

Supplementary data

Figure S1. Phylogenetic analysis of eight pepper groupIII WRKY proteins and *Arabidopsis* and tomato groupIII WRKY proteins. The trees were constructed using MEGA6.06.

A, Phylogenetic tree for groupIII WRKY proteins from pepper, tomato, and *Arabidopsis*.

B, Phylogenetic tree for groupIII WRKY domains from pepper, tomato, and *Arabidopsis*.

Amino acid sequences labeled with red diamonds, yellow circles, and green triangles represent WRKYs from pepper, tomato, and *Arabidopsis*, respectively.

Figure S2. Cd stress and Fe deficiency promotes H₂O₂ accumulation.

A and B, H₂O₂ levels analyzed by DAB staining (brown) at the indicated time points after treatment with 25 μM CdSO₄ and Fe deficient (–Fe) nutrient solution, respectively.

C & D, Leaf yellowing phenotype observed in newly emerging leaves of pepper plants at the eight-leaf stage after Fe deficiency treatment.

E, Excess Cd-triggered H₂O₂ accumulation was inhibited by treatment with 10 mM ascorbic acid (AsA).

Figure S3. GUS expression in transgenic *pCaWRKY41::GUS Arabidopsis* plants under normal growth conditions.

A to F, Photographs of seedlings at 1(A), 3(B), 4 (C), 5 (D), and 6 (E) days after germination (DAG). F, Cleared shoot corresponding to E.

G, Mature leaves and petiole, which may also include vascular bundles.

H and I, Flowers.

J, Siliques.

K, Amino acid sequence analysis of CaWRKY41. A nuclear localization signal and WRKY domain were identified in CaWRKY41; the amino acid sequence is shown in bold and underlined in green. The domain underlined in black contains a highly conserved heptapeptide stretch (WRKYGQY) at its N-terminus, followed by a zinc-finger motif (Cx7Cx23HxC) and C-C-H-C, as indicated by black triangles.

Figure S4. CaWRKY41 is a transcriptional activator localized to the nucleus.

A, Subcellular localization of CaWRKY41-GFP and GFP transiently expressed under the control of the 35S promoter in *N. benthamiana* epidermal cells. DAPI, 4,6-diamidino-2-phenylindole (nucleus stain).

B, Transcriptional activation assay of CaWRKY41 in yeast cells. LacZ reporter gene expression is indicated by blue staining.

C, Growth of 5-week-old WT and *CaWRKY41*-overexpression lines.

D, Leaves isolated from the plants shown in C.

E, Semi-quantitative PCR analysis of *CaWRKY41* expression in eight WT and *CaWRKY41*-overexpression lines.

Figure S5. Analysis of the effects of Cd stress on plant growth using chlorophyll fluorescence imaging before the appearance of visual effects on plant growth.

A & B, Ten-day-old WT, *CaWRKY41-OE1*, and *CaWRKY41-OE4 Arabidopsis* plants grown on ½ MS medium without or with 60 µM CdSO₄. Images of the chlorophyll fluorescence parameter values, *Fv/Fm*, for the plants shown in A and B are shown in C and D, respectively. The data in the images of *Fv/Fm* shown in A and B were mapped to the color palette histograms in E and F, respectively.

Figure S6. Effect of Cd treatment on Zn concentrations in *Arabidopsis*.

A to D, Zn and Fe concentrations in shoots and roots of WT, *CaWRKY41-OE1*, and *CaWRKY41-OE4* plants after 3 and 5 days of treatment with Cd stress. E & F, Seedling growth of WT, *CaWRKY41-OE1*, and *CaWRKY41-OE4 Arabidopsis* plants on ½ MS medium containing 0 and 200 µM ZnSO₄. The data represent the mean ± SE of three biological replicates. Different letters indicate significant differences compared to the control (Tukey's test; lowercase letters indicate $P < 0.05$ and uppercase letters indicate $P < 0.01$).

Figure S7. The *Arabidopsis ocp3* (*overexpressor of cationic peroxidase 3*) mutant shows reduced tolerance to Cd stress.

A, B, and C, Seedling growth of WT and *ocp3* on ½ MS medium containing 0, 30, and 60 µM

CdSO₄. Representative photographs were taken 8 days after germination (DAG).

