Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

Genome-wide identification and expression patterns in response to signals from cadmium of *StCADs* gene family in potato (*Solanum tuberosum* L.)

XinYu Yang[1], HePing Lv[1], Wu Zhang[1], HongJie Liang[1], YanPing Gao[1], YiChen Kang[2,3], YanBin Wu[1], FangFang Wang[1] and Chunyan Xi[1*]

Abstract

Background With the rapid development of the economy and society, soil pollution is becoming more and more serious. Heavy metal cadmium (Cd) pollution is one of the typical problems, which poses a potentially serious threat to crop production and human health. Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in lignin synthesis and plays an important role in plant resistance to external stress. In this study, combined with bioinformatics analysis and expression pattern analysis, the members of the potato CAD family were identified, and their physical and chemical properties, evolutionary characteristics and chromosome location were clarified, as well as their regulatory effects on Cd tolerance.

Results A total of 50 *StCAD* genes belonging to 6 subfamilies were obtained, and all of them were located in the cytoplasm. Members of the same family had similar gene structures and functional domains. The promoter region of each *StCAD* family member contains at least 5 or more abiotic stress response elements, indicating that the family had potential functions in regulating stress. According to the expression pattern analysis, most genes in this family were upregulated after Cd stress, further enhanced CAD activity and significantly promoted lignin accumulation in potato roots.

Conclusion In summary, the *StCAD* family plays an important role in potato response to Cd stress. This study lays a foundation for further studies on the functions of the *StCAD* family and provides candidate genes for Cd resistance molecular breeding in potato.

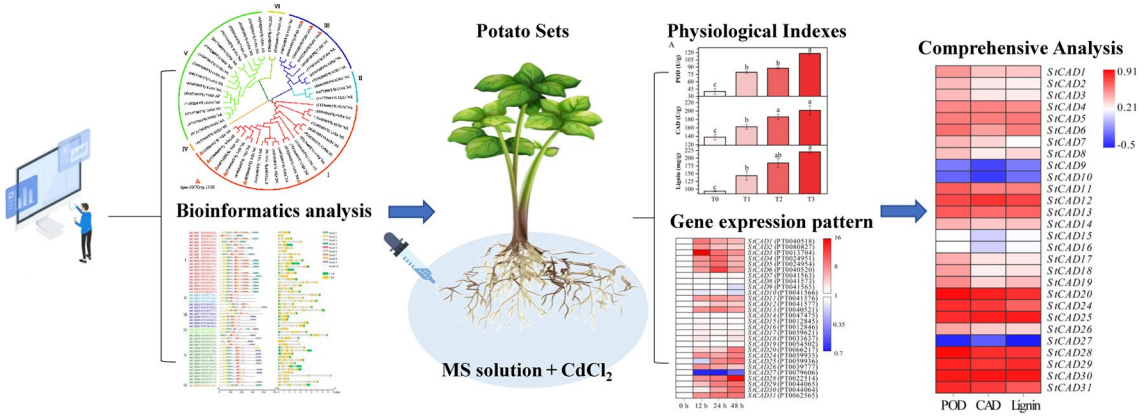
Highlights

1. We obtained 50 *StCAD* genes belonging to 6 subfamilies, all of which were localized in the cytoplasm
2. The promoter region of each *StCAD* family member contains at least 5 or more abiotic stress response elements
3. Most genes of *StCAD* family were upregulated after Cd stress
4. *StCAD* family was beneficial to the enhancement of Cd stress tolerance in potato

Keywords Heavy metal stress, CAD family, Bioinformatics analysis, Gene expressive pattern

*Correspondence:
Chunyan Xi
1228475217@qq.com
Full list of author information is available at the end of the article

Graphical Abstract



Background

In recent years, due to the development of industry and the influence of human activities, the problem of soil pollution has become prominent. Heavy metal pollution is one of the main pollution sources, and Cd pollution accounts for the largest proportion [1, 2]. Cd stress can cause serious harm to plants, causing physiological dysfunction and nutritional disorders [3], leading to cell damage, destruction of photosynthetic systems, protein degradation, DNA damage mutations, and ultimately inhibiting plant growth [4–6]. The growth of crops under Cd stress will lead to a series of problems, such as serious yield reduction and quality reduction [7]. In addition, many studies have shown that crops and vegetables grown on heavy metal contaminated soil will accumulate a large number of heavy metals [8, 9], and the consumption of agricultural products with excessive Cd will induce many diseases including a variety of cancers, which is a serious threat to human health [10, 11].

Potato holds an important position in the world's food industry. It is the primary 'grain and vegetable' crop in China, and its healthy development is of great significance [12]. It is reported that the natural accumulation of Cd in potato tubers is much higher than that in fruits and grains [13]. Therefore, scholars have begun to pay attention to the impact of Cd pollution on potato, have committed to the analysis of potato Cd response mechanism, and have sought solutions [14, 15]. The cell wall of higher plants is the first line of defense against external stress. Previous studies have found that plants will promote the combination of ionic Cd and cell wall components, blocking Cd^{2+} in the cell wall and preventing it from entering the cytoplasm, thereby protecting intracellular metabolic activities [16, 17]. Lignin is an important part of the cell wall, and its accumulation can reduce external damage to plants [18, 19]. Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in its synthesis pathway, which is responsible for catalyzing the conversion of cinnamaldehydes (coniferyl aldehyde, cinnamaldehyde, and coumaraldehyde) into corresponding alcohols, thereby promoting lignin accumulation and participating in plant response to stress [20, 21]. Studies have shown that overexpression of the *SaCAD* gene can improve the tolerance of Arabidopsis to Cd [22]. Kim et al. found that a gene encoding CAD was highly expressed in sweet potato induced by low temperature and other environmental stresses [23]. Park et al. found that CAD activity in rice was significantly induced by UV treatment, suggesting that CAD plays an important role in rice resistance to UV radiation [24]. Hu et al. showed that CAD can enhance plant resistance to adversity by promoting the accumulation of lignin [25]. Our recent transcriptome analysis found that a CAD gene can participate in the response of potato to Cd stress through a targeted regulatory relationship with miRNA, so it is speculated that the CAD family may play an important role in the response of potato to Cd stress [26]. Therefore, we carried out member identification and bioinformatics analysis of the potato CAD family, and explored the expression patterns of *StCAD* family members after Cd stress, focusing on the changes of physiological indicators closely related to CAD genes, which laid a foundation for further clarifying the function of the *StCAD* family. At the same time, it can provide new ideas for later research on Cd tolerance breeding and heavy metal stress relief of potato.

Materials and methods

Plant materials and Cd stress treatment

In this experiment, potato 'Atlantic' tissue culture seedlings were used as experimental materials. Plantlets were grown in MS medium in a culture box, at a temperature of 21 °C, a photoperiod of 16 h light:8 h dark, and a light intensity of 3000 Lx for 20 days of treatment, the seedlings were transferred to a liquid medium supplemented with $CdCl_2$ (5 mmol/L). Plant roots were collected before Cd treatment (T0) and at 12 (T1), 24 (T2), and 48 (T3) h after Cd treatment, washed three times with deionized water, quickly frozen in liquid nitrogen, and then stored in a freezer at -80℃. There were three biological replicates at each time point.

