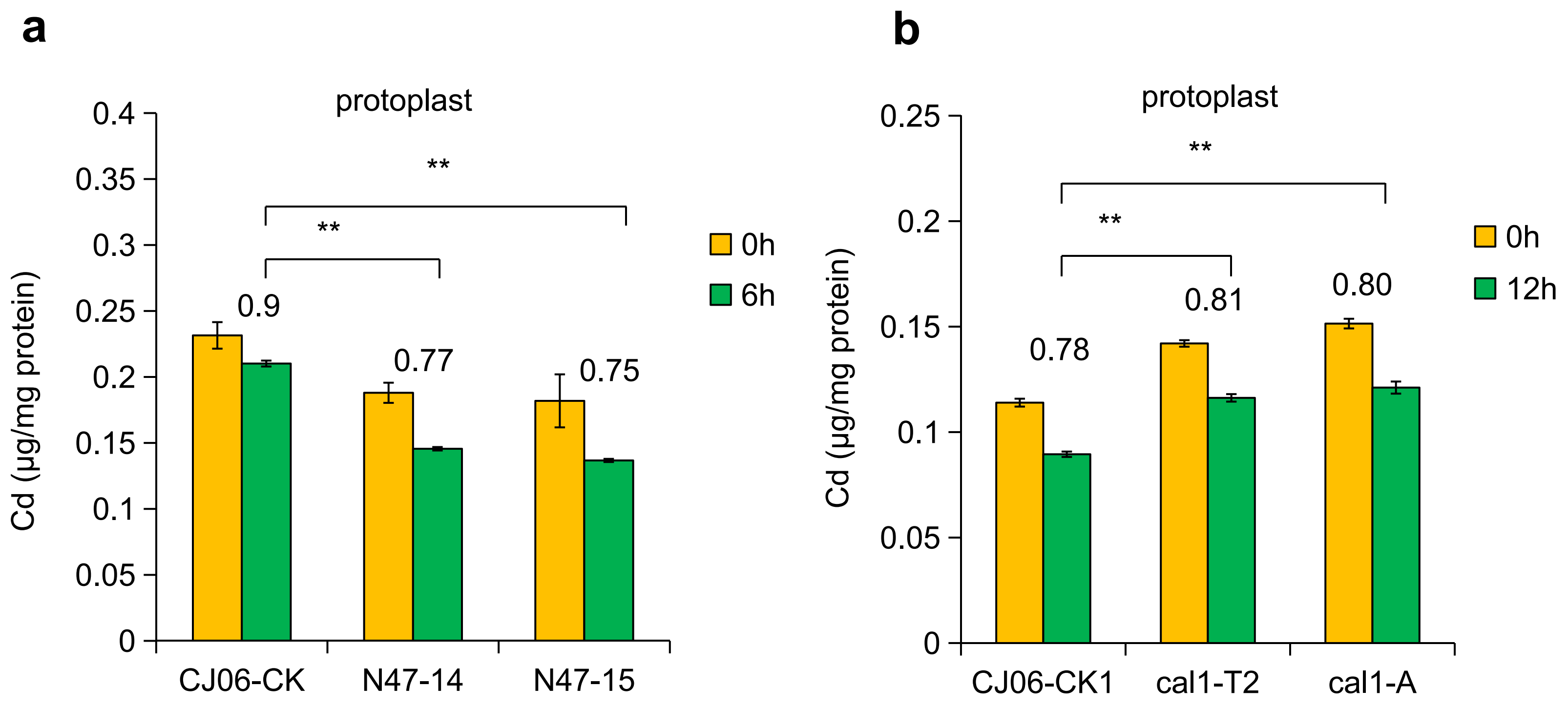
b



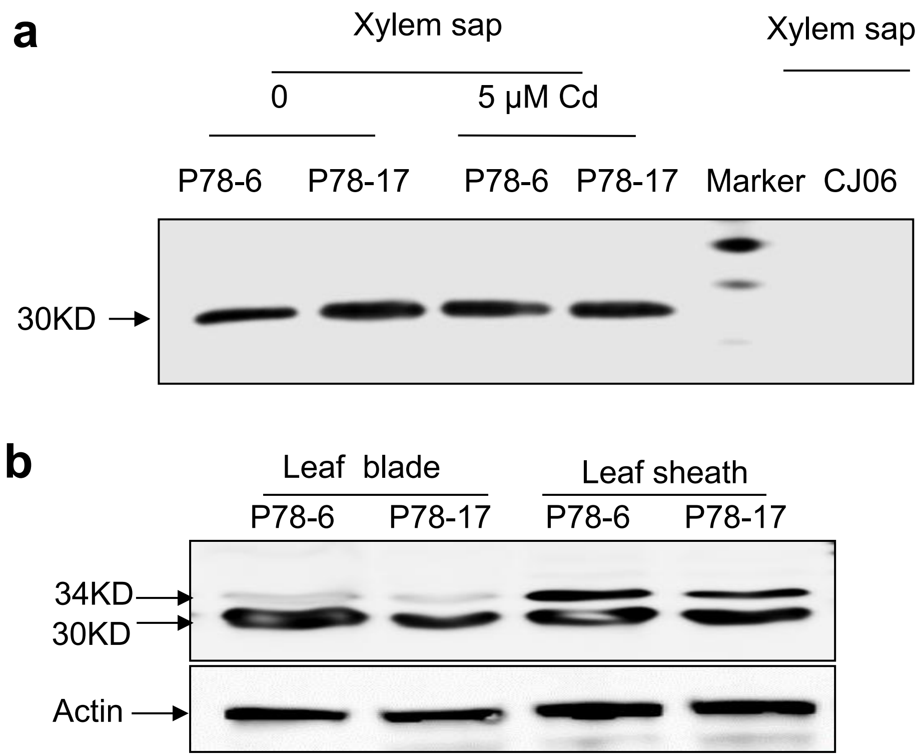
c

Mutants	Dissociation constant/KD(μM)
ΔSPCAL1	53.7
C55A	197.2
C65A	204.1
C75A	96

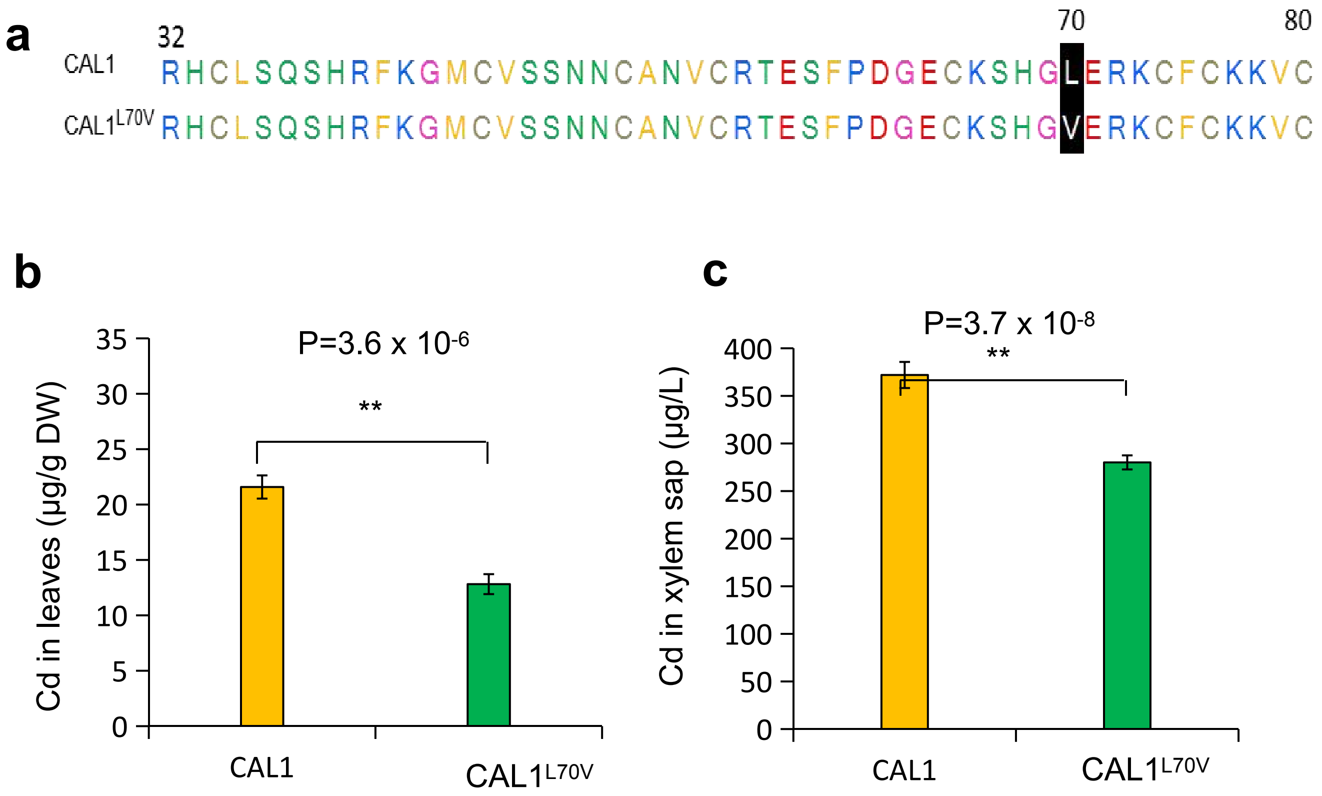
CAL1促进水稻原生质体Cd的外排



