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Over expression of a Functional Sedum alfredii PCS homolog increases Cadmium Tolerance in Arabidopsis

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Abstract

Cadmium would cause environmental pollution and severe injuries to the plants. Metal chelation is an important strategy utilized to combat the damage, while phytochelatins produced from reduced glutathione by phytochelatin synthase (PCS) are vital players. As an essential gene in heavy metal stress, numerous experiments have been performed to reveal the precise role of PCS, whereas in the Sedum alfredii Hance, a Zn/Cd co-hyperaccumulator native to China, studies were relatively rare. We identified a PCS homolog in Sedum alfredii designated SaPCSL (phytochelatin synthase-like) based on the transcriptome data. The bioinformatics analysis indicated that SaPCSL harbored the similar domain composition and located separately in the phylogenetic analysis. Transcription analysis revealed that SaPCSL was preferentially expressed in root and the response of SaPCSL to Cd stress was more prominent in the root than the other two tissues. Heterologous expressing SaPCSL in ycf1 and Arabidopsis confirmed the traits of Cd tolerance and accumulation supported by the better performance of transgenic materials under Cd stress and higher Cd accumulation. Above all, we isolated a PCS homolog in Sedum alfredii and assessed its functions in Cd stress. The findings here could provide theoretical guidance for the application in breeding heavy metal-tolerant plants.

Keywords: Sedum Alfredii; Cd Tolerance; Cd Accumulation; Transgenic Arabidops

Introduction

Heavy metals, such as Cd, lead (Pb), chromium (Cr) and nickel (Ni) originated from either natural or anthropogenic sources, would cause environmental pollution and severe injuries to the animals and plants [1]. While animals could avoid the damage through migration, plants, incapable of movement, have no choice but to endure and adapt. The excess level of a heavy metal would cause several biological and physiological changes including reduced biomass, leaf chlorosis, inhibited root growth, morphological alterations, and more seriously plant death [2]. To combat the injuries, plants evolved numerous protection mechanisms, among which metal chelation is an important pathway [3]. Several high-affinity ligands have been proved to be involved in the chelation of metals in the cytosol including amino acids, organic acids, phytochelatins and metallothioneins [4]. Phytochelatins (PC), non-protein thiols with the general structure (g-Glu-Cys)nGly (n=2 to 11), form metal complexes with a molecular weight of 2.5-3.6 kDa which were then transported to specific compartments such as the vacuole [1].

These cysteine (Cys)-rich polypeptides compounds are produced from reduced glutathione by the enzyme phytochelatin synthase (PCS), which is activated rapidly in the presence of heavy metals such as Cd, Cu, Zn, Ag, Au, Hg and Pb [5]. As a vital gene in heavy metal stress, numerous mutation experiments have been performed to reveal the precise roles of PCS, which show that the resulting plants, lacking PC synthesis, are hypersensitive to Cd but have normal sensitivity towards excess Zn, Ni and Se [6-8]. In Mulberry, two Morus notabilis PCS genes have been characterized and their important roles in heavy metal stress tolerance and accumulation have been illustrated by the transgenic over expression in Arabidopsis and tabacco [9]. Studies concerning

the regulation of PC synthesis and downstream processing of PC–Cd complexes have also been performed to elucidate the possible effects of PCS over expression on Cd and PCs extravacuolar/vacuolar distribution and potential consequences for the efficiency of a plant defense system against toxic cadmium ions [10].

Besides the major metal tolerance mechanism of PC synthesis, its contribution to long-distance element transport especially in monocots also intrigued the researchers' interest. Using rice as a cereal model, researchers examined the physiological roles of Oryza sativa phytochelatin synthase 1 (OsPCS1) in the distribution and detoxification of arsenic (As) and cadmium (Cd), and uncovered the contrasting effects of PC synthesis on these two toxic elements associated with major food safety concerns [11]. The hyperaccumulating ecotype (HE) of Sedum alfredii Hance, a Zn/Cd co-hyperaccumulator native to China, exhibiting remarkable traits of accumulating up to 9,000 μg g-1 Cd and 29,000 μg.g-1 Zn in its shoots without any toxicity symptoms [12,13]. PC synthesis in such a hyperaccumulator was worthy to be studied. We identified a PCS homolog in Sedum alfredii designated SaPCSL (phytochelatin synthase-like) based on a genome-wide analysis of the transcriptome database and assessed the physiological roles of SaPCSL in Cd tolerance.