D, Root elongation in WT and *opc3* in response to Cd stress. Data represent the mean ± SE of three biological replicates, and asterisks indicate a significant difference compared with WT (Student's *t*-test; **P* < 0.05 or ***P* < 0.01).

Figure S8. RT-qPCR analysis of the *ZIP* members involved in Zn uptake.

A to E, Expression of the Zn transporter genes *AtZIP1*, *AtZIP3*, *AtZIP4*, *AtZIP5*, and *AtZIP9*, detected by RT-qPCR analysis in WT, *CaWRKY41-OE1*, and *CaWRKY41-OE4* plants at 0, 6, and 72 hours post treatment with Cd stress. The data represent the mean ± SE of three biological replicates. Different letters indicate significant differences compared to the control (Tukey's test; lowercase letters indicate *P* < 0.05 and uppercase letters indicate *P* < 0.01).

Figure S9. Cd inhibits *Ralstonia solanacearum* growth and *R. solanacearum* infection increases Cd uptake.

A, *CaWRKY41* expression detected by RT-qPCR analysis in pepper plants at the indicated time points post inoculation with *R. solanacearum* FJ150501.

B, *CaWRKY41* expression in *PYL-279* and *PYL-279-wrky41* pepper leaves at the indicated time points post inoculation with *R. solanacearum*. Relative expression of *CaWRKY41* in *PYL-279-wrky41* plants was compared to that of control (*PYL-279*) plants, which was set to 1.

C, *R. solanacearum* growth at 24 or 48 hours post treatment with Cd stress.

D to F, Expression of the defense marker genes *CaPRI*, *CaPR4*, and *CaNPRI*, detected by RT-qPCR analysis in pepper plants at 0, 24, and 48 hours post treatment with 10 or 50 μM Cd. The data represent the mean ± SE of three biological replicates, and asterisks indicate a significant difference compared to the control (Student's *t*-test; **P* < 0.05 or ***P* < 0.01).

G & H, Cd concentration in the roots and leaves of pepper plants at the indicated time points post inoculation with *R. solanacearum*, respectively. The data represent the mean ± SE of three biological replicates. Different letters indicate significant differences compared to the control (Tukey's test; lowercase letters indicate *P* < 0.05 and uppercase letters indicate *P* < 0.01).