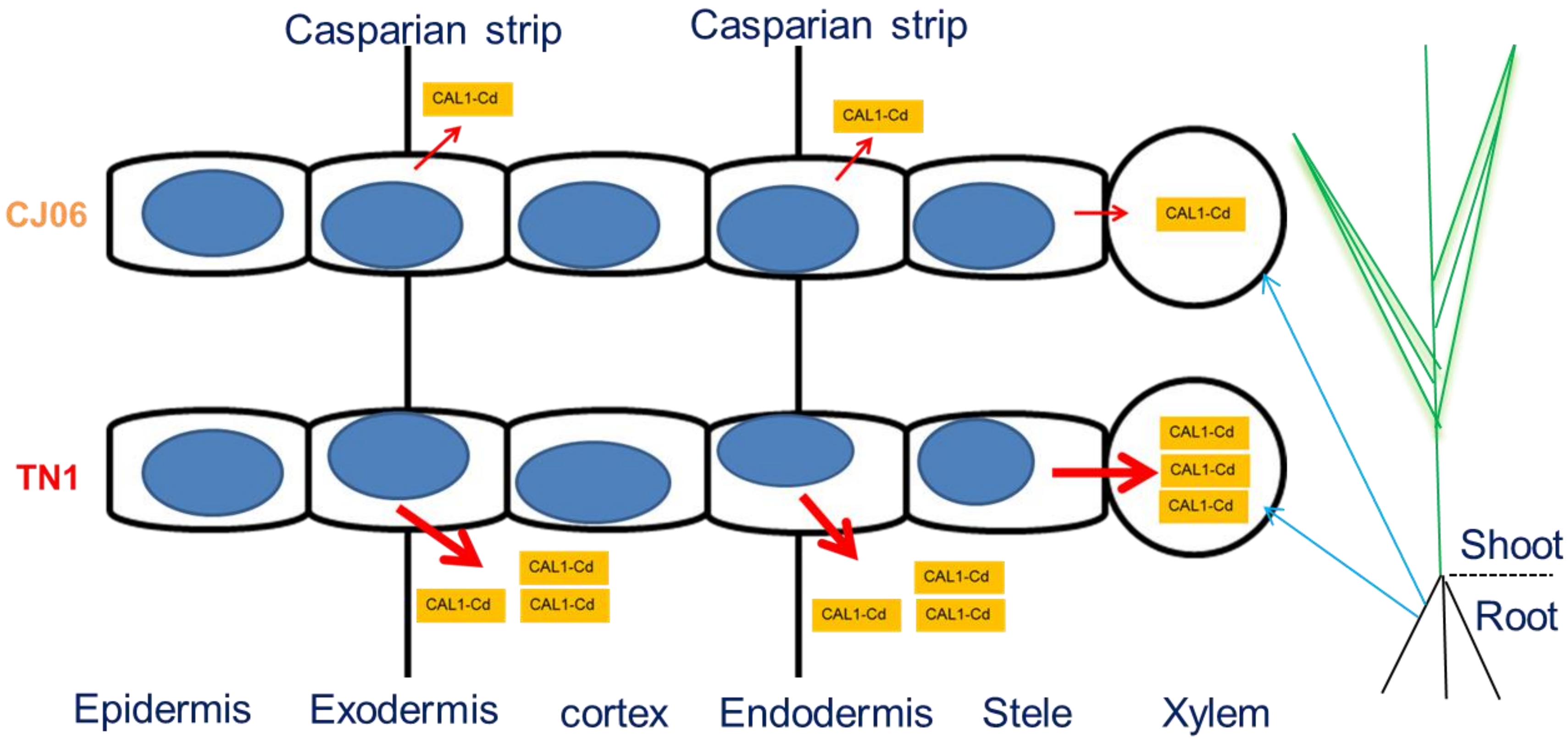
CAL1蛋白成熟形式通过木质部伤流液长途转运并在叶片积累



*CAL1*自然变异降低了水稻叶片和木质部伤流液中Cd含量

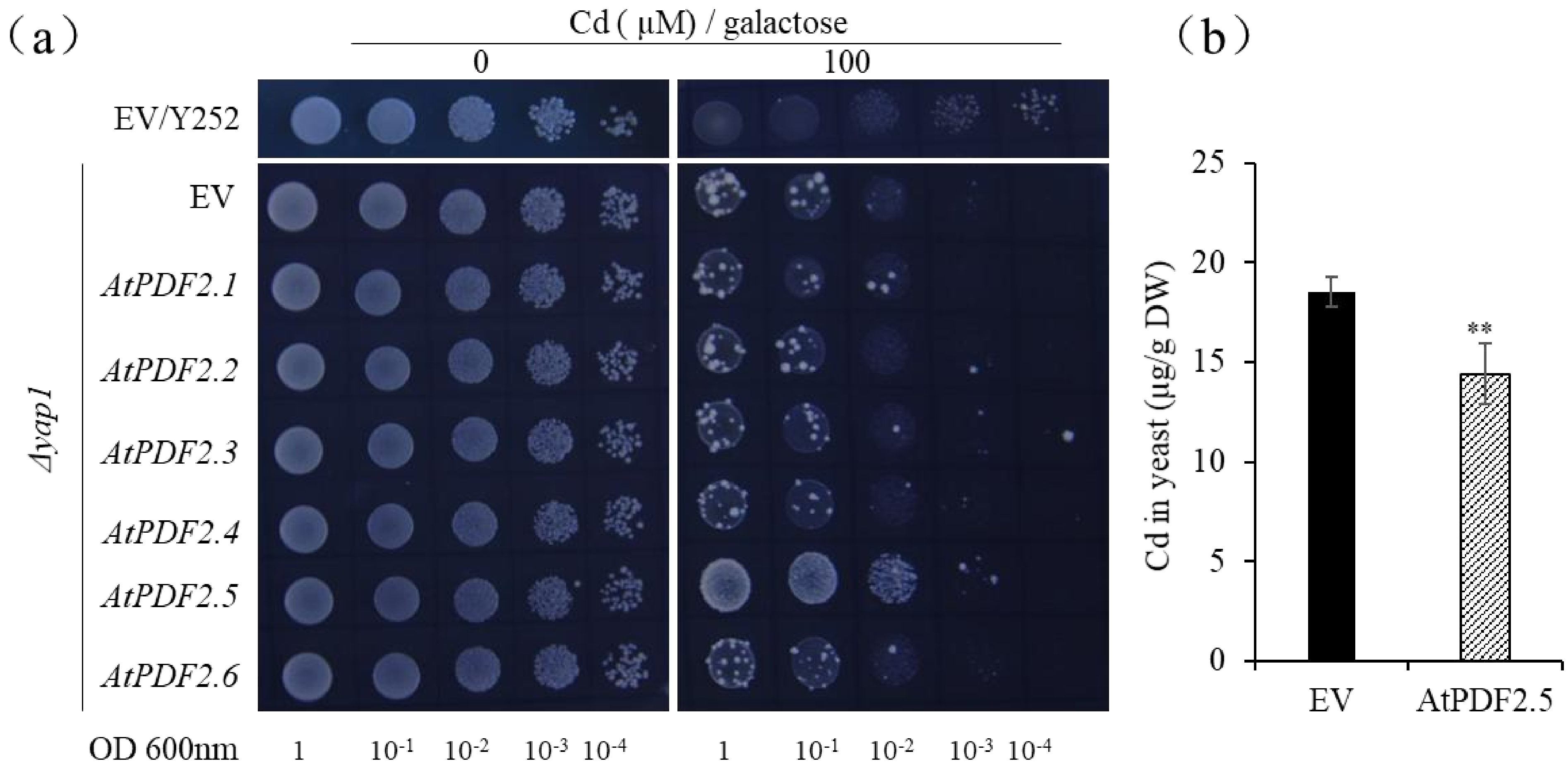


CAL1调控水稻地上部Cd积累的工作模型



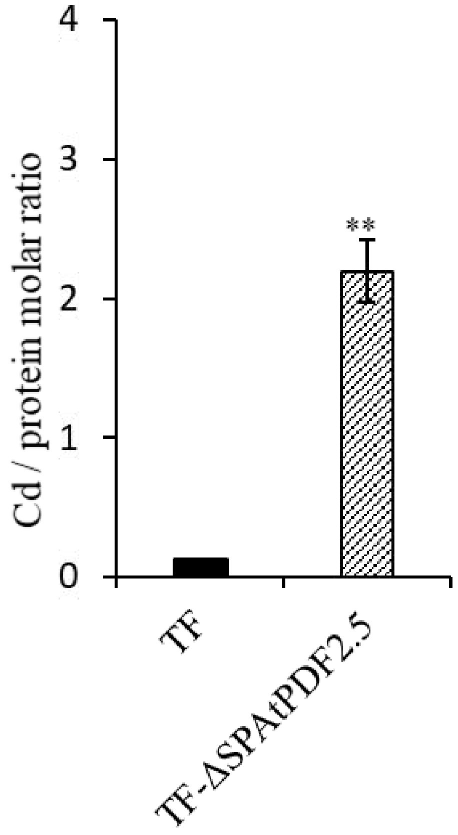
Luo JS et al., 2018, *Nature Communications*

