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Identification and functional analysis of CsZIP9, a ZIP transporter involved in cadmium transport in cucumber (*Cucumis sativus* L.)

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Abstract: Agricultural soil contamination with cadmium (Cd) has become an increasingly serious global issue, and cucumber (*Cucumis sativus* L.) is highly sensitive to Cd stress. Zinc/iron-regulated transporter-like proteins (ZIPs) are involved in the uptake and translocation of divalent cations. However, the functional roles of cucumber ZIP members in Cd transport remain poorly understood. In this study, we cloned and characterised CsZIP9, a ZIP gene from cucumber. Sequence analysis revealed that CsZIP9 contains eight transmembrane domains and shares high similarity with known Cd-transporting ZIPs. Expression analysis showed that CsZIP9 exhibits leaf-preferential expression and is dynamically regulated by Cd stress. Heterologous expression of CsZIP9 in the Cd-sensitive yeast mutant *Δycf1* significantly increased Cd sensitivity, indicating its Cd transport activity. Moreover, overexpression of CsZIP9 in *Arabidopsis* enhanced Cd tolerance, as evidenced by reduced root growth inhibition under Cd treatment, although no direct measurements of Cd accumulation were conducted to support this conclusion. Taken together, these results indicate that CsZIP9 encodes a functional cadmium transporter, offering a promising candidate gene for enhancing Cd tolerance in cucumber.

Keywords: cadmium stress; expression analysis; transgenic *Arabidopsis*; zinc/iron-regulated transporter-like protein (ZIP)

Cadmium (Cd) is recognised as one of the most hazardous heavy metal contaminants in soil environments due to its high toxicity, non-biodegradability, and strong water solubility (Davidova et al. 2024). Because Cd shares high chemical similarity with essential metal elements such as zinc (Zn), iron (Fe), and manganese (Mn), it can be readily taken up by plant roots through

the transport systems of these beneficial elements. In plants, Cd stress triggers a cascade of physiological and biochemical damages, including the induction of reactive oxygen species (ROS) burst, the initiation of lipid peroxidation, the disruption of cell membrane integrity, and the inhibition of various key enzyme activities, thereby interfering with normal plant growth

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and development (Luo et al. 2024; Niu et al. 2025). In addition, Cd can disrupt the uptake, translocation, and internal distribution of essential metal elements, further aggravating nutritional imbalance in plants (Yan et al. 2025). In the absence of dedicated Cd transporters, Cd is taken up and translocated in plants by exploiting the transmembrane transport pathways of essential nutrient metals like Fe, Zn, and Mn (Wu et al. 2021). To date, several transporter families have been identified as being involved in Cd uptake and translocation, such as natural resistance-associated macrophage protein (NRAMP), heavy metal ATPase (HMA), zinc/iron-regulated transporter-like protein (ZIP), ATP-binding cassette (ABC), and yellow stripe like family (YSL) (Liu et al. 2024). Among these, ZIP plays a central role in Zn uptake, distribution, and subcellular trafficking, and also participates in the transport of other divalent metals, including Fe, Cu, Mn, and Cd (Liu et al. 2019; Robe et al. 2025; Yang et al. 2025b).

ZIP proteins are integral membrane transporters characterised by 6–9 transmembrane (TM) domains, with most members containing eight TM helices (TM-I–TM-VIII) (Ajeesh Krishna et al. 2020; Gu et al. 2021). Structurally, the TM domain of ZIP proteins typically consists of eight α -helices arranged as an inner and an outer helical bundle, in which TM-II, TM-IV, TM-V, and TM-VII form the metal transport pathway, while TM-I, TM-III, TM-VI, and TM-VIII face the membrane lipids. The amino acid lengths of ZIP members vary significantly among different plant species, primarily due to the presence of histidine-rich variable loops between TM-III and TM-IV, which are thought to serve as metal-binding sites (Fu et al. 2017; Gao et al. 2022). The most evolutionarily conserved region among ZIPs is TM-IV, which contains amphipathic helices enriched in histidine residues (Palusińska et al. 2020; Wang et al. 2024).

Numerous studies have demonstrated that the ZIP family members play a critical role in Zn transport and homeostasis in plants. In *Arabidopsis*, AtIRT1 was the first ZIP family identified as a major Fe transporter in plants, other ZIP genes, such as AtZIP1, AtZIP3, AtZIP4, AtZIP5, AtZIP9, and AtIRT3, are transcriptionally activated in response to Zn deficiency, enabling rapid enhancement of Zn acquisition capacity upon Zn deficiency (Vert et al. 2002; Lin et al. 2009; Milner et al. 2013; Lee et al. 2021; Ochoa Tufiño et al. 2025). In rice, OsZIP1 acts as a Zn exporter (Liu et al. 2019), OsZIP5 and OsZIP8 mediate Zn uptake and distribution (Lee et al. 2010a, b), OsZIP7