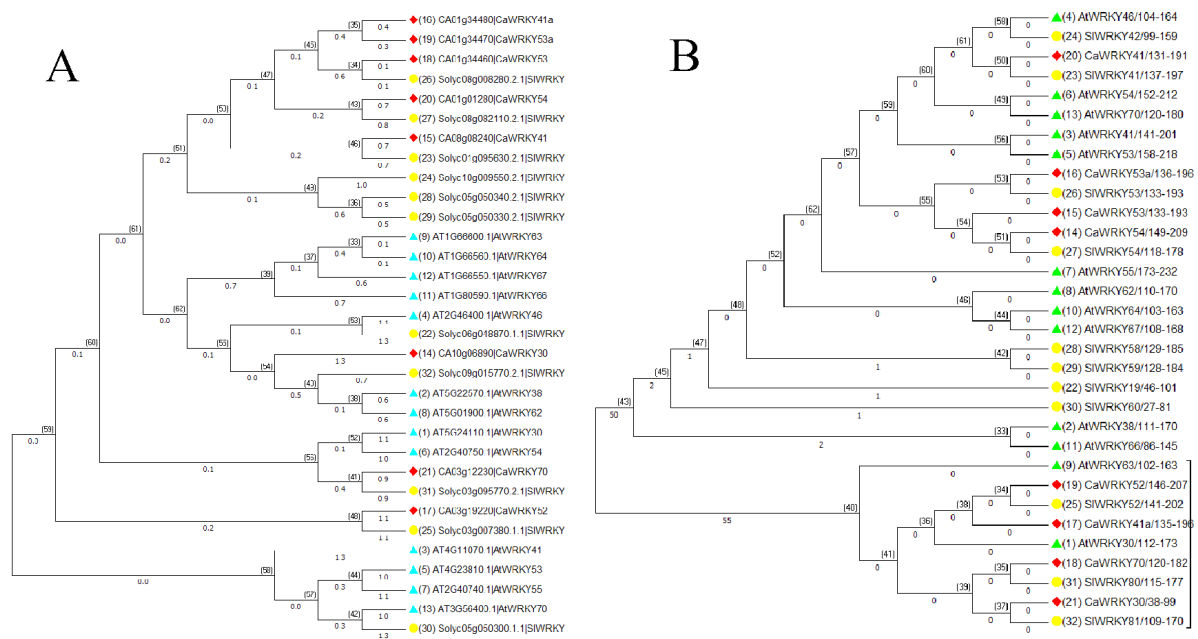


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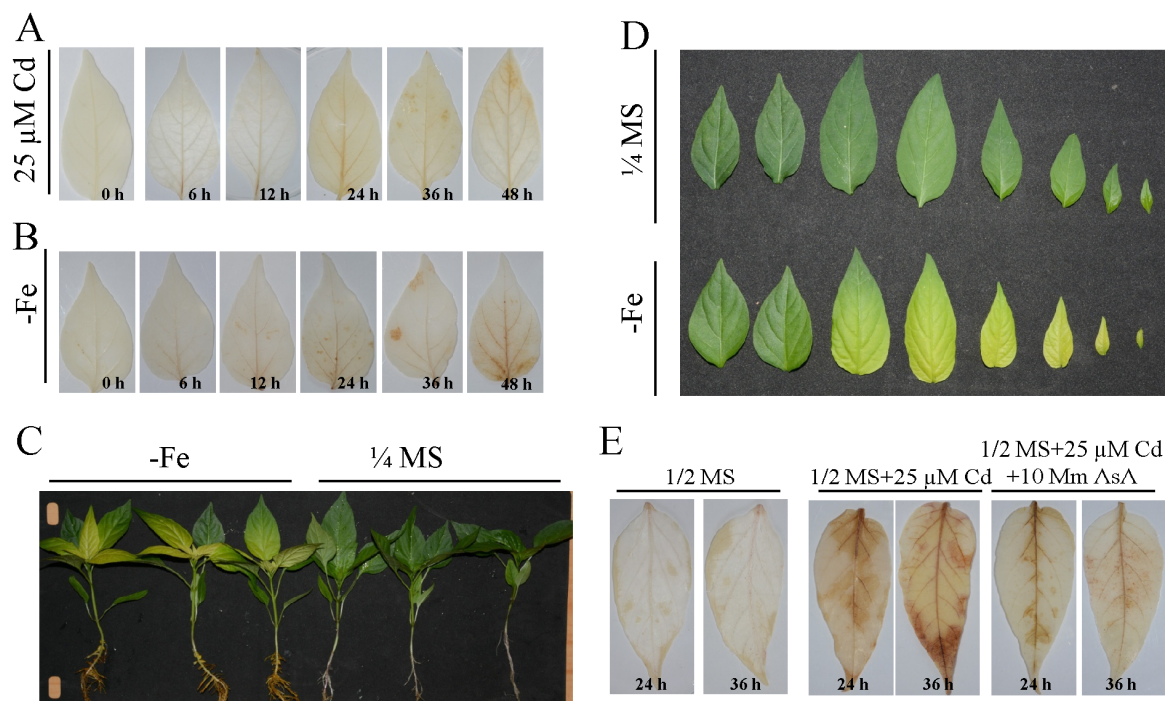
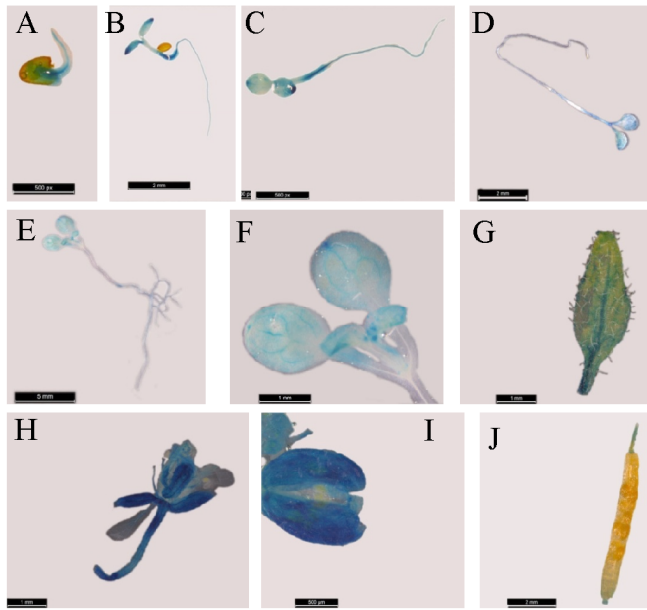


Figure S2. Cd stress and Fe deficiency promotes H₂O₂ accumulation.



