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 H_2O_2 accumulation.

A and B, H_2O_2 levels analyzed by DAB staining (brown) at the indicated time points after treatment with 25 μM $CdSO_4$ 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 H_2O_2 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 $CdSO_4$. 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 $ZnSO_4$. 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 *opc3* on ½ MS medium containing 0, 30, and 60 µM

$CdSO_4$. 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.

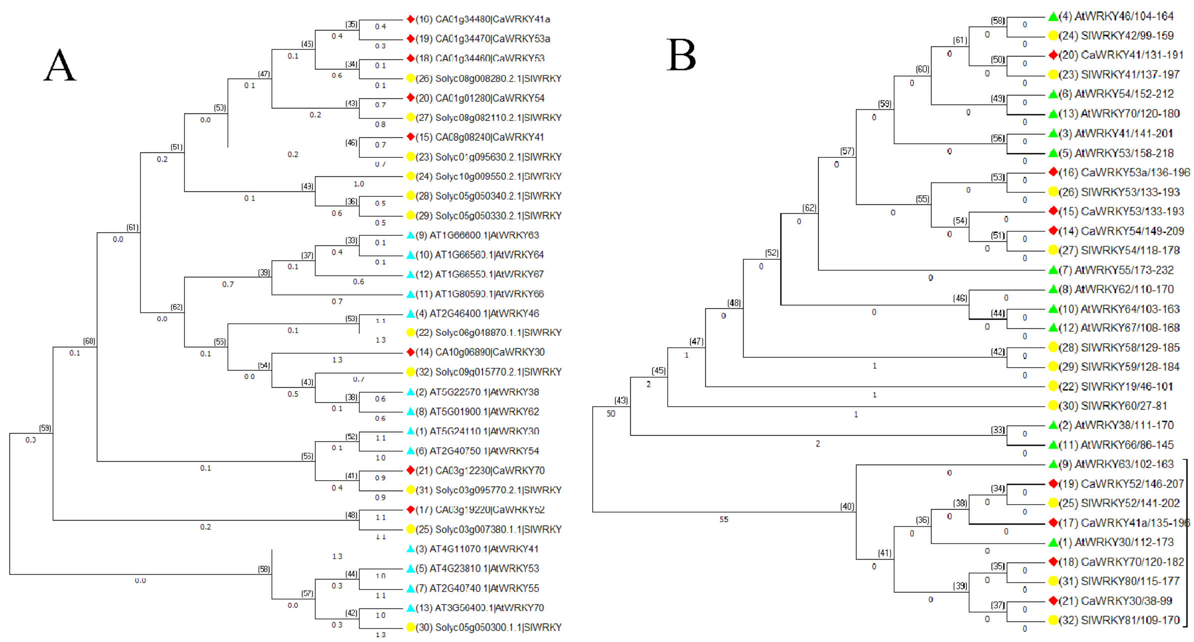
A, *CaWRKY41* expression detected by RT-qPCR analysis in pepper plants at the indicated time points post inoculation with *R. solanacearum* FJ150501.