delivers Zn to developing tissues and grains through xylem loading and inter-vascular transfer (Tan et al. 2019; Gindri et al. 2020), and OsZIP10 transports Zn from the endoplasmic reticulum to the cytosol and promotes radial Zn transport in roots (Tan et al. 2026). ZmZIP5 overexpression increases Zn and Fe in maize roots and shoots while decreasing seed levels, indicating its role in Zn and Fe uptake and root-to-shoot translocation (Li et al. 2019). In addition to Zn, some ZIP transporters also exhibit broad substrate specificity and can transport other transition metals. For instance, OsZIP9 functions as a key influx transporter that mediates the uptake of Zn and Co from the external medium into root cells (Yang et al. 2020), and OsZIP5 acts synergistically with OsZIP9 in mediating Zn and Cd uptake in root (Tan et al. 2020). Both *TpIRT1A* and *TpIRT1B* confer increased sensitivity to Co and Cd in yeast mutants, while overexpression of *TpIRT1B* in *Arabidopsis* results in elevated accumulation of Fe, Mn, Co, and Cd (Jiang et al. 2021a). Collectively, these findings emphasise the functional diversity and substrate specificity of ZIP transporters among different plant species and metal ions.

As a globally important vegetable crop, cucumber (*Cucumis sativus* L.) is recognised for its efficient accumulation of Cd in various tissues (Niu et al. 2025). However, despite the identification of several Cd transporter genes, including *CsNramp5* (Hu et al. 2026), whether additional family members contribute to Cd uptake and translocation remains elusive. Recently, ten ZIP family genes were identified from the cucumber genome, and their structural and evolutionary features were characterised (Wang et al. 2024). However, their Cd-induced expression profiles and Cd transport capacities have yet to be systematically investigated. In the present study, we cloned the *CsZIP9* gene and demonstrated its Cd transport activity using a yeast complementation system. Furthermore, we constructed a plant overexpression vector for *CsZIP9* and generated transgenic *Arabidopsis* plants to preliminarily assess its biological function under Cd stress through phenotypic evaluation. Our results provide a foundation for further studies on the functional roles of ZIP proteins in Cd uptake and translocation in cucumber.

MATERIAL AND METHODS

Plant materials and treatments. Cucumber (inbred line 9930) and *Arabidopsis thaliana* (Col-0) served

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as experimental materials. Various tissue samples, including roots, stems, leaves, flowers, and fruits, were harvested at the appropriate developmental stages according to our previous report (Zhou et al. 2020). Two-week-old cucumber seedlings were exposed to 60 μM CdCl_2 in 1/2 Hoagland solution, and root tissues were harvested at 3, 6, and 9 h post-treatment. Concurrently, control samples (without Cd) were collected at the same time points. All samples were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction.

Arabidopsis seeds were sterilised and placed on 1/2 MS plates. Following a two-day stratification at 4°C , plates were moved to a growth room with 16 h light/8 h dark cycles at 22°C . For Cd tolerance assays, seeds of wild-type (WT) and homozygous T_3 transgenic lines were surface-sterilised and then sown on 1/2 MS medium. Seedlings with uniform root length were transferred to 1/2 MS medium supplemented with 0 or 60 μM CdCl_2 . Root lengths were measured after 7 days of Cd treatment, and all experiments were performed with three biological replicates.

Gene cloning and recombinant vector generation of CsZIP9. Gene-specific primers for *CsZIP9* were designed based on the full-length coding sequence (CDS) available in the Cucurbit Genomics Database (CuGenDB, <http://cucurbitgenomics.org/organism/20>). PCR amplification was performed using the cDNA from cucumber leaves as the template. PCR amplification was performed under the following conditions: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 15 s, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. After PCR amplification, the product was gel-purified and subsequently cloned into the pCambia1304 vector for plant overexpression, as well as into the pYES2 vector for heterologous expression in yeast. Primer sequences are provided in Table S1 in the Electronic Supplementary Material (ESM).

Sequence and structural analysis of CsZIP9. The physicochemical parameters of the *CsZIP9* protein were determined using the ExPASy ProtParam server (<https://web.expasy.org/protparam/>). Subcellular localisation of the *CsZIP9* protein was predicted using Plant-mPLOC (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>). Conserved domain architecture was analysed using the NCBI conserved domain database (CDD, <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Transmembrane helix prediction of the *CsZIP9* protein was performed with the TM-

HMM-2.0 tool (<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>). Multiple sequence alignment was carried out using ClustalW and displayed with GeneDoc, after which a phylogenetic tree was reconstructed using MEGA software based on the Neighbor-Joining (NJ) method.