K

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1  MEKVKVKGLEKKKLLISELTTQGGKEFVKKQ
76  CTGAAAAACAGATTGGCTTACCTGACCTGAAGATGTGATTTACTACTGGGAAAATTTCTCACTACTA
26  LKKKQIGFLASPEECCDLLLGGKILLSSL
151  160      170      180      190      200      210      220
51  GAGAAATATTCTCAATCTTAATTTGAGAGACTTCTCTCTGAGGTTGAAATATATCTTAATTAATCAATCT
51  EKSLSLILNLLKALLLEGGIINANNNSTS
226  235      245      255      265      275      285      295
76  TCATGTTCAATTCATTTCTTGGTAATAATAATAGTCCATGAGTGAAGTTTTGATTTCCCAAGTCATCAT
76  SSCSSISFLGNNSNPMSEVFDSPSHH
301  310      320      330      340      350      360      370
101  TTGGCAAAAATATGGTCTCCAGAGAGAGAAAGAAATCACAAAGGACTAATCAATAAATACCATTTCTGGGCGGGG
101  LDKNNHVSKKRKKSQETFHQITISGTG
376  385      395      405      415      425      435      445
126  CTTGAGGTTCAATGAGATGATTTAGTTGGAGAAATATGGCCGAGAGATATTTAGGGGCTAATCAATCCA
126  LEGSHEDGFFSNWRKYGQKDIILGANNHF
451  460      470      480      490      500      510      520
151  AGGGCTTATTATCGGTGCACACACAGGCATACACAGGGTGTGGCAACAACAAGTCCAAAGATCAGATGGA
151  RAYYRC THRHRTQGC L L A T K Q V Q R S D G
526  535      545      555      565      575      585      595
176  AACTCAACAATCTTTGAGGTAACATACAGAGAGGACAGTTCGCAAGCTGCACAATCAGATATTTCACTT
176  NSTIFEVTTYEGRHSCKAKAQSDIFSSL
601  610      620      630      640      650      660      670
201  AATATCAAAAACCCAGAAACACACAAAAACAAGAACAGAGATGGTAATTTCAACTCTACACAAACCAT
201  NNQKRQKHKKQE Q E N V I F N S T P N H
676  685      695      705      715      725      735      745
226  GCGCGAAGACTTCACATCCACACAAAGAGAGATTTTCACACCATTTTCATTCCTCCACTACACCCCTAAC
226  DAENFNITTKEEVFTPFSPPTPLN
751  760      770      780      790      800      810      820
251  CTAGAGAATTTGAAGAGACAAAATCTTTTGGTATCCATGGTCCATGTTAACGTCGCCAAAATCAAGATTT
251  LENIEETKFFCDSMVMVPLLTSQNKQEF
826  835      845      855      865      875      885      895
276  GGAATGACTACATGACTGCACTGCCAAATTCAGATCTCACTGAATTGATCTGCACCCGACCCCACTCG
276  GMDYMTLHCPNSDLTELEISTPTFTS
901  910      920      930      940      950      960      970
301  ATTCAAATTCCTATTTGGAGATTTGGATTTCTGAGATTTGAACTAATGTAATTTGACTTGA
301  ISNSSEVGDWDLSEDFEPNVIFDIE
976  985
326  GAGTCTTTAGTTAA
326  EFPFS*