Materials and Methods

Plant materials and growth conditions

We collected the hyperaccumulator Sedum alfredii from Quzhou City, Zhejiang Province, China and cultured in a greenhouse on a 16 h light/8 h dark cycle, with an average temperature of 25-28 °C. The consistency of seedlings was ensured through asexual reproduction and those with uniform growth status were used for stress treatment. To analyze the expression of target gene, the S. alfredii seedling were watered with 400 μM CdCl2 for 0 h、0.5 h、6 h、12 h、24 h、48 h、72 h、96 h. Three tissues (roots, stems and leaves) were collected and promptly frozen in liquid nitrogen, followed by RNA extraction for quantitative real-time PCR analyses. All treatments were applied at least three times. Arabidopsis thaliana was also cultured in the greenhouse under the same conditions for transgenic experiments. The transgenic seeds germinated on 1/2 MS agar plates containing 25 mg·L-1 hygromycin after sterilization. Three over expression lines with high expression level of SaPCSL were used in subsequent physiological experiments. The 30 d-old seedlings cultured in Hoagland-Amon solution containing or without 50 μM CdCl2 for 24 h was used to detect Cd2+ flux. For the physiological experiments, the 30 d-old seedling were watered with or without 30 μM CdCl2 for 0 d, 6 d and10 d, while the time intervals were 0 d, 2 d, 5d and 10 d for the measurement of Cd content.

Cloning of SaPCSL gene and bioinformatics analysis

Total RNAs from different tissues were extracted using the Total RNA Purification Kit (Norgen, Thorold, Canada) and transcribed into the first-strand cDNA using the Superscript III First-Strand Synthesis system followed by RNase H treatment (Invitrogen, Carlsbad, CA), respectively. Based on Sedum alfredii transcriptome data, the full-length SaPCSL cDNA was amplified by reverse transcription-PCR (RT-PCR) using gene-specific primers PCS_ORF_L/R (Table 1). The deduced SaPCSL protein sequence and other plant PCSs were aligned using CLUSTALX 2.0. A phylogenetic analysis was constructed using MEGA 5.2 software based on the neighbor-joining method with 1,000 bootstrap replicates.

Assays for SaPCSL tissue expression and Cd-stress profiles

For the tissue expression, expression levels of SaPCSL in three tissues (root, stem and leaf) were examined by means of qRT-PCR with primers RT PCS-L/R (Table 1). For the stress response, abundances of SaPCSL were detected using the templates from the stress-treated tissues. Briefly, RNA samples were reversely transcribed as described above and qRT-PCR reactions were carried out using SYBR premix EX Tag reagent (TaKaRa, DaLian, China) on a 7300 Real-time PCR System (Applied Biosystems, CA, USA). The Sedum alfredii UBC9 gene was used as a control to normalize the relative expression of target genes, and the primer pair for qRT-PCR analysis was UBC9F/R. The amplification procedure and further data analysis were performed according to a previous study [14]. All the experiments were performed three times independently.

Construction of the SaPCSL expression vectors

In order to verify the function of SaPCSL, heterologous expression vectors in yeast and Arabidopsis were constructed using the purified open reading frame of SaPCSL amplified by High Fidelity KOD-Plus DNA Polymerase (Toyobo, Japan) with the specific primers PCS_ORF_L/R (Table 1). The yeast expression vector pYES2.1- SaPCSL was generated using pYES2.1 TOPO vector and plant over expression vector pH2GW7.0-SaPCSL was recombined through Gateway techniques (Invitrogen, Carlsbad, USA). All the positive clones were further sequenced to verify the direction and sequence accuracy.

Heterologous expression of SaPCSL in yeast

The verified pYES2.1- SaPCSL plasmid was transformed into a Cd-sensitive mutant of the Saccharomyces cerevisiae strain Δycf1 (MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YDR135c::kanMX4) by the lithium acetate method, which lacked the ability to compartmentalize Cd into vacuoles [15,16]. To determine the Cd tolerance of SaPCSL, growth of transformed yeast strains under Cd stress were examined using the empty pYES2.0 vector as control. For complementation assays, a series of three 1:10 dilutions from each culture was spotted onto synthetic-galactose-uracil (SG-U) agar plates supplemented with 0, 15 and 20 μM CdCl2 and incubated at 30 °C for three days. Furthermore, the relative growth of transformants in liquid SG-U supplemented with 5 μM $CdCl_2$ were measured at 12 h intervals. The content of Cd was determined in the remaining part of the yeast cells which were dried at 105 °C for 30 min, and then placed at 70 °C until they reached a constant weight. The dried samples were firstly digested with a concentrated acid mixture of HNO_3, $HClO_4$, and H_2SO_4 (volume ratio = 4:1:0.5) at 250 °C for 8 h, then determined by atomic absorption spectrometry (M6, SOLLAR) after dilution with deionized water.