Cis-element analysis of CsZIP9 promoter. The 2 000-bp upstream sequence of the translation start codon (ATG) of *CsZIP9* was retrieved from CuGenDB. The putative promoter region was then submitted to the PlantCARE database (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) for *cis*-element prediction. The identified *cis*-elements were classified into categories including stress-, phytohormone-, and development-related regulation. The distribution and types of *cis*-elements within the promoter region were visualised using the GSDS tool (<http://gsds.gao-lab.org/>).

Heterologous expression of CsZIP9 in yeast and Cd sensitivity assay. The recombinant plasmid pYES2-*CsZIP9*, along with the empty vector control, was separately transformed into the Cd-sensitive yeast strain *Δycf1*. Positive transformants were selected on SD-Ura medium and liquid cultures were grown at 30°C with rotary shaking at 180 rpm until reaching an OD_{600} of 1.0. To evaluate Cd responsiveness, each culture was diluted in a ten-fold series, and small aliquots were applied to SD-Ura plates supplying 0, 30, or 60 μM CdCl_2 . After 2–3 days of incubation at 30°C , the growth patterns were recorded.

Arabidopsis transformation of CsZIP9. The resultant binary construct pCambia1304-*CsZIP9* was transferred into *Agrobacterium tumefaciens* GV3101, which was then used to transform *Arabidopsis thaliana* via the standard floral dip procedure as previously described (Yang et al. 2025a). Hygromycin-resistant transformants were selected on 1/2 MS medium containing 30 mg/L hygromycin, and positive lines were self-pollinated to generate T_3 homozygous populations. To evaluate the transcript levels of *CsZIP9* among independent lines, qRT-PCR was carried out, and two overexpression lines that exhibited significantly elevated expression were selected for subsequent phenotyping.

Total RNA extraction, reverse transcription, and quantitative real-time PCR (qRT-PCR). Total RNA was isolated from cucumber and *Arabidopsis* samples using the RNA-easy Isolation Reagent, and first-strand cDNA was synthesised with the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) according to the recommended pro-

ocols. *CsZIP9* expression was analysed by qRT-PCR according to our established protocols (Yang et al. 2025a), with *CsActin* (for cucumber) and *AtActin* (for *Arabidopsis*) serving as the internal reference genes. The primers were listed in Table S1 in the ESM.

Statistical analysis. Statistical analyses were conducted using SPSS Statistics 23, and differences between groups were evaluated by one-way ANOVA followed by Duncan's multiple range test.

RESULTS

Cloning and sequence analysis of the *CsZIP9* gene. Gene-specific primers were designed according

to the full-length CDS of *CsZIP9*, and PCR amplification was performed using cDNA derived from cucumber leaves as the template. The resulting PCR products were resolved on a 1% agarose gel, yielding a distinct band of approximately 900 bp (Figure 1A). Subsequent sequencing analysis verified that the cloned *CsZIP9* gene fragment comprised 1005 bp, encoding a deduced protein of 334 amino acids (Figure 1B).

Prediction of physicochemical properties showed that the *CsZIP9* protein has a calculated molecular weight of 35.90 kDa and a theoretical isoelectric point of 6.02. The instability index of 26.05 indicates that *CsZIP9* is a stable protein, while its GRAVY