AtPDF2.5能显著增强酵母对镉的抗性，并降低酵母中镉含量



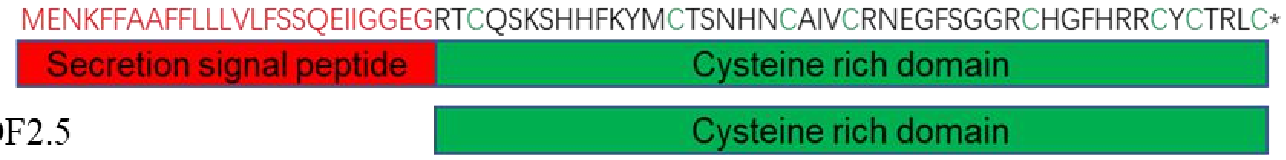
体外纯化的AtPDF2.5蛋白具有镉螯合活性

（a）

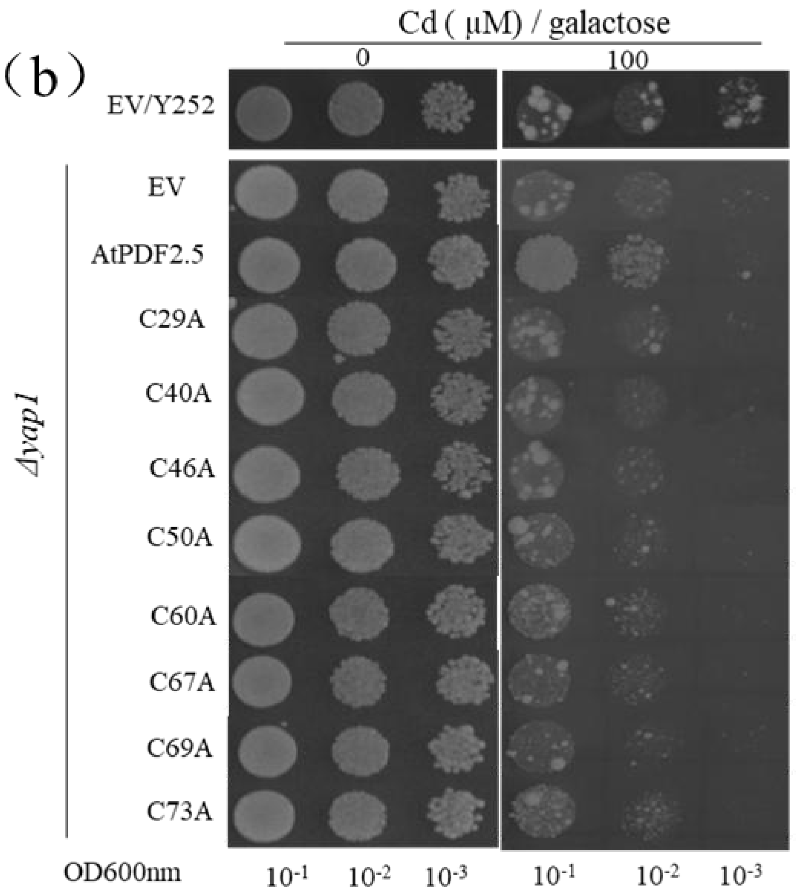


AtPDF2.5蛋白C端Cys的突变显著降低其镉的耐受和螯合

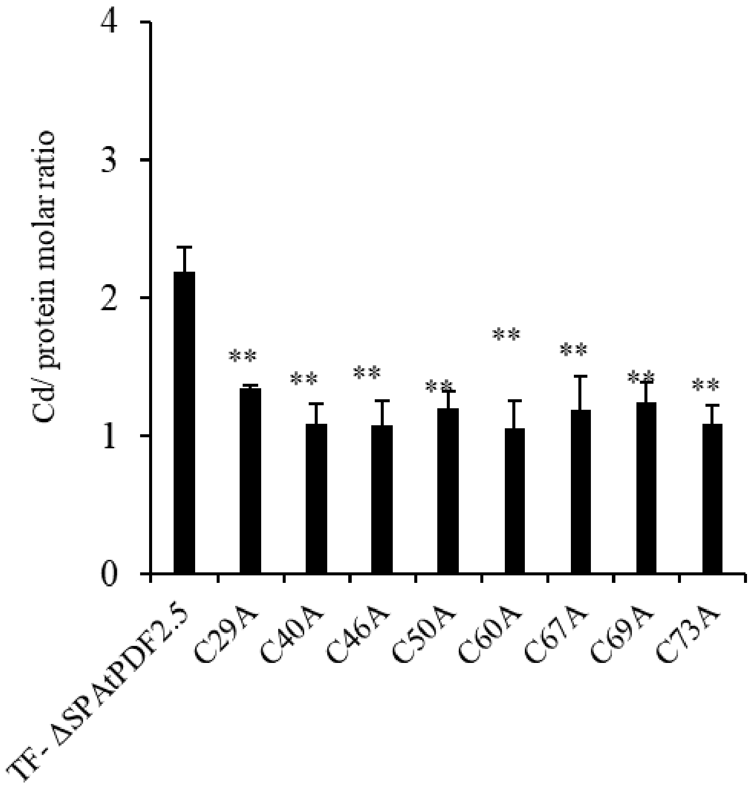
（a）



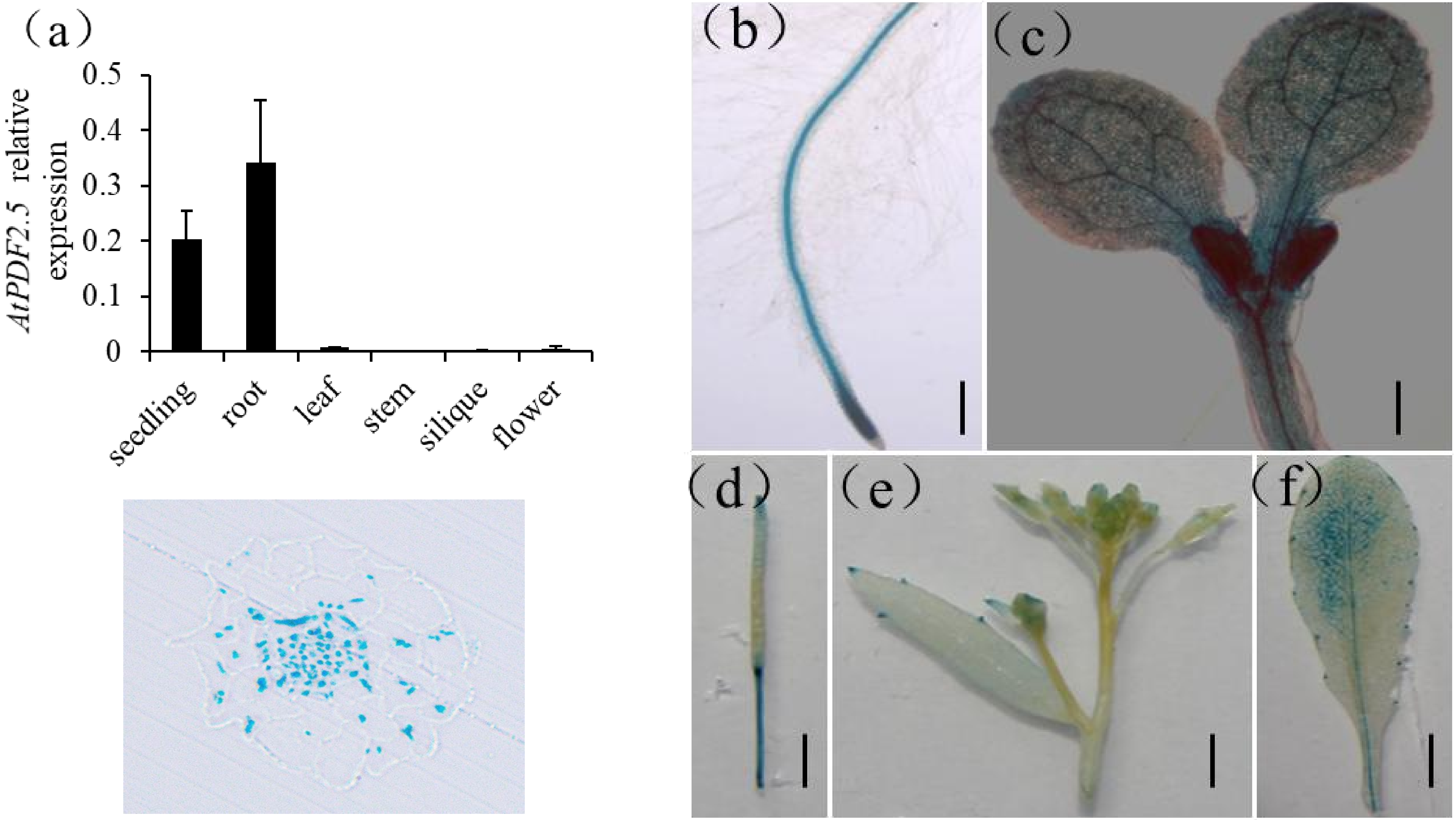
（b）



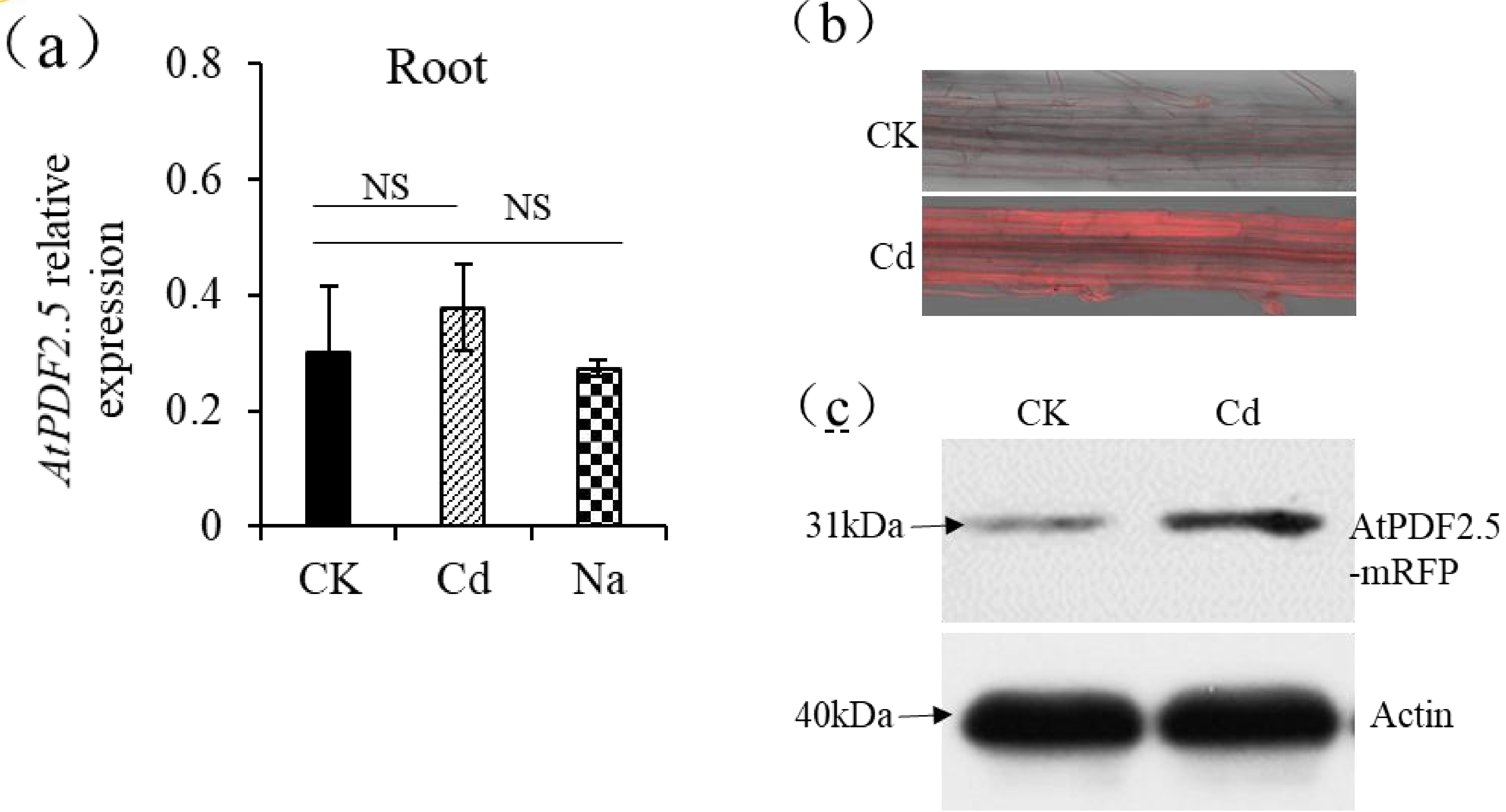
（c）



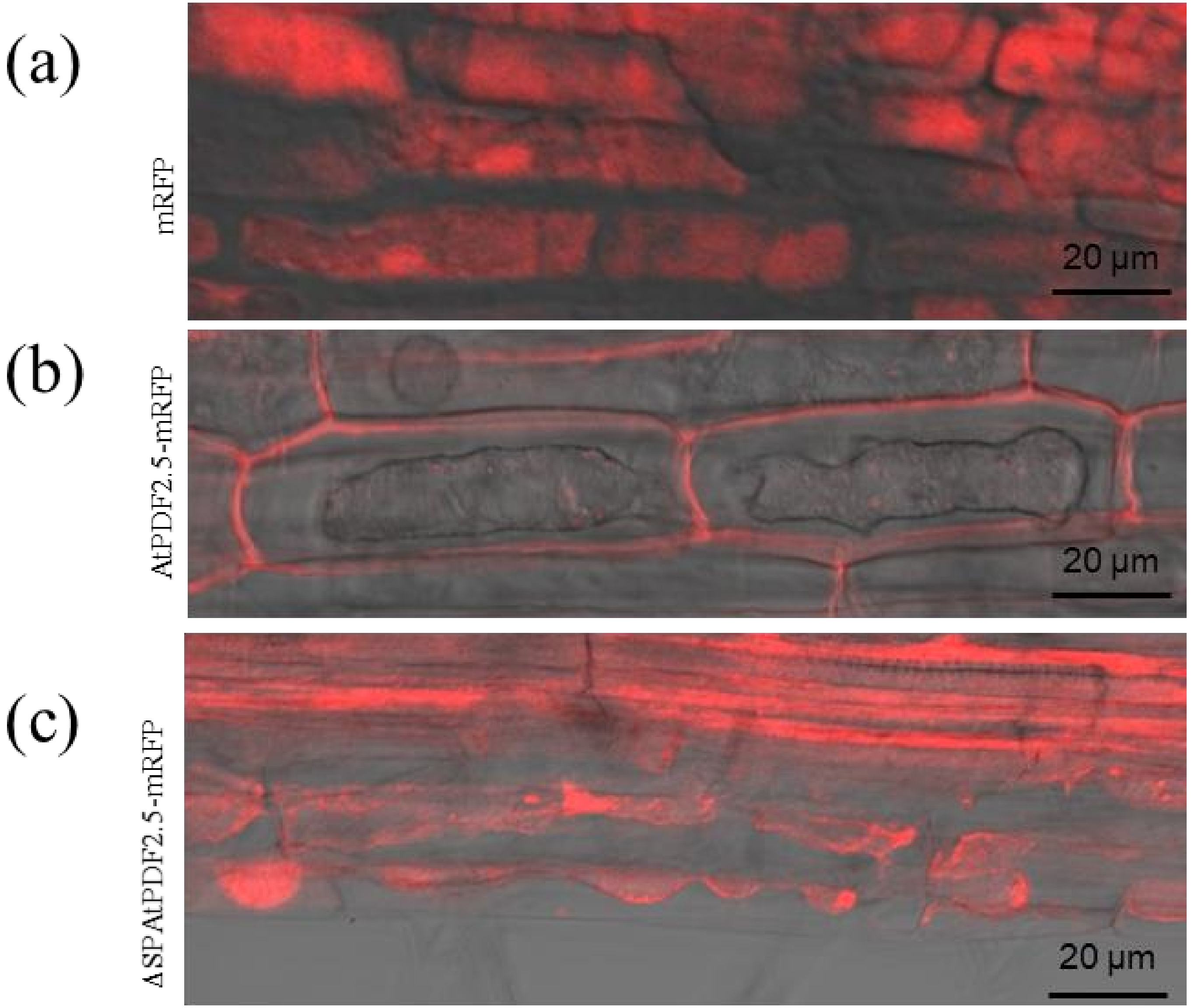
*AtPDF2.5*主要在根中木质部维管组织表达