Transformation and stress treatment of Arabidopsis transformants

A. Thaliana ecotype Columbia plants were transfected by the floral dip method [17] and transgenic lines were screened by hygromycin (Hyg, 20μg · mL-1) resistance, PCR and RT-PCR. The PCR validation used genomic DNA as the template with the Hyg-F/R and RTPCS-L/R primers (Table 1). The SaPCSL expression levels of different transgenic lines were quantified by qRT-PCR. Homozygous lines showing non-segregation from each independent transformant (T3 generation) were screened and three over expression lines (designated OE20, OE25 and OE26) with high transcriptional levels of SaPCSL were used for the stress treatment. In order to study the potential effect of SaPCSL on transgenic lines, seeds from the control and T3 transgenic Arabidopsis were sown on half-strength Murashige and Skoog solid medium. After 7 d, seedlings were transferred to liquid medium for further culture. After 30 d, the plants with consistent growth were transplanted to the nutrient solution containing 30 μM CdCl2. Samples were collected at the 6 days and 10 days, and reduced glutathione (GSH) in the root and aerial parts were measured.

Table 1: All primers used in this experiment.

Primer	Sequence(5'-3')	Description
PCS_ORF_L	ATGGCAATGGCGGGTTTGTA	Gene specific amplification
PCS_ORF_R	TCAGACTTCAAATTTCTGCTTCACA	Gene specific amplification
M 13-F	TCAAATTTCTGCTTCAC	
M 13-R	CAGGAAACAGCTATGACC	
RTPCS-F	TGCACTTGCGTCAACAGCTTC	qRT-PCR
RTPCS-R	GGCTCCGCACCGTTACATTT	qRT-PCR
UBC9-F	TGGCGTCGAAAAGGATTCTGA	qRT-PCR
UBC9-R	CCTTCGGTGGCTTGAATGGATA	qRT-PCR
Hyg-F	TTATCGGCACTTTGCATCG	
Hyg-R	TTATCGGCACTTTGCATCG	

H_2O_2 and superoxide anion accumulations were exhibited through histochemical staining using 3,3-diaminobenzidine [18] solution (1 mg · mL-1, DAB-HCl, pH 3.8) and NBT (1 mg · mL-1). Images of leaves were taken using a stereoscopic microscope (LEICA M125, Germany) after chlorophyll elimination. To test the characteristic of SaPCSL's Cd accumulation in A. thaliana, roots and aerial parts were collected individually for the Cd concentration analysis at day 0, day 2, day 5 and day 10. The roots were resorbed by dipping in 1 mM EDTA for 30 min, and then washed three times with distilled water. The samples were treated as above and the Cd concentration was measured using the acid digests. All the experiment was performed three times, with each replicate consisting of at least 12 plants.

Net Cd^{2+} Efflux Measurements

Using the NMT system (NMT100 Series, Younger, USA LLC, Amherst, MA, USA), the net Cd^{2+} fluxes in the roots of Arabidopsis were noninvasively measured by the Younger USA NMT Service Centre (Xuyue, Beijing). Before measuring flux, the roots were balanced in the testing liquid (0.05 mM $CdCl_2$, 0.1 mM KCl, 0.02 mM $CaCl_2$, 0.02 mM $MgCl_2$, 0.5 mM NaCl, 0.1 mM Na_2SO_4 and 0.3 mM MES, pH 5.7) for 15 min. After that, by a Cd^{2+}-selective microelectrode, transmembrane Cd^{2+} flux of roots corresponding to different lines (120 μm to root apex) were measured for 15 min. All of the measurements were repeated at least six times independently.

Results and Discussion

SaPCSL sequence characteristics

In order to identify the functions of PCS in Sedum alfredii, we searched against the transcriptome data and obtained a transcript encoding a PCS homolog designated as SaPCSL. The open reading frame was 1,668 bp in length, encoding a protein of 555 amino-acid residues with a molecular weight of 62 kDa and a pI of 7.45 (Figure 1A). Domain prediction results showed that SaPCSL had the same domains as PCSs from other plants, including a highly conserved N-terminal domain and a variable Cys-rich region at C-terminal domain (Figure 1B). In all eukaryotes, five conserved cysteine residues of PCS are involved in metal recognition, which were also found in SaPCSL [19,20]. To clarify the phylogenetic relationships among PCSs, a phylogenetic tree was constructed using amino acids of SaPCSL and PCSs of other species (Figure 1C). The detailed abbreviations and species were listed in Table 2. Phylogenetic analysis showed that SaPCSL located separately but more closely to the clade of NtPCS from Nicotiana tabacum.