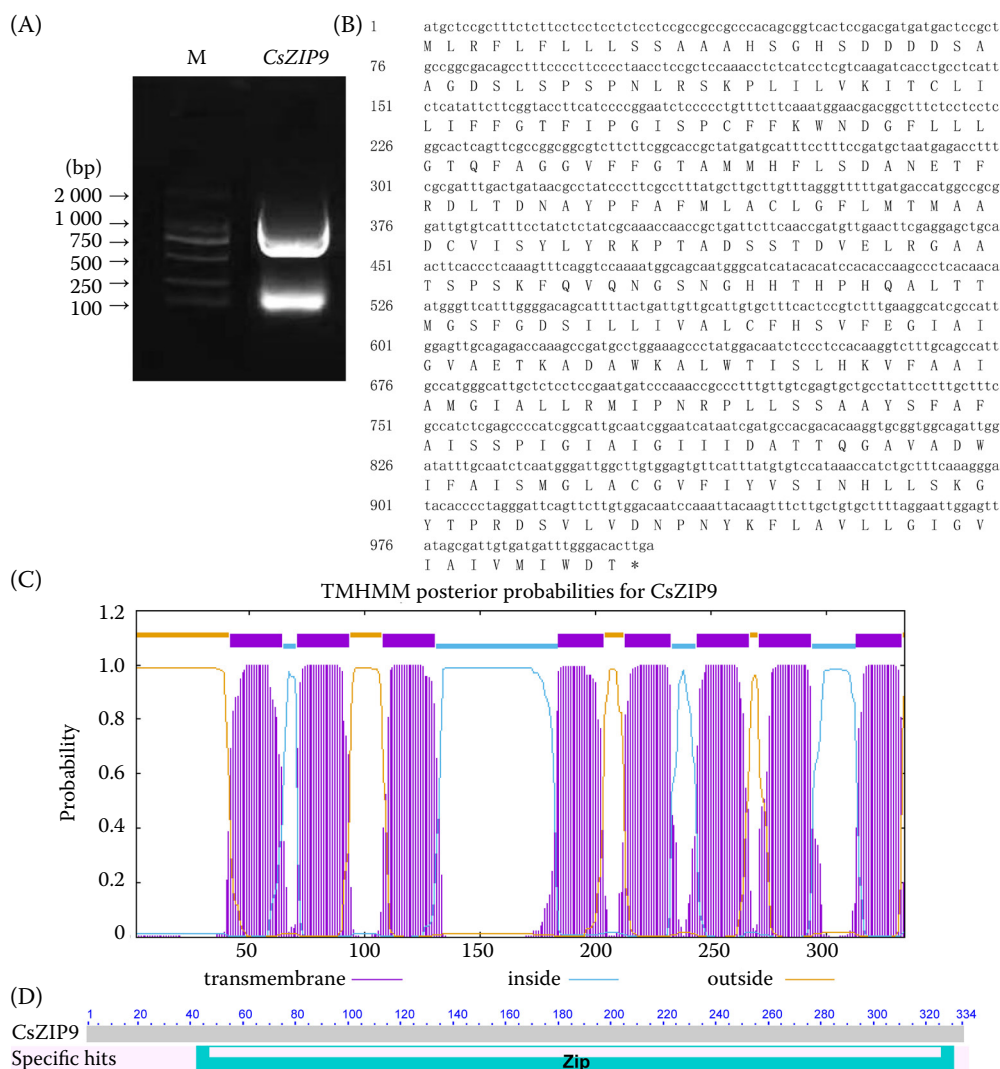


Figure 1. Cloning and structural characterisation of the cucumber *CsZIP9* gene: (A) agarose gel electrophoresis of the *CsZIP9* PCR product (M: 2000 bp DNA ladder), (B) amino acid sequence deduced from the coding sequence (CDS) of *CsZIP9*, (C) prediction of transmembrane helices and topology of *CsZIP9* using TMHMM-2.0, (D) identification of conserved domains in *CsZIP9* using the NCBI conserved domain database (CDD) online tool

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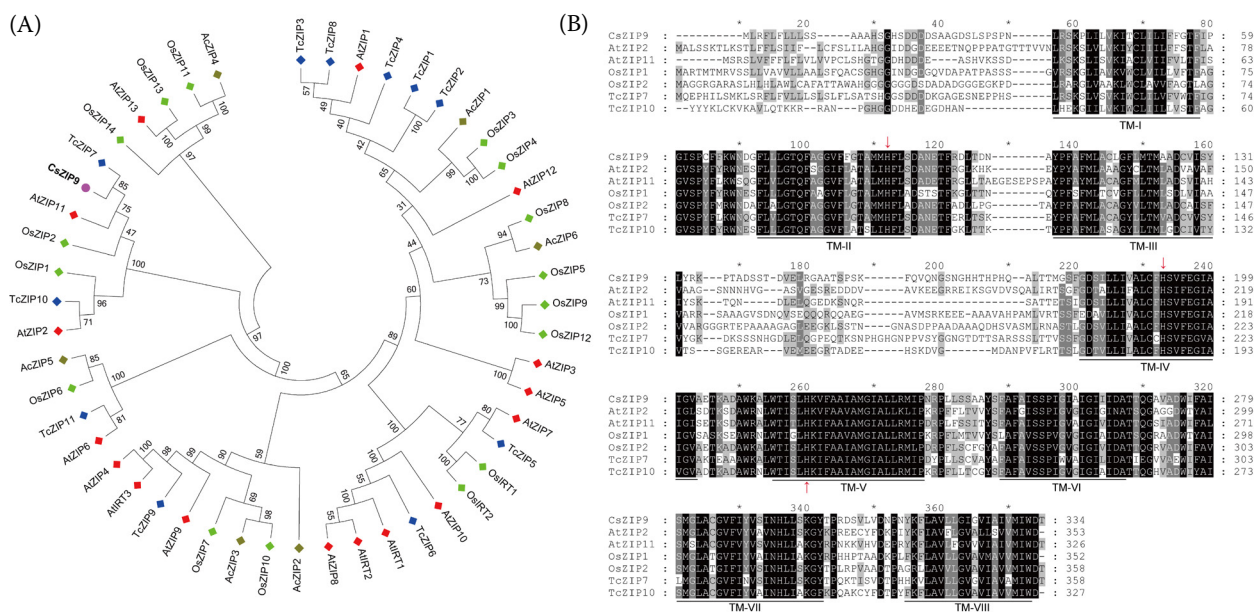


