

RESEARCH ARTICLE

Open Access



RsWRKY15–*RsPDR12* module regulates Cd uptake and accumulation by promoting Cd efflux in radish (*Raphanus sativus* L.)

Shilin Ma¹, Zhihe Yang¹, Bingshuang Li¹, Liang Xu¹, Yan Wang¹, Yinbo Ma², Xiaoli Zhang¹, Jingxue Li¹, Kai Wang¹, Xinyu Zhang¹ and Liwang Liu^{1*}

Abstract

Cadmium (Cd), a non-essential and toxic heavy metal, accumulates in radish taproot and adversely affects radish safety and quality. Pleiotropic drug resistance (PDR) proteins play a crucial role in heavy metal uptake and accumulation in plants. However, the *PDR* gene-mediated regulation of Cd accumulation remains unexplored in radish. *RsPDR12* gene expression significantly increased under Cd stress. *RsPDR12* demonstrated Cd transport activity and decreased Cd accumulation in yeast cells, and its overexpression reduced root Cd concentration by Cd²⁺ efflux in *Arabidopsis*. *RsPDR12* alleviated Cd stress by enhancing membrane permeability and reactive oxygen species (ROS) scavenging in radish plants. RsWRKY15 was identified as the upstream regulatory factor of *RsPDR12*. Dual-luciferase assay demonstrated that RsWRKY15 bound to the *RsPDR12* promoter to activate its transcription. *RsWRKY15* expression and promoter activity were significantly induced under Cd stress. *RsWRKY15* overexpression mitigated oxidative damage and reduced root Cd concentration in radish and *Nicotiana benthamiana* plants, respectively. These findings advance understanding of the molecular mechanism underlying the RsWRKY15–*RsPDR12* mediated regulatory network of Cd accumulation and support the genetic improvement of low Cd-accumulation cultivars in radish breeding programs.

Keywords *Raphanus sativus*, Cd uptake and accumulation, *RsPDR12*, RsWRKY15, NMT

Core

Cd accumulates in the radish taproot, compromising the safe production of radish. *RsPDR12* demonstrated significant Cd transport activity and reduced Cd accumulation in yeast cells. *RsPDR12* overexpression decreased

Cd accumulation by mediating Cd²⁺ efflux in roots. RsWRKY15 binds to the *RsPDR12* promoter to activate its transcription. *RsWRKY15* overexpression reduces Cd accumulation by decreasing Cd²⁺ influx in roots under Cd Stress. The RsWRKY15–*RsPDR12* module regulates Cd accumulation by promoting Cd efflux in roots.

*Correspondence:

Liwang Liu
nauliulw@njau.edu.cn

¹ National Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Key Laboratory of Horticultural Crop Biology and Genetic Improvement (East China) of MOAR, Zhongshan Biological Breeding Laboratory, College of Horticulture, Nanjing Agricultural University, Nanjing 211800, People's Republic of China

² College of Horticulture and Landscape Architecture, Yangzhou University, Yangzhou 225009, People's Republic of China

Gene & accession numbers

Information of genes in this study can be found in the database (<https://bigd.big.ac.cn>) under the accession numbers: *RsPDR12*(Rsa1g010600); *RsWRKY15*(Rsa4g037000).



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Cadmium (Cd) is one of the most toxic heavy metal (HM) pollutants due to its long half-life and non-biodegradability. Its pervasive toxicity affects various tissues and organs in plants, animals and humans (Sheng et al. 2019; McLaughlin et al. 2021; Tang et al. 2024). Cd contamination has become a significant problem, severely impacting agricultural productivity by industrial and natural factors (Zhao et al. 2021; Yokosho et al. 2021). Under Cd stress, reactive oxygen species (ROS) are triggered in plants, resulting in cellular oxidative damage and disruption of physiological and biochemical processes (Song et al. 2017; Lu et al. 2020). Consequently, plants exhibit restricted growth, decreased photosynthetic rates, impaired respiration, leaf yellowing, inhibited root growth, and potential mortality (Han et al. 2019; Zhao et al. 2021). Understanding the molecular mechanisms of HM accumulation in plants is therefore essential for mitigating HM stress.

Under HM stress, specific transporters including ATP-binding cassettes (ABCs), P1B ATPases, and natural resistance-associated macrophage proteins (NRAMPs) respond and perform crucial functions in HM uptake, accumulation, and translocation (Ziegler et al. 2017; Liu et al. 2020; Ashraf et al. 2021; Zheng et al. 2020). ABC transporters function as essential membrane transport proteins (Kuromori et al. 2017; Gani et al. 2021). Pleiotropic drug resistance (PDRs) proteins, members of the ABCG subfamily of the ABC family, transport HMs and other substrates, including phytohormones and lipids (Lefèvre et al. 2018; Xie et al. 2021; Zhang et al. 2020). Compared with wild-type (WT) plants, *AtPDR8*-overexpressing (OE) *Arabidopsis* exhibited reduced Cd accumulation and decreased Cd content, while Cd accumulation increased in *pdr8* mutant plants (Kim et al. 2007). Lead (Pb) accumulation in *Arabidopsis* is regulated by the expression of *AtPDR12* and other enzyme-related genes (Fan et al. 2016). The *Arabidopsis pdr12* mutant shows sensitivity to Pb, while *AtPDR12* overexpression reduces Pb accumulation in plants (Lee et al. 2005). However, the molecular mechanism by which PDRs influence HM accumulation in radish taproots remains unclear.

Transcription factors (TFs), including MYBs, WRKYs, bZIPs, and HSFs, are essential components of the gene expression regulatory network under HM stress (Agarwal et al. 2006; Zhang et al. 2019, 2023a; Tang et al. 2019, 2024; Sheng et al. 2019; Han et al. 2019). WRKYs, containing one or two conserved WRKY domains—comprising the WRKYGQK core sequence and a C₂H₂- or C₂HC-type zinc finger motif in the N-terminal—constitute one of the largest TF families in higher plants (Rushton et al. 2010). WRKY TFs regulate plant responses to biotic and abiotic stresses by activating or

inhibiting the transcription of downstream functional genes by binding to the W-box [(T)(T)TGAC(C/T)] element in their target genes promoter sequences (Han et al. 2019). In *Arabidopsis*, Cd accumulation decreased with *AtWRKY13* overexpression, while increased in the *AtWRKY13* loss-of-function mutant. ChIP-qPCR and yeast one-hybrid (Y1H) assays demonstrated that *AtWRKY13* binds to the W-box region in the *AtPDR8* gene promoter, facilitating Cd²⁺ removal from the cytoplasm by activating *AtPDR8* transcription and positively regulating plant Cd accumulation (Sheng et al. 2019). Conversely, maize *ZmWRKY4* mediates the Cd stress response by positively regulating the expression of *ZmSOD4* and *ZmAPX* genes (Hong et al. 2017).

Radish (*Raphanus sativus* L.), a member of the Brassicaceae family, is an economically significant root vegetable crop. Under Cd stress, the radish taproot accumulates more Cd compared with other organs (Xu et al. 2012; Sheng et al. 2019). Because the taproot constitutes the main edible portion of radish, Cd accumulation presents a potential risk to human health by food chain contamination (Zhao and Wang 2020). *PDR* genes play a crucial role in plant HM absorption and accumulation. Although *PDR* gene family members can reduce Cd content under Cd stress, the biological function and regulatory mechanism governing Cd uptake and accumulation in radish remain unclear. Pb treatment can alter the methylation levels of *RsPDR12* in radish, affecting its expression (Tang et al. 2021). Previous transcriptome data revealed significant induction of *RsPDR12* gene expression under Cd stress (Zhang et al. 2023b). We evaluated *RsPDR12* expression under Cd stress by Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) analysis and investigated its function in yeast, *Arabidopsis*, and radish. The upstream TF *RsWRKY15*, which regulates *RsPDR12*, was identified using a Y1H library to examine the transcriptional regulatory network of the TF-*RsPDR12* module under Cd stress. In addition, *RsWRKY15* function under Cd stress was characterized in *N. benthamiana* and radish, leading to the proposal of the *RsWRKY15*-*RsPDR12* module as a regulator of Cd uptake and accumulation in radish taproots. These findings illuminate the *PDR*-mediated regulatory network under Cd stress in radish, contributing to Cd uptake and accumulation and supporting the development of low Cd-accumulation cultivars in radish breeding programs.

Results

Expression profiling and promoter activity analysis of *RsPDR12* under Cd treatment

Consistent with transcriptome data, RT-qPCR analysis revealed that *RsPDR12* expression levels in the radish taproot were induced and peaked at 24 h under Cd

treatment (Fig. 1A). The coding sequence (CDS) of *RsPDR12* comprised 4245 bp and encoded 1414 amino acids, exhibiting 93.53% similarity with AtPDR12 (Fig. S1). Subcellular localization analysis revealed that the GFP-tagged *RsPDR12* protein localized to the plasma membrane (Fig. 1B).

The *RsPDR12* promoter-driven GUS activity demonstrated greater intensity compared with control under Cd treatment (Fig. 1C). The luciferase (LUC) signal of the *RsPDR12* promoter increased upon leaf exposure to Cd treatment (Fig. 1D and E), and the relative LUC intensity

of the *RsPDR12* promoter showed significant enhancement (Fig. 1F), indicating marked activation of *RsPDR12* promoter activity under Cd stress.

Cd transport activity of *RsPDR12* in yeast cells

To investigate the Cd transport activity of *RsPDR12* in yeast, the empty vector and *RsPDR12*-pYES2 were transformed into yeast strain BY4741. In the presence of the transcription inhibitor glucose, no significant growth differences were observed between yeast cells containing the empty vector and those expressing *RsPDR12*,