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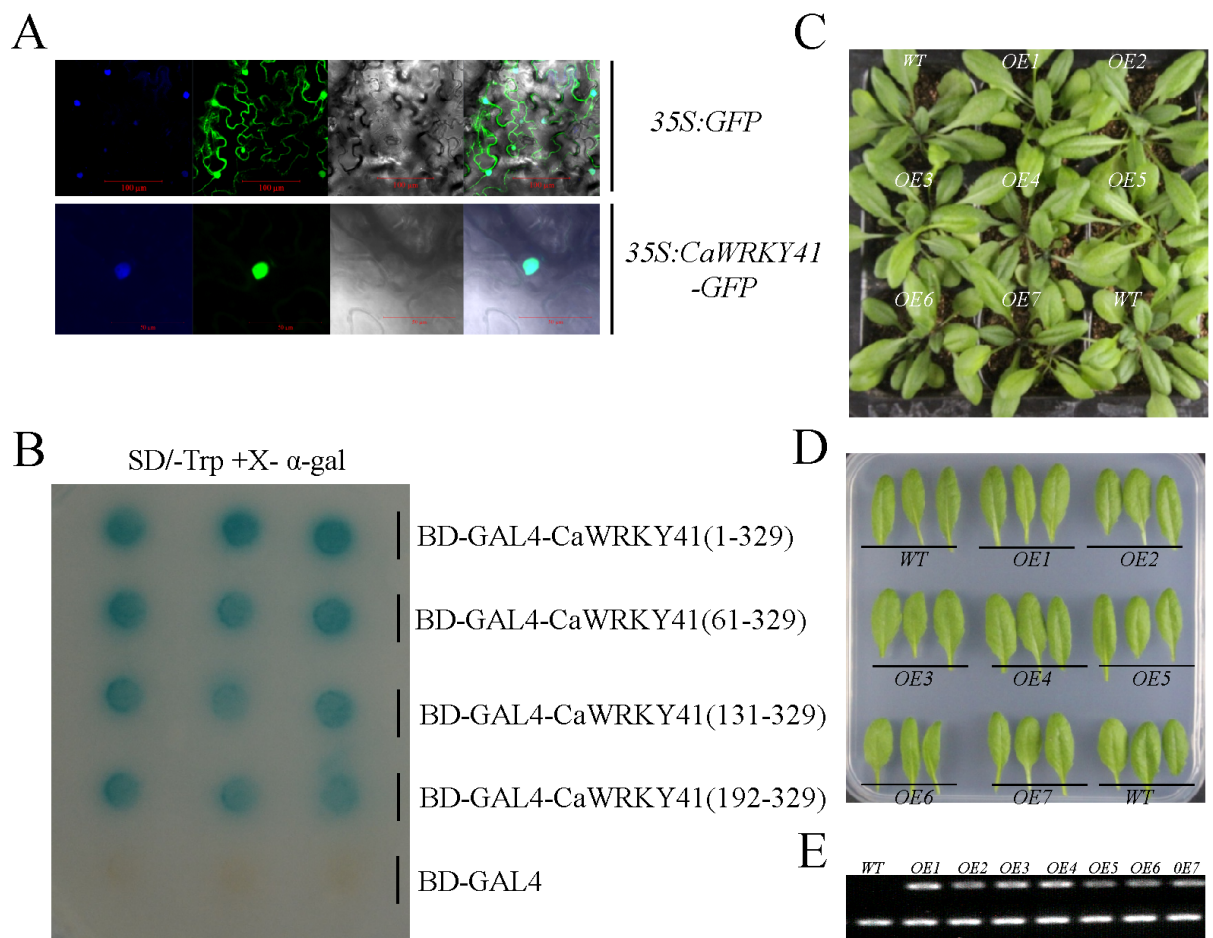


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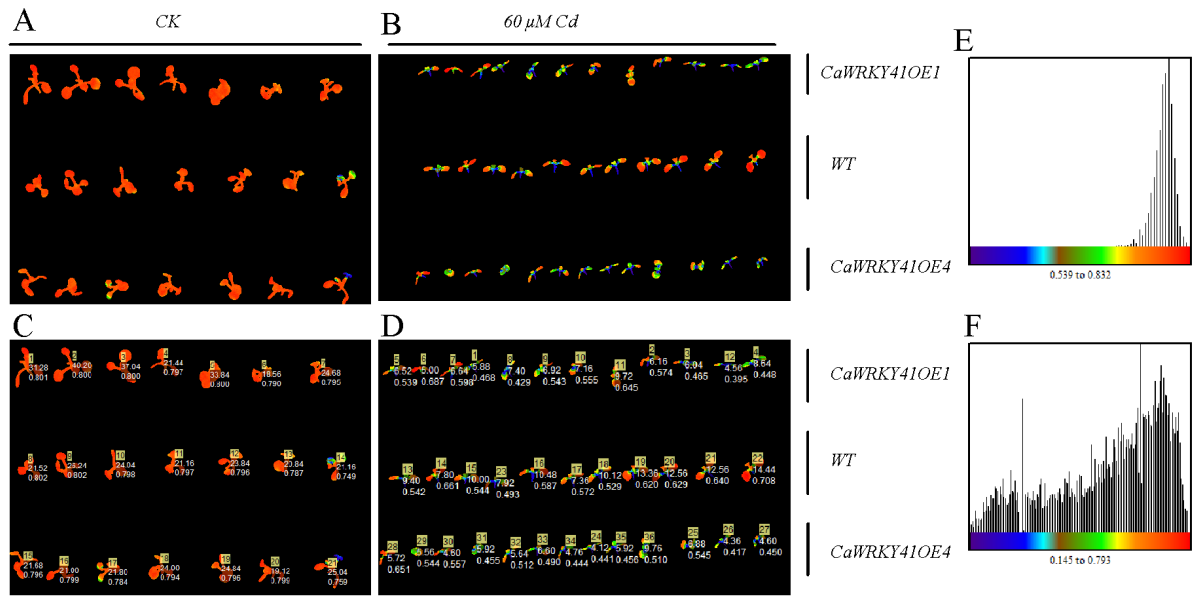


