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THE CINNAMYL ALCOHOL DEHYDROGENASE (CAD) GENE FAMILY IN FLAX (*LINUM USITATISSIMUM* L.): INSIGHT FROM EXPRESSION PROFILING OF *CADS* INDUCED BY ELICITORS IN CULTURED FLAX CELLS

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Abstract: Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in the biosynthesis of lignin and lignans as it catalyzes the final step of monolignol biosynthesis, using NADPH as a cofactor. In higher plants, CAD is encoded by a multigene family consisting of three major classes. Based on the recently released flax (*Linum usitatissimum* L.) whole-genome sequences, in this study we identified six CAD family genes that contain an ADH_N domain and an ADH_zinc_N domain, which suggests that the putative flax CADs (LuCADs) are zinc-dependent alcohol dehydrogenases and members of the plant CAD family. In addition, expression analysis using quantitative real-time PCR revealed spatial variations in the expression of *LuCADs* in different organs. Comparative analysis between LuCAD enzymatic activity and *LuCAD* transcripts indicates that the variation of LuCAD enzymatic activities by elicitors is reflected by transcription of *LuCADs* in flax suspension-cultured cells. Taken together, our genome-wide analysis of CAD genes and the expression profiling of these genes provide valuable information for understanding the function of CADs, and will assist future studies on the physiological role of monolignols associated with plant defense.

Key words: elicitation; cinnamyl alcohol dehydrogenase; suspension-cultured cells; flax; monolignol

INTRODUCTION

Phenylpropanoids are a widespread class of plant-derived natural products derived from the carbon skeleton of phenylalanine. They regulate a wide range of physiological processes, including defense against herbivores and pathogen attack, pigmentation of flowers and fruits, UV-B protection and secondary cell wall biogenesis [1-3]. In plants, the phenylpropanoid pathway is linked to the biosynthesis of major secondary metabolites, such as flavonoids, lignins and lignans [2]. Phenylpropanoid-based polymers like lignin, which is the second most abundant plant biopolymer, contribute to the stability and robustness of plants against mechanical or environmental damage [2]. In addition, plant lignans (phenylpropanoid dimers) are thought to play a role in plant defense against insects, due to their antifeedant activity [4], with intensive investigations also carried out on their pharmacological and nutritional effects on humans [5].

The biosynthesis of monolignol, e.g. coniferyl alcohol, which is a common precursor of lignin and lignan, is initiated with the deamination of L-phenylalanine by phenylalanine ammonia lyase to form trans-cinnamic acid [6]. Then, trans-cinnamic acid is converted to cinnamaldehyde, via the action of various enzymes, including cinnamic acid 4-hydroxylase, 4-coumarate:coenzyme A ligase, p-coumarate 3-hydroxylase, caffeic acid O-methyltransferase and cinnamoyl-CoA reductase [6]. In the last step of monolignol biosynthesis, cinnamaldehydes are converted into hydroxycinnamyl alcohols (monolignols), like coniferyl alcohols, via the action of cinnamyl alcohol dehydrogenase (CAD, EC 1.1.1.195). CAD has been purified from the xylem tissue with extensive lignifica-

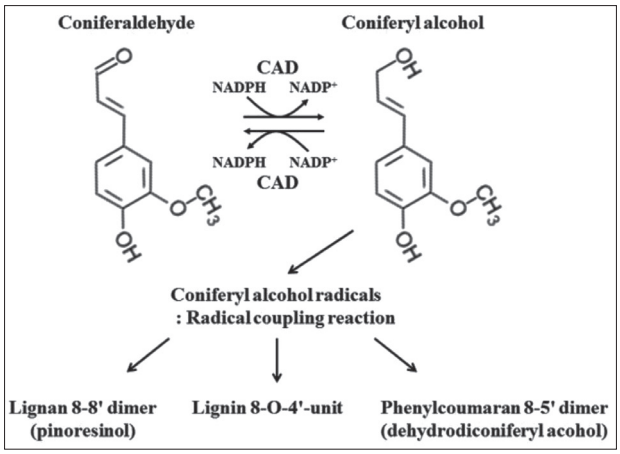

Fig. 1. Enzymatic reaction catalyzed by cinnamyl alcohol dehydrogenase (CAD) and coniferyl alcohol-derived products.