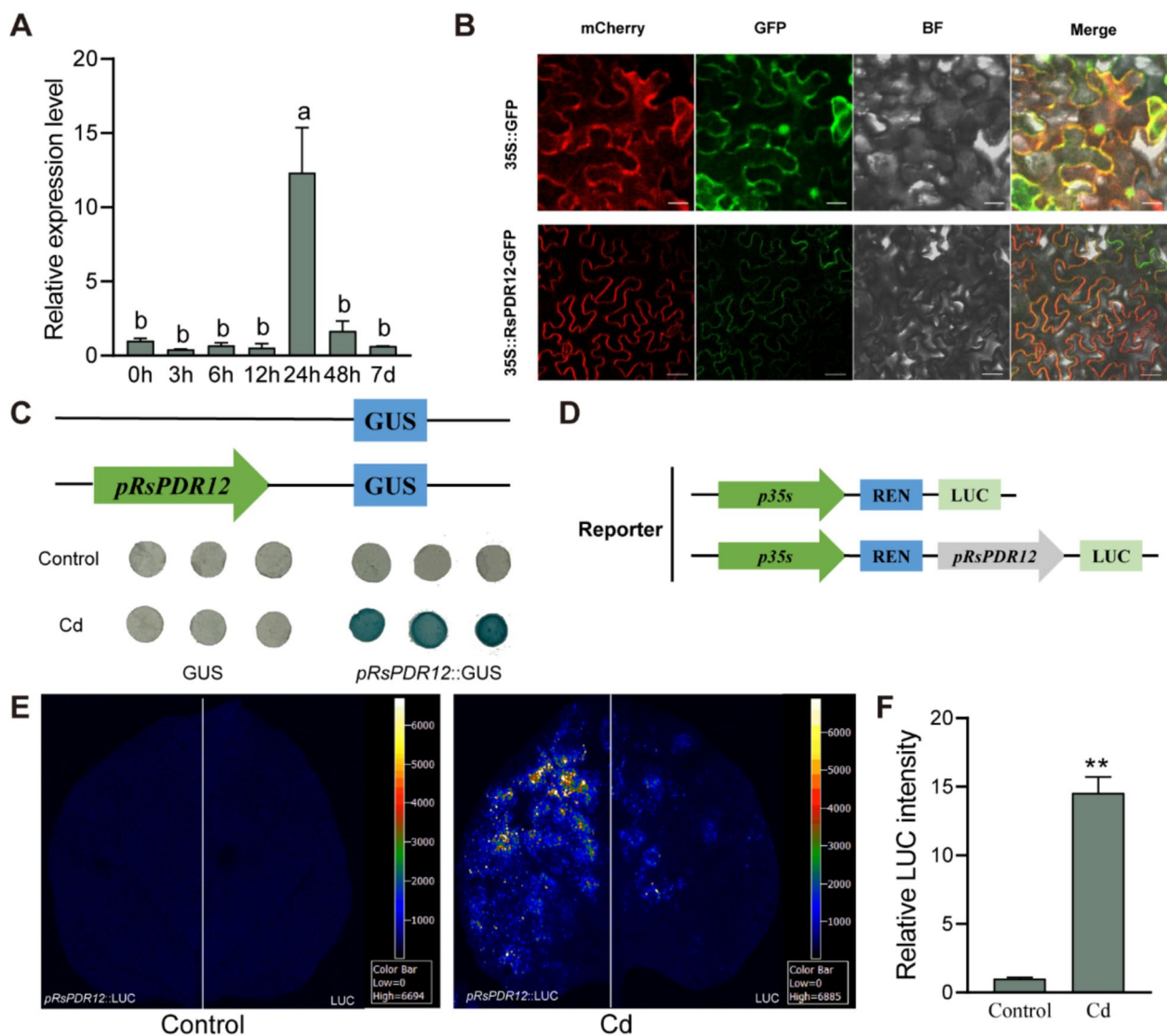


Fig. 1 Characterization and promoter activity analysis of *RsPDR12*. **A** Expression profiling of the *RsPDR12* gene in radish roots under Cd treatment (0.1 mM CdCl₂). Different letters above the bars indicate significant differences ($P < 0.05$). **B** The subcellular localization of *RsPDR12* in *N. benthamiana* leaves. The PM-mCherry was used as a membrane marker. Scale bar = 50 μm. **C** GUS staining analysis of *pRsPDR12* in *N. benthamiana* leaves under Cd treatment (0.27 mM CdCl₂, 8 h). **D** Structures of the report vector. **E** LUC assay of *pRsPDR12* in *N. benthamiana* leaves under Cd treatment (0.27 mM CdCl₂, 8 h). **F** Relative LUC intensity of the *pRsPDR12* promoter under Cd treatment. Data are the mean ± SD of three replicates (t -test; ** $P < 0.01$)

and growth was inhibited as the CdCl_2 concentration increased (Fig. 2A). In the presence of the transcription inducer galactose, no significant growth differences were observed between yeast cells with pYES2 and *RsPDR12*-pYES2 under normal conditions. However, under 55 and 82 μM CdCl_2 treatments, yeast cells carrying the empty vector exhibited significantly inhibited growth compared with those expressing *RsPDR12* (Fig. 2A). The Cd content was lower in yeast cells expressing *RsPDR12* than in cells with the empty vector after 0.27 mM CdCl_2 treatment for 12 h (Fig. 2B), demonstrating that *RsPDR12* exhibits Cd transport activity and decreases Cd accumulation in yeast.

RsPDR12 overexpression reduces Cd accumulation

To examine the function of *RsPDR12* in *Arabidopsis* under Cd stress, the overexpression lines *RsPDR12*-OE-9 and *RsPDR12*-OE-18 were selected for further analysis (Fig. 3A). Under normal conditions, both *RsPDR12*-OE lines and WT plants demonstrated normal growth on Murashige and Skoog (MS) plates. However, under Cd treatment, the *RsPDR12*-OE-9 and

RsPDR12-OE-18 lines displayed longer root length, higher fresh weight, and lower Cd content compared with WT (Fig. 3B–E). Dithizone staining revealed that *RsPDR12*-OE-9 and *RsPDR12*-OE-18 lines exhibited fewer red–black Cd–dithizone precipitates than WT under Cd treatment (Fig. 3F). Thus, *RsPDR12* overexpression enhances growth and reduces Cd accumulation in *Arabidopsis*.

The Cd-fluorescent probe assay revealed lower fluorescence in the root systems of *RsPDR12*-OE lines compared with WT (Fig. 3G), indicating reduced Cd concentration in *RsPDR12*-OE lines. In addition, non-invasive micro-test technology (NMT) measured the dynamic changes in Cd^{2+} fluxes in the root elongation zone (Fig. 3H). The transient and average Cd^{2+} fluxes in *RsPDR12*-OE roots were positive (efflux), while those in WT roots were negative (influx), indicating that *RsPDR12* promotes Cd^{2+} efflux from plant roots under Cd treatment (Fig. 3I and J). These results suggest that *RsPDR12* reduces Cd uptake and accumulation in plants by facilitating Cd^{2+} efflux under Cd stress.

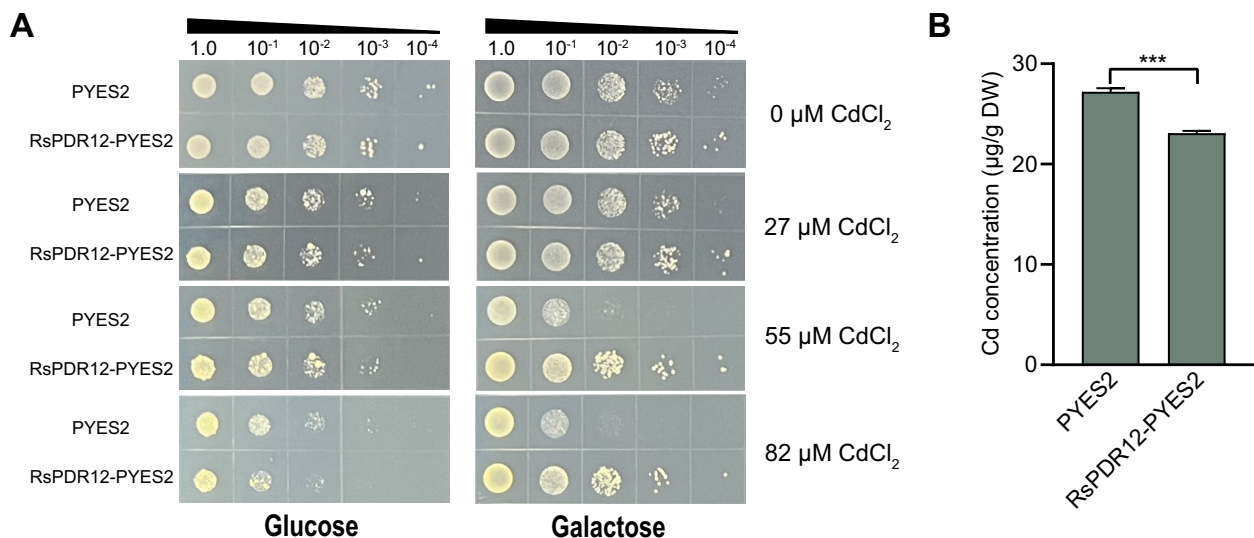


Fig. 2 Transport ability of the *RsPDR12* gene under Cd treatment. **A** Dot assay of yeast cells expressing *RsPDR12*-pYES2 or the empty vector (pYES2) on medium containing glucose, galactose, and Cd. **B** Cd concentrations in yeast expressing *RsPDR12* under Cd treatment (0.27 mM CdCl_2) (t-test; *** $P < 0.001$)

(See figure on next page.)

Fig. 3 *RsPDR12* reduces Cd accumulation in *Arabidopsis* plants. **A** Expression level of *RsPDR12* in WT and *RsPDR12*-OE lines. **B** Phenotype changes in WT and *RsPDR12*-OE lines under Cd treatment (50 μM CdCl_2). Bar = 1 cm. **C** Changes in the root length and **(D)** fresh weight. **E** Cd content of WT and *RsPDR12*-OE lines under Cd treatment (50 μM CdCl_2). Data are presented as the mean $\pm$ SD, $n = 3$. Bars with different lowercase letters are significantly different at $P < 0.05$. **F** Dithizone staining of WT and *RsPDR12*-OE lines under Cd treatment (50 μM CdCl_2). **G** Cadmium content detection of *RsPDR12*-overexpressing *Arabidopsis* lines with Cd-fluorescent analysis. Bar = 50 μm . **H** Testing position of the probe in the roots. **I** Transient Cd^{2+} fluxes measured every 6 s in 14-d-old seedlings of *RsPDR12*-overexpressing *Arabidopsis* and WT exposed to 0.1 mM Cd for 8 h. **J** Average Cd^{2+} fluxes for 5 min. Data are the mean $\pm$ SD ($n = 5$). Bars indicate significant differences between the roots of *RsPDR12*-OE *Arabidopsis* and WT at **** $P < 0.0001$

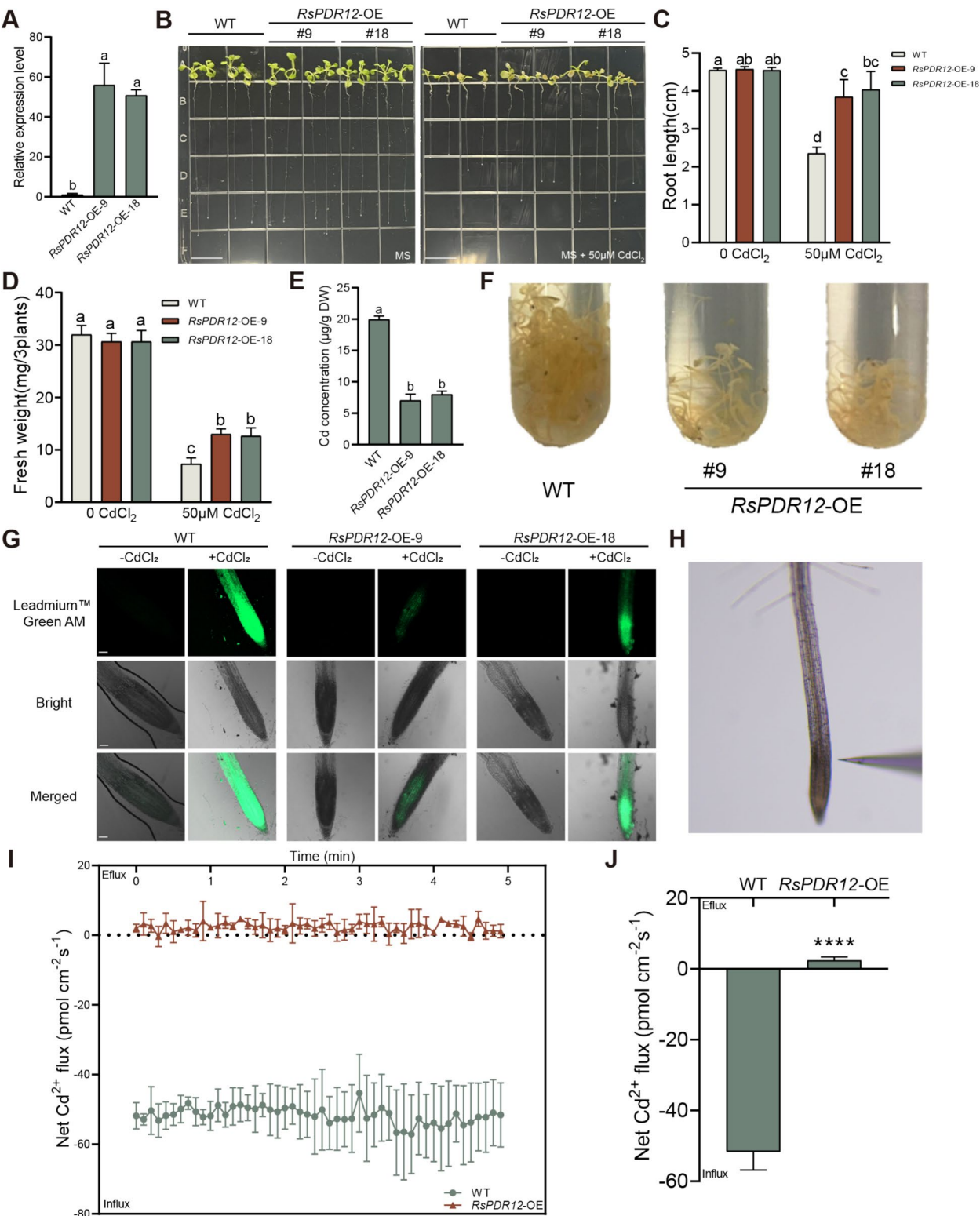


Fig. 3 (See legend on previous page.)