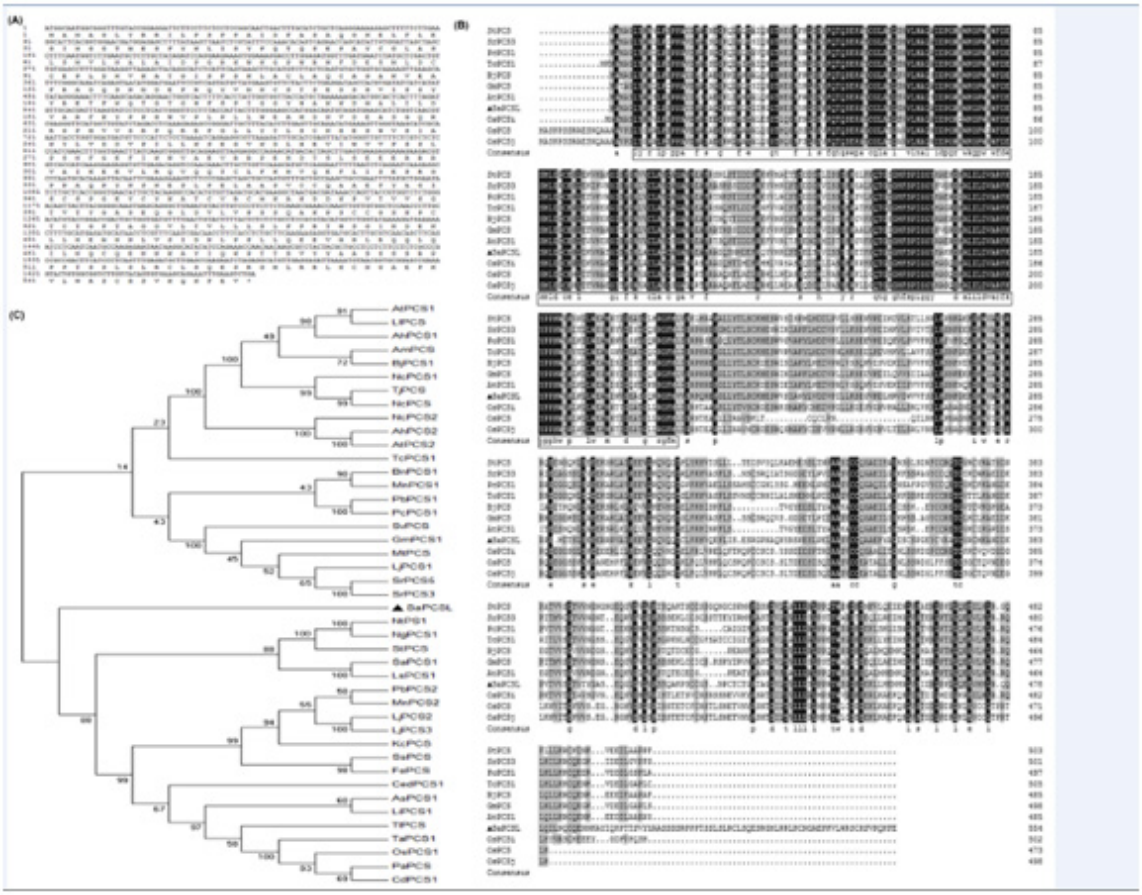

Figure 1: Phylogenetic analysis and multiple alignment of SaPCSL.
A: Nucleotide and deduced amino acid sequences of Sedum alfredii SaPCSL cDNA.
B:Multiple alignment of thirteen amino acid sequences including StPCS (Solanum tuberosum,CAD68110.1), SrPCS3 (Sesbania rostrata,ACT87977.1), PcPCS1 (Pyrus calleryana,AGS56990.1), TcPCS1 (Theobroma cacao,EOX94380.1), BjPCS (Brassica juncea,CAC37692.1), GmPCS (Glycine max,AAL78384.1), AtPCS (Arabidopsis thaliana,AAD16046.1), OsPCSi (Oryza sativa Indic,AOA52643.1),OsPCS (Oryza_sativa,AAO13349.2),OsPCSj (Oryza sativa Japonica,BAW83511.1); (C) The phylogenetic analysis of SaPCSL with other PCSs. The Genebank accession numbers and species names are listed in table 2. SaPCSL wasmarked with black triangle.

Table 2: Names and accession numbers of PCSs used in phylogenetic tree.