tion, although non-lignified tissue also exhibited CAD activity [7,8]. This indicates that CAD activity is directly involved in lignification, but is also required for the synthesis of non-lignin products such as lignans (Fig. 1) [6,9]. The altered expression of CAD genes in various plants resulted in the variation of monolignol biosynthesis, leading to changes in lignin composition, but total lignin content was not affected [10-12]. This might be due to the incorporation of other phenolic products that compensate for monolignols in lignin as well as other members of the CAD gene family with redundant function [13]. In addition, *CAD* double mutation in Arabidopsis (*cad4*/*cad5*) resulted in a phenotype with a limp floral stem at maturity as well as weakened vasculature/mechanical integrity in accordance with the reduction in proper lignin deposition [14,15], supporting the functional redundancy of the CAD gene family.

Flax (*Linum usitatissimum* L.) belongs to the Linaceae family, and is grown as either an oilseed crop (oil from the seed of linseed varieties) or as a fiber crop (fiber derived from the stem of fiber varieties) [16,17]. In addition, flax is a particularly rich source of secoisolariciresinol diglycoside (SDG), a natural dietary lignan of flaxseeds now available in dietary supplements [16]. SDG is converted by intestinal bacteria in the colon of humans to the mammalian lignans enterodiol and enterolactone [18]. Although monolignols are necessary for lignin biosynthesis, the generation of a low-lignin flax plant by CAD gene silencing has begun to gain interest as a way of increasing flax fiber fineness [19]. This indicates that CAD manipulation is a useful tool to improve the quality of flax as a medicinal or fiber crop. However, the CAD gene family in flax is poorly identified and understood. To better understand the gene function and to address physiological questions about lignin/lignan biosynthesis, it is imperative to include a genome-wide analysis in identifying potential candidate genes encoding CAD in flax.

In this study, we identified flax genes potentially encoding six CADs. A comprehensive phylogenetic analysis of the CAD gene family with related CAD proteins from other species indicated that six LuCADs were classified into two groups. In addition, the expression profiles and activation pattern of LuCADs in elicitor-treated flax-cell suspensions suggested that LuCADs are involved in the methyl jasmonic acid (MeJA)-related defense signaling pathway. Our systematic analysis provides a foundation for further functional dissection of CAD genes in flax, and could help to elucidate the function of the CAD gene family in higher plants.

MATERIALS AND METHODS

Retrieval of putative flax CAD genes

To obtain members of the flax gene family, *L. usitatissimum*, the genome sequence (Phytozome v9.1; http://www.phytozome.net/search.php?method=Org_Lusitatissimum) was queried by nine Arabidopsis and 12 rice CAD protein sequences using the Basic Local Alignment Search Tool (BLAST) algorithms, BLASTp and tBLASTn. The amino acid sequence of candidate genes was analyzed to examine the presence of the characteristic ADH_N domain (PF08240) and ADH_zinc_N domain (PF00107) using SMART (http://smart.embl-heidelberg.de/) and Pfam. A nomenclature system for CAD used in this study (generic name: LuCAD1 to LuCAD6) was provisionally applied to distinguish each CAD gene according to scaffold number.

Protein analysis: sequence, subcellular localization and phylogeny

To identify the sequence conservation and functional homology of CAD in flax and other plants, multiple-sequence alignments of all putative CADs were carried out using ClustalW (http://bioinformatics.ubc.ca/resources/tools/clustalx), which were manually corrected. In addition, the amino acid sequences of putative LuCAD proteins were analyzed to calculate the molecular weight (MW) and theoretical isoelectric point (pI) using the Compute pI/Mw tool (http://web.expasy.org/compute_pi/), and to predict subcellular localization using PLantLoc web services (http://cal.tongji.edu.cn/PlantLoc/index.jsp).

Phylogenetic analysis was performed using the Phylogeny.fr server (http://www.phylogeny.fr) in 'one click' mode to infer the evolutionary history of CADs in the higher plants as previously described [20]. Bootstrap branch support values (BP) were estimated in PHYML 3.0 with 100 replicates by default.