ROS accumulation was reduced in *RsPDR12*-OE radish

RsPDR12 was transiently overexpressed and silenced in radish cotyledons (Fig. 4A). 3,3'-Diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) staining showed no significant differences among EV, *RsPDR12*-OE, and *RsPDR12*-RNAi cotyledons under normal conditions (Fig. 4B and C). Under Cd stress, *RsPDR12*-OE cotyledons displayed lighter histochemical staining compared with EV cotyledons, whereas *RsPDR12*-RNAi cotyledons exhibited deeper staining than EV cotyledons (Fig. 4B and C).

The levels of hydrogen peroxide (H_2O_2), superoxide (O_2^-), malondialdehyde (MDA) and proline were not significantly different among EV, *RsPDR12*-OE and *RsPDR12*-RNAi cotyledons under normal conditions (Fig. 4D–G). However, under Cd treatment, the levels of H_2O_2 , O_2^- , and MDA in *RsPDR12*-OE cotyledons were lower than those in EV cotyledons, while they were significantly elevated in *RsPDR12*-RNAi cotyledons (Fig. 4D–F). Conversely, proline content was significantly increased in *RsPDR12*-OE cotyledons and decreased in *RsPDR12*-RNAi cotyledons under Cd stress (Fig. 4G).

These results demonstrate that *RsPDR12* overexpression reduces ROS accumulation and membrane permeability, presumably due to decreased Cd uptake and accumulation under Cd stress in radish.

RsWRKY15 binds to the *RsPDR12* promoter and activates its transcription

To investigate the regulation of *RsPDR12* under Cd stress, a 510-bp upstream promoter sequence of *RsPDR12* was isolated, and the direct upstream activator was identified using Y1H screening against a radish cDNA library (Fig. S2). The TF *RsWRKY15* was identified by the Y1H library approach. The Y1H assay subsequently confirmed *RsWRKY15* binding to the FL and S2 fragments of the *RsPDR12* promoter. Notably, three tandem repeats of the W-box (W1; core sequence: GAGTCACA) significantly activated the lacZ reporter gene. However, the interaction ceased when the W-box sequence was altered from GTCA to ATCC, indicating that *RsWRKY15* functions as an upstream regulator of *RsPDR12* by directly binding to the specific W1 in its promoter region (Fig. 5A). DLA results demonstrated that the LUC signal and activity significantly increased when *35S::RsWRKY15*

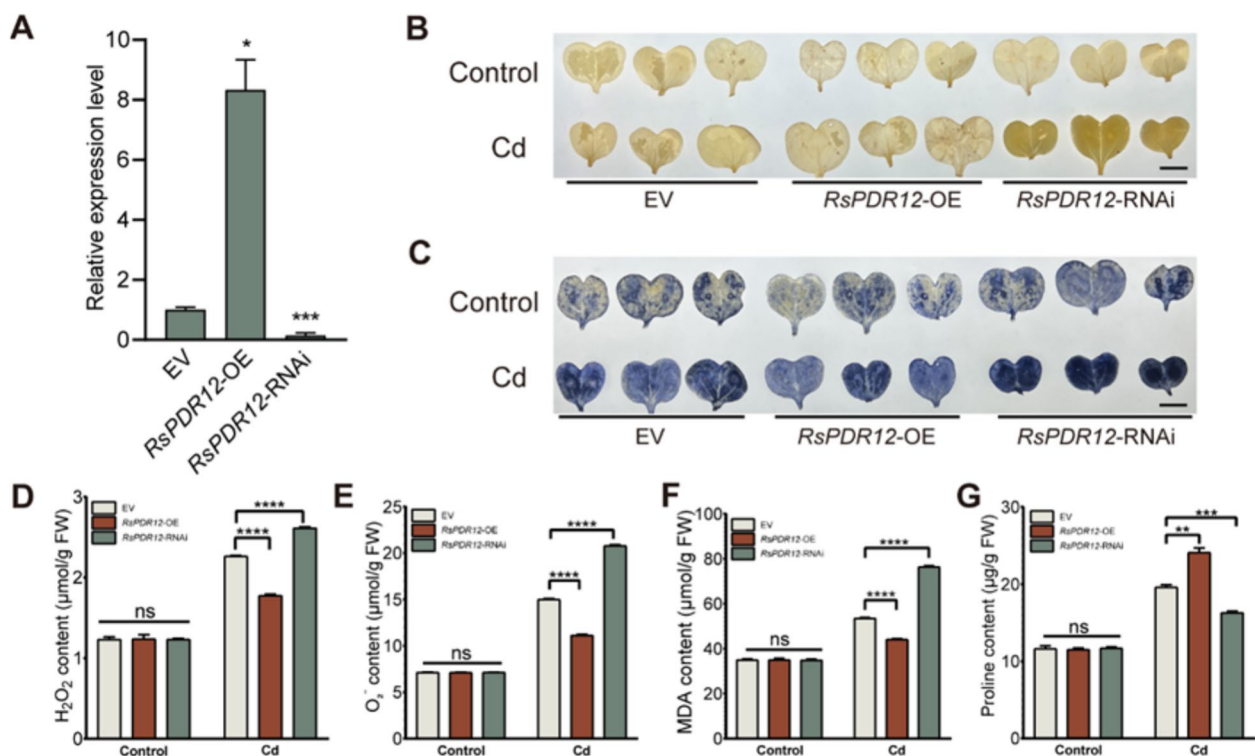


Fig. 4 ROS accumulation was reduced in *RsPDR12*-overexpressing radish. **A** Expression profile of *RsPDR12* in *RsPDR12*-OE and *RsPDR12*-RNAi radish cotyledons. **B** DAB and **C** NBT staining of radish cotyledons transiently infected with EV, *RsPDR12*-OE, and *RsPDR12*-RNAi under control and Cd stress (0.27 mM CdCl_2 , 8 h). Scale bar = 1 cm. **D** H_2O_2 , **E** O_2^- , **F** MDA, and **G** proline content in EV, *RsPDR12*-OE, and *RsPDR12*-RNAi radish plants under control conditions and Cd stress (0.27 mM CdCl_2 , 8 h). Data are the mean $\pm$ SD of three replicates (t-test; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant)

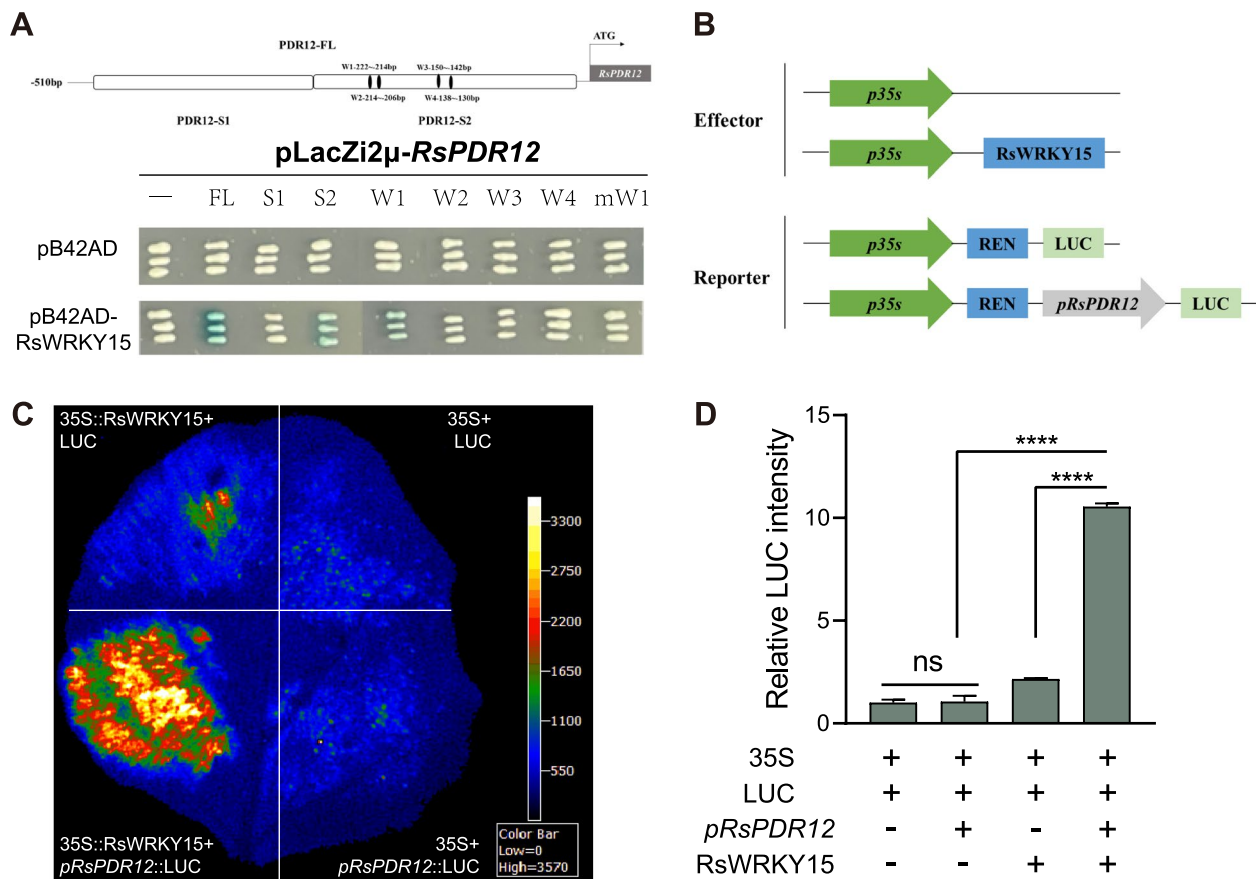


Fig. 5 RsWRKY15 activates *RsPDR12* transcription by directly binding to its promoter. **A** RsWRKY15 binds to the W1 motifs of *RsPDR12* promoter in a Y1H assay. **B** Generation of the effector and reporter constructs for DLA. **C** Transient expression assays of the empty vector, RsWRKY15, and *RsPDR12* promoter in *N. benthamiana* leaves. **D** Relative LUC intensity from the empty vector, RsWRKY15, and *RsPDR12* promoter. Data are the mean $\pm$ SD of three replicates (t-test; **** $P < 0.0001$; ns, not significant)

was co-expressed with *pRsPDR12::LUC* (Fig. 5C and D), confirming RsWRKY15 as an upstream TF regulating *RsPDR12* gene expression.