Gene	Accession N	Species
AtPCS1	AAD16046.1	Arabidopsis thaliana
LlPCS	ACL00594.3	Leucaena leucocephala
AhPCS1	AAS45236.1	Arabidopsis halleri
AmPCS	AEW23125.1	Amaranthus tricolor
BjPCS1	CAC37692.1	Brassica juncea
NcPCS1	AAT07467.1	Noccaea caerulescens
TjPCS	BAB93119.1	Thlaspi japonicum
NcPCS	BAB93120.1	Noccaea caerulescens
NcPCS2	ABY89660.1	Noccaea caerulescens
AhPCS2	ADZ24787.1	Arabidopsis halleri
AtPCS2	AAK94671.1	Arabidopsis thaliana

PbPCS1	AEY68568.1	Pyrus betulifolia
PcPCS1	AGS56990.1	Pyrus calleryana
BnPCS1	AHC98018.1	Boehmeria nivea
MnPCS1	AMR70492.1	Morus notabilis
GmPCS1	AAL78384.1	Glycine max
SvPCS	AFM38979.1	Sophora viciifolia
MtPCS	XP 013449920.1	Medicago truncatula
SrPCS5	AAY83876.1	Sesbania rostrata
SrPCS3	ACT87974.1	Sesbania rostrata
LjPCS1	AAT80342.1	Lotus japonicus
TcPCS1	EOX94380.1	Theobroma cacao
SaPCS	AHB86971.1	Sedum alfredii
SaPCS1	ACU44656.1	Sonchus arvensis
LsPCS1	AAU93349.1	Lactuca sativa
StPCS	CAD68110.1	Solanum tuberosum
NtPS1	AAO74500.1	Nicotiana tabacum
NgPCS1	ABX10958.1	Nicotiana glauca
SsPCS	AGC82138.1	Suaeda salsa
FePCS	BAF75863.1	Fagopyrum esculentum
KcPCS	ADK61091.1	Kandelia cande
PbPCS2	AHM93477.1	Pyrus betulifolia
MnPCS2	AMR70493.1	Morus notabilis
LjPCS2	AAT80341.1	Lotus japonicus
LjPCS3	AAY81941.1	Lotus japonicus
CdPCS1	ADR10438.1	Ceratophyllum demersum
AsPCS1	AAO13809.1	Allium sativum
LiPCS1	ALT55649.1	Iris sp. ser. Hexagonae
TlPCS	AAG22095.3	Typha latifolia
TaPCS1	AAD50592.1	Triticum aestivum
OsPCS1	AAO13349.2	Oryza sativa
PaPCS	AFU06381.1	Phragmites australis
CdPCS1	AAO13810.2	Cynodon dactylon

Tissue and Cd-stress expression pattern of SaPCSL in Sedum alfredii

To further elucidate the roles of SaPCSL, we firstly isolated total RNA from different tissues of Sedum alfredii and analyze the transcript abundance of SaPCSL by quantitative real-time RT-PCR with SaPCSL-specific primers (Table 1). We observed that SaPCSL was ubiquitously expressed in Sedum alfredii and preferentially expressed in root (Figure 2A). Correspondingly, the response of SaPCSL to Cd stress was more prominent in the root than the other two tissues. We found the relative expression levels in roots increased before 24 h after $CdCl_2$ treatment and exhibited peak expression at 96 h (Figure 2B). As shown in the Figure 2C, the expression of SaPCSL in stem was significantly decreased at 0.5 h and then enhanced with the maximum expression level of 72 h. However, when it came to 96 h, it drops sharply and was significantly lower than the initial value. In leaves, the expression was significantly decreased at the early stage, and maintained the tendency through the whole treatment (Figure 2D).