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 *CaPR1*, *CaPR4*, and *CaNPR1*, 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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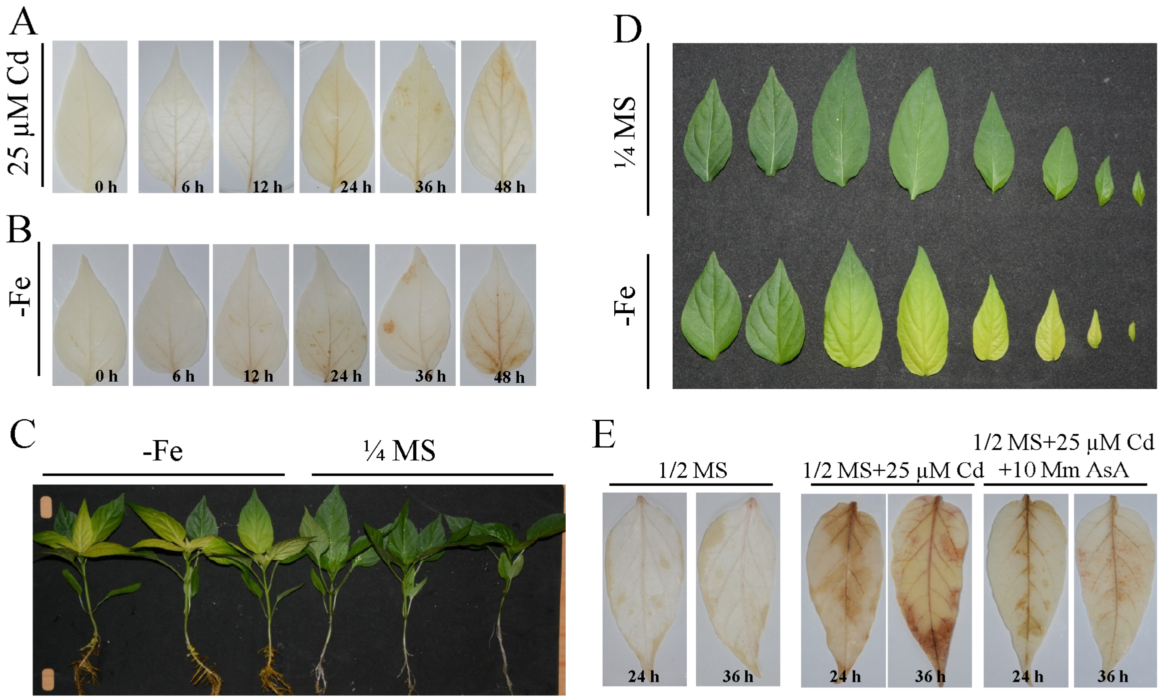

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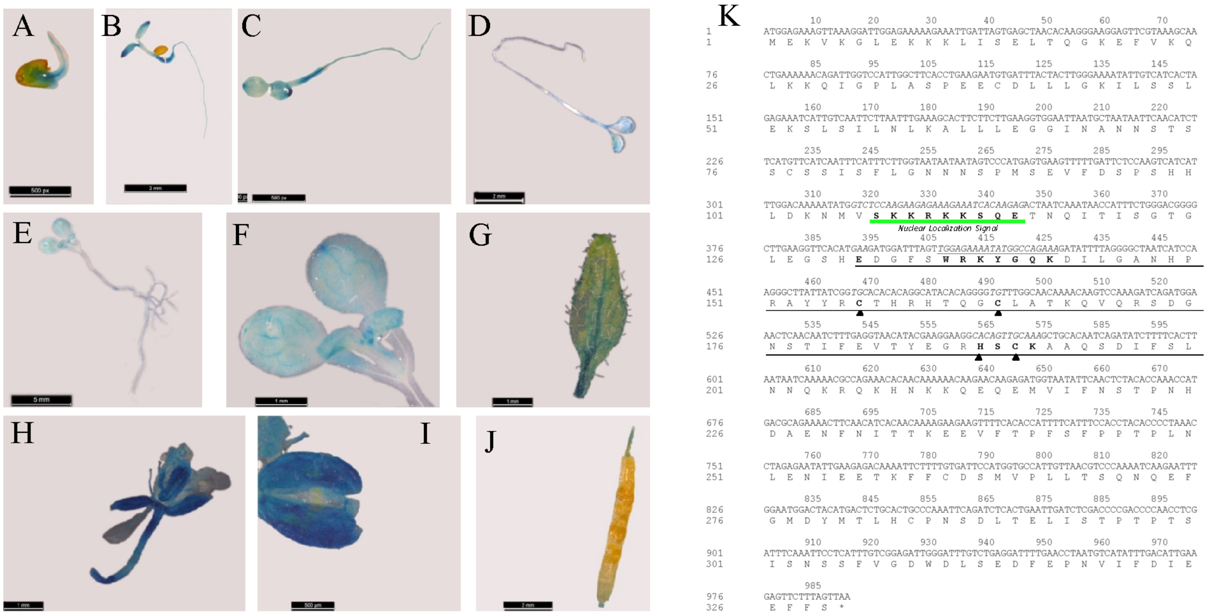