Expression profiling and promoter activity of the *RsWRKY15* gene under Cd treatment

The complete CDS of *RsWRKY15* is 963 bp and encodes 320 amino acids, containing a conserved WRKYGQK domain and a C₂H₂ zinc finger motif at the C terminus (Fig. S3), both characteristic features of the WRKY TF family. RT-qPCR analysis revealed that the *RsWRKY15* expression level reached its maximum in radish at 24 h under Cd treatment (Fig. 6A). Subcellular localization analysis demonstrated that the RsWRKY15 protein was localized in the nucleus (Fig. 6B).

The GUS activity of *RsWRKY15* exhibited greater intensity compared with control under Cd treatment (Fig. 6C). The LUC signal and relative intensity of leaves containing *pRsWRKY15::LUC* increased following Cd exposure,

demonstrating that the promoter activity of *RsWRKY15* was strongly induced by Cd stress (Fig. 6D–F).

RsWRKY15 overexpression reduces Cd accumulation in *N. benthamiana* plants

To investigate the function of *RsWRKY15* under Cd treatment, overexpression lines *RsWRKY15*-OE-1 and *RsWRKY15*-OE-3 were selected for functional analysis (Fig. 7A). Under normal conditions, both *RsWRKY15*-OE lines and WT exhibited normal growth on MS plates, while the *RsWRKY15*-OE-1 and *RsWRKY15*-OE-3 lines demonstrated longer roots, higher fresh weight, and lower Cd content than WT plants under Cd treatment (Fig. 7B–E). Dithizone staining revealed that *RsWRKY15*-OE lines contained fewer red-black Cd-dithizone precipitates (Fig. 7F), indicating that *RsWRKY15* overexpression enhanced growth vigor and reduced Cd accumulation.

Cd fluorescence probe staining analysis results showed reduced fluorescence in the root systems of

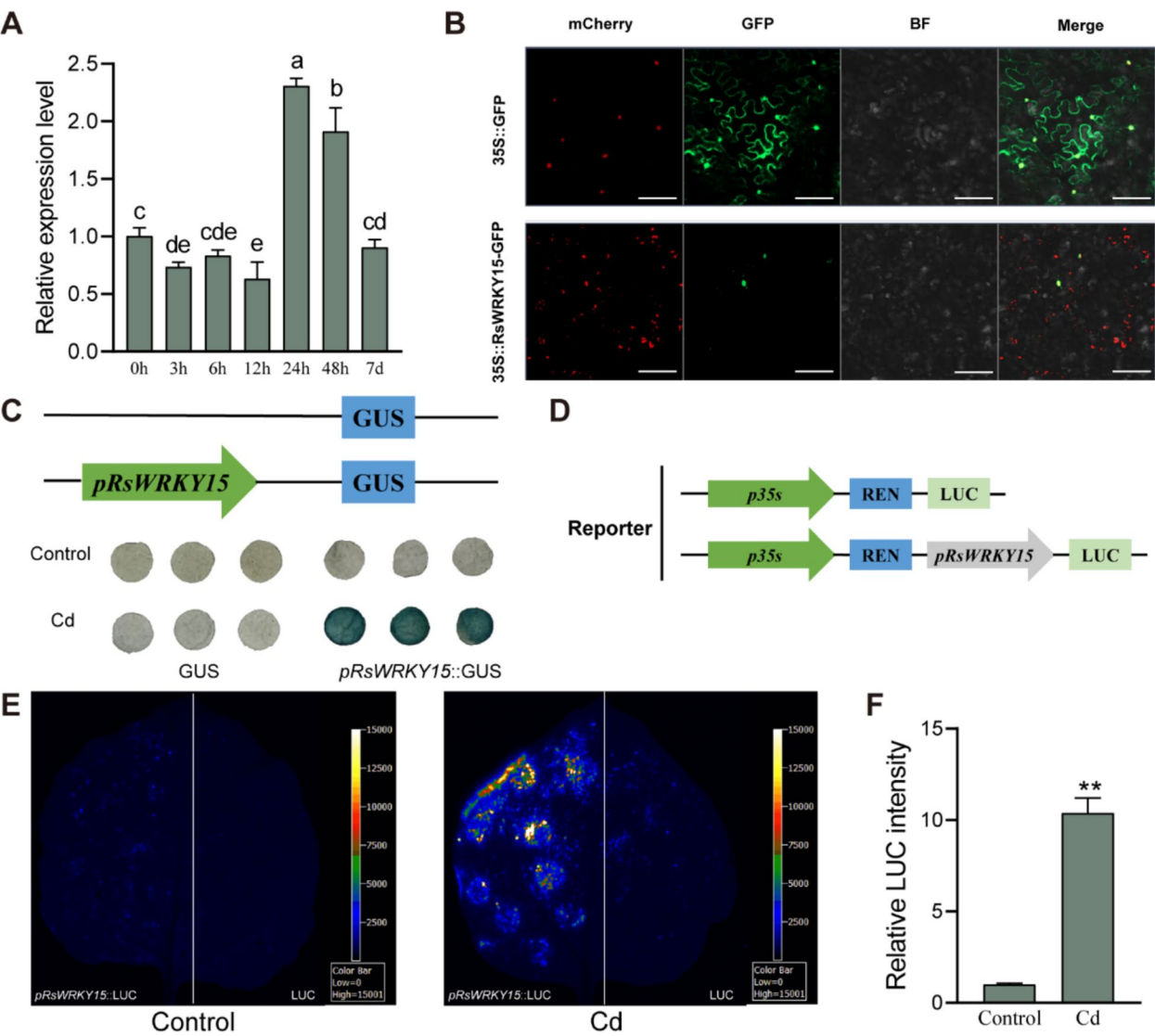


Fig. 6 Characterization and differential expression profile of *RsWRKY15*. **A** Expression profiling of the *RsWRKY15* gene in radish roots under Cd treatment (0.1 mM CdCl₂). Different letters above the bars indicate significant differences ($P < 0.05$). **B** Subcellular localization of *RsWRKY15* in *N. benthamiana* leaves. pSuper::NF-YA4-mCherry was used as a nuclear marker. Scale bar = 50 μ m. **C** GUS staining analysis of *pRsWRKY15* in *N. benthamiana* under control conditions and Cd treatment (0.27 mM CdCl₂, 8 h). **D** Structure of the report vector. **E** LUC assay of *pRsWRKY15* in *N. benthamiana* leaves under control conditions and Cd treatment (0.27 mM CdCl₂, 8 h). **F** Relative LUC intensity of the *pRsWRKY15* promoter under Cd treatment. Data are the mean $\pm$ SD of three replicates (t -test; ** $P < 0.01$)

(See figure on next page.)

Fig. 7 *RsWRKY15* reduces Cd accumulation in *N. benthamiana* plants. **A** Expression level of *RsWRKY15* in WT and *RsWRKY15*-OE lines. **B** Phenotype changes under Cd treatment (55 μ M CdCl₂). Bar = 1 cm. **C** Root length. **D** Fresh weight. **E** Cd content under Cd treatment (55 μ M CdCl₂). Data are presented as the mean $\pm$ SD, $n = 3$. Bars with different lowercase letters are significantly different at $P < 0.05$. **F** Dithizone staining under Cd treatment (55 μ M CdCl₂). **G** Cd content detection with Cd fluorescence. Bar = 50 μ m. **H** Testing position of the probe in roots. **I** Transient Cd²⁺ fluxes measured every 6 s in 21-d-old seedlings of *RsWRKY15*-OE lines and WT under Cd treatment (0.1 mM Cd for 8 h). **J** Average Cd²⁺ fluxes in 5 min. Data are the mean $\pm$ SD ($n = 5$). Asterisks indicate significant differences between the roots of *RsWRKY15*-OE and WT at $P < 0.001$

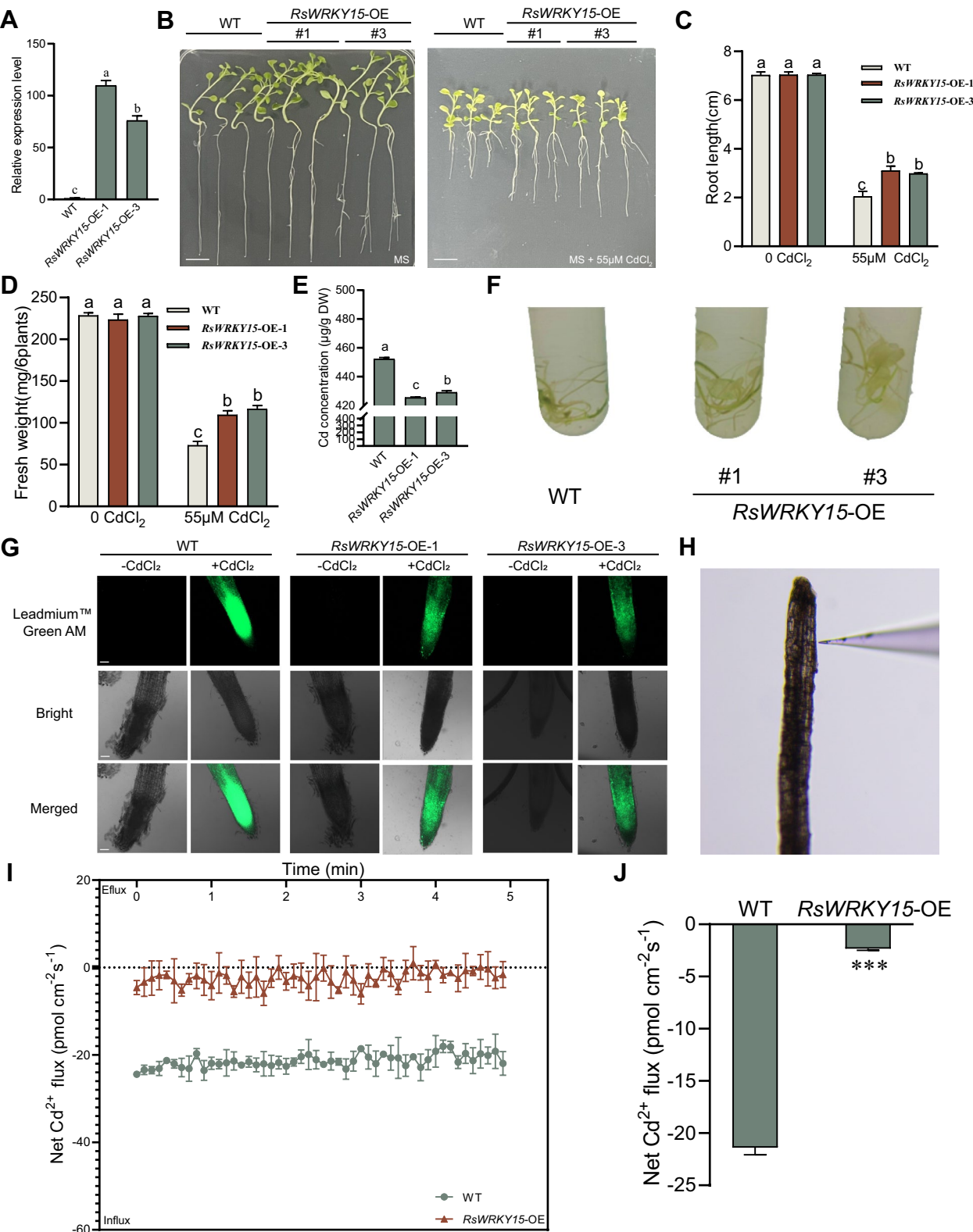


Fig. 7 (See legend on previous page.)