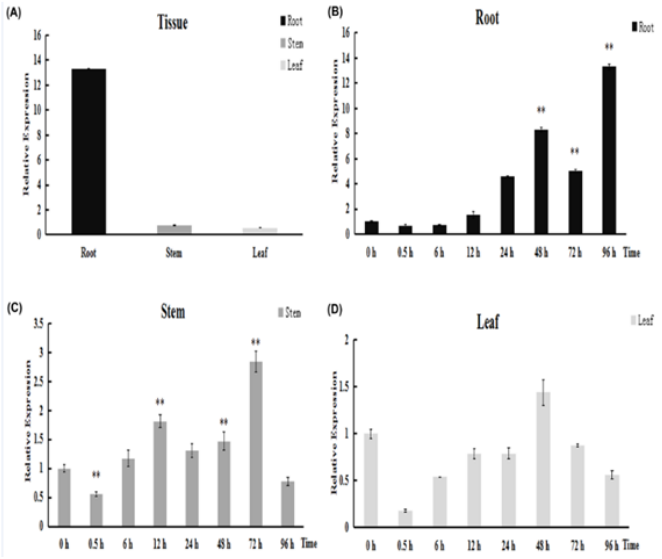

Figure 2: Expression patterns of water culture seedlings in Sedum alfredii with or without Cd treatment.
A: The expression of SaPCSL in different tissues under normal conditions.
B: The temporal and spatial expression patterns of root under Cd stress.
C: The temporal expression patterns of stem under Cd stress.
D: The temporal expression patterns of leaf under Cd stress. The time of exposure is indicated on the x-axis; the y-axis indicates the mRNA expression relative to UBC which was selected as an internal reference gene. The normalized mRNA levels without treatment (y-axis "Relative mRNA expression") were set arbitrarily to 1. Vertical bars represent means ± SD (n = 3). Student's t-tests were applied to evaluate statistical significance and significant differences ($P < 0.05$ and $P < 0.01$) are indicated by * and ** respectively.

SaPCSL increased the cadmium tolerance and cadmium accumulation of yeast

The cadmium resistance experiment of transformed yeast was used to verify whether SaPCSL could increase the cadmium tolerance of the Cd sensitive strain. The SaPCSL- pYES2.1 and pYES2.1 were transformed into yeast (ycf1) separately, and the growth status of transformants was observed. We found both of these yeasts were inhibited in SG-U medium containing 15 μM and 20 μM $CdCl_2$. But the yeast that expressed the SaPCSL had a better growth than the yeast strain containing pYES2.1 which was more strongly inhibited (Figure 3A). The growth curve of transgenic yeasts under cadmium treatment was also tested. We observed that the two yeasts increased exponentially after 24 hours. And the growth rate of SaPCSL-ycf1 was significantly greater than the pYES2.1-ycf1 (Figure 3B). And we determined the cadmium content of two kinds of yeast after a week in SG-U medium containing 5 μM $CdCl_2$ (Figure 3C). The data showed that the cadmium content of SaPCSL-ycf1 was about 2.5 times as high as that the pYES2.1-ycf1 (Figure 3C). All these results indicated that the expression of SaPCSL increased the uptake of cadmium by yeast.

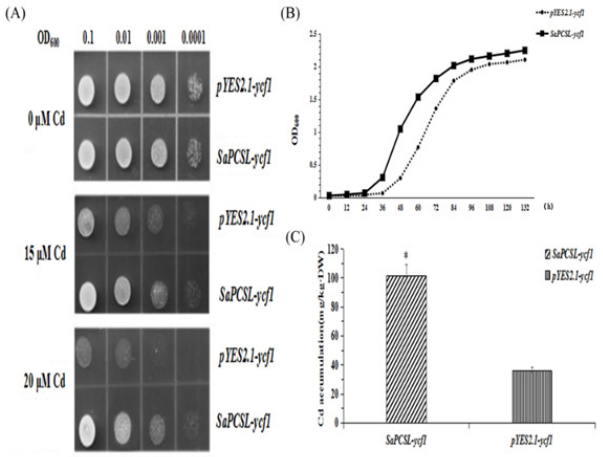

Figure 3: Analysis of cadmium tolerance and accumulation in yeast
A: Tolerance analysis of SaPCSL-ycf1 and pYES2.1-ycf1 under 0 μM, 15 μM, 20 μM CdCl2 treatment.
B: The growth curves of the two yeasts were measured under cadmium treatment.
C: Determination of cadmium accumulation in SaPCSL-ycf1 and pYES2.1-ycf1 after 1 week of treatment with 5 μM CdCl2.

Heterologous expression of SaPCSL in Arabidopsis

To study the function of SaPCSL in plants, SaPCSL was over expressed in Arabidopsis, and three transgenic lines (OE20、OE25 、OE26) were subjected to CdCl2 treatment for 6 and 10 days. Phenotype changes were observed compared with untreated control. Without Cd stress, the selected plants flourished with no divergences (Figure 4A). When they were applied to the stress treatment, both transgenic lines and WT showed chlorosis while wild type was more serious (Figure 4B). Cd toxicity was correlated with oxidative injuries and NBT and DAB staining reflected the effects of Cd toxicity on the production of ROS to a certain extent. Under normal conditions, no differences of the O2- and H2O2 levels were observed between transgenic and WT plants. However, under Cd stress conditions, the accumulation of O2- and H2O2 in transgenic lines was obviously lower than that in WT plants illustrated by the production of dark blue (NBT) or deep brown [18] products (Figure 4C, 4D).