Identification of StCAD gene family members

The Hidden Markov Models (HMM) of cinnamyl alcohol dehydrogenase (CAD) protein domains ADH_N and ADH_zinc_N, PF08240 and PF00107, were downloaded from the Pfam database (http://www.sanger.ac.uk/Software/Pfam/). Using PF08240 and PF00107 as probes, the HMMER3.0 software package was used to search the protein sequences predicted by the potato reference genome (http://spuddb.uga.edu/) and the potato transcriptome constructed by our laboratory. The search results were submitted to the NCBI conserved domain database (http://www.ncbi.nlm.nih.gov/cdd/) for verification. The potato reference genome sequence and gff3 structure annotation file were downloaded from the Ensemble database (http://plants.ensembl.org/Solanum_tuberosum/Info/Index) to verify the CAD gene family members and subsequent analysis.

Bioinformatics analysis of StCAD gene

Mega 7 software [27] was used to construct the phylogenetic tree of *StCAD* family members by the ML method, bootstrap 1000 times, and the family members were divided into groups according to the branch of the phylogenetic tree. The amino acid sequences of each gene were submitted to the ExPASy website (https://web.expasy.org/protparam/) to output various physical and chemical properties, and the subcellular localization analysis was performed using the CELLO website (http://cello.life.nctu.edu.tw/). The motif analysis was performed by the meme tool (http://alternate.meme-suite.org/tools/meme) to identify conserved motifs. The gff3 file of the *StCAD* gene was input into TBtools software [28] to realize the visualization of chromosome position and exon–intron structure. The 2-kb promoter sequence upstream of the *StCAD* gene coding region was extracted and submitted to the PlantCARE database for cis-acting element prediction and visualization based on TBtools.

qRT-PCR analysis of StCAD gene in response to Cd stress

According to the sequencing results of previous studies (NCBI accession number: SRP314907) [26], the expression patterns of some *StCAD* genes under Cd stress were analyzed by qRT-PCR. Samples were taken at 0 h, 12 h, 24 h and 48 h under Cd stress. According to the manufacturer's instructions, the total RNA of the samples was extracted by RNAout kit (160,906–50, Tiandz, Beijing), RNA-gel in Additional file 1. Reverse transcription of mRNA was performed using the kit FastKing gDNA Dispelling RT SuperMix provided by Tiangen Biochemical Technology Co., Ltd. (Beijing, China), and actin was used as the mRNA reference gene. The obtained cDNA was detected on a fluorescence quantitative PCR instrument using the TB Green Premix Ex Taq II kit, and the relative expression level was calculated according to the $2^{-\Delta\Delta Ct}$ method [29]. Each group had 3 biological replicates, and each reaction had 3 technical replicates. The primer sequence is shown in Additional file 2.

Measurement of physiological indices

The cinnamyl alcohol dehydrogenase (CAD) activity, peroxidase (POD) activity, and lignin content were determined using the kits BC4170, BC0090 and BC4200 provided by Beijing Solarbio Science Technology Co., Ltd (Beijing, China). The specific prescription determination method refers to the instructions in the kit.

Data analysis

SPSS 19.0 software was used for variance analysis. Least significant difference (LSD) and Student–Newman–Keuls (SNK) methods were used to investigate the difference at the $P \leq 0.05$ level. Data analysis and mapping were performed using Origin 2018 software.

Results

Identification of StCAD gene family members

Through protein sequence analysis and verification, 50 members of the potato CAD family were finally obtained, named *StCAD1–StCAD50*, with isoelectric points ranging from 5.31 to 9.18. Most CAD proteins (76%) have isoelectric points less than 7, suggesting that they may be acidic proteins. The molecular weight is between 20,848.99 to 46,165.99 Dalton; the amino acid sequence size ranges from 190 to 433 aa. The total number of atoms is between 2923 and 6480. Most of the instability coefficient (92%) was below 40, suggesting that it was a stable protein. Subcellular localization prediction of all genes showed that all genes were located in the cytoplasm (Table 1).

Table 1 Physicochemical properties and subcellular localization of proteins encoded by *StCADs*

Gene name	Trans ID	Theoretical pI	Molecular weight/ Dalton	Number of amino acids/aa	Total number of atoms	Instability index	Grand average of hydropathicity	Aliphatic index	Subcellular localization
StCAD1	*PT0040518*	6.27	38,953	357	5479	18.63	−0.073	90.25	Cytoplasmic
StCAD2	*PT0080827*	7.87	22,957.8	213	3270	23.48	0.141	101.56	Cytoplasmic
StCAD3	*PT0013704*	6.13	39,120.2	357	5512	17.05	−0.04	91.07	Cytoplasmic
StCAD4	*PT0024951*	6.65	26,432.2	243	3703	28.14	−0.12	85.66	Cytoplasmic
StCAD5	*PT0024954*	8.48	29,932.4	269	4179	23.61	−0.21	80.63	Extracellular; periplasmic; cytoplasmic
StCAD6	*PT0040520*	8.04	33,783.1	308	4731	26.01	−0.131	79.61	Cytoplasmic
StCAD7	*PT0041563*	6.35	27,097.8	249	3766	29.89	−0.079	81.29	Extracellular; cytoplasmic
StCAD8	*PT0041573*	6.79	34,678.2	322	4901	30.06	0.026	91.65	Cytoplasmic
StCAD9	*PT0041565*	6.16	28,228.6	258	3940	25.65	−0.045	82.65	Cytoplasmic
StCAD10	*PT0041566*	6.26	39,209.4	360	5513	26.19	−0.055	87.66	Periplasmic; cytoplasmic
StCAD11	*PT0041576*	6.37	38,819	362	5491	25.97	0.027	92.33	Cytoplasmic
StCAD12	*PT0041577*	6.27	38,753.7	361	5476	25.94	−0.015	92.03	Cytoplasmic
StCAD13	*PT0040521*	5.46	23,122.5	209	3207	28.56	−0.072	82.79	Extracellular; periplasmic; cytoplasmic
StCAD14	*PT0047475*	5.84	38,952.8	361	5483	34.76	0.007	93.42	Cytoplasmic
StCAD15	*PT0012845*	5.62	41,876.4	385	5888	24.92	0.193	95.65	Cytoplasmic
StCAD16	*PT0012846*	5.47	38,596.5	356	5427	24.59	0.156	94.93	Cytoplasmic
StCAD17	*PT0059621*	8.3	39,913.9	363	5600	25.12	−0.124	84.78	Periplasmic; cytoplasmic
StCAD18	*PT0033637*	6.11	39,504.4	361	5552	25.06	−0.136	89.61	Cytoplasmic
StCAD19	*PT0054502*	7.54	39,479.4	361	5542	30.58	−0.076	86.61	Cytoplasmic
StCAD20	*PT0066217*	5.7	38,933	358	5462	25.23	−0.052	90.22	Cytoplasmic
StCAD21	*PT0057743*	5.88	32,914	310	4679	38.39	0.051	95.92	Cytoplasmic
StCAD22	*PT0057744*	7.53	34,655.1	326	4938	42.75	0.058	97.78	Cytoplasmic
StCAD23	*PT0055608*	6.46	34,848.1	328	4934	30.2	0.088	95.05	Cytoplasmic
StCAD24	*PT0059935*	6.78	28,422.5	266	3947	49.98	0.014	75.85	Periplasmic; cytoplasmic
StCAD25	*PT0059936*	7.89	46,166	433	6480	41.6	0.041	86.27	Cytoplasmic
StCAD26	*PT0039777*	7.17	39,473.3	368	5607	33.18	0.001	101.31	Cytoplasmic
StCAD27	*PT0079606*	6.48	41,033	389	5866	24.14	0.009	94.69	Periplasmic
StCAD28	*PT0022514*	8.93	35,199	331	5058	25.98	0.034	102.48	Periplasmic; cytoplasmic
StCAD29	*PT0044065*	9.18	35,636.6	333	5118	23.45	0.005	99.52	Periplasmic; cytoplasmic
StCAD30	*PT0044064*	9.1	34,618.1	330	4955	30.32	0.011	92.49	Periplasmic
StCAD31	*PT0062565*	8.63	34,560.1	330	4957	30.64	0.055	100.46	Periplasmic; cytoplasmic
StCAD32	*PT0065063*	6.61	38,663.1	356	5473	24.21	0.143	99.92	Cytoplasmic
StCAD33	*PT0081907*	6.32	37,323.6	347	5473	24.21	0.143	99.92	Cytoplasmic
StCAD34	*PT0038524*	6.46	35,518.2	327	5015	28.45	0.113	99.2	Cytoplasmic
StCAD35	*PT0038525*	6.36	41,099.7	378	5805	29.34	0.045	97.67	Cytoplasmic
StCAD36	*PT0038526*	6.01	32,906.1	305	4644	29.08	0.086	101.25	Cytoplasmic
StCAD37	*PT0038529*	6.37	34,614.2	321	4917	22.6	0.152	103.81	Cytoplasmic
StCAD38	*PT0047759*	6.08	39,408.8	361	5547	36.97	0.093	95.22	Cytoplasmic
StCAD39	*PT0047760*	5.85	41,103.7	375	5803	34.01	0.016	96.6	Cytoplasmic
StCAD40	*PT0063937*	6.19	41,032	380	5743	29.09	−0.098	81.5	Cytoplasmic