RsWRKY15-OE lines compared with WT, indicating decreased Cd concentration in *RsWRKY15*-OE lines (Fig. 7G). Furthermore, NMT measured the dynamic changes in Cd^{2+} fluxes in the root elongation zone (Fig. 7H). The transient and average Cd^{2+} fluxes in roots of *RsWRKY15*-OE lines decreased under Cd treatment compared with WT (Fig. 7I and J), suggesting that *RsWRKY15* reduces Cd accumulation in plants by decreasing Cd^{2+} influx under Cd stress.

The *NtPDR12* expression level was significantly upregulated, exhibiting a 6.09-fold increase compared with WT (Fig. S4).

ROS accumulation is reduced in *RsWRKY15*-OE radish

RsWRKY15 underwent transient OE and silencing in radish cotyledons (Fig. 8A). The Cd content significantly decreased in *RsWRKY15*-OE and increased in *RsWRKY15*-RNAi compared with EV in radish cotyledons (Fig. S5). DAB and NBT staining revealed no significant differences between OE-EV, *RsWRKY15*-OE, RNAi-EV, and *RsWRKY15*-RNAi cotyledons under normal conditions (Fig. 8B and C). Under Cd stress, *RsWRKY15*-OE cotyledons displayed lighter

histochemical staining compared with OE-EV cotyledons, while *RsWRKY15*-RNAi cotyledons exhibited deeper histochemical staining compared with RNAi-EV cotyledons (Fig. 8B and C).

Under normal conditions, no significant changes were observed in the levels of H_2O_2 , O_2^- , MDA and proline between *RsWRKY15*-OE and OE-EV, and *RsWRKY15*-RNAi and RNAi-EV cotyledons (Fig. 8D–G). However, under Cd treatment, *RsWRKY15*-OE cotyledons showed lower levels of H_2O_2 , O_2^- , and MDA than OE-EV cotyledons, while these levels significantly increased in *RsWRKY15*-RNAi cotyledons compared with RNAi-EV cotyledons (Fig. 8D–F). Conversely, under Cd stress, proline content was notably higher in *RsWRKY15*-OE cotyledons and reduced in *RsWRKY15*-RNAi cotyledons compared with OE-EV and RNAi-EV cotyledons (Fig. 8G). *RsPDR12* expression was elevated in *RsWRKY15*-OE cotyledons compared with OE-EV cotyledons, while showing significant reduction in *RsWRKY15*-RNAi cotyledons compared with RNAi-EV cotyledons (Fig. S6).

RsWRKY15 overexpression can reduce ROS accumulation, likely by decreased Cd uptake and accumulation under Cd stress in radish.

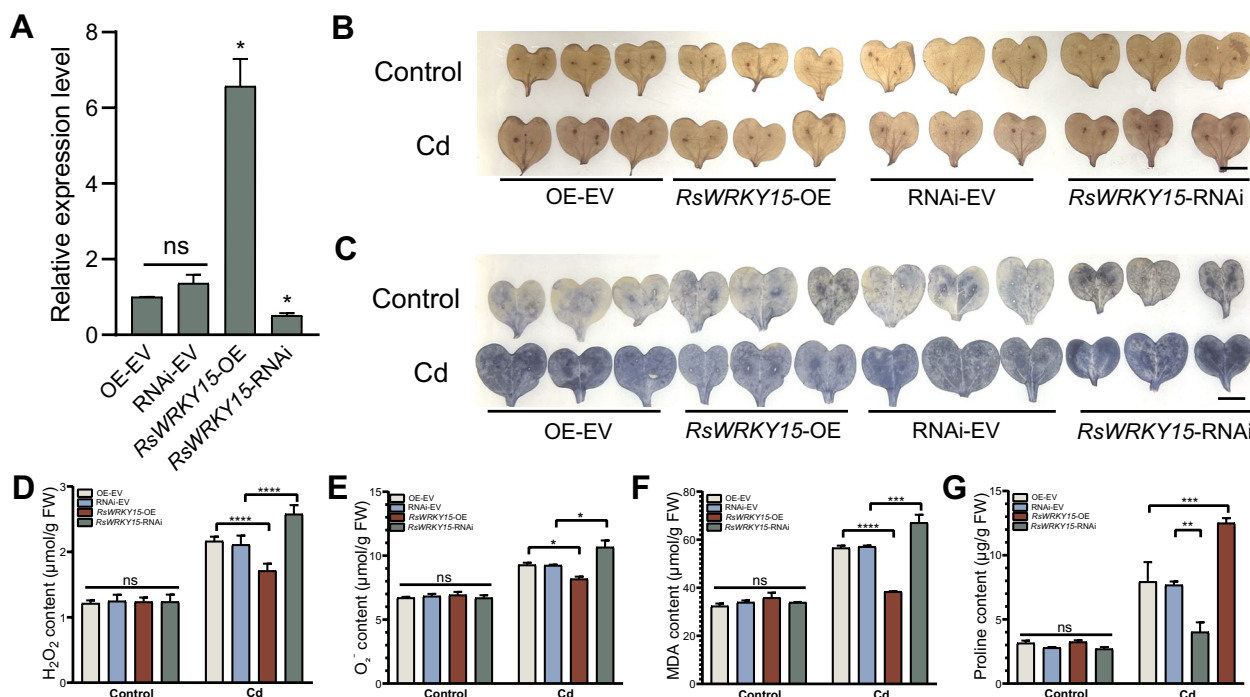


Fig. 8 ROS accumulation was reduced in *RsWRKY15*-overexpressing radish. **A** *RsWRKY15* expression in OE-EV, RNAi-EV, *RsWRKY15*-OE, and *RsWRKY15*-RNAi radish cotyledons. **B** DAB and (**C**) NBT staining of radish cotyledons transiently transformed with the OE-EV, RNAi-EV, *RsWRKY15*-OE, and *RsWRKY15*-RNAi under Cd stress (0.27 mM CdCl_2 , 8 h). Scale bar = 1 cm. **D** H_2O_2 , **E** O_2^- , **F** MDA and (**G**) proline contents, respectively, in OE-EV, RNAi-EV, *RsWRKY15*-OE, and *RsWRKY15*-RNAi radish plants under Cd stress (0.27 mM CdCl_2 , 8 h). Data are the mean $\pm$ SD of three replicates (t-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant)

Discussion

RsPDR12 is a vital participant in reducing Cd accumulation under Cd stress

Stress induced by Cd, Pb, and other HMs is an increasingly significant environmental concern, affecting both plant productivity and growth potential while presenting serious risks to human health (Matsuda et al. 2009; Zhao et al. 2021; Chen et al. 2024; Guo et al. 2024; Wang et al. 2024a, b). Several ABCG subfamily transporters—including *AtABCG36/AtPDR8*, *RsABCG36/RsPDR8*, *OsABCG40/OsPDR9*, *AtABCG40/AtPDR12*, *AhPDR33*, and *OsPDR20*—are potential mediators in plant responses to HM stress (Fan et al. 2016; Ziegler et al. 2017; Sheng et al. 2019; Gupta et al. 2019; Ashraf et al. 2021; Zhang et al. 2023b; Wang et al. 2024; Li et al. 2024). The Cd efflux pumps, *AtABCG36/AtPDR8* and *RsABCG36/RsPDR8* demonstrate capacity to reduce Cd accumulation in *Arabidopsis* and radish (Kim et al. 2007; Sheng et al. 2019; Zhang et al. 2023b). *RsPDR8* expression shows dramatic upregulation under both Cd and Pb stresses (Zhang et al. 2023b). In this investigation, *RsPDR12* expression increased under Cd treatment in radish, with *RsPDR12* localizing to the plasma membrane (Fig. 1). Given that *AtPDR12* similarly localizes to the plasma membrane and responds to Pb stress, *RsPDR12* likely plays a comparable positive role in managing Cd stress. *OsABCG36/OsPDR9* functions as a Cd efflux pump, transporting Cd from rice root cells, as demonstrated by heterologous yeast and genetic transformation in rice (Fu et al. 2019). In addition, yeast cells transformed with *RsPDR12* exhibited enhanced growth and decreased Cd content compared with cells containing the empty vector (Fig. 2). While *AtPDR8* overexpression in *Arabidopsis* reduces Cd accumulation (Kim et al. 2007), *AtPDR12* overexpression decreases Pb accumulation by regulating enzyme-related genes (Lee et al. 2005; Fan et al. 2016). In the present study, *RsPDR12*-OE lines demonstrated more vigorous growth and significantly reduced Cd content compared with WT plants under Cd treatment, indicating that *RsPDR12* overexpression reduces Cd accumulation and enhances Cd²⁺ efflux under Cd stress compared with WT plants (Fig. 3).

TF *RsWRKY15* reduces Cd accumulation in plants

In plants, TFs mediate various metal stress perception and coping mechanisms (Liu et al. 2017; Zhu et al. 2018; Li et al. 2023). The WRKY TF family represents one of the most significant regulatory families involved in HM stress responses (Hong et al. 2017; Sheng et al. 2019; Han et al. 2019; Cai et al. 2020; Jia et al. 2021; Li et al. 2023; Zhang et al. 2023a). In *Arabidopsis*, overexpression and loss-of-function of *AtWRKY12* correlated with

increased and decreased Cd accumulation by the phytochelatin synthesis pathway (Han et al. 2019). *AtWRKY13* overexpression decreased Cd accumulation, while loss-of-function of *AtWRKY13* resulted in increased Cd accumulation and sensitivity in *Arabidopsis* (Sheng et al. 2019). *AtWRKY33* responds to Cd stress and effectively reduces Cd accumulation (Zhang et al. 2023a). Similarly, *AtWRKY45* diminishes Cd accumulation (Li et al. 2023). *ZmWRKY4* appears to regulate the expression of *ZmSOD4* and *ZmAPX* genes under Cd stress (Hong et al. 2017). *GmWRKY142* overexpression decreased Cd uptake and accumulation in soybean (Cai et al. 2020), while *GmWRKY172* overexpression increased Cd accumulation and reduced seed Cd content (Xian et al. 2023). *TaWRKY70* overexpression decreased Cd accumulation in wheat (Jia et al. 2021). *PyWRKY75* overexpression enhanced Cd accumulation and chlorophyll content of poplar (Wu et al. 2022). These findings indicate that WRKY-mediated responses to Cd stress are conserved across plant species. Although, evidence regarding the potential role of WRKYs in Cd stress response remains limited in radish, *CmWRKY15-1* is involved in biotic stress resistance in chrysanthemum (Chen et al. 2023). This study initially identified the function of *RsWRKY15* in facilitating decreased Cd accumulation under Cd stress in radish. *RsWRKY15* expression increased under Cd treatment, and *RsWRKY15*-OE lines exhibited more robust growth and significantly lower Cd content under Cd stress. NMT demonstrated that *RsWRKY15* overexpression reduced Cd accumulation in plants by decreasing Cd²⁺ influx under Cd stress. These results suggest that *RsWRKY15* plays a positive role in managing Cd stress in plants.