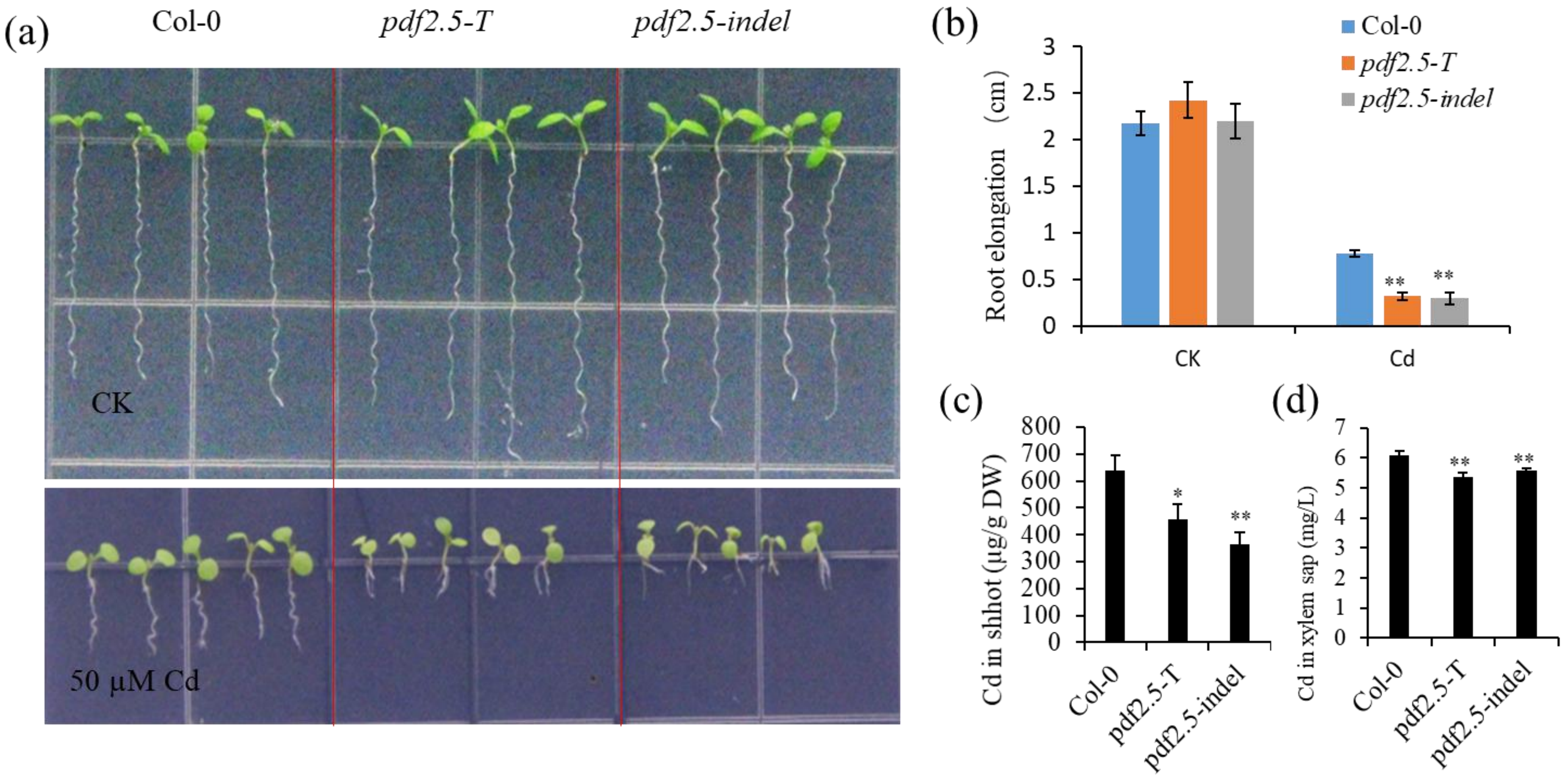
AtPDF2.5在蛋白水平受到镉的调控



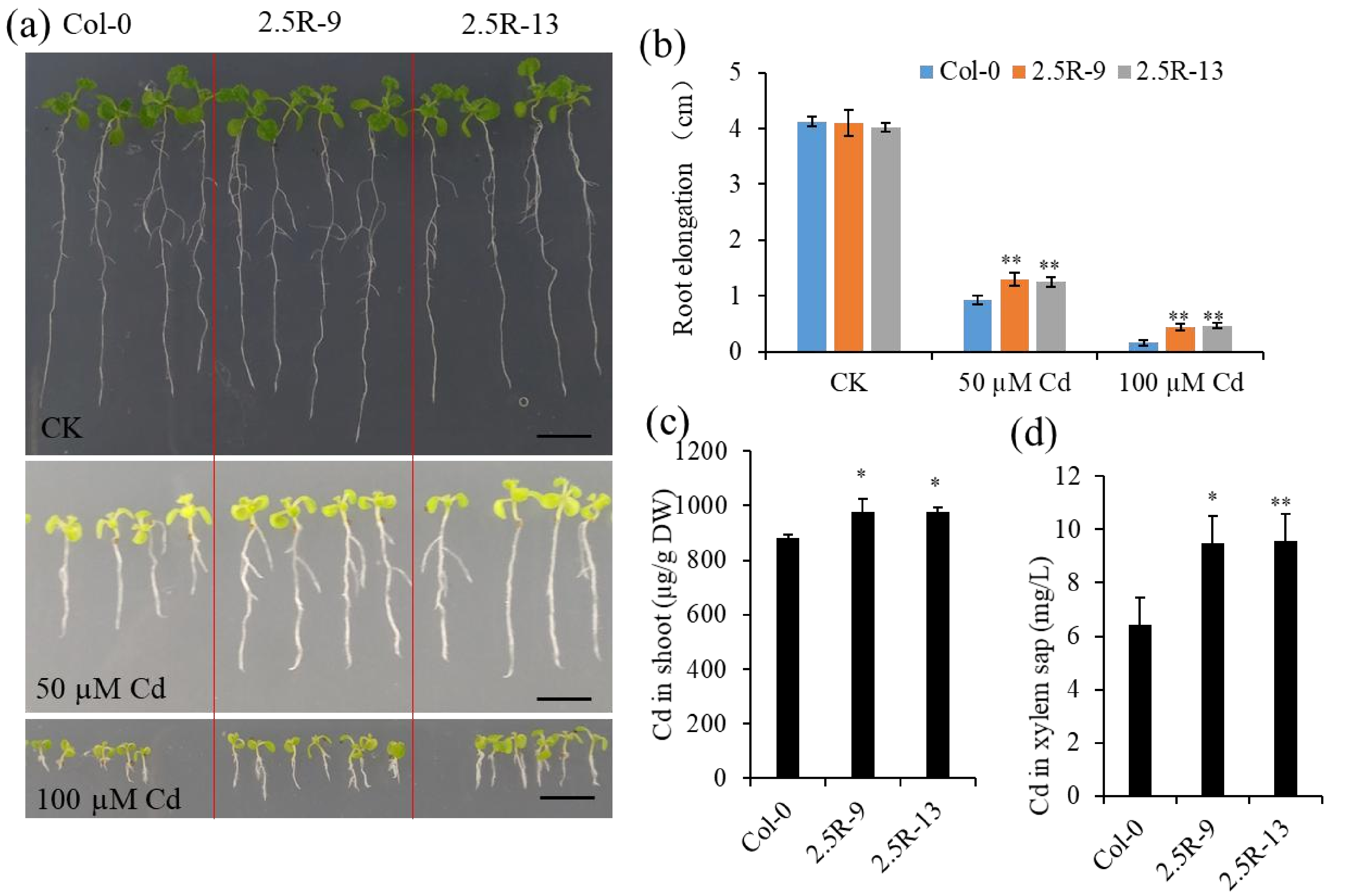
AtPDF2.5定位于质外体体细胞壁依赖N端信号肽



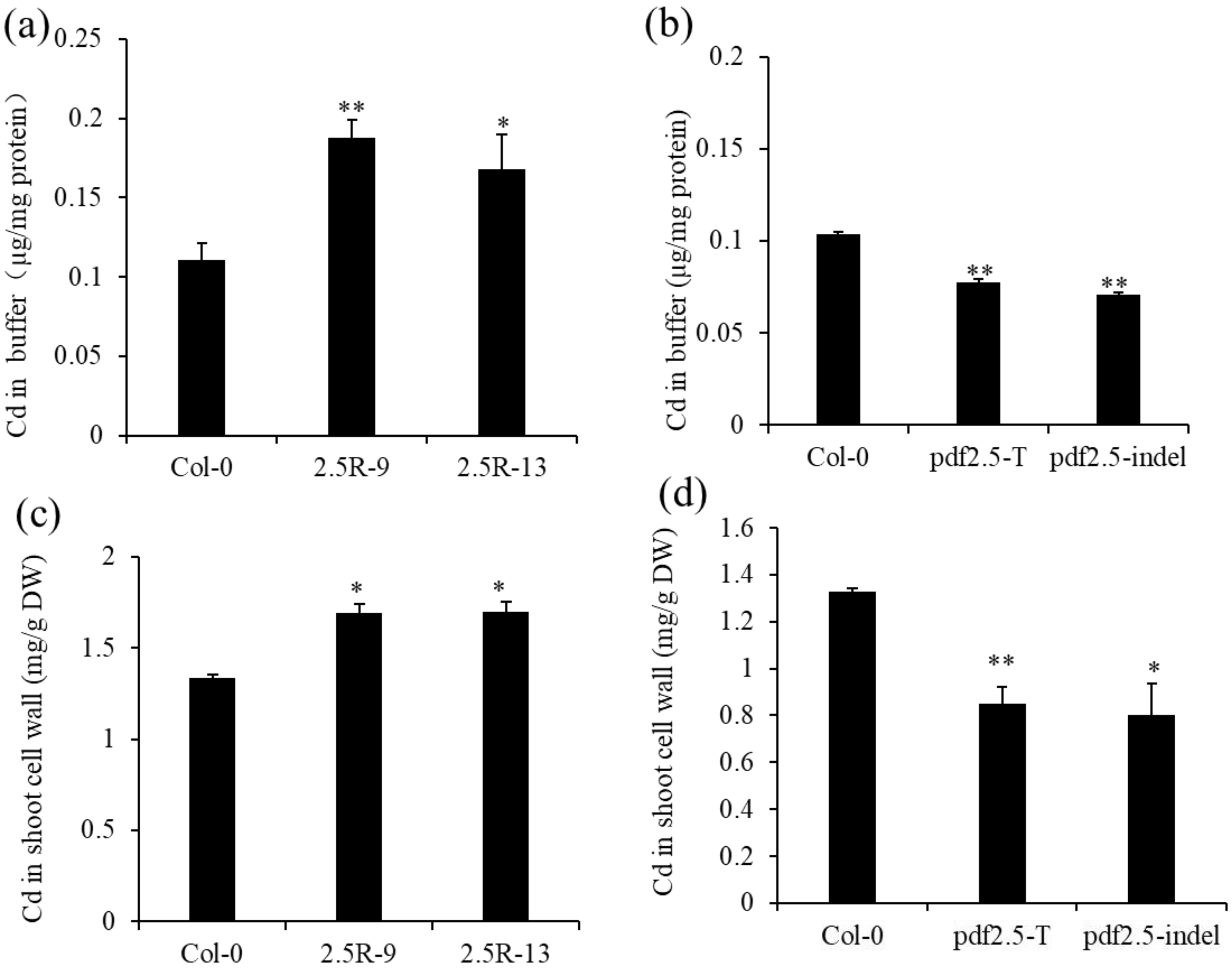
*AtPDF2.5*的突变显著降低植物对镉的耐受和积累



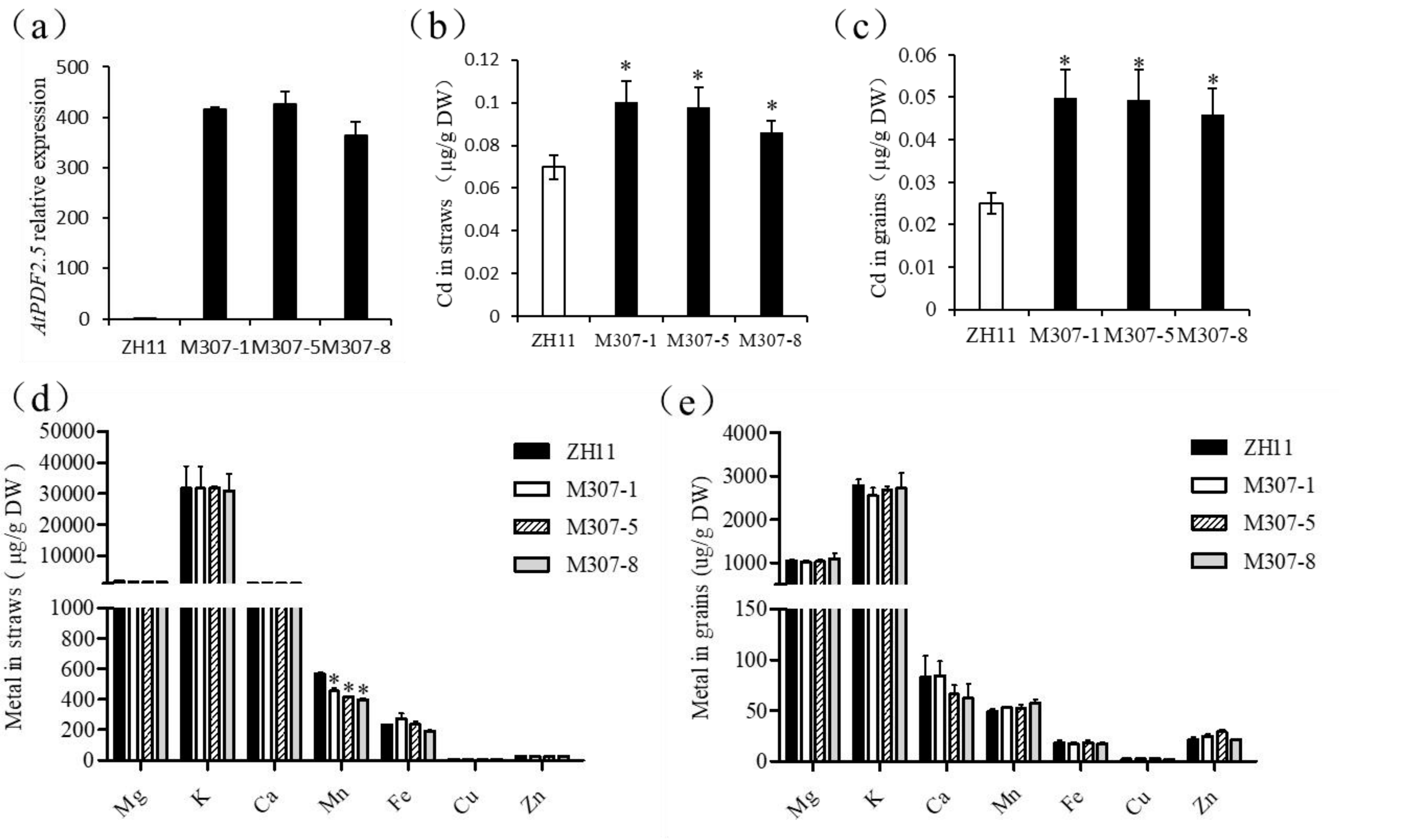
*AtPDF2.5*过表达显著增强植物对镉的耐受和积累



*AtPDF2.5*促进原生质体镉的外排并积累在细胞壁



异源过表达*AtPDF2.5*增加水稻中镉的累积

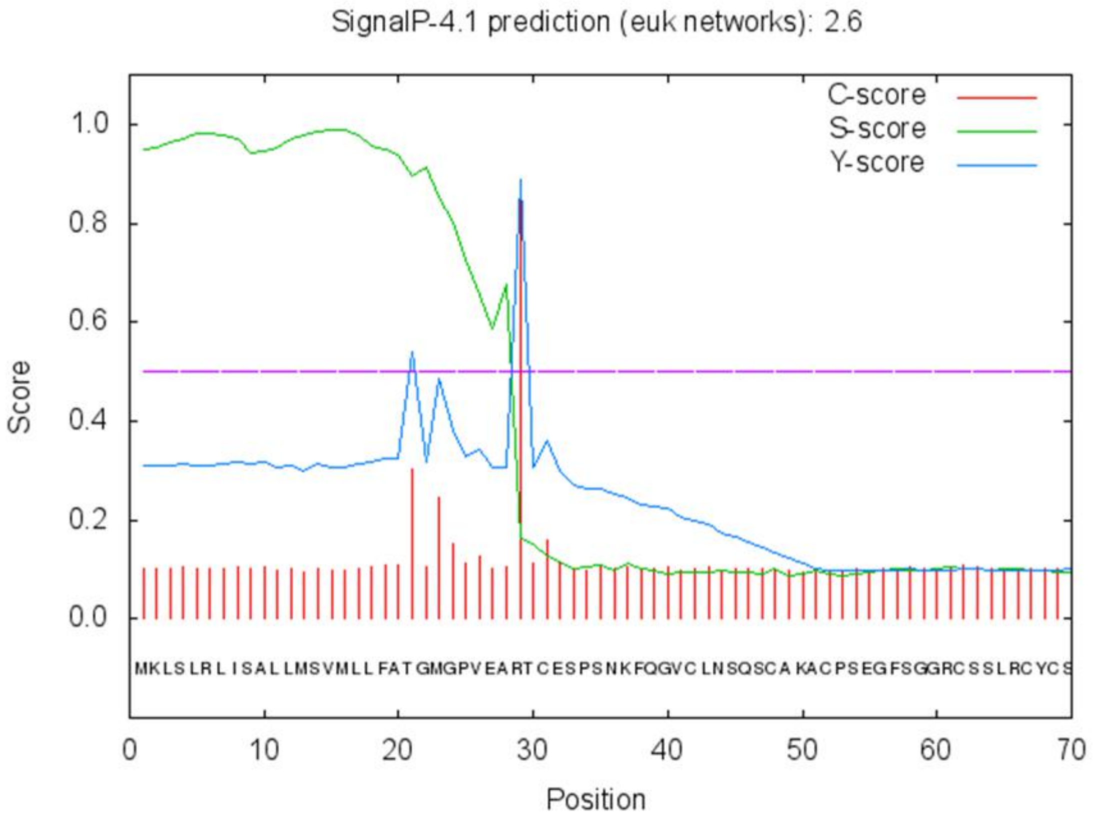