Table 1 (continued)

Gene name	Trans ID	Theoretical pI	Molecular weight/ Dalton	Number of amino acids/aa	Total number of atoms	Instability index	Grand average of hydropathicity	Aliphatic index	Subcellular localization
StCAD41	*PT0079071*	5.92	41,156.5	380	5783	29	0.047	88.21	Cytoplasmic
StCAD42	*PT0019942*	6.32	40,643.8	380	5708	24.89	0.06	87.39	Cytoplasmic
StCAD43	*PT0031884*	6.64	41,505.1	381	5852	25.95	92.05	0.009	Cytoplasmic
StCAD44	*PT0075616*	6.09	20,849	190	2923	38.02	−0.01	83.05	Periplasmic; cytoplasmic
StCAD45	*PT0075618*	6.13	42,316.9	381	5956	26.4	0.066	88.64	Cytoplasmic
StCAD46	*PT0025617*	6.61	42,309	389	5971	28.16	0.062	90.88	Cytoplasmic
StCAD47	*PT0079173*	5.78	34,124	316	4754	31.67	−0.122	78.35	Cytoplasmic
StCAD48	*PT0079174*	5.31	23,927.5	223	3331	38.51	0.05	81.26	Cytoplasmic
StCAD49	*PT0079175*	5.37	41,651.9	387	5841	30.8	0	86.67	Cytoplasmic
StCAD50	*PT0065246*	9.11	41,511	381	5901	40.35	−0.075	95.89	Periplasmic; cytoplasmic

PT is the abbreviation of PGSC0003DMT40

Evolutionary analysis of the StCAD gene family

Phylogenetic analysis of the potato CAD family revealed that 50 genes belong to 6 subfamilies, of which the first subfamily includes 20 genes, the second subfamily includes 3 genes, the third subfamily includes 8 genes, the fourth subfamily includes 2 genes, the fifth subfamily includes 16 genes, and the sixth subfamily includes 1 gene. There are 9 differentially upregulated genes in the CAD family obtained by previous sequencing, which are in the first subfamily and the third subfamily. The 50 genes of potato CAD family are closely related to the 24 genes of Arabidopsis CAD family (Fig. 1 and Additional file 3).

Structural characteristics analysis of StCAD gene family

The intron–exon and motif visualization analysis were performed using the software TBtools. Figure 2 shows that the number and length of exons in the *StCAD* family are different, and the number of exons varies from 3 to 10. Among them, *StCAD40*, *StCAD43*, *StCAD45*, *StCAD49*, and *StCAD50* have the largest number of exons, 10, and *StCAD2*, *StCAD4*, *StCAD5*, *StCAD7*, *StCAD9*, and *StCAD13* have the least number of exons, only 3.

Conserved motif analysis of the *StCAD* protein showed that the members of the *StCAD* family were highly conserved at the 5' end. The conserved motifs contained in the family members were Motif 1, Motif 2, Motif 3 and Motif 5. In the same subgroup, the number and type of protein-conserved motifs were similar. Among them, subfamily I contained all conserved motifs except Motif 9 which contained more conserved motifs than other subfamilies. We speculate that the gene structure of subfamily I may be more complex than other subfamilies. In addition, some motifs have obvious subfamily specificity. Motif 1 and Motif 10 only appeared in Subfamily I and Subfamily VI, and Motif 7 only appeared in Subfamily I (Fig. 2 and Additional file 4).

Chromosome localization of the StCAD gene family

Chromosome localization analysis of potato CAD family genes showed that each gene was irregularly distributed on 12 chromosomes. Among them, chromosomes 3, 4, 9, 11, and 12 were more distributed. Five genes were distributed on chromosomes 3, 9, and 12, respectively. Six genes were distributed on chromosome 4, and 13 genes were distributed on chromosome 11. The tandem repeat gene clusters were formed on chromosomes 3, 4, 9, and 11 (Fig. 3).

Cis-element analysis of StCAD gene promoter related to stress

In this study, a 2-kb sequence upstream of the translation initiation site of the CAD gene was extracted and submitted to the PlantCare online website for cis-element prediction. Figure 4 shows 18 major abiotic stress response elements. The results showed that the 50 CAD genes contained multiple phytohormone response elements, including auxin response element (TGA-element), auxin response element (AuxRR-core), salicylic acid response element (TCA-element), gibberellin response element (GARE-motif), abscisic acid responsive element (ABRE);: methyl jasmonate response element (TGACG-motif and CGTCA-motif); methyl jasmonate response element. These CAD genes were mainly distributed in subfamilies I, III and V. In addition, most CAD genes were also found to contain cis-elements in response to stress and stress signals, such as stress response element (STRE)