RsWRKY15–*RsPDR12* reduces Cd accumulation

Several strategies including minimizing HM accumulation, transport, and detoxification, have been investigated in plants. However, limited WRKY–ABCG/PDR interactions have demonstrated significant roles under Cd stress. WRKY TFs regulate gene expression under abiotic stress by binding to the W-box [(T)(T)TGAC(C/T)] in target gene promoters (Han et al. 2019). In *Arabidopsis*, *AtWRKY13* directly targets *AtPDR8* to effectively decrease Cd accumulation (Sheng et al. 2019). *AtPSE1* enhances Pb accumulation primarily by activating genes involved in PC synthesis and, to a lesser extent, by activating the transcription of the ABC transporter gene *AtPDR12/AtABCG40* (Fan et al. 2016). *AtWRKY45* decreases Cd accumulation by activating the transcription of *AtPCS1* and *AtPCS2* in *Arabidopsis* (Li et al. 2023). *AtWRKY12* overexpression suppresses *AtGSH1* expression and increases Cd accumulation in *Arabidopsis* (Han et al. 2019). In soybean, *GmWRKY142*

decreases Cd uptake and accumulation by activating the transcription of *GmTCDT1*, *GmCDT1-1*, and *GmCDT1-2* (Cai et al. 2020). In wheat, *TaWRKY70* overexpression reduces Cd accumulation by positively regulating *TaCAT5* expression (Jia et al. 2021). While the WRKY–PDR module has been studied in several plant species, specific WRKY members vary among species (He et al. 2019; Sheng et al. 2019; Zhang et al. 2025). In radish, *RsWRKY75* activates *RsPDR8* transcription by direct promoter binding, thereby reducing Cd accumulation in roots (Zhang et al. 2025). In *Arabidopsis*, *AtWRKY13* similarly reduces Cd accumulation by activating *AtPDR8* expression (Sheng et al. 2019). In addition, *AtWRKY33* activates *AtPDR12* expression, conferring tolerance to *Botrytis cinerea* in *Arabidopsis* (He et al. 2019). This study reveals that *RsWRKY15* binds to the *RsPDR12* promoter and activates its transcription to reduce Cd accumulation in radish taproots (Fig. 5). The overexpression of *RsPDR12* and *RsWRKY15* decreased Cd accumulation in roots. Thus, the transcriptional regulatory network of the *RsWRKY15*–*RsPDR12* module positively regulates Cd uptake and accumulation in radish taproots. Based on these results, a hypothetical model of the *RsWRKY15*–*RsPDR12* module conferring reduced Cd uptake and accumulation is proposed (Fig. 9). Our study demonstrates that *NtPDR12* was upregulated in *RsWRKY15*-OE lines, corresponding with decreased Cd accumulation.

Thus, the module is conserved across species, potentially facilitating the development of elite cultivars with low Cd accumulation. *RsWRKY75* reduces Cd uptake and accumulation by activation of *RsPDR8* and *RsAPX1* transcription in radish (Zhang et al. 2025). Cd stress response typically involves multiple gene networks. For instance, NAC102 directly binds to the *WAKL1* promoter and induces its transcription, facilitating pectin degradation and reducing Cd binding (Han et al. 2023). These findings suggest that the *RsWRKY15*–*RsPDR12* module operates in conjunction with other regulatory networks under Cd stress, providing insight into the *RsPDR12*-mediated regulatory network in radish taproots under Cd stress.

Materials and methods

Plant materials, growth conditions and Cd treatments

The germinating seeds of a radish advanced inbred line ‘NAU-YH’ were cultivated in a 2:1 mixture of soil and vermiculite under controlled growth conditions with a photoperiod cycle of 14 h light at 25 °C and 10 h dark at 16 °C. Three-week-old radish seedlings were transferred to 1/2 Hoagland nutrient solution, which was renewed every 3 days (Xu et al. 2020). After 6 days, radish plants of uniform size were subjected to 0.1 mM CdCl₂ treatment for 3, 6, 12, 24, 48 h and 7 days, with untreated plants serving as controls. Radish roots were harvested after Cd treatment, immediately frozen in liquid nitrogen, and

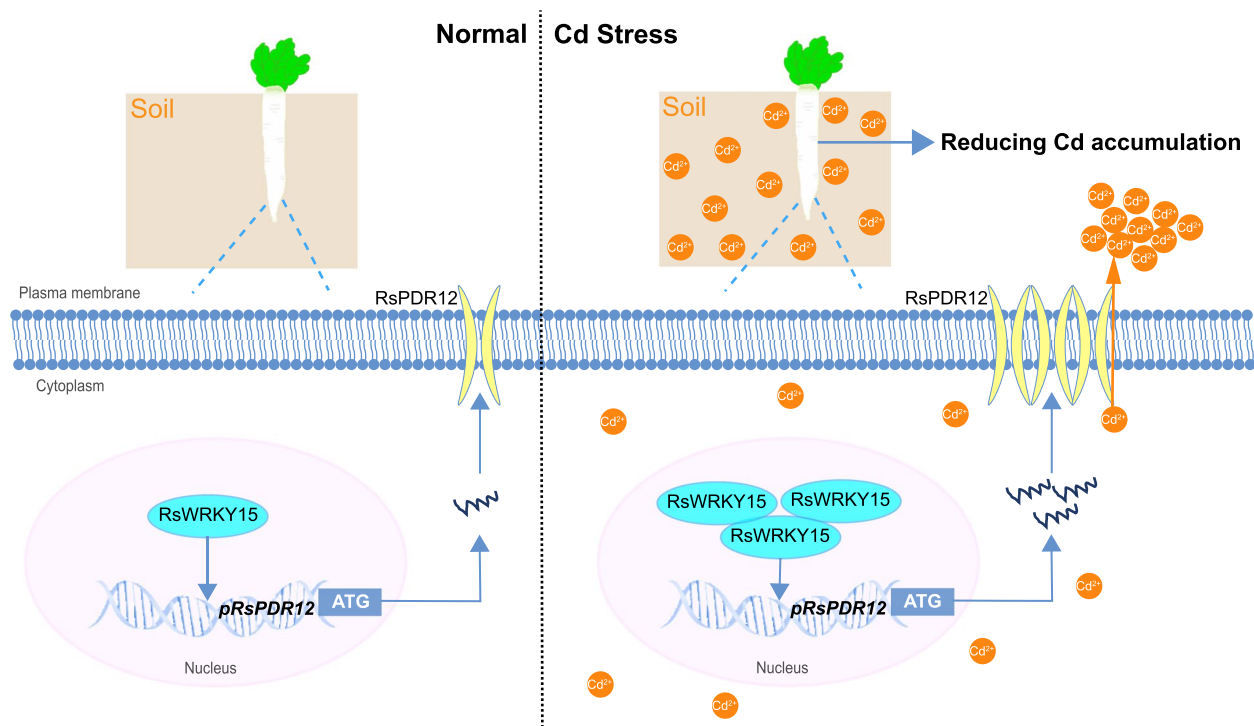


Fig. 9 Putative model of Cd uptake and accumulation regulated by *RsWRKY15*–*RsPDR12* module in the radish taproots

stored at -80°C for subsequent analysis (Zhang et al. 2023b).

Seeds of WT (Col-0) and *RsPDR12*-OE *Arabidopsis* lines, along with WT and *RsWRKY15*-OE *N. benthamiana* lines were sterilized and cultivated on MS medium at 24°C under CdCl_2 treatment.

RNA extraction and RT-qPCR analysis

Total RNA was extracted using a Total RNA Kit (Tiangen, Beijing, China). cDNA synthesis was performed with the HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). PCR was performed on a LightCycler® 480 System (Roche, Mannheim, Germany) (Xu et al. 2020). *RsActin* in radish, *AtActin* in *Arabidopsis*, or *NtActin* in *N. benthamiana* functioned as internal reference genes (Xu et al. 2012). Relative expression levels were determined by the $2^{-\Delta\Delta\text{CT}}$ method with three biological and three technical replicates (Livak and Schmittgen 2001). All primers used in this study are listed in Supplementary Table S1.

Promoter activity analysis of *RsPDR12* and *RsWRKY15*

The upstream sequences of the ATG site were isolated as the promoter regions of *RsPDR12* and *RsWRKY15*. These promoters were fused with the pGreenII-0800-LUC and pCAMBIA1391-GUS vectors to produce the recombinant plasmids *pRsPDR12::LUC*, *pRsPDR12::GUS*, *pRsWRKY15::LUC* and *pRsWRKY15::GUS*. The plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 (pSoup) and infiltrated into *N. benthamiana* leaves. Plants were maintained under normal conditions for 2 days, then exposed to 0.27 mM CdCl_2 for 8 h, and analyzed using a chemiluminescence imaging system (ChemiScope 6000, Clinx Science Instruments, China) and GUS staining (Wang et al. 2022). Relative LUC intensity was determined using a living fluorescence imager (Lb985, Berthold, Germany) (He et al. 2023a).

Subcellular localization of *RsPDR12* and *RsWRKY15*

The *RsPDR12* and *RsWRKY15* CDSs, excluding the stop codon, were inserted into the pCAMBIA1300-GFP vector to generate *35S::RsPDR12::GFP* and *35S::RsWRKY15::GFP* constructs. These were transformed into *A. tumefaciens* strain GV3101 and infiltrated into *N. benthamiana* leaves (Wang et al. 2022). PM-mCherry and pSuper::NF-YA4-mCherry proteins served as plasma membrane and nuclear markers, respectively (Yang et al. 2022). GFP and mCherry signals were detected under a laser confocal microscope (LSM800, Zeiss, Germany) after 48 h (Dong et al. 2023).

Analysis of Cd transport activity of *RsPDR12* in yeast

The *RsPDR12* CDS was inserted into the pYES2 vector. The empty vector and *RsPDR12*-pYES2 were transformed into yeast strain BY4741. The transformed yeast cells underwent serial $10\times$ dilutions and were spotted onto yeast nitrogen base (YNB)-Ura solid medium containing 0, 27, 55 and $82\text{ }\mu\text{M CdCl}_2$, with 2% glucose (expression suppressor) or 2% galactose (expression inducer). Plates were incubated at 30°C for 3 days. To measure Cd content, yeast cells were cultured in YNB-Ura medium with 2% galactose for 20 h, then exposed to 0.27 mM CdCl_2 for 8 h. Cd content was determined using an inductively coupled plasma mass spectrometer (ICP-MS; NexION-5000G; Perkin Elmer, USA) (Zhang et al. 2023b; Gaddam et al. 2024).

Ectopic transformation of *RsPDR12* and *RsWRKY15*

A. tumefaciens strain GV3101 containing *35S::RsPDR12* was introduced into *Arabidopsis* by the floral-dip method (Clough and Bent 1998), while *A. tumefaciens* strain GV3101 containing *35S::RsWRKY15* was introduced into *N. benthamiana* plants by the leaf-disc method (Wu et al. 2014). *RsPDR12*-OE *Arabidopsis* lines and *RsWRKY15*-OE *N. benthamiana* lines underwent hygromycin screening. Sterilized seeds of WT and two *RsPDR12*-OE *Arabidopsis* lines (OE-9 and OE-18) were cultivated on MS medium containing $50\text{ }\mu\text{M CdCl}_2$, while WT and two *RsWRKY15*-OE lines (OE-1 and OE-3) were cultivated on MS medium containing $55\text{ }\mu\text{M CdCl}_2$. For histochemical localization of Cd, *N. benthamiana* WT and *RsWRKY15*-OE plants were submerged in 1.56 mM dithizone dye solution for 6 h. The red–black Cd–dithizone precipitates were observed after washing plants with dH_2O (Zhu et al. 2016). The Cd content of WT, *RsPDR12*-OE, and *RsWRKY15*-OE plants was determined using ICP-MS (NexION-5000G, Perkin Elmer, USA) (Xu et al. 2020; Liu et al. 2022).