Figure 2. Evolutionary and structural characterisation of CsZIP9: (A) phylogenetic tree illustrating the phylogenetic relationship of CsZIP9 within the ZIP proteins in diverse plant species, (B) multiple sequence alignment of CsZIP9 with closely related ZIP homologs

The eight putative TMs are underlined; three highly conserved histidine residues, localised in TM-II, TM-IV, and TM-V, are highlighted with red arrows; the accession numbers and corresponding amino acid sequences of all ZIP members analysed in the phylogenetic tree were provided in Table S2 in the ESM

score of 0.617 suggests that it is a hydrophobic protein. Subcellular localisation prediction showed that CsZIP9 is localised to the plasma membrane. TM-HMM-2.0 analysis revealed that CsZIP9 possesses eight transmembrane helices, and both its N- and C-termini are located on the extracellular side of the membrane (Figure 1C). Domain analysis using NCBI CDD revealed a conserved ZIP domain (PF02535) spanning amino acid residues 43 to 330 (Figure 1D).

Phylogenetic and sequence conservation analysis of CsZIP9. To further investigate the evolutionary relationship and functional conservation of CsZIP9, multiple sequence alignment and phylogenetic analysis were performed. The deduced amino acid sequence of CsZIP9 was aligned with ZIP protein sequences from other plant species, including *Theobroma cacao* (Pacheco et al. 2023), *Areca catechu* (An et al. 2021), *Arabidopsis* and rice (Zheng et al. 2018). Phylogenetic analysis

showed that CsZIP9 clusters closely with several ZIP members known to be involved in metal ion transport, such as TcZIP7/10, AtZIP2/11, and OsZIP1/2 (Figure 2A). Multiple sequence alignment revealed that CsZIP9 shares high sequence similarity with ZIP proteins from other plant species, particularly within the transmembrane domains (Figure 2B). Conserved histidine residues were identified in TM-II, TM-IV, and TM-V, regions often associated with metal ion binding and transport in other ZIP transporters, and their high sequence conservation among plant ZIPs implies a potential function in metal coordination.

Analysis of *cis*-elements in the CsZIP9 promoter.

To identify potential *cis*-elements in the promoter region of CsZIP9, a 2 000-bp sequence upstream of the start codon was analysed using the online PlantCARE database (Figure 3). The analysis revealed that the CsZIP9 promoter contains a variety

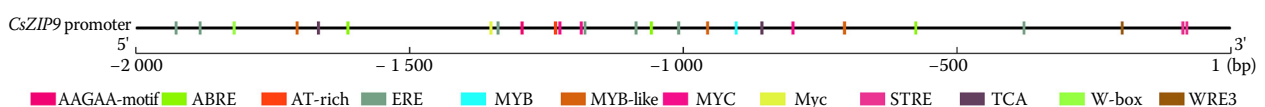


Figure 3. Distribution of *cis*-elements in the 2 000-bp upstream region of CsZIP9

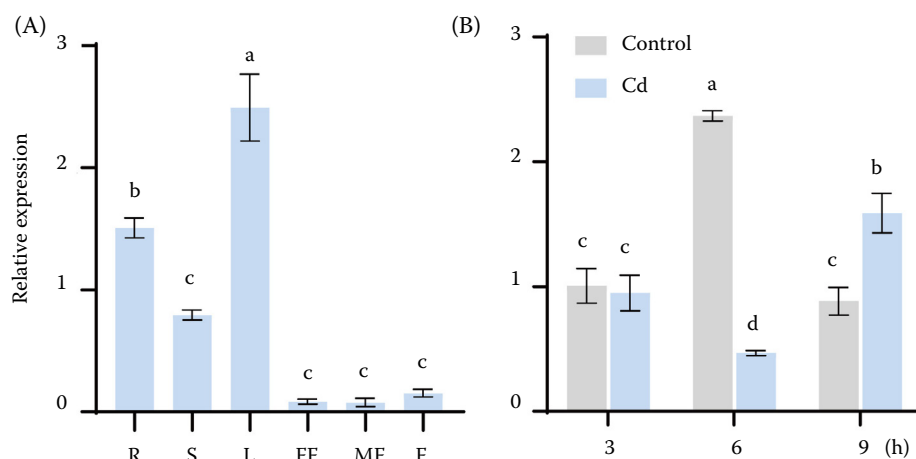


Figure 4. Expression analysis of *CsZIP9* in different tissues and under Cd stress: (A) relative expression levels of *CsZIP9* in various cucumber tissues, including roots, stems, leaves, fruits, male and female flowers, (B) time-course expression of *CsZIP9* in response to Cd treatment at 0, 3, 6, and 9 h

R – root; S – stem; L – leaf; FF – female flower; MF – male flower; F – fruit; data are shown as mean $\pm$ SE ($n = 3$); different letters above the bars indicate significant differences ($P < 0.05$)

of stress-responsive elements, including W-box, WRE3, AT-rich, MYB, MYB-like, MYC, Myc, and STRE motifs. In addition, several hormone-responsive elements were identified, including three abscisic acid (ABA) responsive elements (ABRE), as well as *cis*-elements associated with growth and development. These results suggest that *CsZIP9* may be involved in both growth and development and abiotic stress responses in cucumber.