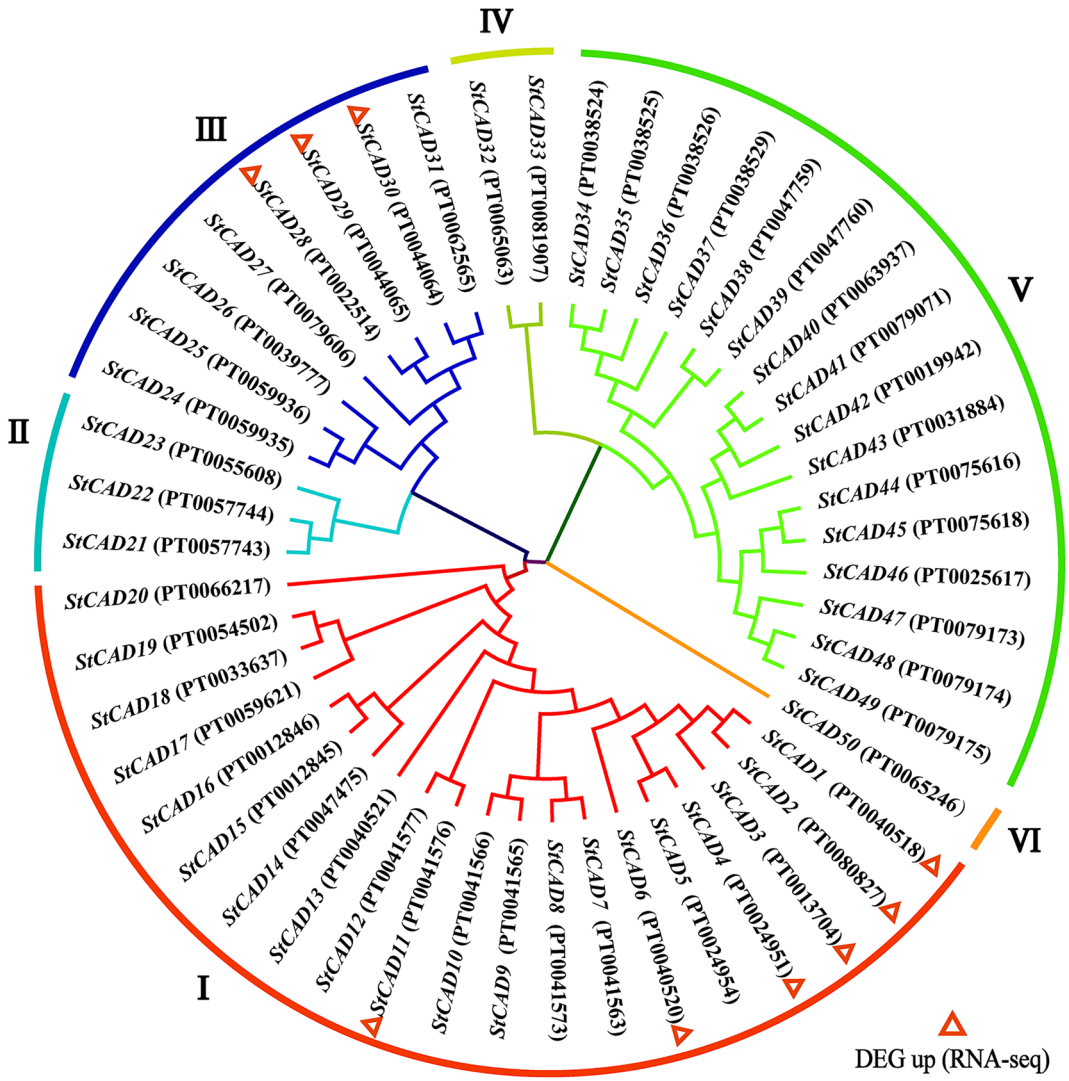


Fig. 1 Phylogenetic tree of potato *StCADs* gene family

in 28 genes and antioxidant response element (ARE) in 39 genes. In summary, the *StCAD* family has potential functions in regulating hormones and stress, especially closely related to abiotic stress.

Analysis of expression patterns of the StCAD gene family in response to Cd stress

Based on the previous research results, the potato CAD gene family may respond to abiotic stress. There are 9 differentially upregulated genes in the CAD family obtained by pre-sequencing, which are in the first subfamily and the third subfamily. Therefore, we analyzed the expression patterns of subfamily I and III. Cd stress was performed on potatoes, and samples were taken at different time points. The expression characteristics of *StCAD* in plants were verified by real-time PCR (Fig. 5 and Additional file 5). Except for *StCAD9*, *StCAD10* and *StCAD27*, other genes were upregulated after Cd stress. *StCAD3* changed the most at 12 h of Cd stress, which was 16.61 times higher than that before stress. *StCAD6* changed the most at 24 h of Cd stress, which was 12.18 times higher. *StCAD28* changed the most at 48 h of Cd stress, which was 15.41 times higher. The expression levels of *StCAD7*, *StCAD8*, *StCAD14*, *StCAD15*, *StCAD16* and *StCAD19* did not change significantly after stress. Based on this, we concluded that potato CAD gene family members have undergone functional differentiation during evolution.

Fig. 2 Gene structure and motif analysis of *StCADs* in potato. The different line colors of gene IDs showed the classification of CADs based on phylogeny tree

Analysis of physiological characteristics related to the StCAD gene

The activities of POD, CAD and lignin in potato were significantly changed after Cd stress. The activities of POD, CAD and lignin in potato were significantly changed after Cd stress. The POD activity increased with the increase of Cd stress time, and the POD activity of the T3 treatment was significantly higher than that of other treatments. CAD activity increased significantly after Cd stress and CAD activity of the T0 treatment was significantly lower than that of other treatments. The lignin content showed a gradually increasing trend, and the lignin content of the T0 treatment was the lowest, while that of the T3 treatment was significantly higher than that of T0 and T1 (Fig. 6A). To explore the relationship between the *StCAD* gene and related physiological indicators, correlation analysis was conducted on the *StCAD* gene, POD and CAD activities and lignin content, and the results showed that except for *StCAD9*, *StCAD10* and *StCAD27*, the other genes were positively correlated with physiological indicators (Fig. 6B).

Discussion

Lignin is widely regarded as an important secondary metabolite involved in plant stress resistance, which can enhance the mechanical strength and stress resistance of plants, is conducive to the transport of minerals and water in plants and the defense of an adverse external

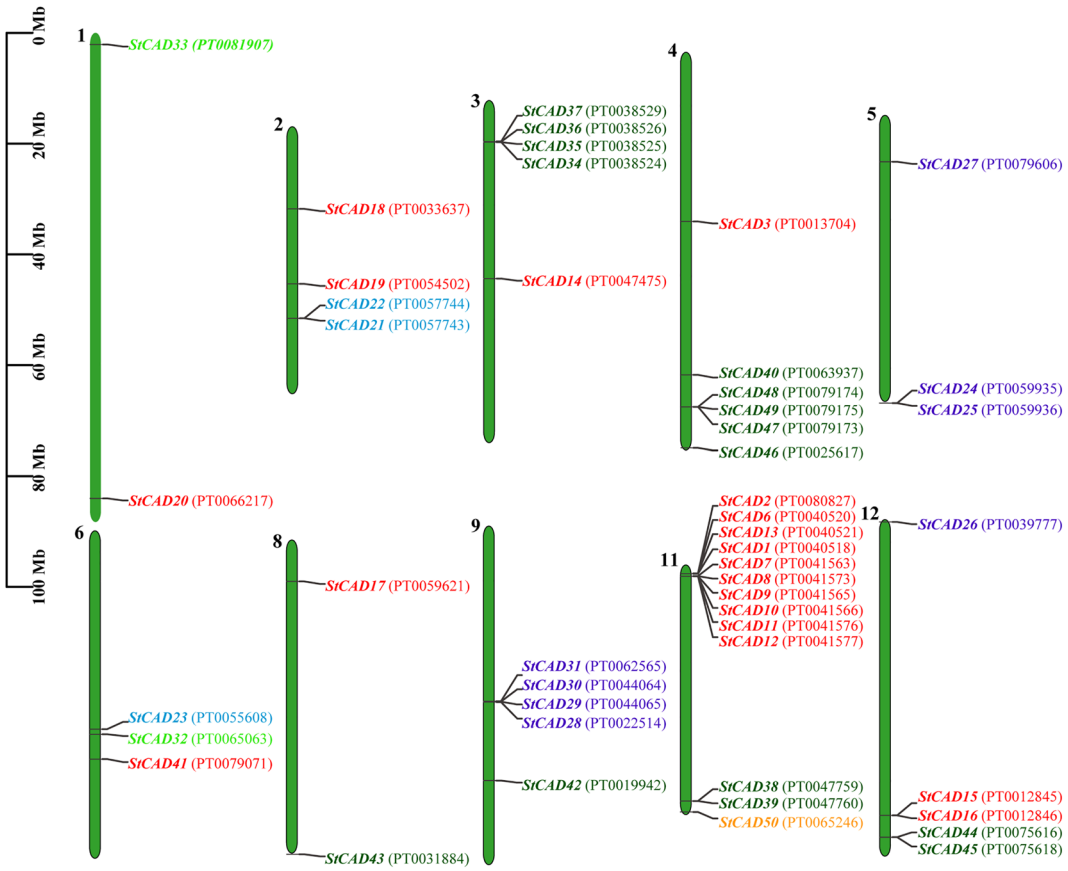


Fig. 3 Mapping of the *StCADs* gene family on potato chromosomes. The different line colors of gene IDs showed the classification of CADs based on phylogeny tree