Cd^{2+} flux measurement in roots using a Cd-fluorescent probe and NMT

The net Cd^{2+} flux of WT, *RsPDR12*-OE (*RsPDR12*-OE-9 and *RsPDR12*-OE-18), and *RsWRKY15*-OE (*RsWRKY15*-OE-1 and *RsWRKY15*-OE-3) was measured using the Cd-fluorescent probe Leadmium™ Green AM (Invitrogen, Carlsbad, CA, USA). The plants were washed with distilled water, incubated with the Cd-fluorescent probe for 1 h, followed by washing with 0.9% NaCl solution. Signal detection was performed using a laser confocal microscope (LSM800, Zeiss, Germany) (Zhang et al. 2021; Gaddam et al. 2024).

The net Cd^{2+} flux of WT, *RsPDR12*-OE (*RsPDR12*-OE-9 and *RsPDR12*-OE-18), and *RsWRKY15*-OE

(*RsWRKY15*-OE-1 and *RsWRKY15*-OE-3) was also measured using NMT (NMT100 Series, Younger USA LLC, Amherst, MA, USA) with iFluxes/imFluxes v.1.0. Microelectrodes were prepared using backfilling solution (10 mM $\text{Cd}(\text{NO}_3)_2 + 0.1$ mM KCl) and selective liquid ion-exchange cocktails (Wu et al. 2019; Tan et al. 2024). The microelectrodes were calibrated with 0.05, 0.1 and 0.5 mM Cd^{2+} , and Cd^{2+} flux was measured under 0.1 mM Cd^{2+} treatment (Zhang et al. 2021).

Transient transformation of *RsPDR12* and *RsWRKY15* in radish

RsPDR12 and *RsWRKY15* CDSs were inserted into the pCAMBIA1300-GFP and pTCK303 vectors to produce *35S::RsPDR12*, *RNAi::RsPDR12*, *35S::RsWRKY15*, and *RNAi::RsWRKY15* constructs, which were introduced into *A. tumefaciens* strain GV3101. Two-week-old radish cotyledons underwent transient transformation (He et al. 2023b). Radish plants received CdCl_2 treatment for 8 h, and H_2O_2 and O_2^- levels were detected by DAB and NBT staining (He et al. 2023a). MDA and proline levels were measured according to a previous report (Thirumalaikumar et al. 2018).

cDNA library screening and Y1H assay

The *RsPDR12* promoter was integrated into the pAbAi vector, and subsequently transferred into yeast strain Y1H Gold. The Y1H Gold containing pAbAi-*pRsPDR12* was used to screen the radish yeast library. Yeast cells were cultured on solidified dextrose (SD)/–Leu medium supplemented with 300 ng/mL AbA, followed by PCR detection and sequencing. The *RsWRKY15* CDS was inserted into the pB42AD vector, while the *RsPDR12* promoter sequences (510 bp upstream from the start codon), two truncated fragments (S1, –510 to –285 bp; S2, –284 to –1 bp) of the *RsPDR12* promoter, and the 3×Motif (W1, –222 to –214 bp; W2, –214 to –206 bp; W3, –150 to –142 bp; W4, –138 to –130 bp) along with 3×m-Motif (mW1, GAATCCCA) were incorporated into the pLacZi2μ vector. Both plasmids were co-transferred into yeast strain EGY48. Yeast cells of positive clones were transferred and cultured in SD/–Trp/–Ura medium containing X-gal at 30 °C for 2–3 days (Dong et al. 2022). The pB42AD and pLacZi2μ vectors were used as negative controls.

Dual-luciferase reporter assay

The *RsPDR12* promoter was inserted into the pGreenII-0800-LUC vector to generate *pRsPDR12::LUC*. The *RsWRKY15* CDS was incorporated into the pCAMBIA1300-GFP vector to produce *35S::RsWRKY15*, *35S::RsWRKY15*, *pRsPDR12::LUC*, and the empty vector were transformed into *A. tumefaciens* strain GV3101

(pSoup) and introduced into *N. benthamiana* leaves. The infiltrated leaves were maintained under controlled growth conditions for 2 days and analyzed using a chemiluminescence imaging system (ChemiScope 6000, Clinx Science Instruments, China). The relative LUC intensity was determined using a living fluorescence imager (Lb985, Berthold, Germany) (Ying et al. 2023).

Statistical analysis

Statistical analysis was performed using Duncan's multiple comparison test ($P < 0.05$) or Student's *t*-test using SPSS v.21.0 (IBM, New York, NY, USA). The results are presented as mean ± standard deviation of three biological replicates.

Abbreviations

DLA	Dual-luciferase reporter assay
GFP	Green fluorescence protein
GUS	β-Glucuronidase
LUC	Firefly luciferase
NMT	Non-invasive micro-test technology
PDR	Pleiotropic drug resistance protein
ROS	Reactive oxygen species
TF	Transcription factor
Y1H	Yeast one-hybrid

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43897-025-00195-7>.

Additional file 1: Supplementary Figure. S1 Amino acid sequence alignment of *RsPDR12* and *AtPDR12*. At, *Arabidopsis thaliana* (AT1G15520). Supplementary Figure. S2 The upstream transcription factors of *RsPDR12* were screened by yeast one-hybrid library. (A) Self-activation detection of *RsPDR12* gene promoter. (B) Colony detection electrophoresis results via Y1H library screening with *RsPDR12* promoter. Supplementary Figure. S3 Amino acid sequence alignment of *RsWRKY15* and *AtWRKY15*. At, *Arabidopsis thaliana* (AT2G23320). Supplementary Figure. S4 The relative expression level of *RsPDR12* gene in OE-EV, RNAi-EV, *RsWRKY15*-OE, and *RsWRKY15*-RNAi radish cotyledons using the RT-qPCR analysis. Data are presented as the mean ± SD, $n = 3$. Bars with different lowercase letters are significantly different at $P < 0.05$. Supplementary Figure. S5 The Cd content in radish cotyledons under Cd treatment (0.27 mM CdCl_2 , 8 h). (A) The Cd content of EV, *RsPDR12*-OE and *RsPDR12*-RNAi under Cd treatment. (B) The Cd content of OE-EV, RNAi-EV, *RsWRKY15*-OE and *RsWRKY15*-RNAi under Cd treatment. Data are the mean ± SD of three replicates (*t*-test; ** $P < 0.01$; *** $P < 0.001$). Supplementary Figure. S6 The relative expression level of *NtPDR12* gene in WT and *RsWRKY15*-OE tobacco lines using the RT-qPCR analysis. Data are presented as the mean ± SD, $n = 3$. Bars with different lowercase letters are significantly different at $P < 0.05$.

Additional file 2: Supplementary Table 1. The list of primer pairs used in this study.

Acknowledgements

Not applicable.

Authors' contributions

L.L. and S.M. designed the research. S.M. performed the data analysis and wrote the paper. S.M., Z.Y. and B.L. performed experiments. L.X., Y.W. and Y.M. contributed powerful analytical tools. L.L., J.L., K.W., X.Z., X.Z. and Y.M. critically reviewed the final draft of the manuscript and helped with the revision of the paper. All authors read and approved the final paper.

Funding

This work was funded by grants from the National Natural Science Foundation of China (32172579), Jiangsu Agricultural Science and Technology Innovation Fund [CX (23)1013], the Key Technology R & D Program of Jiangsu Province (BE2023366), Jiangsu Seed Industry Revitalization Project [JBGS(2021)071, 2024-SJ-261] and the Project Founded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Data Availability

The data will be available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors approve the manuscript and consent to the publication of the work.

Competing interests

The authors declare that they have no competing interests.