Analysis of gene structure and cis-elements in LuCAD promoter regions

The information on *LuCAD* gene features, including introns and exons, was obtained from Phytozome v9.1. The intron phases of different introns were analyzed using Wise 2.0 (http://www.ebi.ac.uk/Tools/Wise2). For this, amino acid and corresponding full-length DNA sequences were aligned to determine the position of introns as described [21]. For *cis*-element analysis, 1000-bp upstream sequences of *LuCAD* genes were downloaded from Phytozome v9.1 and compared with known cis-regulatory elements in the PLACE database collection (http://www.dna.affrc.go.jp/PLACE/).

Plant material and suspension culture

Flax seeds (golden variety) were obtained from Danong Co. Ltd in South Korea. Seeds were germinated on basal Murashige and Skoog (MS) medium [22] containing 0.8% agar. Cotyledons and hypocotyl segments from six-day-old seedlings were used as explants for callus formation. Explants were cultivated for 3-5 weeks on the solid MS medium, supplemented with 3% sucrose, 0.5 mg/L NAA (1-naphthaleneacetic acid) and 2 mg/L BAP (6-benzylaminopurine). To establish suspension cultures, the yellow and friable calli were resuspended in liquid MS medium supplemented with the abovementioned growth regulators. The cells in suspension were subcultured every two weeks, and incubated on a rotary shaker set to 120 rpm in darkness at 25°C.

Preparation and treatment of elicitors

The four elicitors used in the elicitation experiment were chitosan, pectin, salicylic acid (SA) and MeJA. Chitosan (crab shell chitosan, Sigma-Aldrich) was prepared according to the procedure described by Ahmed and Baig [23], and pectin (from apple, Sigma-Aldrich) was dissolved in 1 N acetic acid. The pH of the elicitor solutions was adjusted to 5.8 with 1 M NaOH before autoclaving. SA and MeJA were dissolved in 10% methanol. One-week-old flax cell cultures were treated with elicitors at the following concentrations unless indicated differently: 50 mg/L chitosan, 50 mg/L pectin, 1 mM SA and 50 μM MeJA. The cells were harvested at different time points, snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Determination of CAD activity

Soluble proteins were extracted by grinding cultured cells in extraction buffer (100 mM Tris-HCl pH 7.5, 2% polyethylene glycol 6000, 5 mM DTT, 2% polyvinylpolypyrrolidone and Protease Inhibitor Cocktail (Roche)). After a 2-h incubation on ice, the crude extract was centrifuged (15 min, 10000 g) at 4°C and the supernatant was used in the assays. Protein concentration was determined according to the method described by Bradford [24] with bovine serum albumin as the standard. CAD activity was determined by the forward reaction of the reduction of coniferaldehyde to coniferyl alcohol as described [7]. The assay was carried out at 30°C in 1 mL of reaction buffer (100 mM Tris-HCl pH 7.5, 67 μM NADPH and 100 μM coniferaldehyde)

Table 1. Gene catalog and nomenclature of cinnamyl alcohol dehydrogenases in *L. usitatissimum*.

Name	Gene ID	Location	CDS (bp)	AA	Intron Nr.	pI	kDa	Subcellular localization
LuCAD1	Lus10002089	scaffold575: 36005 - 37909	1083	360	3	6.83	39.1	CYT
LuCAD2	Lus10025706	scaffold605: 59751 - 61237	1074	357	3	6.41	38.4	CYT
LuCAD3	Lus10002812	scaffold810: 34274 - 36245	1083	360	4	5.53	39.4	CWL, CYT
LuCAD4	Lus10027864	scaffold1143: 684684 - 686446	1080	359	4	5.63	39.2	CWL, CYT
LuCAD5	Lus10014104	scaffold1247: 422144 - 423510	1077	358	3	5.41	38.9	EXC
LuCAD6	Lus10019811	scaffold1491: 126477 - 127844	1077	358	3	5.41	39.0	CHL, EXC

Plant proteins can be localized in the chloroplast (CHL), cell wall (CWL), cytoplasm (CYT) and extracellular space (EXC).

containing 200 µL of crude enzyme preparation. The enzymatic reduction of cinnamaldehydes was determined by the change in absorbance at 340 nm using the molar extinction coefficient (18.6×10^3 $_M^{-1}$ cm^{-1} for coniferaldehyde and 5.3×10^3 $_M^{-1}$ cm^{-1} for NADPH) [7,25]. An assay without sample protein was used as a control. The activity was expressed as nkatal mg^{-1} protein (1 nkatal is defined as the amount of enzyme that converts 1 nmole of substrate mg^{-1} protein s^{-1}).