environment, and plays an important role in the process of plant growth [30, 31]. The biosynthesis of lignin is very complex, involving many enzymes and multi-step reactions [32, 33]. Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in lignin synthesis, which can catalyze the last step of the lignin specific synthesis pathway and play an important regulatory function in lignin biosynthesis [34]. Many studies have shown that CAD family genes are closely related to lignin biosynthesis and lignin deposition [35–37].

In this study, members of the potato CAD family were identified, and gene structure, protein physicochemical form, phylogenetic evolution and expression pattern under Cd stress were analyzed. In recent years, scholars at home and abroad have gradually paid more attention to CAD gene families. CAD genes have been identified in a variety of plants such as *Arabidopsis thaliana*, and their number and species have been determined [38]. The *Arabidopsis* CAD family contains 12 genes, including 18 in grape, 12 in rice, and 17 in alfalfa. In addition, 12 CAD genes in the Arabidopsis CAD family were found, and 6 of them could catalyze 5 cinnamaldehydes to produce cinnamyl alcohol, which further promoted the synthesis of lignin [39]. We have identified 50 CAD family genes in potato, which is relatively large compared with other species, which may be related to the large potato genome and gene replication [40]. The analysis of physical and chemical properties showed that *StCADs* may be a class of acidic proteins, and most of the stability coefficient (92%) was below 40, suggesting that *StCADs* were a class of stable proteins. Subcellular localization prediction revealed that all the genes were located in the cytoplasm, indicating that they play a role in the cytoplasm. Evolutionary analysis and gene structure analysis showed that genes with close genetic relationship often had similar gene structures, which may be due to the fragment replication of CAD family members in the process of evolution, which played an important role in the amplification of family members. The study of the multiple replications of potato CAD family genes can help us understand the process of potato polyploidy better. The number of exons/introns was diverse between *StCAD* family members. It was stated that intron number can impact the speed of gene expression and genes with less number of

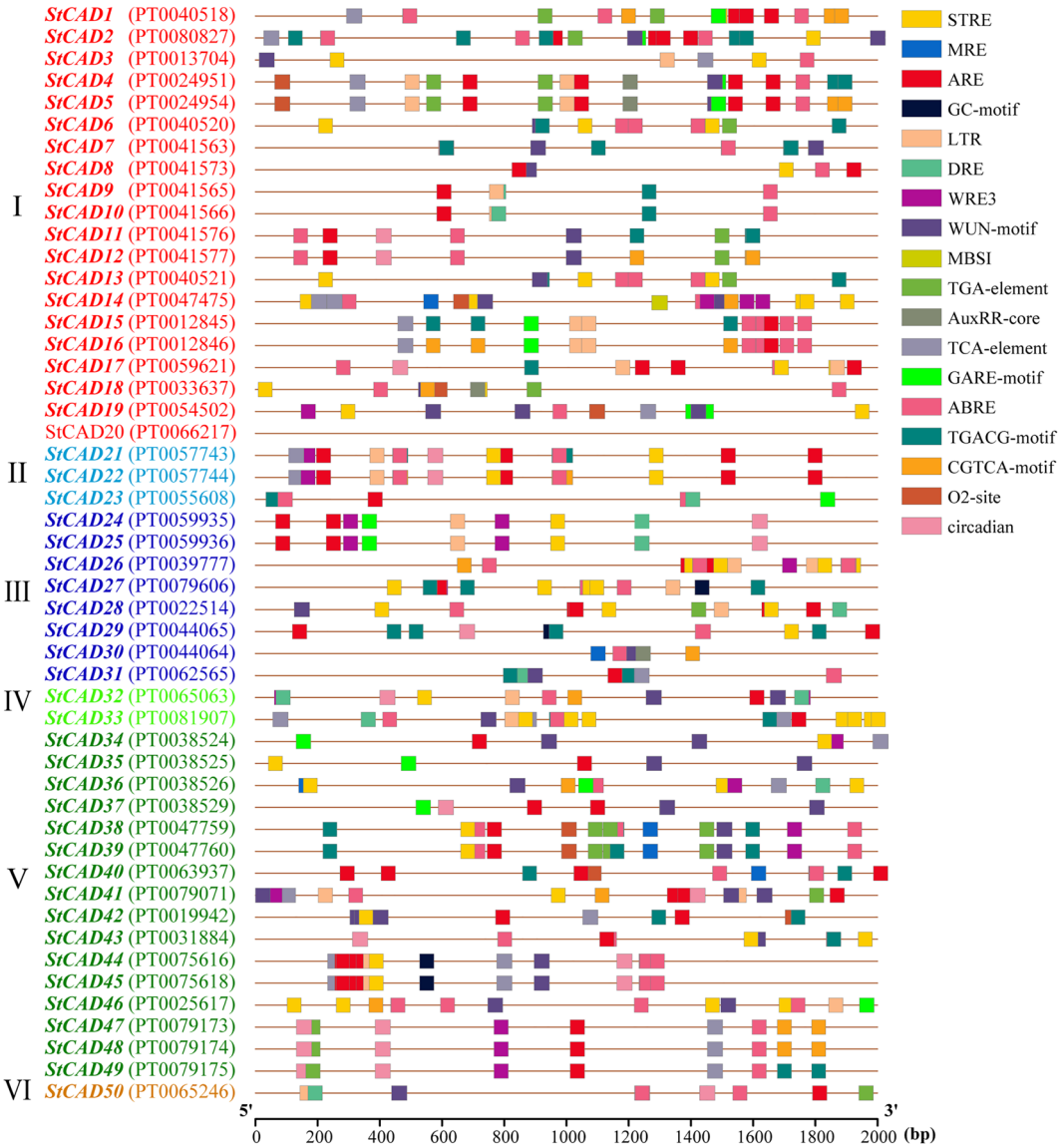


Fig. 4 Analysis of cis-acting elements of potato CAD family genes. STRE: stress response element; MRE: metal response element; ARE: antioxidant response element; GC-motif: hypoxia response element; LTR: low temperature response element; DRE: dehydration response element; WRE3: trauma-induced response element; WUN-motif: trauma-induced response element; MBSI: defense and pressure response components; TGA-element: auxin response element; AuxRR-core: auxin response element; TCA-element: salicylic acid response element; GARE-motif: gibberellin response element; ABRE: abscisic acid responsive element; TGACG-motif: methyl jasmonate response element; CGTCA-motif: methyl jasmonate response element; O2-site: zein metabolic regulatory elements; circadian: circadian response element

introns can leave the nucleus faster to start the translation process [41, 42]. Based on this, *StCAD* family members have had different evolutionary processes and their genetic structure has been affected.