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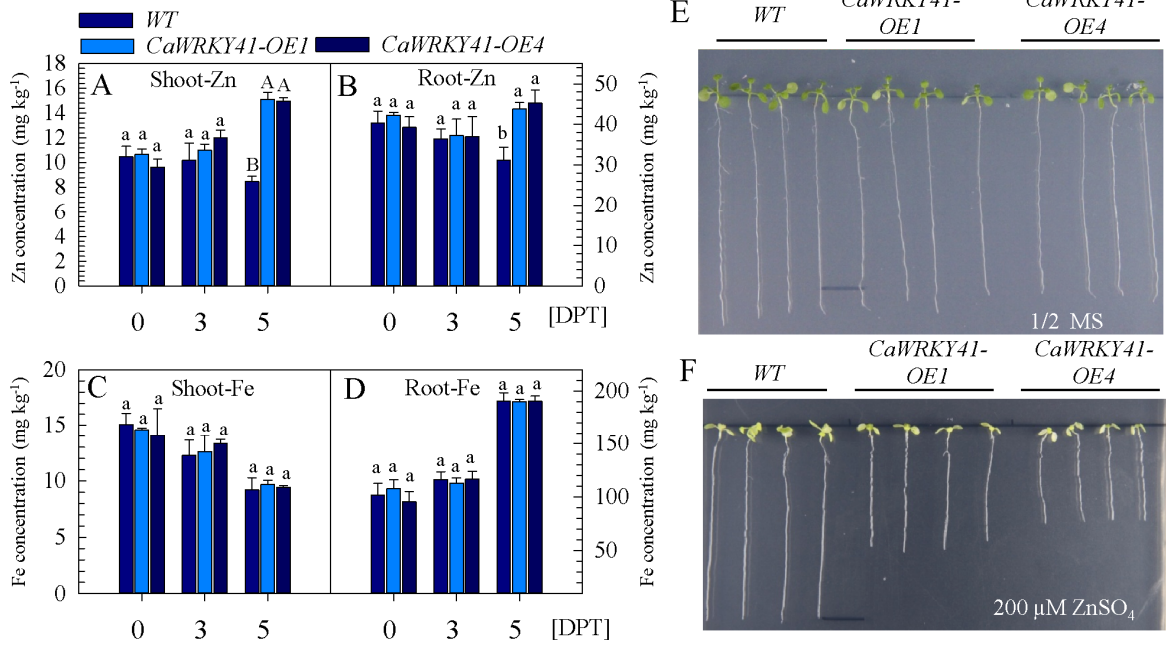