Tissue-specific and Cd-responsive expression of *CsZIP9*. The expression pattern of *CsZIP9* was analysed by qRT-PCR. As shown in Figure 4A, *CsZIP9* exhibited distinct tissue-specific expression, with the highest transcript level in leaves, moderate levels in roots and stems, and extremely low expression in flowers and fruits. Furthermore, the expression of *CsZIP9* was significantly regulated by Cd stress

in a time-dependent manner (Figure 4B). After 6 h of Cd treatment, its expression was markedly suppressed to the lowest level; however, at 9 h post-treatment, a recovery of transcript accumulation was observed. These results suggest that *CsZIP9* may function differently from typical uptake transporters in response to Cd stress in cucumber.

Heterologous expression of *CsZIP9* sensitises yeast cells to Cd stress. The recombinant vector pYES2-*CsZIP9* was transformed into the Cd-sensitive yeast mutant $\Delta ycf1$ to analyse its possible function. As shown in Figure 5, yeast cells expressing *CsZIP9* exhibited severe growth inhibition under Cd stress, suggesting that *CsZIP9* also possesses Cd transport activity. Its function may facilitate Cd uptake or accumulation, thereby rendering the yeast cells more sensitive to Cd stress.

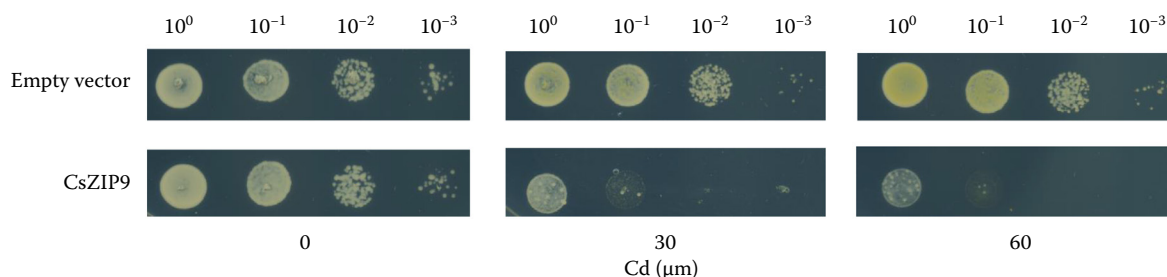


Figure 5. Functional characterisation of *CsZIP9* in the Cd-hypersensitive yeast mutant $\Delta ycf1$; transformants harbouring pYES2 (empty vector) or pYES2-*CsZIP9* were serially diluted tenfold and spotted onto SD-Ura solid medium with or without Cd to assess Cd sensitivity

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Overexpression of *CsZIP9* alleviates Cd-induced root growth inhibition in *Arabidopsis*. Transgenic *Arabidopsis* plants overexpressing *CsZIP9* were successfully obtained via *Agrobacterium*-mediated transformation. As shown in Figure 6A, positive seedlings capable of normal root growth on selection medium were identified through hygromycin resistance screening. These positive seedlings were further cultivated, and T₃ generation seeds were harvested for subsequent analysis. qRT-PCR analysis of overexpression (OE) lines showed that OE6 and OE8 had significantly higher *CsZIP9* transcript levels than the WT and other transgenic lines (Figure 6B), and thus were selected for subsequent functional assays.

Cd treatment significantly suppressed root elongation in both WT and *CsZIP9*-overexpressing lines

(Figure 6C). Notably, the overexpression lines exhibited a markedly lower degree of root growth inhibition compared to the WT control (Figure 6D). These findings demonstrate that *CsZIP9* overexpression enhances *Arabidopsis* tolerance to Cd stress, allowing for relatively better root growth performance under Cd exposure.