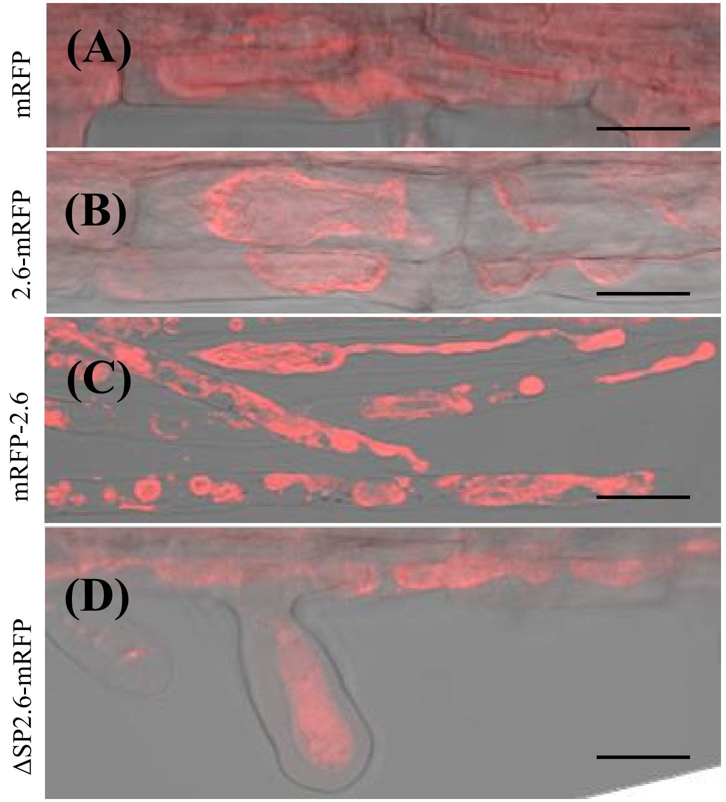
小结一

1) 体外实验表明AtPDF2.5具有镉螯合活性，异源表达能显著增强酵母对镉的抗性，并降低了酵母中的镉含量；
2) *AtPDF2.5*主要在根中木质部维管组织表达，并在蛋白水平受到镉的调控，亚细胞定位于质外体空间细胞壁；
3) AtPDF2.5正调控拟南芥对镉的抗性积累，其突变显著降低植物对镉的抗性和积累，其过表达则显著增强植物对镉的抗性和积累；
4) AtPDF2.5促进了原生质体镉的外排，显著增加镉在细胞壁中的积累；
5) 异源过表达显著增加镉在水稻中的累积，而对其它元素的积累没有影响，说明AtPDF2.5在植物修复中具有应用前景。

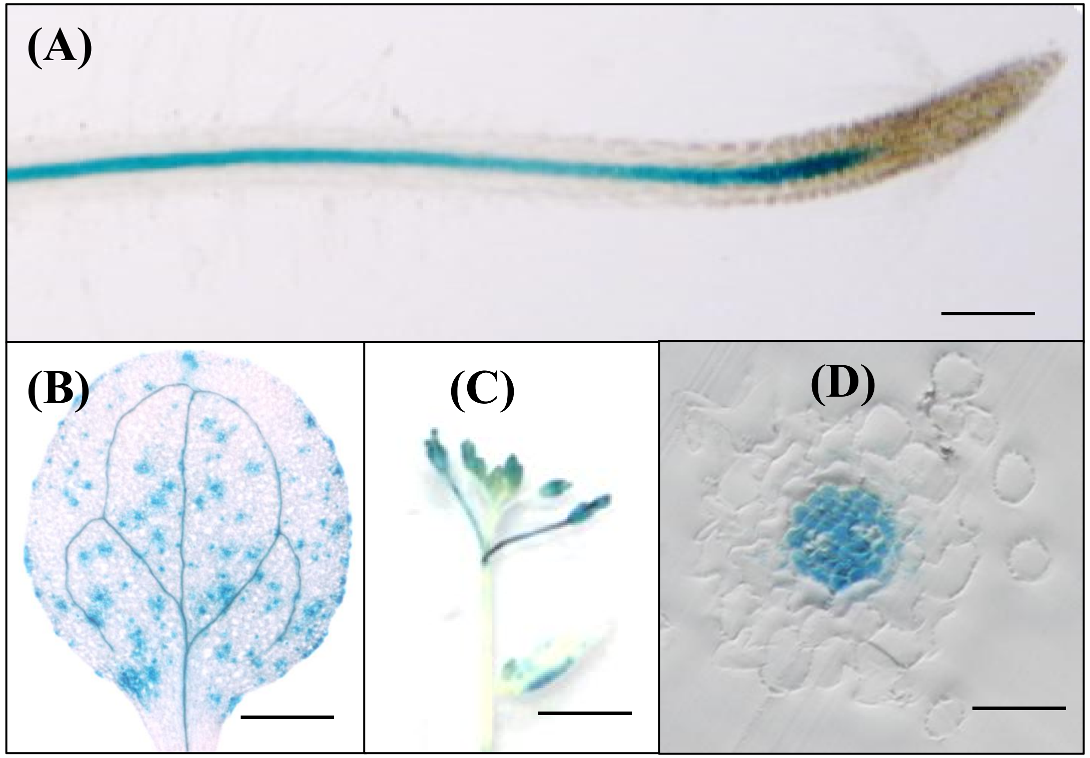
Luo JS et al., 2019, *Plant Cell & Environment*