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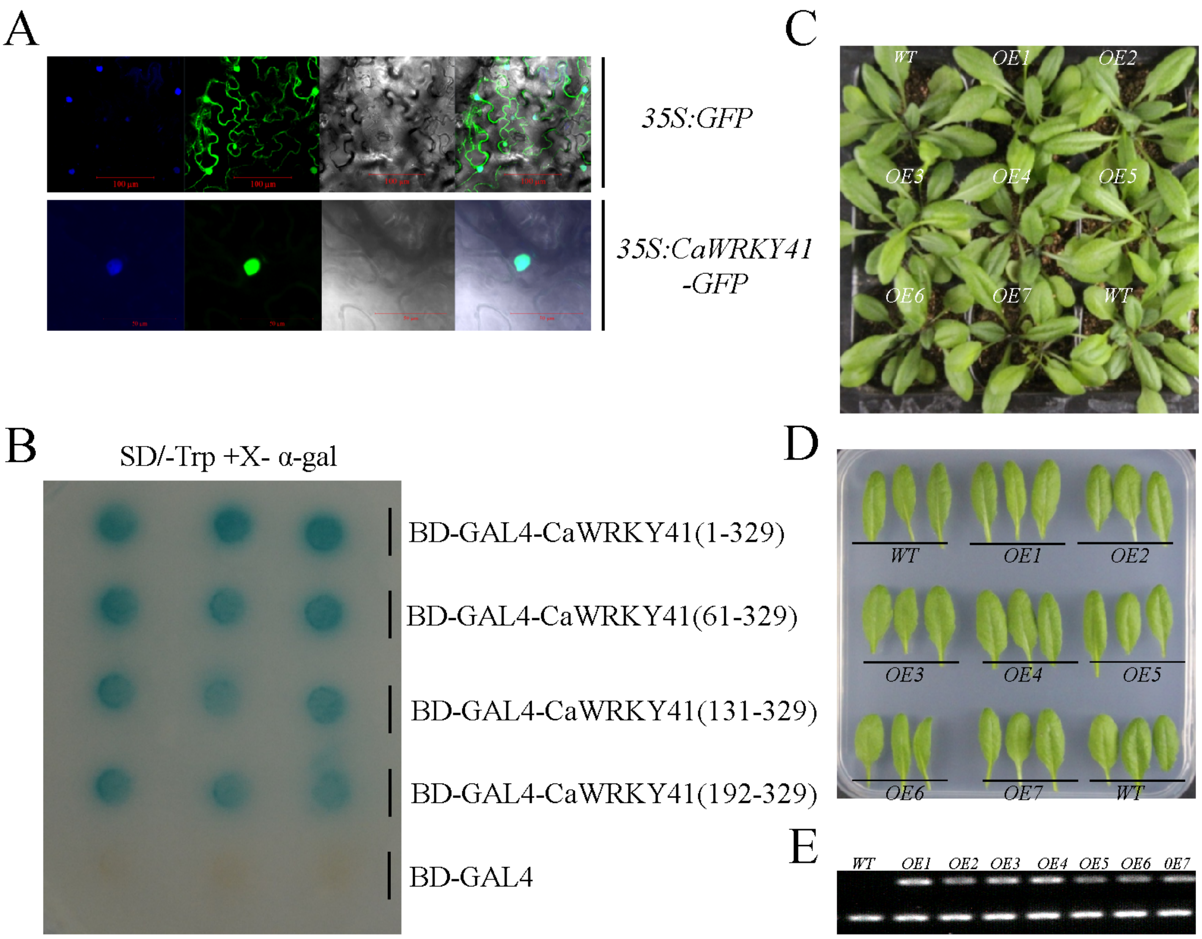