DISCUSSION

Plants have evolved sophisticated transport systems and corresponding genes to secure efficient acquisition of essential metal ions. Cd is a non-essential and highly phytotoxic metal that severely hampers plant growth and development. Given the absence of specific Cd transporters, Cd uptake in plants re-

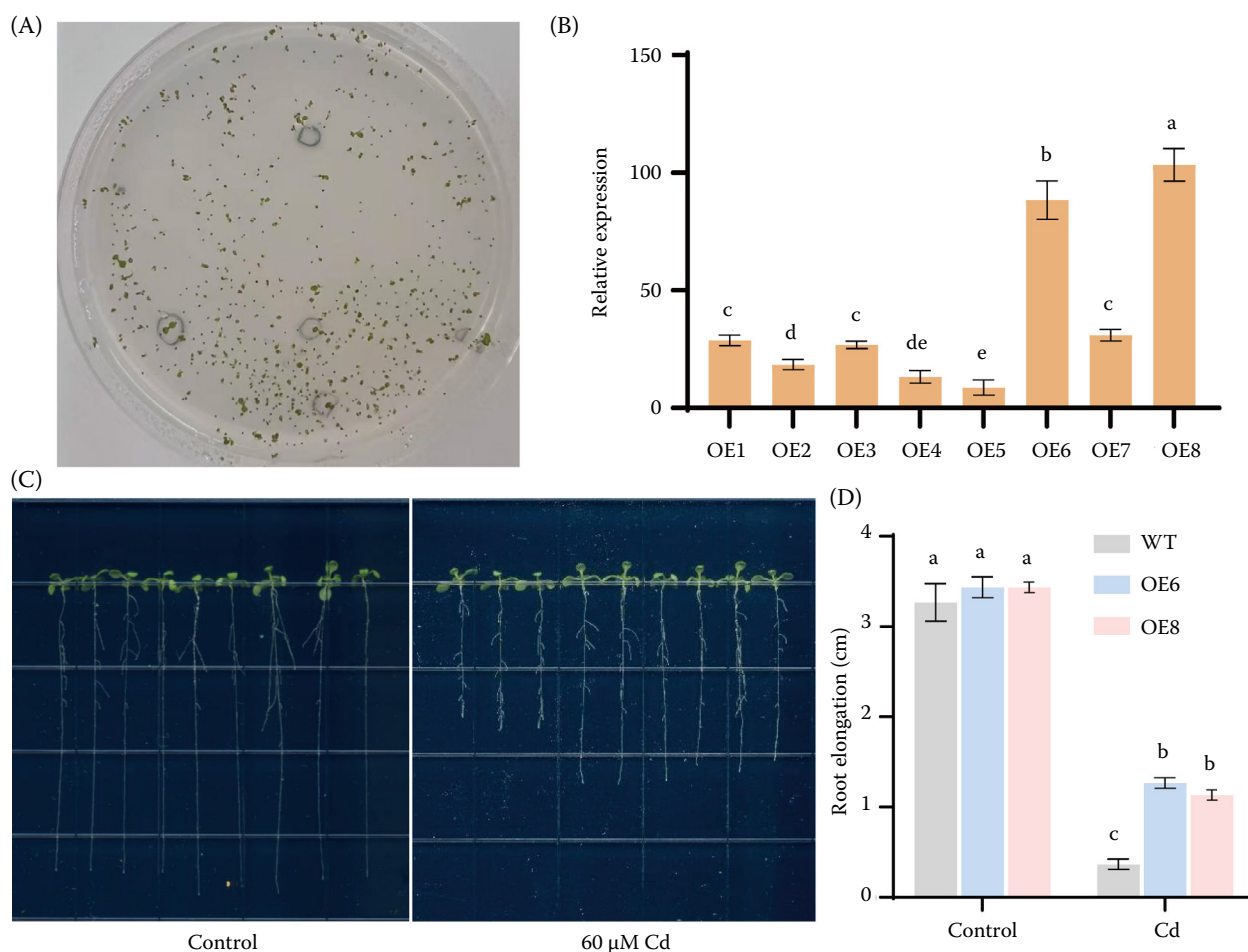


Figure 6. Identification and phenotypic analysis of *CsZIP9* transgenic *Arabidopsis* lines: (A) screening of positive transgenic seedlings on hygromycin-containing medium, (B) relative expression levels of *CsZIP9* in different transgenic lines determined by qRT-PCR, (C) phenotypes of WT and *CsZIP9*-overexpressing lines under Cd stress, (D) root length of WT and *CsZIP9*-overexpressing lines under Cd treatment

OE – overexpression lines; WT – wild-type; data are presented as mean $\pm$ SE ($n = 3$); different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$)

lies on the transport pathways of essential elements like Fe, Zn, and Mn, with ZIP transporters playing a key role in this process (Yang et al. 2025b). In the present study, we successfully cloned the *CsZIP9* gene, a member of the ZIP family from cucumber. Sequence analysis revealed that *CsZIP9* contains a 1 005-bp open reading frame encoding a protein of 334 amino acids, which harbours a conserved ZIP domain and 8 TM domains, with both the N- and C-termini located on the extracellular side (Figures 1 and 2B). These structural features are highly consistent with the canonical architecture of functionally characterised ZIP transporters across various plant species (Gu et al. 2021; Ahmad et al. 2023; Zhang et al. 2024), which typically possess 6–9 TM domains and a histidine-rich variable loop implicated in metal coordination. Phylogenetic analysis further demonstrated that *CsZIP9* is closely clustered with several ZIP members that have been implicated in divalent cation transport, including Zn and Cd (Figure 2A). The conserved structural domains and phylogenetic proximity suggest that *CsZIP9* may share functional similarities with these homologous transporters, particularly regarding its potential involvement in metal ion homeostasis.