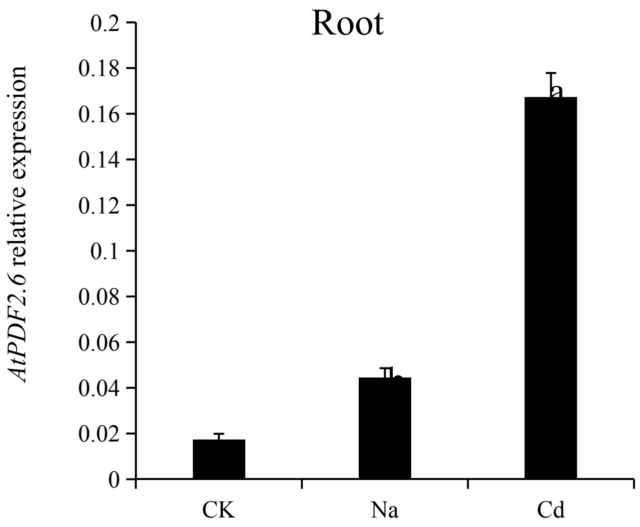
AtPDF2.6定位于细胞质



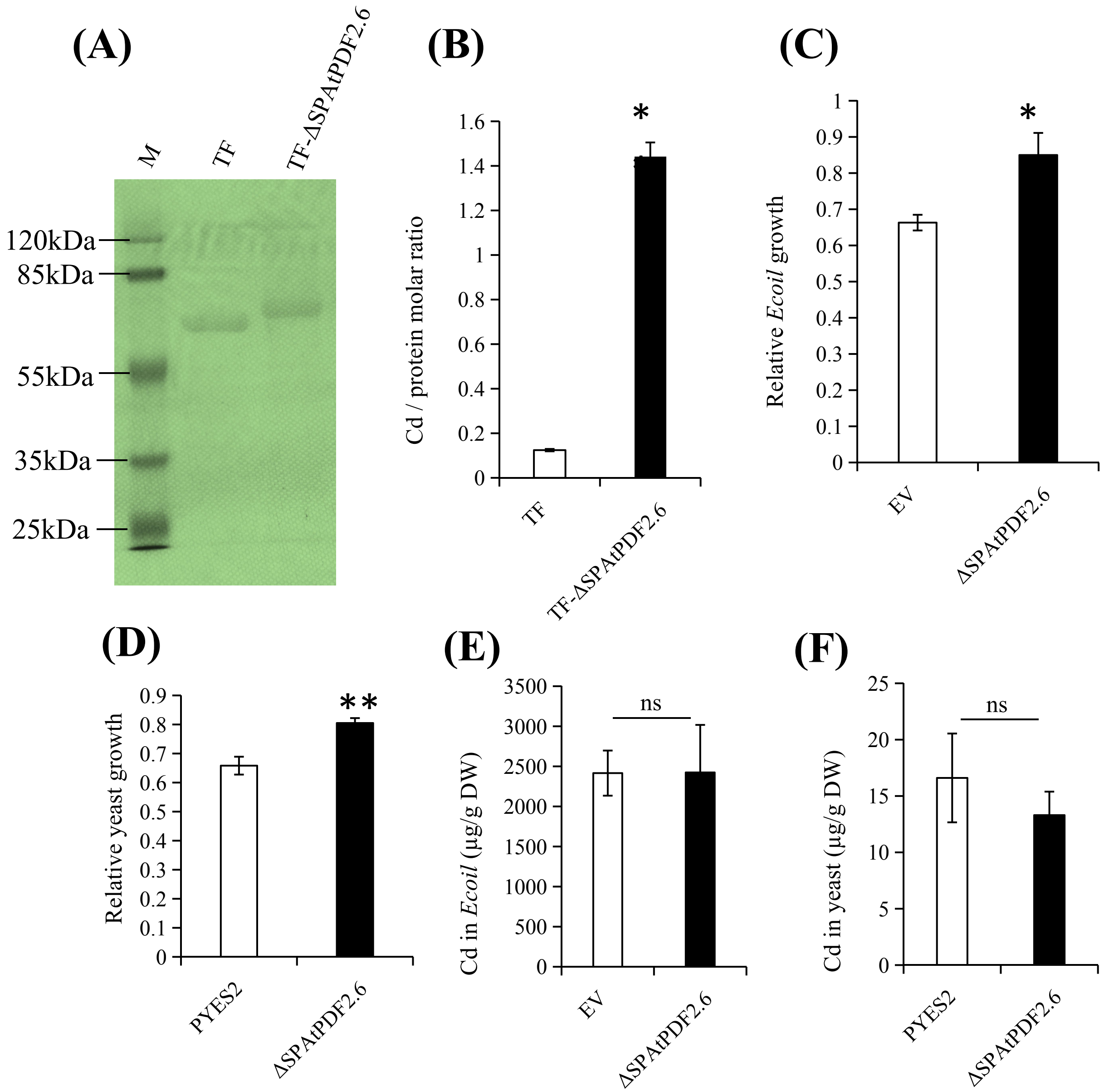


*AtPDF2.6*主要在根中维管组织表达,并受Cd显著诱导

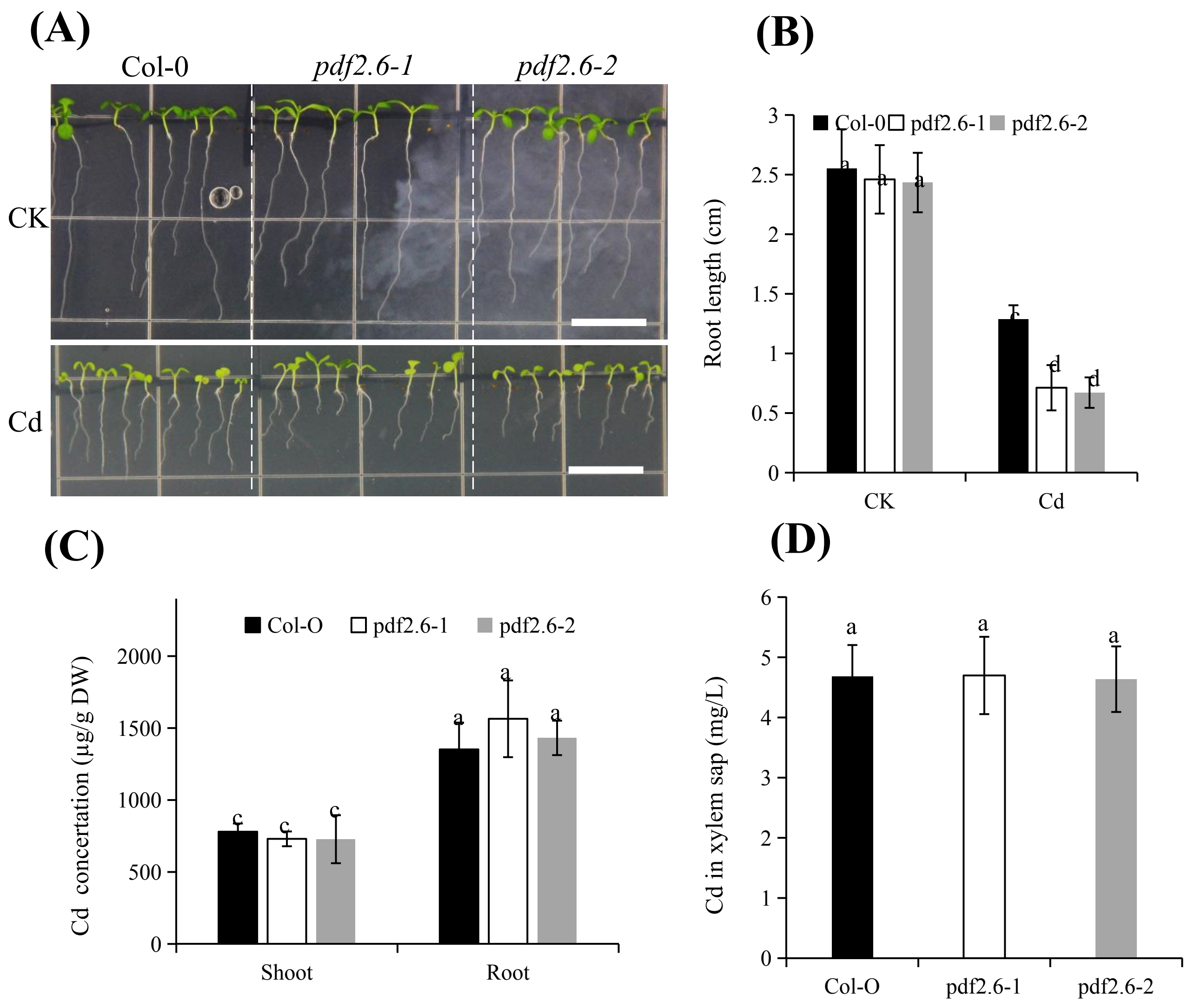




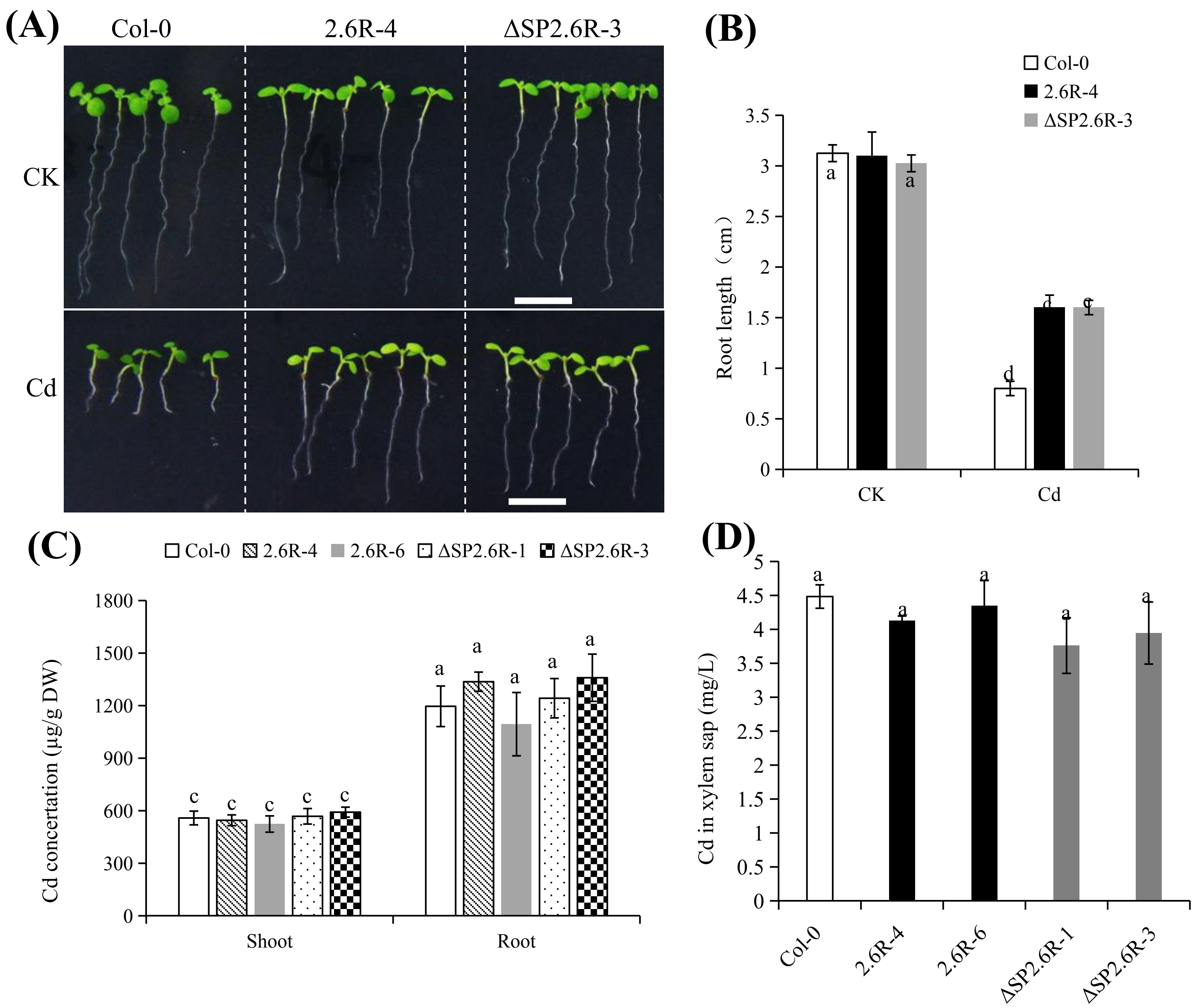
AtPDF2.6具有Cd螯合活性



*AtPDF2.6*的突变显著降低植物对镉的耐受



*AtPDF2.6*过表达显著增强植物对镉的耐受和积累



小结二

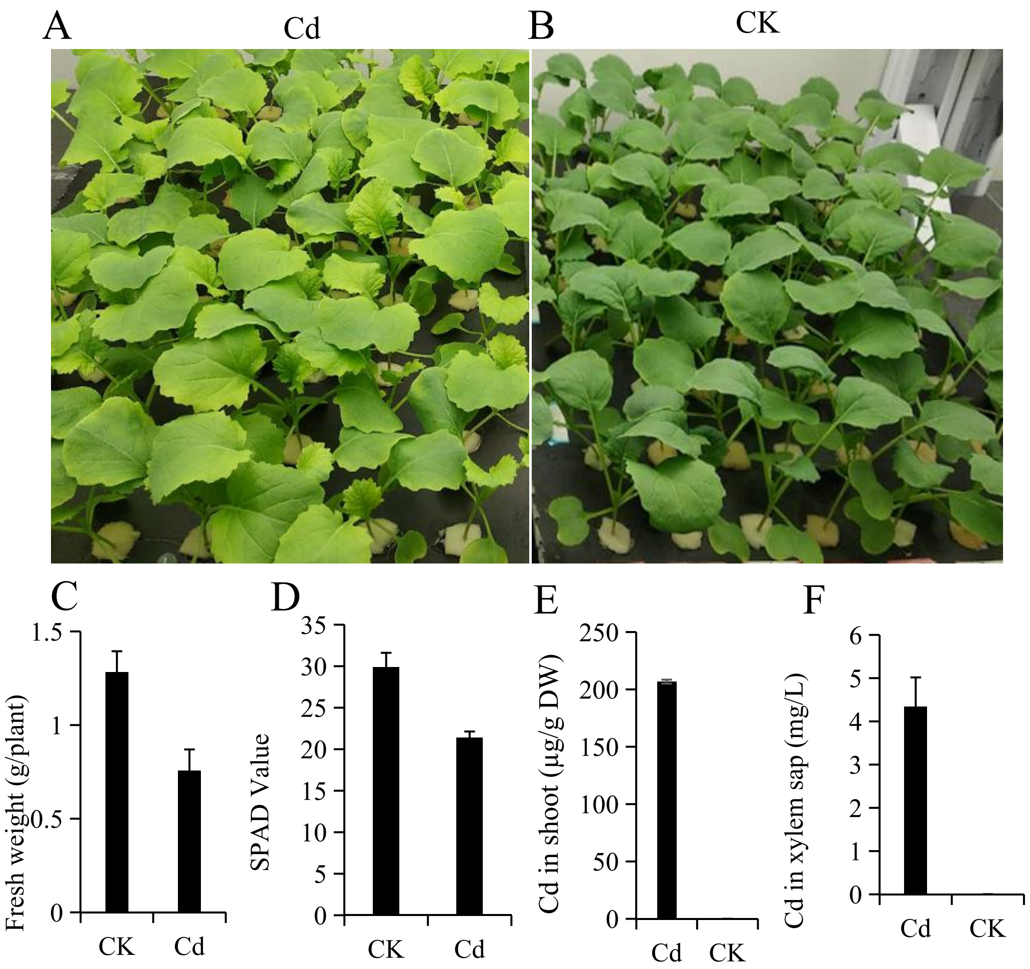
1) *AtPDF2.6*主要在根中木质部维管组织表达，并在转录水平受到镉的调控，亚细胞定位于细胞质；