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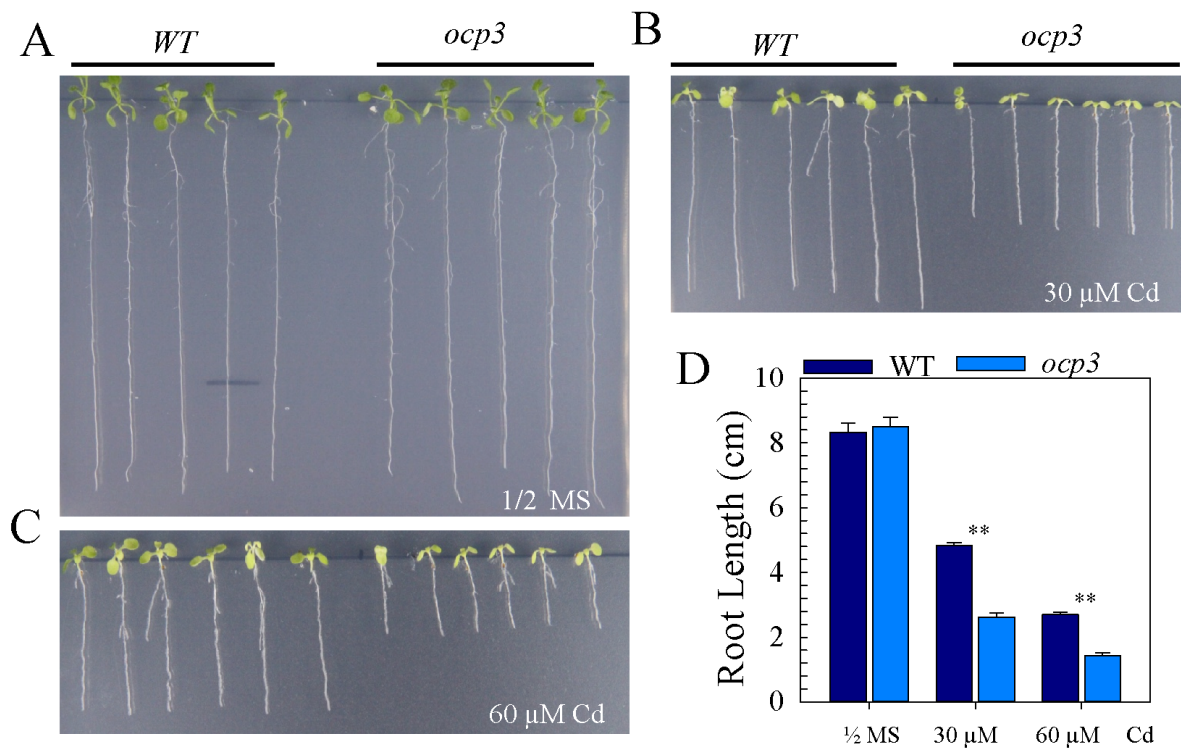


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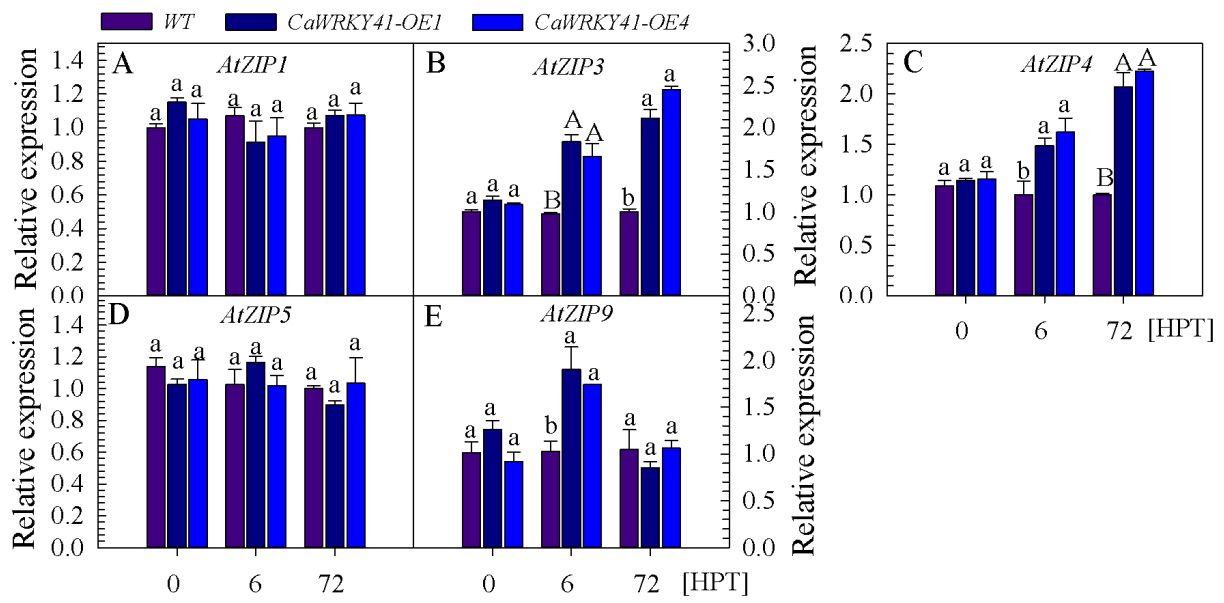