The cis-element analysis of potato CAD family genes showed that the promoter region was rich in cis-elements related to plant response to stress, including metal response elements, salicylic acid response elements and abscisic acid response elements, suggesting that it may play an important role in response to abiotic stress. There is evidence that the CAD activity in *Miscanthus sinensis* increases when it is subjected to cold stress [43]. Liu et al. found that drought could induce *CmCAD2* and *CmCAD3* expression and promote lignin biosynthesis in melon [44]. CAD activity and gene expression in barley leaves were induced to increase after cold and freezing treatment [45]. Previous studies have shown that CAD is involved in the regulation of low temperature stress by

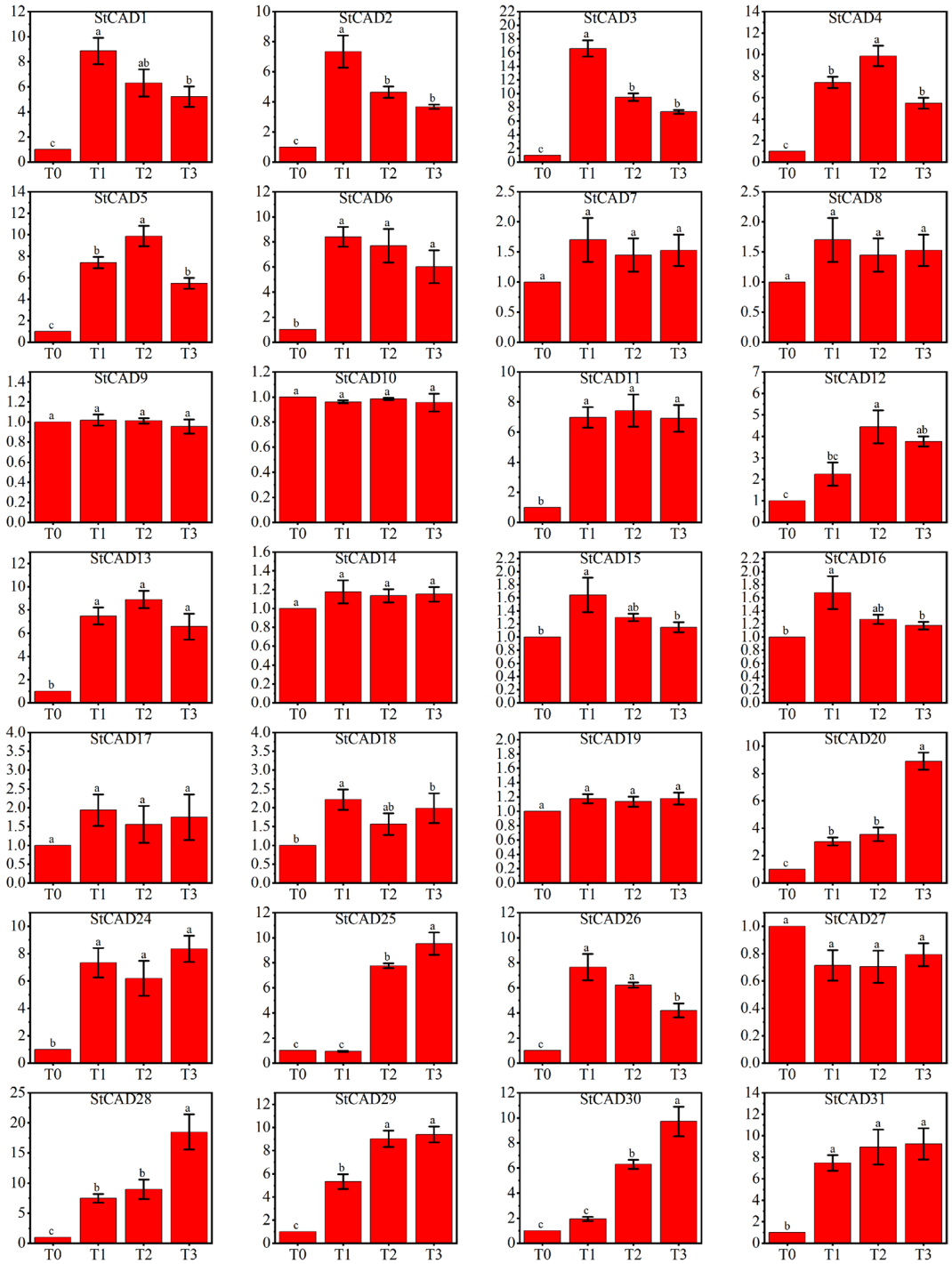


Fig. 5 Expression changes of CAD family genes in potato under cadmium stress

abscisic acid, which improves the cold resistance of sweet potato, and *IbCAD1* is also involved in the mechanical damage response regulated by jasmonic acid and salicylic acid [23]. In our study, the expression pattern showed that most CAD genes were upregulated after Cd stress, indicating that *StCAD* family genes responded to Cd stress, which was consistent with previous studies. Many studies have shown that CAD family genes help plants resist stress by participating in lignin synthesis. Jourdes et al. found that the lignin content of plants was greatly

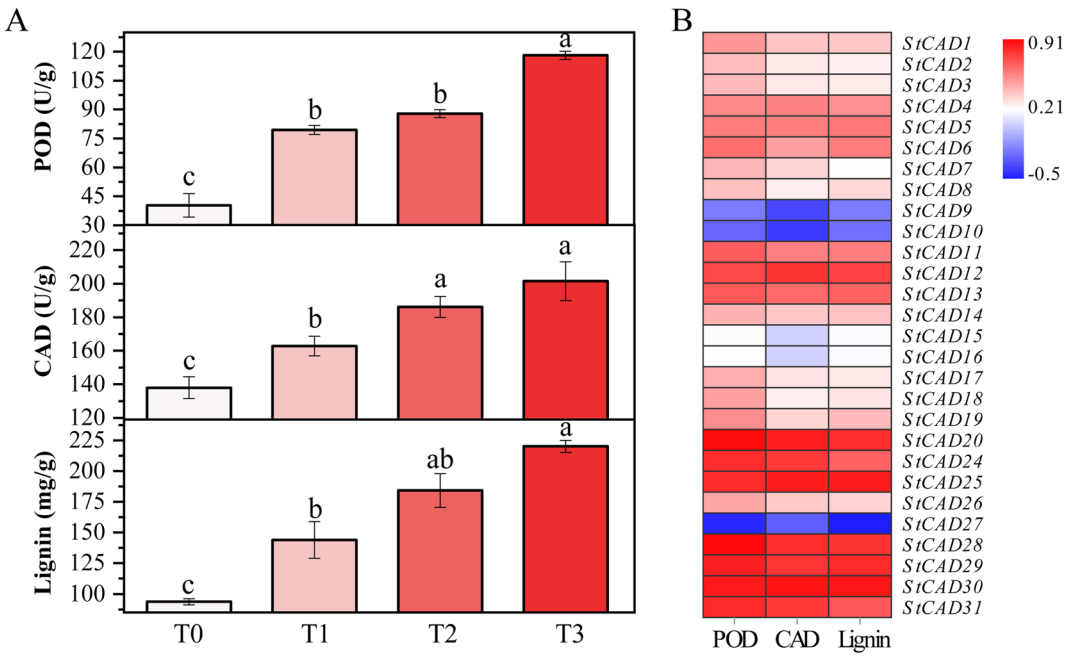


Fig. 6 Analysis of *StCAD* gene related physiological characteristics. **A**: *StCAD* gene related physiological indicators; **B**: correlation between gene expressions value of StCADs and physiological indexes

reduced after double mutation of the *AtCAD4* and *AtCAD5* genes in Arabidopsis thaliana, and even lodging stems appeared [46]. Eudes et al. found that *AtCAD1* played a compensatory role in lignin synthesis and participated in the regulation of lignification in *AtCAD4* and *AtCAD5* double mutants of Arabidopsis thaliana [47]. In the study of Populus tomentosa, it was found that the *PtCAD9* gene may be involved in the defense mechanism of lignin [48]. In this study, after Cd stress, most of the genes in *StCAD* were upregulated, and CAD, POD activity and lignin content were significantly increased, moreover, most CAD genes were positively correlated with lignin content, which was similar to previous studies, indicating that CAD gene expression was closely related to lignin content. The *StCAD* family plays an important role in potato response to Cd stress, which is conducive to the improvement of potato Cd stress tolerance.