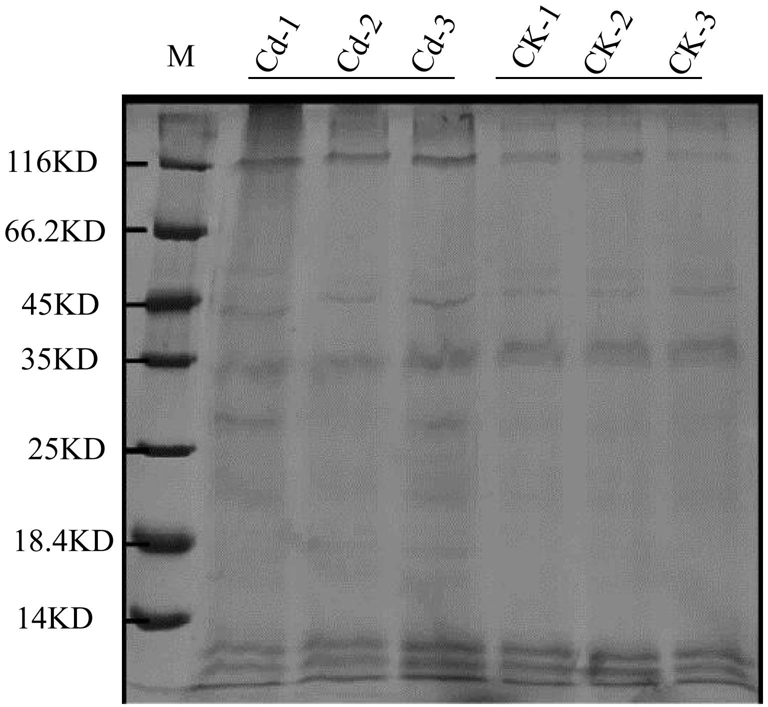
2) 体外实验表明AtPDF2.6具有镉螯合活性，异源表达能显著增强酵母对镉的抗性，并降低了酵母中的镉含量；

3) AtPDF2.6正调控拟南芥对镉的抗性，其突变显著降低植物对镉的抗性，其过表达则显著增强植物对镉的抗性。

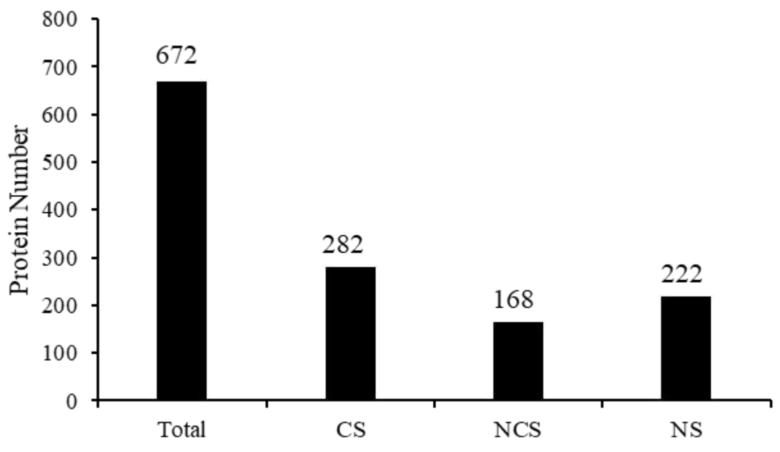
Luo JS et al., 2019, *Plant Molecular Biology*

Cd胁迫条件下油菜木质部伤流液蛋白变化

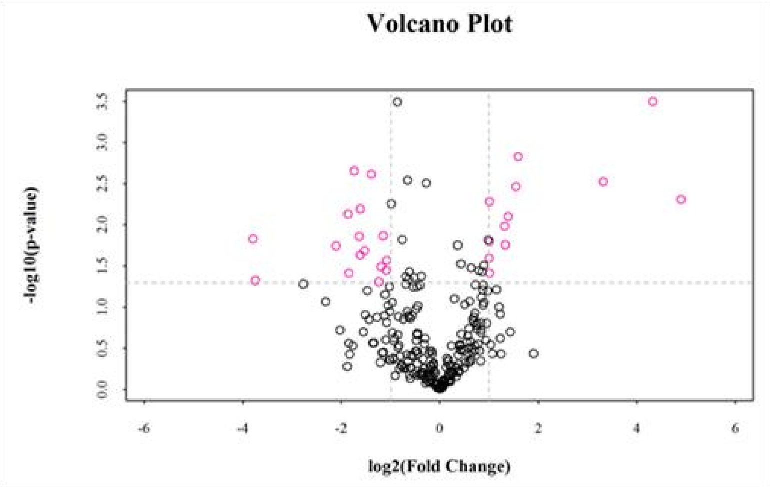




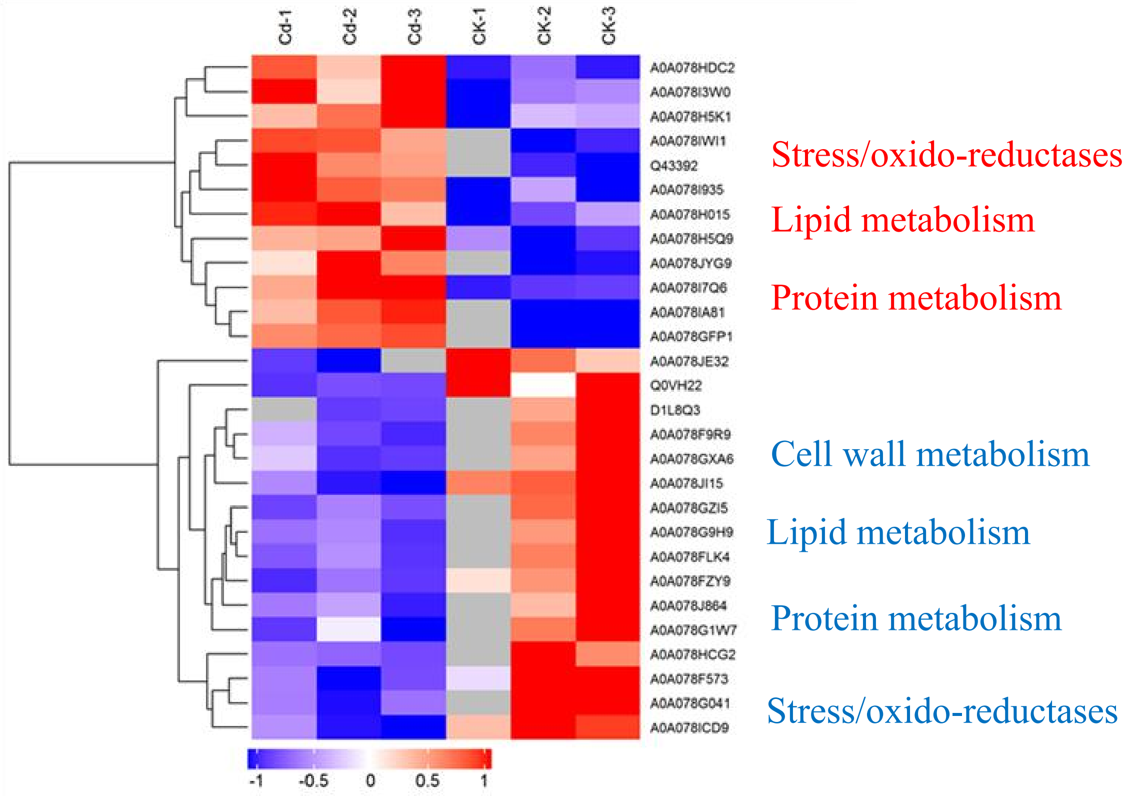
Cd胁迫条件下油菜木质部伤流液中发生显著变化的蛋白



a



b



油菜特异响应Cd的木质部伤流液中鉴定到植物防御素蛋白

Table 3 List of identified proteins unique present in Cd explored xylem sap samples from *Brassica napus*.
SecretomeP column indicates results from subcellular classification as classical (CS) and non-classical secretory (NCS) proteins.

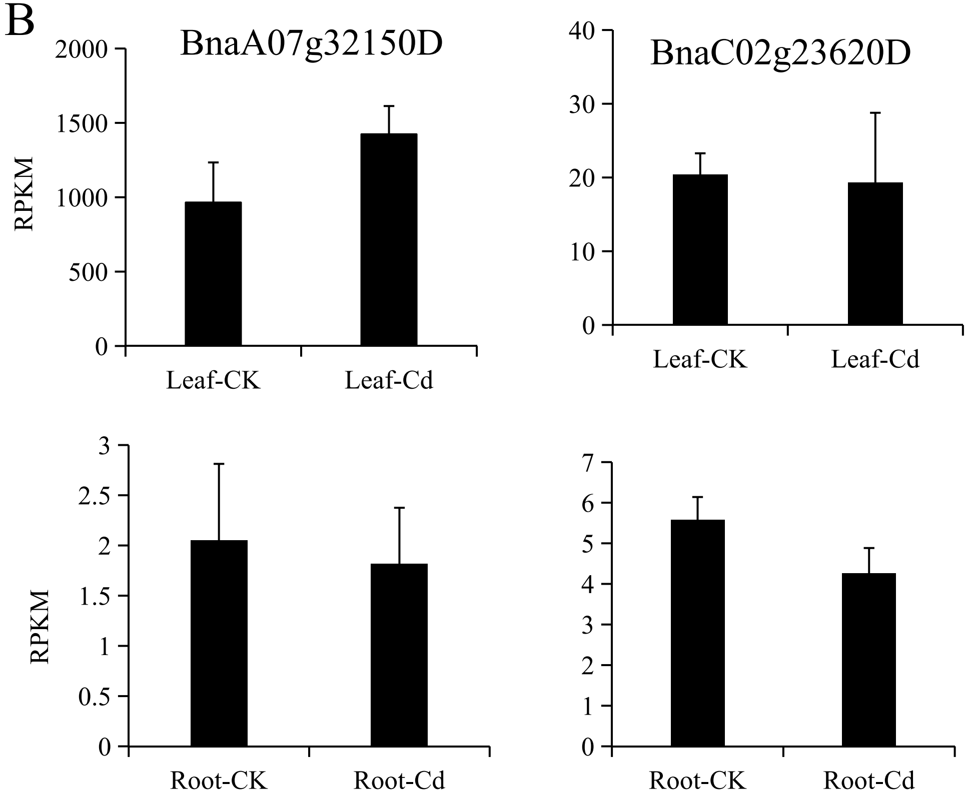
Accession [a]	Similar to Arabidopsis	Description	Secretome P [b]	Functional classification
A0A078JU82	AT3G59010	Pectin methylesterase	CS	
A0A078FM34	AT5G41870	Pectin lyase-like superfamily protein	CS	
A0A078JIG4	AT1G02460	Pectin lyase-like superfamily protein	NCS	
A0A078FM34	AT5G41870	Pectin lyase-like superfamily protein	CS	
A0A078JIG4	AT1G02460	Pectin lyase-like superfamily protein	NCS	Cell wall metabolism
A0A078I1Z2	AT1G68560	Encodes a bifunctional alpha-l-arabinofuranosidase/ beta-d-xylosidase	CS	
A0A078F3L6	AT5G20950	Encodes a beta-glucosidase involved in xyloglucan metabolism	CS	
A0A078FKS9	AT5G66630	DA1-related protein 5	NCS	
A0A078IG80	AT3G26380	Glycoside Hydrolase (GH27) family	CS	
A0A078IQ70	AT5G26000	Member of Glycoside Hydrolase Family 1.	CS	
A0A078H1R6	AT4G20830	Encodes an oligogalacturonide oxidase	CS	
A0A068F4Y8	AT4G33720	CAP superfamily protein	CS	
A0A078J6D0	AT2G43570	Chitinase	CS	
A0A078GI16	AT4G36010	Pathogenesis-related thaumatin superfamily protein	CS	
A0A078FK91	AT5G44420	PDF1.2A, plant defensin	CS	Stress/oxido-reductases
A0A078IPF4	AT5G20630	Encodes a germin-like protein.	CS	
A0A078GTU7	AT3G26060	Encodes periredoxin Q	NCS	
A0A078G4Z5	AT3G28200	Peroxidase superfamily protein	CS	
A0A078G4Z5	AT3G28200	Peroxidase superfamily protein	CS	
A0A078JER4	AT1G59560	Encodes a chloroplast-localized putative RING-type ubiquitin E3 ligase.	CS	
A0A078JCD8	AT1G52950	Nucleic acid-binding, OB-fold-like protein	NCS	
A0A078GRU8	AT1G23110	Fold protein	NCS	
A0A078FN41	AT5G56710	Ribosomal protein L31e family protein	NCS	
A0A078JLL9	AT3G56340	Ribosomal protein S26e family protein	NCS	Protein synthesis /degradation
A0A078J609	AT5G58290	26S proteasome AAA-ATPase subunit RPT3 (RPT3) mRNA	NCS	
A0A078HUC8	AT3G09630	Ribosomal protein L4/L1 family	NCS	
A0A078IP32	AT3G19760	Eukaryotic initiation factor 4A-III	NCS	
A0A078FQZ9	AT4G09800	Encodes a ribosomal protein S18C, a constituent of the small subunit of the ribosomal complex	NCS	
A0A078J757	AT2G29960	Encodes a cyclophilin protein that exhibits peptidylprolyl cis/trans-isomerase and protein refolding activities	CS	
A0A078H6L6	AT2G36620	RPL24A encodes ribosomal protein L24	NCS	