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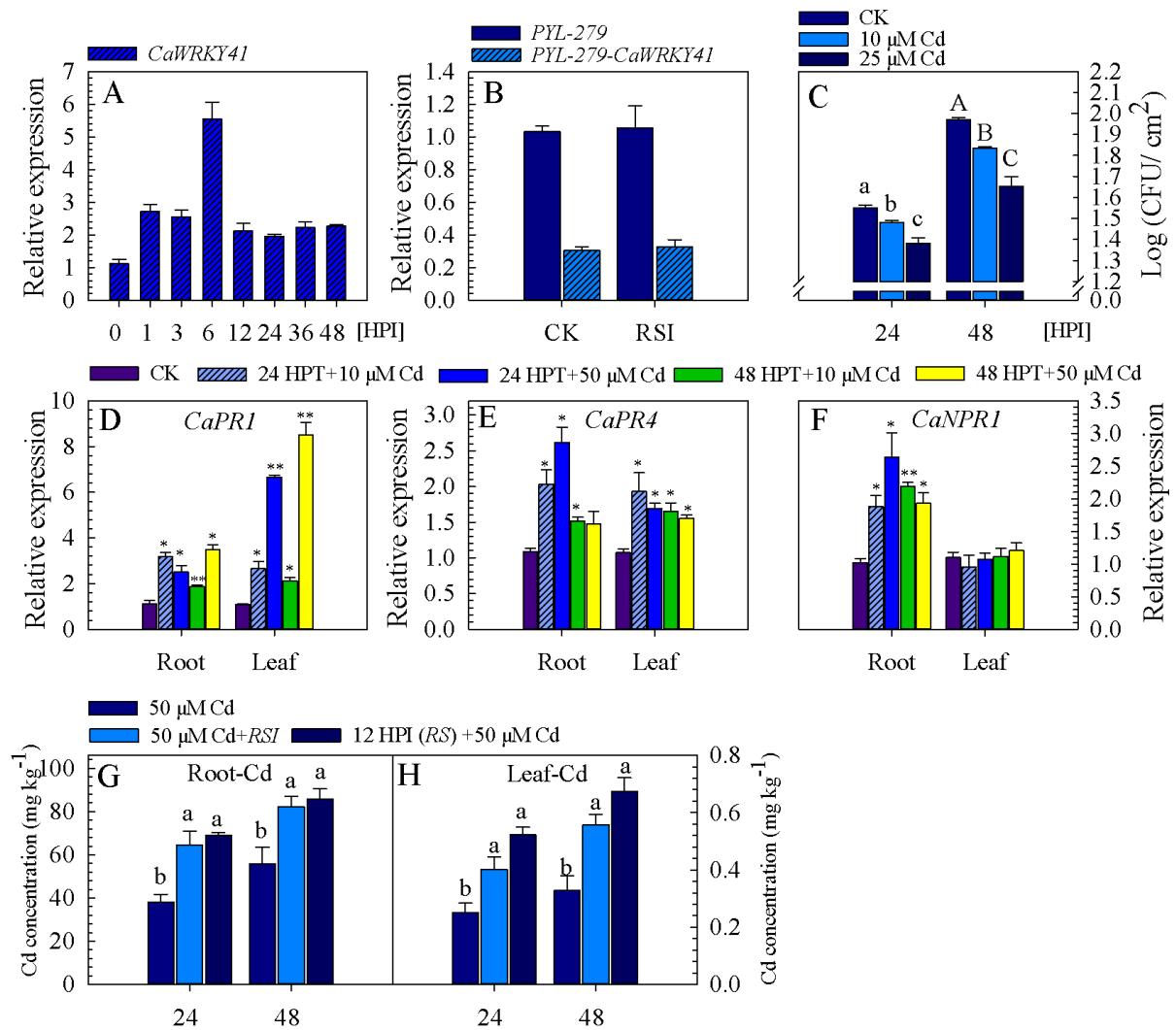


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