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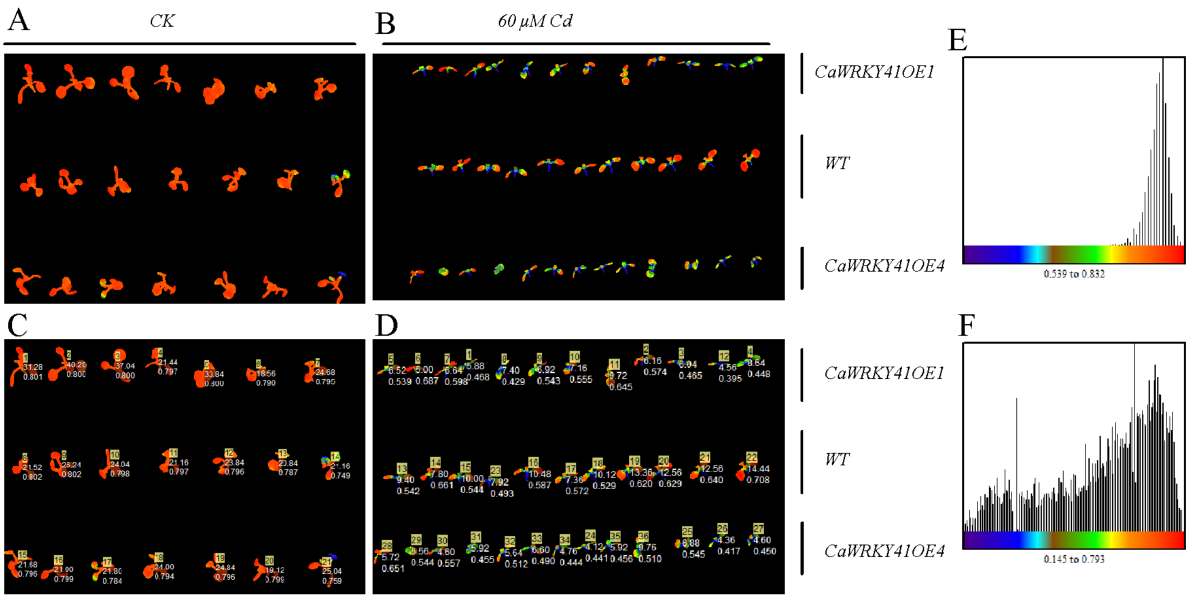

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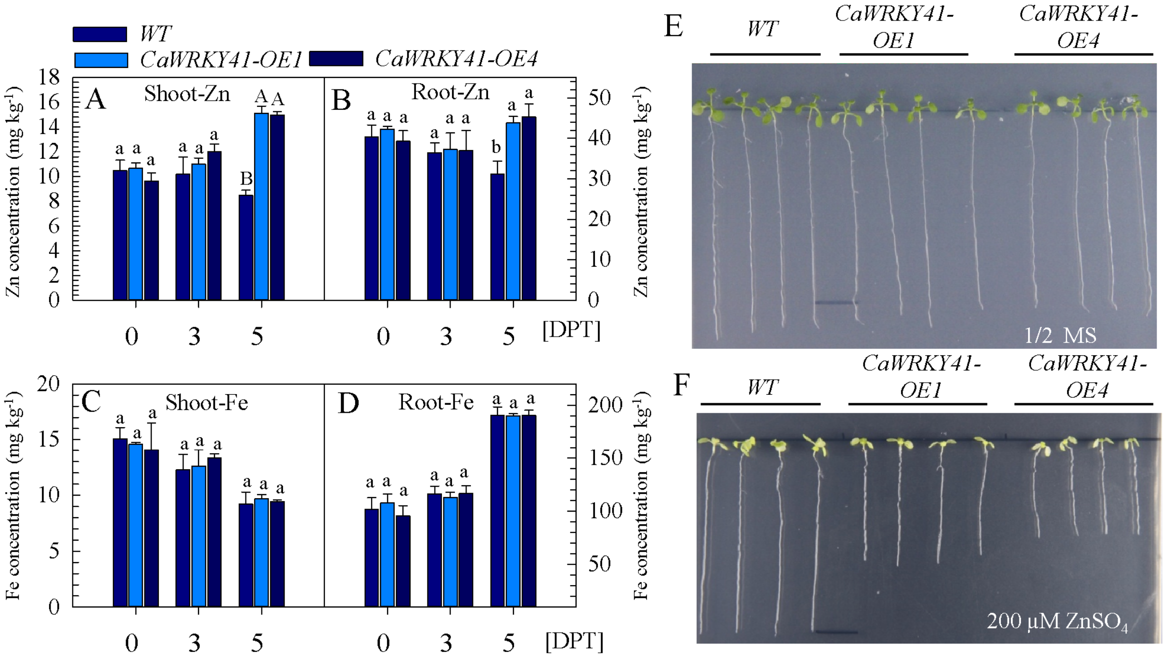