Conclusions

A total of 50 potato *StCAD* genes were identified, and subcellular localization prediction found that all genes were located in the cytoplasm, the 50 genes were divided into 6 subfamilies, and the homologous genes had similar structures. Chromosomal localization analysis found that all genes were irregularly distributed on 12 chromosomes, and cis-element analysis found that the *StCAD* family has potential functions in regulating hormones and stress, especially closely related to abiotic stress. Analysis of the expression patterns of CAD genes under Cd stress showed that most of the genes were upregulated after Cd stress, and the related activities of CAD, POD and lignin were also significantly increased, indicating that the *StCAD* family responded to Cd stress and played an important defense role in potato response to Cd stress, although the downstream regulation mechanism of transcription level still needs to be further studied. The genes that play an active role in the response of potato to cadmium stress were obtained in our study, which provides a new idea for the analysis of the mechanism of potato response to cadmium stress, and is very beneficial to the development of cadmium-tolerant potato breeding in the future.

Abbreviations

CAD	Cinnamyl alcohol dehydrogenase
POD	Peroxidase
LSD	Least significant difference
SNK	Student–Newman–Keuls
PT	PGSC0003DMT40
STRE	Stress response element
MRE	Metal response element
ARE	Antioxidant response element
GC-motif	Hypoxia response element
LTR	Low temperature response element
DRE	Dehydration response element
WRE3	Trauma-induced response element
WUN-motif	Trauma-induced response element
MBSI	Defense and pressure response components
TGA-element	Auxin response element

AuxRR-core	Auxin response element
TCA-element	Salicylic acid response element
GARE-motif	Gibberellin response element
ABRE	Abscisic acid responsive element
TGACG-motif	Methyl jasmonate response element
CGTCA-motif	Methyl jasmonate response element
O2-site	Zein metabolic regulatory elements
circadian	Circadian response element.
qRT-PCR	Quantitative real-time polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00543-7.

Additional file 1. RNA gel photograph.

Additional file 2. Primer sequences for quantification of StCADs genes.

Additional file 3. Evolutionary analysis of CAD gene families in potato and Arabidopsis Thaliana.

Additional file 4. Motif sequences.

Additional file 5. Heat map of CAD family gene expression in potato under cadmium stress.

Acknowledgements
The authors would also like to thank the Charlesworth Group (https://www.cwauthors.com) for linguistic assistance during the preparation of this manuscript.

Author contributions
XY: conceptualization, original draft preparation, funding acquisition. HL: investigation. WZ: formal analysis, project administration. HL: data curation. YG: validation. YK: visualization. YW: supervision. FW: methodology. CX: review and editing.

Funding
This work was supported by Doctoral Foundation of Gansu Academy of Agricultural Sciences (2023GAAS38); National Natural Science Foundation of China (32360488, 32201810); Natural Science Foundation of Gansu Province (23JRRA1339, 22JR5RA858). Key Project of Modern Agricultural Science and Technology Regional Innovation Center for Support System of Gansu Province (2021GAAS52);

Availability of data and materials
The datasets generated and analyzed during the current study are available in the NCBI repository, https://www.ncbi.nlm.nih.gov/sra/?term=SRP314907, the accession number is SRP314907. The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Institute of Potato, Gansu Academy of Agricultural Sciences, Lanzhou 730070, Gansu, China. [2]Horticulture College of Gansu Agricultural University, Lanzhou 730070, Gansu, China. [3]State Key Laboratory of Aridland Crop Science (Gansu Agricultural University), Lanzhou 730070, Gansu, China.

Received: 28 December 2023 Accepted: 4 February 2024
Published online: 21 February 2024

References

1. Xu YC, Chu LL, Jin QJ, Wang YJ, Chen X, Zhao H, Xue ZY. Transcriptome-wide identification of mirnas and their targets from typha angustifolia by rna-seq and their response to cadmium stress. PLoS ONE. 2015;10: e0125462.
2. Liu R, Zhang ZH, Shen JC, Wang ZH. Analysis of metal content and vertical stratification of epiphytic mosses along a Karst Mountain highway. Environ Sci Pollut R. 2018;25:29605–13.
3. He SY, Yang XE, He ZL, Baligar VC. Morphological and physiological responses of plants to cadmium toxicity: a review. Pedosphere. 2017;27:421–38.
4. DalCorso G, Farinati S, Maistri S, Furin A. How plants cope with cadmium: staking all on metabolism and gene expression. J Integr Plant Biol. 2008;10:1268–80.
5. Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett. 2010;8:199–216.
6. Shahid MA, Balal RM, Khan N, Zotarelli L, Liu GD, Sarkhosh A, Fernandez-Zapata JC, Nicolas JJM, Garcia-Sanchez F. Selenium impedes cadmium and arsenic toxicity in potato by modulating carbohydrate and nitrogen metabolism. Ecotoxicol Environ Saf. 2019;180:588–99.
7. Michal M, Marek V, Alexander L. Plant cell responses to cadmium and zinc. Plant Cell Mongr. 2014;22:209–46.
8. Zarcinas BA, Pongsakul P, Mclaughlin MJ. Heavy metals in soils and crops in Southeast Asia. Tailand Enviro Geochem Hlth. 2004;26:359–71.
9. Kumar SR, Agrawal M, Marshall F. Heavy metals contamination of soil and vegetables in suburban areas of Varanasi. India Ecotox Environ Safe. 2007;66:258–66.
10. Bertin G, Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie. 2006;88:1549–59.
11. Nawrot T, Plusquin M, Hogervorst J, Roels HA, Celis H, Thijs L, Vangronsveld J, Van Hecke E, Staessen JA. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol. 2006;7:119–26.
12. Andrivon D. Potato facing global challenges: how, how much, how well. Potato Res. 2018;60:389–400.
13. Lai JL, Liu ZW, Chen LI, Luo XG. Analysis of accumulation and phytotoxicity mechanism of uranium and cadmium in two sweet potato cultivars. J Hazard Mater. 2020;409: 124997.
14. Yang XY, Kang YC, Liu YH, Shi MF, Zhang WN, Fan YL, Yao YH, Li H, Qin SH. Integrated analysis of miRNA-mRNA regulatory networks of potato (*Solanum tuberosum* L.) in response to cadmium stress. Ecotox Environ Safe. 2021;224: 112682.
15. Kang Y, Yao Y, Liu Y, Shi M, Zhang W, Zhang R, Li H, Qin S, Yang X. Exogenous Glutathione enhances tolerance of the potato (*Solanum tuberosum* L.) to cadmium stress by regulating the biosynthesis of phenylpropanoid and the signal transduction of plant hormones. Chem Bio Techno Agric. 2023;10:24.
16. Xu SS, Lin SZ, Lai ZX. Cadmium impairs iron homeostasis in Arabidopsis thaliana by increasing the polysaccharide contents and the iron-binding capacity of root cell walls. Plant Soil. 2015;392:71–85.
17. Esposito S, Sorbo S, Conte B, Basile A. Effects of heavy metals on ultrastructure and HSP70S induction in the aquatic moss Leptodictyum riparium Hedw. Int J Phytoremediat. 2012;14:443–55.
18. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. Annu Rev Plant Biol. 2003;54:519–46.
19. Bonawitz ND, Chapple C. The genetics of lignin biosynthesis: connecting genotype to phenotype. Annu Rev Genet. 2010;44:337–63.
20. Hirano K, Aya K, Kondo M, Okuno A, Morinaka Y, Matsuoka M. OsCAD2is the major CAD gene responsible for monolignol biosynthesis in rice culm. Plant Cell Rep. 2012;31:91–101.
21. Youn B, Camacho R, Moinuddin S, Lee C, Davin LB, Lewis NG, Kang C. Crystal structures and catalytic mechanism of the Arabidopsis cinnamyl alcohol dehydrogenases AtCAD5 and AtCAD4. Org Biomol Chem. 2006;4:1687–97.