Received: 30 November 2024 Accepted: 9 July 2025

Published online: 12 February 2026

References

- Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, et al. A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J Biol Chem*. 2006;281:36–45.
- Ashraf MA, Akihiro T, Ito K, Kumagai S, Sugita R, Tanoi K, et al. ATP binding cassette proteins ABCG37 and ABCG33 function as potassium-independent cesium uptake carriers in *Arabidopsis* roots. *Mol Plant*. 2021;14:664–78.
- Cai Z, Xian P, Wang H, Lin R, Lian T, Cheng Y, et al. Transcription factor GmWRKY142 confers cadmium resistance by up-regulating the cadmium tolerance 1-like genes. *Front Plant Sci*. 2020;11:724.
- Chen Q, Kuang A, Wu H, Liu D, Zhang X, Mao H. Physiological response of *CmWRKY15-1* to chrysanthemum white rust based on TRV-VIGS. *Front Plant Sci*. 2023;14:1140596.
- Chen W, Shi Y, Wang C, Qi X. AtERF13 and AtERF6 double knockout fine-tunes growth and the transcriptome to promote cadmium tolerance in *Arabidopsis*. *Gene*. 2024;911:148348.
- Clough S-J, Bent A-F. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J*. 1998;16:735–43.
- Dong J, Wang Y, Xu L, Li B, Wang K, Ying J, et al. RsCLE22a regulates taproot growth through an auxin signaling-related pathway in radish (*Raphanus sativus* L.). *J Exp Bot*. 2023;74:233–50.
- Fan T, Yang L, Wu X, Ni J, Jiang H, Zhang Q, et al. The *PSE1* gene modulates lead tolerance in *Arabidopsis*. *J Exp Bot*. 2016;67:4685–95.
- Fu S, Lu Y, Zhang X, Yang G, Chao D, Wang Z, et al. The ABC transporter OsABCG36 is required for Cd tolerance in rice. *J Exp Bot*. 2019;70:5909–18.
- Gaddam S-R, Sharma A, Trivedi P-K. MiR397b-LAC2 module regulates cadmium stress response by coordinating root lignification and copper homeostasis in *Arabidopsis thaliana*. *J Hazard Mater*. 2024;465:133100.
- Gani U, Vishwakarma RA, Misra P. Membrane transporters: the key drivers of transport of secondary metabolites in plants. *Plant Cell Rep*. 2021;40:1–18.
- Guo N, Ling H, Yu R, Gao F, Cao Y, Tao J. Expression of *Saix suchowensis* SsIRT9 enhances cadmium accumulation and alters metal homeostasis in tobacco. *J Hazard Mater*. 2024;463:132958.
- Gupta B-B, Selter L-L, Baranwal V-K, Arora D, Mishra S-K, Sirohi P, et al. Updated inventory, evolutionary and expression analyses of G (PDR) type ABC transporter genes of rice. *Plant Physiol Biochem*. 2019;142:429–39.
- Han GH, Huang RN, Hong LH, Xu JX, Hong YG, Wu YH, et al. The transcription factor NAC102 confers cadmium tolerance by regulating WAKL11 expression and cell wall pectin metabolism in *Arabidopsis*. *J Integr Plant Biol*. 2023;65(10):2262–78.
- Han Y, Fan T, Zhu X, Wu X, Ouyang J, Jiang L, et al. WRKY12 represses *GSH1* expression to negatively regulate cadmium tolerance in *Arabidopsis*. *Plant Mol Biol*. 2019;99:149–59.
- He Y, Xu J, Wang X, He X, Wang Y, Zhou J, et al. The *Arabidopsis* Pleiotropic Drug Resistance transporters PEN3 and PDR12 mediate camalexin secretion for resistance to *Botrytis cinerea*. *Plant Cell*. 2019;31:2206–22.
- He Q, He M, Zhang X, Zhang X, Zhang W, Dong J, et al. RsvQ4-RsWRKY26 module positively regulates thermotolerance by activating *RsHSP70-20* transcription in radish (*Raphanus sativus* L.). *Environ Exp Bot*. 2023a;214:105467.
- He Q, Zhang X, He M, Zhang X, Ma Y, Zhu Y, et al. Genome-wide characterization of RsHSP70 gene family reveals positive role of *RsHSP70-20* gene in heat stress response in radish (*Raphanus sativus* L.). *Plant Physiol Biochem*. 2023b;199:107710.
- Hong C, Cheng D, Zhang G, Zhu D, Chen Y, Tan M. The role of *ZmWRKY4* in regulating maize antioxidant defense under cadmium stress. *Biochem Biophys Res Commun*. 2017;482:1504–10.
- Jia Z, Li M, Wang H, Zhu B, Gu L, Du X, et al. *TaWRKY70* positively regulates *TaCAT5* enhanced Cd tolerance in transgenic *Arabidopsis*. *Environ Exp Bot*. 2021;190:104591.
- Kim D-Y, Bovet L, Maeshima M, Martinoia E, Lee Y. The ABC transporter *AtPDR8* is a cadmium extrusion pump conferring heavy metal resistance. *Plant J*. 2007;50:207–18.
- Kuromori T, Sugimoto E, Ohiraki H, Yamaguchi-Shinozaki K, Shinozaki K. Functional relationship of *AtABCG21* and *AtABCG22* in stomatal regulation. *Sci Rep*. 2017;7:12501.
- Lee M, Lee K, Lee J, Noh EW, Lee Y. *AtPDR12* contributes to lead resistance in *Arabidopsis*. *Plant Physiol*. 2005;138:827–36.
- Lefèvre F, Fourmeau J, Pottier M, Baijot A, Cornet T, Abadía J, et al. The *Nicotiana tabacum* ABC transporter NtPDR3 secretes O-methylated coumarins in response to iron deficiency. *J Exp Bot*. 2018;69:4419–31.
- Li F, Deng Y, Liu Y, Mai C, Xu Y, Wu J, et al. *Arabidopsis* transcription factor WRKY45 confers cadmium tolerance via activating *PCS1* and *PCS2* expression. *J Hazard Mater*. 2023;460:132496.
- Li H, Li C, Sun D, Yang ZM. OsPDR20 is an ABCG metal transporter regulating cadmium accumulation in rice. *J Environ Sci (China)*. 2024;136:21–34.
- Liu L, Zhao L, Chen P, Cai H, Hou Z, Jin X, et al. ATP binding cassette transporters *ABCG1* and *ABCG16* affect reproductive development via auxin signaling in *Arabidopsis*. *Plant J*. 2020;102:1172–86.
- Liu S, Li L, Deng Y, Bai Y, Sun C, Huang S, et al. BrPNAC895 and BrPABI449 coregulate the transcription of the afflux-type Cd transporter *BrpHMA2* in *Brassica parachinensis*. *Hortic Res*. 2022;9:uhac044.
- Liu Y, Yu X, Feng Y, Zhang C, Wang C, Zeng J, et al. Physiological and transcriptome response to cadmium in cosmos (*Cosmos bipinnatus* Cav.) seedlings. *Sci Rep*. 2017;7:14691.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*. 2001;25:402–8.
- Lu Q, Chen S, Li Y, Zheng F, He B, Gu M. Exogenous abscisic acid (ABA) promotes cadmium (Cd) accumulation in *Sedum alfredii* Hance by regulating the expression of Cd stress response genes. *Environ Sci Pollut Res Int*. 2020;27:8719–31.
- Matsuda T, Kuramata M, Takahashi Y, Kitagawa E, Youssefian S, Kusano T. A novel plant cysteine-rich peptide family conferring cadmium tolerance to yeast and plants. *Plant Signal Behav*. 2009;4:419–21.
- McLaughlin MJ, Smolders E, Zhao FJ, Grant C, Montalvo D, Chapter one-managing cadmium in agricultural systems. *Adv Agron*. 2021; 166: 1–129.
- Rushton PJ, Somssich IE, Ringler P, Shen Q. WRKY transcription factors. *Trends Plant Sci*. 2010;15:247–58.
- Sheng Y, Yan X, Huang Y, Han Y, Zhang C, Ren Y, et al. The *WRKY* transcription factor, *WRKY13*, activates *PDR8* expression to positively regulate cadmium tolerance in *Arabidopsis*. *Plant Cell Environ*. 2019;42:891–903.
- Song Y, Wang Y, Mao W, Sui H, Yong L, Yang D, et al. Dietary cadmium exposure assessment among the Chinese population. *PLoS ONE*. 2017;12:e0177978.
- Tan C, Nie W, Liu Y, Wang Y, Yuan Y, Liu J, et al. Physiological response and molecular mechanism of *Quercus variabilis* under cadmium stress. *Plant Physiol Biochem*. 2024;211:108724.
- Tang M, Xu L, Wang Y, Cheng WL, Luo X, Xie Y, et al. Genome-wide characterization and evolutionary analysis of heat shock transcription factors (HSFs) to reveal their potential role under abiotic stresses in radish (*Raphanus sativus* L.). *BMC Genomics*. 2019;20:772.

- Tang M, Xu L, Wang Y, Dong J, Zhang X, Wang K, et al. Melatonin-induced DNA demethylation of metal transporters and antioxidant genes alleviates lead stress in radish plants. *Hortic Res.* 2021;8(1):124.
- Tang M, Zhang X, Xu L, Wang Y, Chen S, Dong J, et al. Genome- and transcriptome-wide characterization of ZIP gene family reveals their potential role in radish (*Raphanus sativus* L.) response to heavy metal stresses. *Sci Hortic.* 2024;324:112564.
- Thirumalaikumar VP, Devkar V, Mehterov N, Ali S, Ozgur R, Turkan I, et al. NAC transcription factor JUNGBRUNNEN1 enhances drought tolerance in tomato. *Plant Biotechnol J.* 2018;16:354–66.
- Wang K, Xu L, Wang Y, Ying J, Li J, Dong J, et al. Genome-wide characterization of homeodomain-leucine zipper genes reveals RsHDZ17 enhances the heat tolerance in radish (*Raphanus sativus* L.). *Physiol Plant.* 2022;174:e13789.
- Wang Q, Li X, Li Z, Sun Q, Li C, Zhao X, et al. Molecular characterization of pleiotropic drug resistance (PDR) genes involved in tolerance of cadmium in peanut (*Arachis hypogaea* L.). *Ecotoxicol Environ Saf.* 2024a;288:117324.
- Wang Y, Huang X, Li X, Lu Q, Wu T, Shao M, et al. The plasma membrane-localized transporter VrNramp5 of *Vigna radiata* L. governs cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Environ Exp Bot.* 2024b;220:105712.
- Wu S, Shi K, Hu C, Guo J, Tan Q, Sun X. Non-invasive microelectrode cadmium flux measurements reveal the decrease of cadmium uptake by zinc supply in pakchoi root (*Brassica chinensis* L.). *Ecotoxicol Environ Saf.* 2019;168:363–8.
- Wu W, Cheng Z, Liu M, Yang X, Qiu D. C3HC4-type RING finger protein NbZFP1 is involved in growth and fruit development in *Nicotiana benthamiana*. *PLoS ONE.* 2014;9:e99352.
- Wu X, Chen Q, Chen L, Tian F, Chen X, Han C, et al. A WRKY transcription factor, PyWRKY75, enhanced cadmium accumulation and tolerance in poplar. *Ecotoxicol Environ Saf.* 2022;239:113630.
- Xian P, Yang Y, Xiong C, Guo Z, Alam I, He Z, et al. Overexpression of *GmWRKY172* enhances cadmium tolerance in plants and reduces cadmium accumulation in soybean seeds. *Front Plant Sci.* 2023;14:1133892.
- Xie X, Cao P, Wang Z, Gao J, Wu M, Li X, et al. Genome-wide characterization and expression profiling of the PDR gene family in tobacco (*Nicotiana tabacum*). *Gene.* 2021;788:145637.
- Xu L, Zhang F, Tang M, Wang Y, Dong J, Ying J, et al. Melatonin confers cadmium tolerance by modulating critical heavy metal chelators and transporters in radish plants. *J Pineal Res.* 2020;69:e12659.
- Xu Y, Zhu X, Gong Y, Xu L, Wang Y, Liu L. Evaluation of reference genes for gene expression studies in radish (*Raphanus sativus* L.) using quantitative real-time PCR. *Biochem Biophys Res Commun.* 2012;424:398–403.
- Yang Z, Yang F, Liu J, Wu H, Yang H, Shi Y, et al. Heavy metal transporters: functional mechanisms, regulation, and application in phytoremediation. *Sci Total Environ.* 2022;809:151099.
- Ying J, Wang Y, Xu L, Yao S, Wang K, Dong J, et al. RsGLK2.1-RsNF-YA9a module positively regulates the chlorophyll biosynthesis by activating RHEMA2 in green taproot of radish. *Plant Sci.* 2023;334:111768.
- Yokosho K, Yamaji N, Ma J. Buckwheat FeNramp5 mediates high manganese uptake in roots. *Plant Cell Physiol.* 2021;62:600–9.
- Zhang C, Tong C, Cao L, Zheng P, Tang X, Wang L, et al. Regulatory module WRKY33-ATL31-IRT1 mediates cadmium tolerance in *Arabidopsis*. *Plant Cell Environ.* 2023a;46:1653–70.
- Zhang H, Jing W, Zheng J, Jin Y, Wu D, Cao C, et al. The ATP-binding cassette transporter OsPDR1 regulates plant growth and pathogen resistance by affecting jasmonates biosynthesis in rice. *Plant Sci.* 2020;298:110582.
- Zhang P, Wang R, Ju Q, Li W, Tran LSP, Xu J. The R2R3-MYB transcription factor MYB49 regulates cadmium accumulation. *Plant Physiol.* 2019;180:529–42.
- Zhang X, Ma Y, Lai D, He M, Zhang X, Zhang W, et al. RsPDR8, a member of ABCG subfamily, plays a positive role in regulating cadmium efflux and tolerance in radish (*Raphanus sativus* L.). *Plant Physiol Biochem.* 2023b;205:108149.
- Zhang X, Ma Y, Zhang W, Ji M, Dong J, Lai D, et al. RsWRKY75 promotes ROS scavenging and cadmium efflux via activating the transcription of RsAPX1 and RsPDR8 in radish (*Raphanus sativus* L.). *Plant Cell Rep.* 2025;44:65.
- Zhang Y, Sa G, Zhang Y, Hou S, Wu X, Zhao N, et al. *Populus euphratica* annexin1 facilitates cadmium enrichment in transgenic *Arabidopsis*. *J Hazard Mater.* 2021;405:124063.
- Zhao F, Tang Z, Song J, Huang X, Wang P. Toxic metals and metalloids: uptake, transport, detoxification, phytoremediation, and crop improvement for safer food. *Mol Plant.* 2021;15:27–44.
- Zhao F, Wang P. Arsenic and cadmium accumulation in rice and mitigation strategies. *Plant Soil.* 2020;446:1–21.
- Zheng P, Cao L, Zhang C, Pan W, Wang W, Yu X, et al. MYB43 as a novel substrate for CRL4PRL1 E3 ligases negatively regulates cadmium tolerance through transcriptional inhibition of HMAs in *Arabidopsis*. *New Phytol.* 2020;234:884–901.
- Zhu H, Ai H, Cao L, Sui R, Ye H, Du D, et al. Transcriptome analysis providing novel insights for Cd-resistant tall fescue responses to Cd stress. *Ecotoxicol Environ Saf.* 2018;160:349–56.
- Zhu H, Chen C, Xu C, Zhu Q, Huang D. Effects of soil acidification and liming on the phytoavailability of cadmium in paddy soils of central subtropical China. *Environ Pollut.* 2016;219:99–106.
- Ziegler J, Schmidt S, Strehmel N, Scheel D, Steffen A. *Arabidopsis* transporter ABCG37/PDR9 contributes primarily highly oxygenated coumarins to root exudation. *Sci Rep.* 2017;7:3704.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.