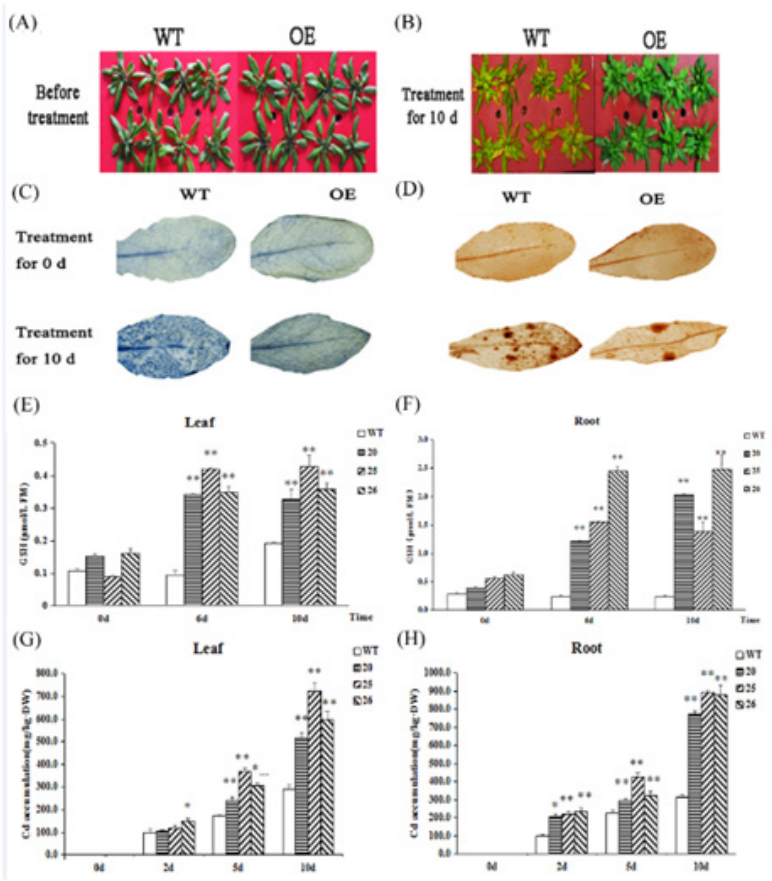

Figure 4:Analysis of cadmium tolerance and accumulation in different lines-WT (wild type); 20, 25 and 26 (over expression lines).
A: Under normal condition, the growth of over expressed lines is consistent with that of WT.
B: After 10 days of 30 μM CdCl2 treatment, WT showed chlorosis, and transgenic lines grew better than WT.
C: The results of NBT staining showed that WT accumulated more negative oxygen ions in the treatment of Cd.
D: DAB staining results showed that WT accumulated more H2O2 in the treatment of Cd. (E, F) Determination of GSH content in leaves and roots treated with 30 μM CdCl2 for 0, 6 and 10 days. (G, H) Determination of Cd accumulation in tow lines for 0, 2, 5 and 10 days.

Additionally, we also compared with GSH contents in WT plants and transgenic plants after stress. The GSH contents were significantly elevated in transgenic Arabidopsis plants, implying a more active process of PC synthesis (Figure 4E, 4F). Furthermore, over expression of SaPCSL remarkably increased the Cd accumulation in the roots and aerial parts (Figure 4G, 4H), suggesting the potential role of SaPCSL in Cd detoxification. Non-invasive micro-test (NMT) was used to investigate the Cd^{2+} uptake in the root tips of transgenic lines and WT plants. Under normal conditions, the net Cd^{2+} fluxes of OE and WT were stable into the cells without Cd treatment, displaying a relatively small divergence (Figure 5A). With 50 μM $CdCl_2$ supplementation for 24 h, the Cd^{2+} influxes of OE lines were higher than those of WT plants (Figure 5B). These results indicated that a greater Cd uptake capacity existed in the transgenic plants than in WT.

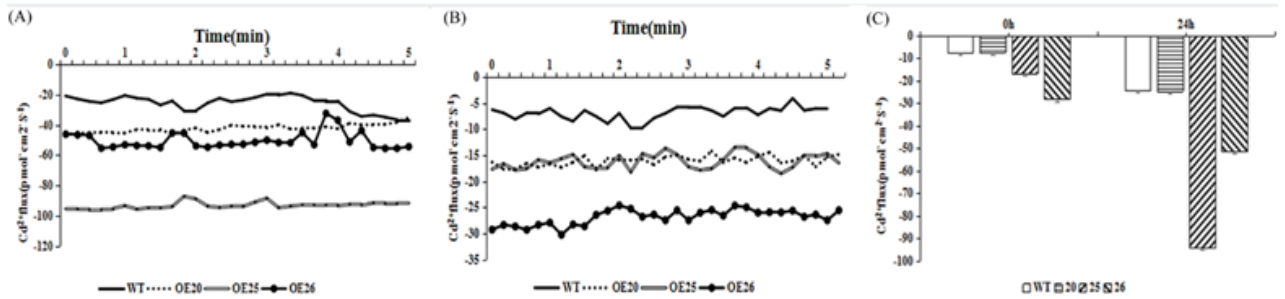

Figure 5: Non-invasive micro-test (NMT) in the root tips of different lines (WT, 20, 25, 26 lines).
A: Determination of Cd2+ fluxes of WT and over expression lines under normal conditions.
B: Determination of Cd2+ fluxes of different lines under 50 μM CdCl2 stress for 24 h. (C) Measurement of average Cd2+ fluxes of different lines under 50 μM CdCl2 stress for 0 and 24 h.