油菜防御素基因的表达模式

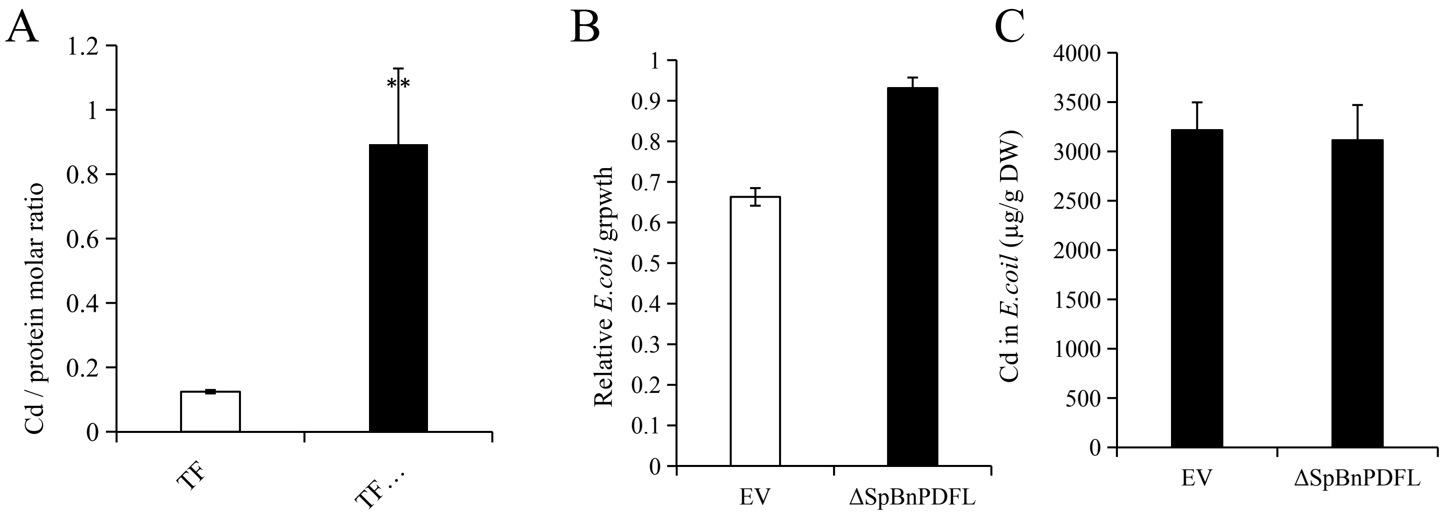
A

Gene ID	Peptide sequence	Description
BnaA07g32150D	SSGTWSGVCGNNNACK	Defensin-like protein
	LEGAQHGSCNYVFPAHK	
BnaC02g23620D	CNNAVPTPK	Defensin-like protein

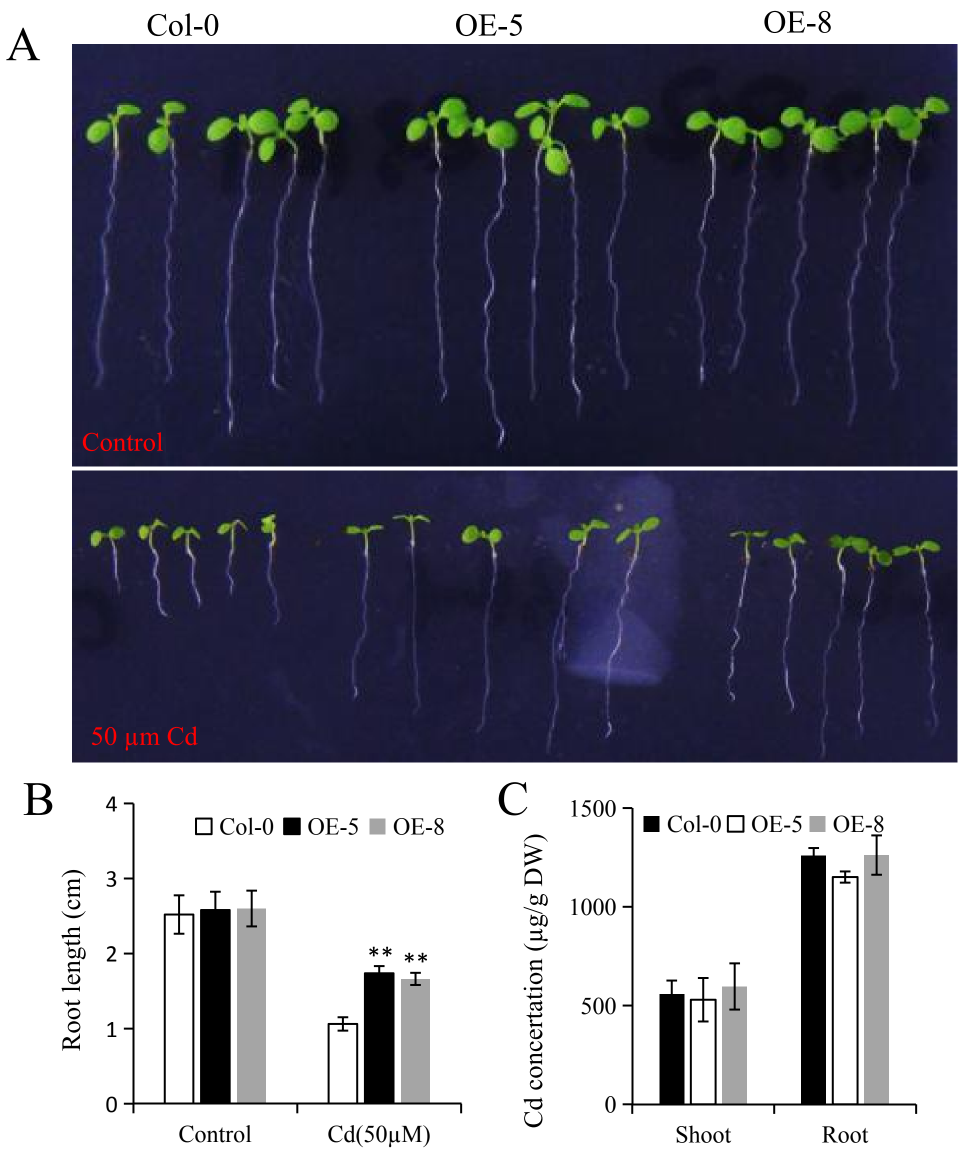
B



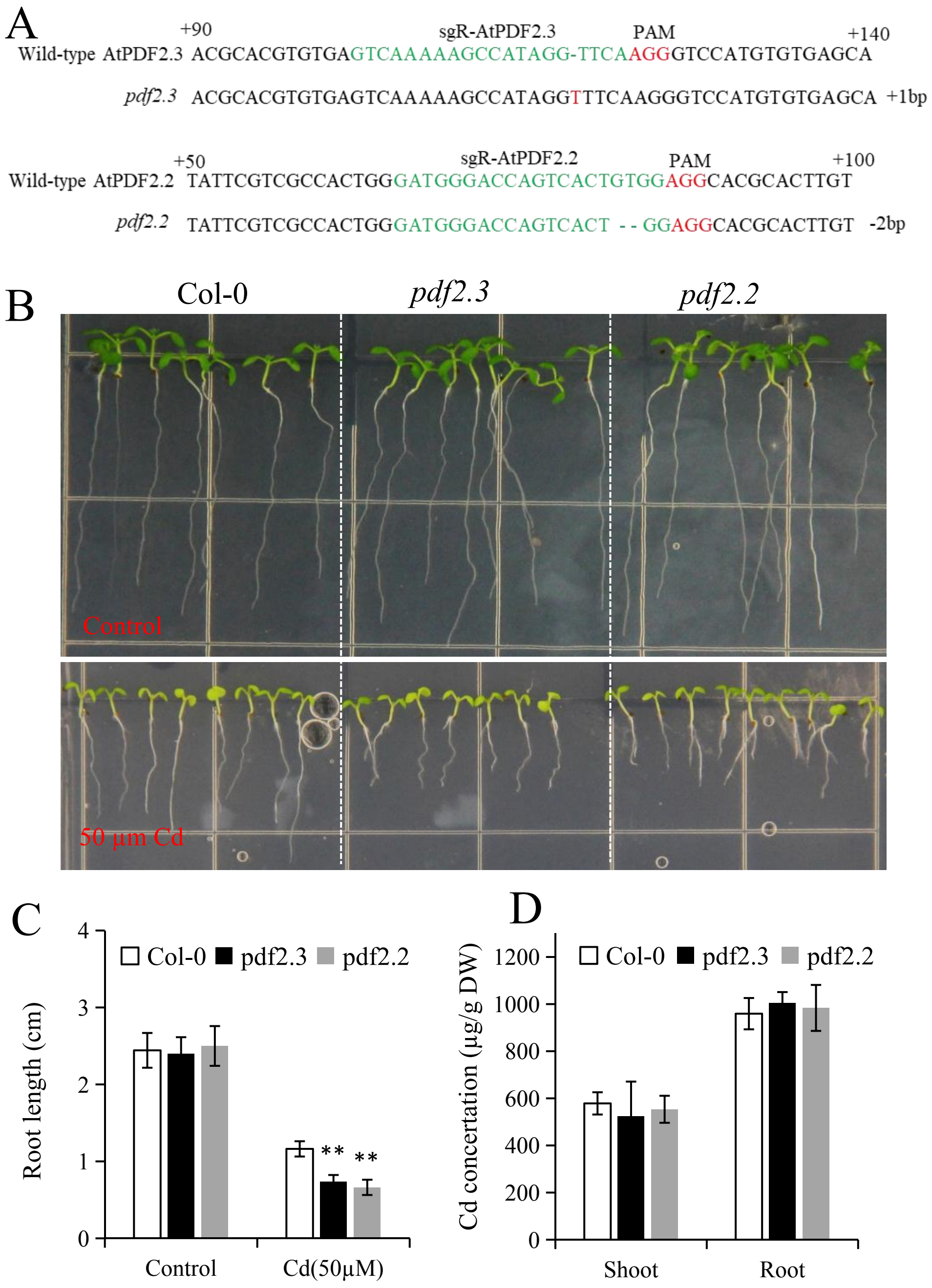
BnPDFL具有Cd螯合活性



*BnPDFL*异源表达能增强拟南芥对Cd的耐受性



*BnPDFL*在拟南芥中的同源基因*AtPDF2.2*和*AtPDF2.3*的突变显著降低了植物对Cd的耐受性



小结三

通过木质部伤流液蛋白分析，我们鉴定到了油菜中一些响应镉胁迫发生显著变化的蛋白，并验证了油菜防御度基因在植物镉耐受中发挥重要作用。

Luo JS & Zhang ZH. 2019, *BMC Plant Biology*

致谢

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谢谢大家