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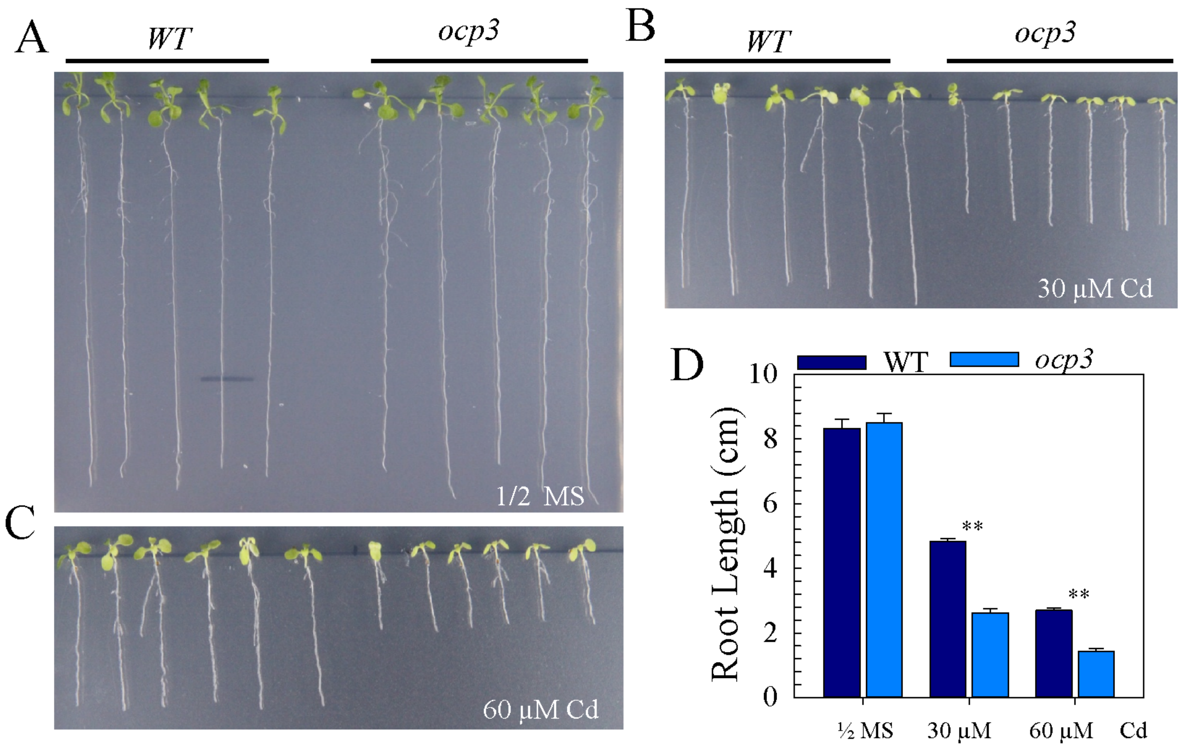

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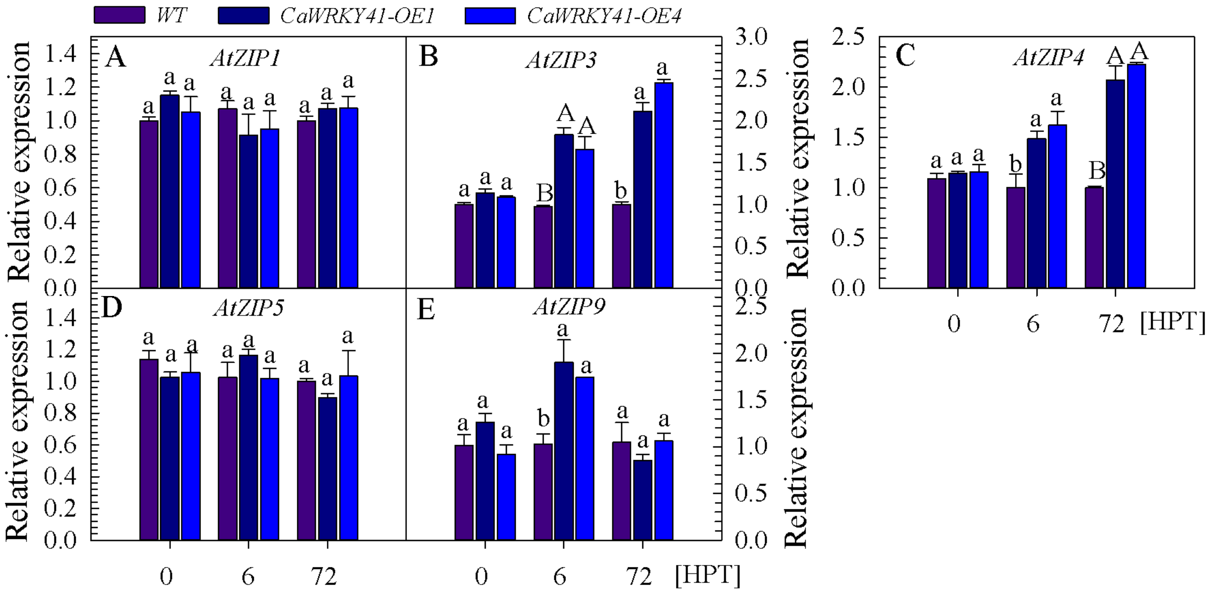