22. Qiu WM, Song XX, Han XJ, Liu MY, Qiao GR, Zhuo RY. Overexpression of Sedum alfredii cinnamyl alcohol dehydrogenase increases the tolerance and accumulation of cadmium in Arabidopsis. Environ Exp Bot. 2018;155:566–77.
23. Kim Y, Bae JM, Huh G. Transcriptional regulation of the cinnamyl alcohol dehydrogenase gene from sweetpotato in response to plant developmental stage and environmental stress. Plant Cell Rep. 2010;29:779–91.
24. Park HL, Kim TL, Bhoo SH, Lee TH, Lee SW, Cho MH. Biochemical characterization of the rice cinnamyl alcohol dehydrogenase gene family. Molecules. 2018;23:2659.
25. Hu Y, Li WC, Xu YQ, Li GJ, Liao Y, Fu FL. Differential expression of candidate gene for lignin biosynthesis under drought stress in maize leaves. J Appl Genet. 2009;50:213–23.
26. Yang XY, Kang YC, Liu YH, Shi MF, Zhang WN, Fan YL, Yao YH, Li H, Qin SH. Integrated analysis of miRNA-mRNA regulatory networks of potato (*Solanum tuberosum* L.) in response to cadmium stress. Ecotoxicol Environ Saf. 2021;224:112682–112682.
27. Kumar S, Stecher G, Tamura K. Mega 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.
28. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He YH, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13:1194–202.
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402–8.
30. Espineira JM, Novo UE, Gómez Ros LV, Carrión JS, Merino F, Ros BA, Poma F. Distribution of lignin monomers and the evolution of lignification among lower plants. Plant Biol. 2011;13:59–68.
31. Dowd PF, Sarath G, Mitchell RB, Saathoff AJ, Vogel KP. Insect resistance of a full sib family of tetraploid switchgrass *Panicum virgatum* L. with varying lignin levels. Genet Resour Crop Evol. 2013;60:975–84.
32. Pusztahelyi T, Holb IJ, Pócsi I. Secondary metabolites in fungus–plant interactions. Front Plant Sci. 2015;6:573.
33. Ralph J, Lundquist K, Brunow G, Lu F, Boerjan W. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. Phytochem Rev. 2004;3:29–60.
34. Pandey B, Pandey VP, Dwivedi UN. Cloning, expression, functional validation and modeling of cinnamyl alcohol dehydrogenase isolated from xylem of Leucaena leucocephala. Protein Expr Purif. 2011;79:197–203.
35. Ferreira SS, Simões MS, Carvalho GG, De Lima LGA, Svartman RMA, Cesarino L. The lignin toolbox of the model grass Setaria viridis. Plant Mol Biol. 2019;101:235–55.
36. Victor C, Marcal S, Charles H, Hua C, Pedro F, Alexander AM, Jorge APP, Jacqueline G. Genome-wide analysis of the lignin toolbox of Eucalyptus grandis. New Phytol. 2015;206:1297–313.
37. HIrano K, Aya K, Kondo M, Okuno A, Morinaka Y, Matsuoka M. OsCAD2 is the major CAD gene responsible for monolignol biosynthesis in rice culm. Plant Cell Rep. 2012;31:91–101.
38. Ruan YL, Llewellyn DJ, Furbank RT. Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development. Plant Cell. 2003;15:952–64.
39. Sibout R, Séguin A. Cinnamyl alcohol dehydrogenase-c and -d are the primary genes involved in lignin biosynthesis in the floral stem of Arabidopsis. Plant Cell. 2005;17:2059–76.
40. Xue Z, Duan L, Liu D, Guo J, Ge S, Dicks J, OMaille P, Osbourn A, Qi X. Divergent evolution of oxidosqualene cyclases in plants. New Phytol. 2012;193:1022–38.
41. Heidari P, Puresmaeli F, Vafaee Y, Ahmadizadeh M, Ensani M, Ahmadinia H. Comparative analysis of phospholipase D (PLD) gene family in *Camelina sativa* and *Brassica napus* and its responses in Camelina seedlings under salt stress. Agronomy. 2023;13:2616.
42. Yaghobi M, Heidari P. Genome-wide analysis of aquaporin gene family in Triticum turgidum and its expression profile in response to salt stress. Genes. 2023;14:202.
43. Domon JM, Baldwin L, Acket S, Caudeville E, Arnoult S, Zub H, Gillet F, Lejeune-Henaut I, Brancourt-Hulmel M, Pelloux J, Rayon C. Cell wall compositional modifications of Miscanthus ecotypes in response to cold acclimation. Phytochemistry. 2013;85:51–61.
44. Liu W, Jiang Y, Wang CH, Zhao LL, Jin YZ, Xing QJ, Li M, Lv TH, Qi HY. Lignin synthesized by CmCAD2 and CmCAD3 in oriental melon (*Cucumis melo* L.) seedlings contributes to drought tolerance. Plant Mol Bio. 2020;103:689–704.
45. Janská A, Aprile A, Zámečník J, Cattivelli L, Ovesná J. Transcriptional responses of winter barley to cold indicate nucleosome remodelling as a specific feature of crown tissues. Funct Integr Genomic. 2011;11:307–25.
46. Jourdes M, Cardenas CL, Laskar DD, Moinuddin SG, Davin LB, Lewis NG. Plant cell walls are enfeebled when attempting to preserve native lignin configuration with poly-p-hydroxycinnamaldehydes: evolutionary implications. Phytochemistry. 2007;68:1932–56.
47. Eudes A, Pollet B, Sibout R, Do CT, Séguin A, Lapierre C, Jouanin L. Evidence for a role of AtCAD1 in lignification of elongating stems of Arabidopsis thaliana. Planta. 2006;225:23–39.
48. Barakat A, Bagniewska-Zadworna A, Choi A, Plakkat U, DiLoreto D, Yellanki P, Carlson J. The cinnamyl alcohol dehydrogenase gene family in populus: phylogeny, organization, and expression. BMC Plant Biol. 2009;9:26.

Publisher's Note