Discussion

Heavy metal stress such as Cd would cause multiple deteriorative disorders such as oxidation of protein, redox imbalance, and ultimately resulting in the activation of programmed cell death (PCD) [21]. Plants employ numerous strategies to cope with the injuries, among which chelation is a vital procedure [22]. Hyperaccumulators count a lot as a model for the illustrating heavy metal uptake, translocation and detoxification [23]. Sedum alfredii, a Cd-hyperaccumulator, has been deemed to be a good plant material for plant restoration [24,25]. Until now, numerous studies have been performed to uncover its traits of hyperaccumulation and tolerance, and characterized several genes closely related to metal uptake [26- 28], detoxification [29], ROS scavenging [30] and other responsive members [31]. MTs, a peptide involved in metal chelation have been well researched and the expression of the SaMT2 gene in yeast and tobacco could improve the resistance and tolerance of cadmium [29]. However, the functions of another member, PCS, an enzyme vital for the PC synthesis, still needed to be studied. In this study, we identified and characterized a PCS homolog to assess its relationship with Cd tolerance and accumulation.

The bioinformatics analysis indicated that the sequences of the N-terminal domains of SaPCSL included conservation of five cysteine residues, whereas their C-terminal domains exhibited greater variation. Phylogenetic analysis showed that SaPCSL located separately with no obvious clustering with other PCSs, which may infer the occurrence of sequential variation which was evolved under the severe heavy metal contaminated surroundings. Combined with the tissue-expression profiles, SaPCSL showed a dominant expression in the root which was the first barrier for the abiotic stress. This may suggest its roles in chelating metal, protecting root cells from injuries different from those responsible for metal sequestration, translocation and storage in leaf. Many studies have reported that over expression of PCSs in plants can improve the heavy-metal (HM) tolerance and accumulation through the enhanced PCs content, while some others have shown contrary findings.

For example, A. thaliana over expressing AtPCS1 caused Cd^{2+} hypersensitivity, despite an increase in the content of PCs was detected. Zhao et al. suggested that the different relationships between heavy-metal tolerance and PCSs among various transgenic plants might be attributed to sequence variations of PCS genes, and/or differences in downstream processing of rHM-PC complexes. Hence, whether the SaPCSL could confer the HM tolerance is worthy to be studied. In this study, expressing SaPCSL in ycf1 compensated the defective of Cd tolerance, supported by the experimental proofs including better performance under Cd stress and accumulating higher levels of Cd. This indicated that SaPCSL could respond to the deletion mutation of the yeast ycf1 strain, and enhance the tolerance and accumulation ability of the yeast to Cd. It also inferred that SaPCSL may be involved in the accumulation of Cd in the cell.

The traits of enhancing cadmium tolerance and accumulation were further assessed through over expression of SaPCSL in Arabidopsis. In this study, transgenic Arabidopsis and wild-type were all damaged after Cd treatment by observing the phenotypic changes, but more severe injuries were detected on leaves of wild-type plants. Meanwhile, transgenic lines exhibited higher GSH contents implying a more active PC synthesis. We also observed lowered O2- and H_2O_2 contents in transgenic Arabidopsis under Cd stress. As is well known, Cd induces both cytotoxic or genotoxic stresses directly or indirectly through ROS damage. The lower ROS levels in transgenic seedlings may be the enhanced detoxification contributed by over expression of SaPCSL. The functions of SaPCSL were further highlighted by the results that the rapid Cd^{2+} net influx and higher accumulation of cadmium in transgenic Arabidopsis thaliana. Higher cadmium accumulation along with better tolerance proved the significance of SaPCSL in Sedum alfredii, which also displayed its potential appliance in phytoremediation. Above all, we isolated a PCS homolog in Sedum alfredii, studied the transcription response to Cd stress, and finally characterized its functions in Cd tolerance and accumulation by heterologous expression. The findings here could enrich genes related to metal chelation and provide theoretical guidance for the application in breeding heavy metal-tolerant crop plants.

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