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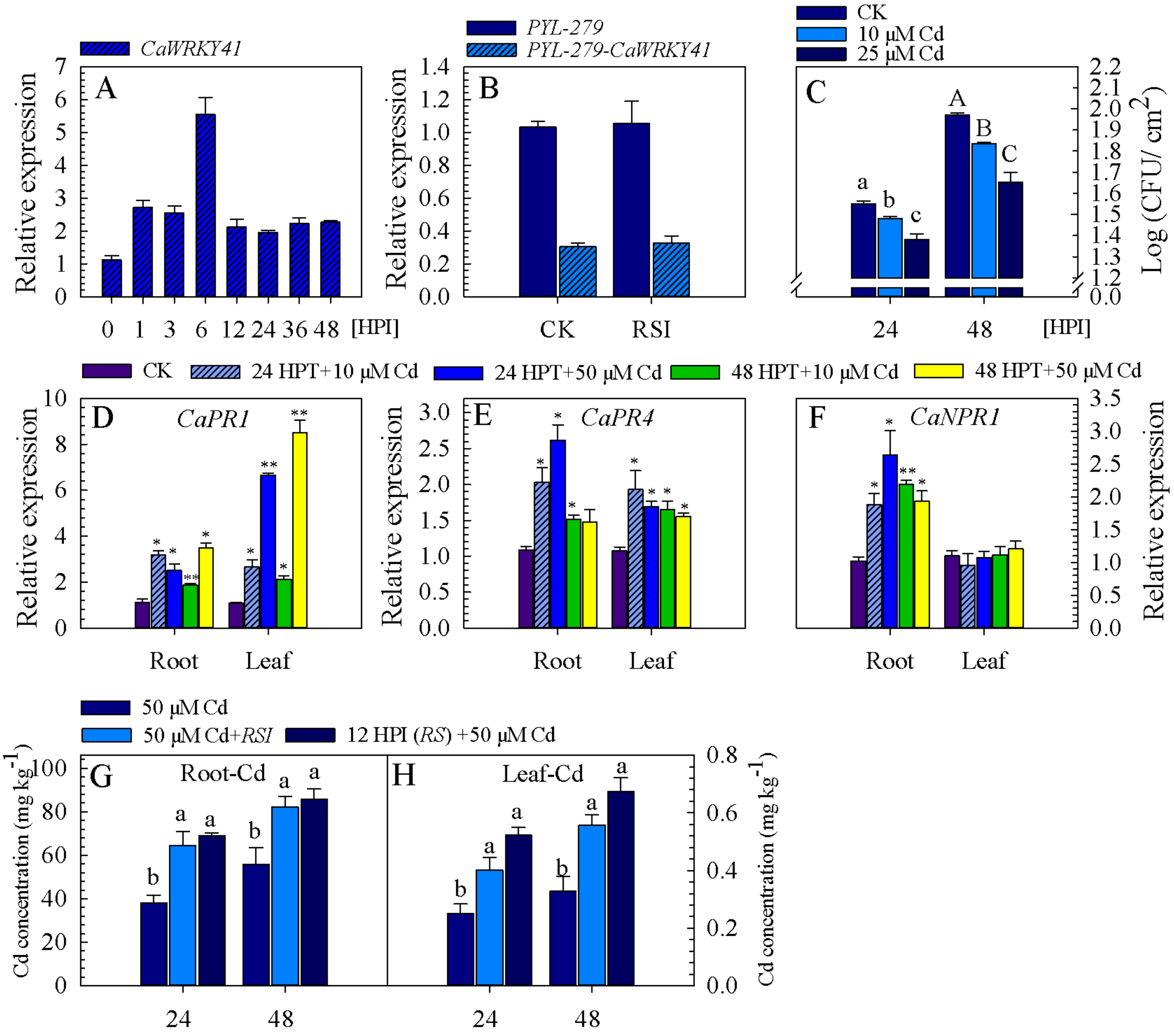

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