The *cis*-elements within the promoter play a central role in the transcriptional regulation of genes, serving as key determinants of the spatial-temporal specificity and strength of gene expression. *Cis*-element analysis revealed the presence of development-related *cis*-elements in the *CsZIP9* promoter, implying its potential involvement in plant growth and developmental regulation (Figure 3). Expression analysis by qRT-PCR revealed that *CsZIP9* exhibited a predominant expression pattern in leaves, with moderate levels in roots and stems, and extremely low transcript abundance in other tissues (Figure 4A). Root expression of this gene indicates a possible role in soil Cd uptake, whereas its abundant expression in aboveground tissues, particularly in leaves, implies a critical role in Cd translocation and aboveground accumulation. This leaf-preferential expression pattern has been reported for some ZIP members in other plant species (Ajeesh Krishna et al. 2020; Zhang et al. 2024), suggesting that *CsZIP9* may be more closely associated with metal redistribution or physiological processes within photosynthetic tissues rather than root acquisition. Additionally, the promoter region of *CsZIP9* contains multiple ABRE (ABA-responsive) elements, drought-responsive MYB/MYC elements, as well as disease- and wound-related elements such

as W-box and WRE3 (Figure 3). These findings suggest that *CsZIP9* may be regulated by MYB/MYC transcription factors and ABA signalling, and may participate in plant responses to both abiotic and biotic stresses. However, these regulatory relationships remain hypothetical and require experimental validation through promoter activity assays and transcription factor interaction studies in future work. In addition, *CsZIP9* expression was dynamically regulated under Cd stress (Figure 4B), and similar expression patterns have been documented for many ZIP genes in response to heavy metal exposure (Palusińska et al. 2020; Gao et al. 2022), implying a temporal coordination of metal transport and detoxification processes. Collectively, these expression characteristics suggest that *CsZIP9* may function at the interface of metal homeostasis and stress adaptation, particularly in root-to-shoot Cd uptake and translocation.

Functional characterisation via heterologous expression in the Cd-sensitive yeast mutant *Δycf1* demonstrated that *CsZIP9* expression significantly increased yeast sensitivity to Cd stress (Figure 5), providing direct evidence that *CsZIP9* possesses Cd transport activity. This observation is consistent with previous reports on many plant ZIP transporters, such as BjZIP1 (Li et al. 2025), OsZIP2 (Li et al. 2024), OsZIP5 and OsZIP9 (Tan et al. 2020), which have been shown to confer Cd hypersensitivity when expressed in yeast mutants. Such a phenotype typically indicates that these ZIP transporters mediate Cd influx and intracellular accumulation, thereby exacerbating metal toxicity in yeast. In contrast, overexpression of *CsZIP9* in *Arabidopsis* led to enhanced Cd tolerance, as evidenced by significantly reduced root growth inhibition under Cd stress compared to WT plants (Figure 6). The seemingly contradictory results between yeast and plant systems may be reconciled by considering the distinct roles of *CsZIP9* in Cd uptake and root-to-shoot translocation, which ultimately alleviates Cd-induced root elongation inhibition in *Arabidopsis*. Similar results have been reported for several ZIP transporters. Under Cd treatment, *OsZIP7* knockout mutants showed higher Cd levels in roots and basal regions but lower levels in young leaves at the seedling stage, along with increased total Cd accumulation compared to WT plants (Tan et al. 2019). Heterologous expression of *OsZIP5* and *OsZIP9* in yeast enhanced Cd sensitivity, whereas their corresponding rice knockout mutants showed impaired Cd uptake (Tan

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et al. 2020). Overexpression of *SmZIP* in tobacco enhances Cd uptake and root-to-shoot translocation, leading to altered subcellular distribution and chemical speciation of Cd, thereby alleviating Cd toxicity in transgenic plants (Jiang et al. 2021b). Collectively, these findings indicate that *CsZIP9* possesses Cd transport activity, warranting further investigation into its potential utility in breeding and phytoremediation.

CONCLUSION

In this study, we cloned and characterised *CsZIP9*, a cucumber *ZIP* gene whose transcript is predominantly accumulated in leaves and dynamically regulated by Cd stress. Both heterologous expression in yeast and overexpression in *Arabidopsis* confirmed its ability to mediate Cd transport. However, the precise function of *CsZIP9* in Cd uptake and its substrate specificity toward other divalent cations, such as Zn, Fe, Mn, and Co, requires further validation through experiments including the generation of *CsZIP9* knockout or overexpression lines in cucumber.

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