Quantitative real-time PCR analysis

Total RNA was extracted using the RNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions, and was reverse-transcribed into cDNA using the QuantiTect® Reverse Transcription kit (Qiagen) in accordance with the manufacturer's recommendations.

Quantitative real-time PCR (qRT-PCR) was performed using the QuantiSpeed SYBR kit (PhileKorea Technology) in the ECO™ Real-time PCR system (Illumina) with default parameters. The expression levels of different genes were normalized to the constitutive expression level of flax actin [17]. Specific primer pairs used in qRT-PCR are listed in Supplementary Table 1.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and the means were compared via Duncan's multiple range tests at $p<0.05$, which served to determine the significance of the means. All experiments were repeated at least three times and all data were expressed as means±standard deviation.

RESULTS

The CAD gene family in flax

To identify the complete CAD gene family in flax, all scaffold sequences in the Phytozome v9.1 database were screened using the CAD proteins from Arabidopsis (AtCAD) and rice (OsCAD). The redundant sequences were removed, resulting in a total of six putative CAD genes from flax (Table 1). All the putative LuCAD proteins had an ADH_N domain and ADH_zinc_N domain (Fig. 2), which were specific to zinc-dependent alcohol dehydrogenases and members of the plant CAD family [26,27]. The predicted molecular weight and theoretical pI of all CADs ranged from 38.4 kDa/5.41 to 39.4 kDa/6.83, respectively (Table 1). In addition, the predicted protein localization of LuCAD proteins varied from the cytoplasm, the cell wall and extracellular space (Table 1). Four of the six LuCAD proteins are predicted to be cytosolic proteins (Table 1), and differential subcellular localization between LuCAD proteins indicates that they might appear to function redundantly in the biosynthesis of hydroxycinnamyl alcohols.

In order to explore the phylogenic relationships among the CAD family in plants, a phylogenetic tree was constructed based on the alignment of the amino acid sequences. The phylogenetic tree divided the CADs into three major classes, and LuCADs were classified into two classes (Supplementary Fig. 1). Class I and class II consisted of 4 and 2 LuCAD genes, respectively.

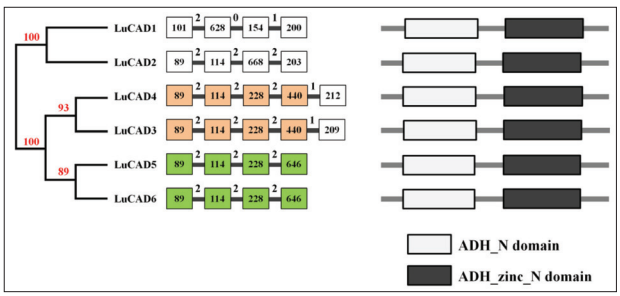

Fig. 2. Phylogenetic analysis, intron-exon structures and domain distribution of CAD gene family in flax. Default values were used except for 100 bootstraps. Numbers in boxes are the nucleotide lengths of each exon, and the connecting thin boxes indicate the positions of the introns. The numbers above the introns indicate the phase of the intron. Exons with different colors are conserved in length between each duplicated flax CAD pair. The conserved domains in the putative LuCADs were analyzed with the SMART domain search program (http://smart.embl-heidelberg.de/).

Intron-exon structure and multiple sequence alignment of LuCAD genes

To gain further insight into the structural diversity of LuCAD genes, we compared the exon/intron organization in the coding sequence of each LuCAD gene. The coding sequences of all the *LuCADs* are disrupted by three to four introns, and the mean number of introns per gene is 3.3 in LuCADs (Fig. 2). The genetic relatedness of *LuCADs* was determined by analyzing the exonic structures, including number of nucleotides in the exons and the conserved intron phases. As shown in Fig. 2, *LuCAD3/4* and *LuCAD5/6* exhibited four conserved exons. *LuCAD3* and *4* exhibited the intron-exon structure found only in *CADs* in eudicots [13], whereas one individual intron may be missing from other *LuCADs*. In addition, introns 1-3 from the LuCAD genes, except *LuCAD1*, are phase 2 introns, which lie between the second and third nucleotides of adjoining codons [28].

All of the flax CADs contain the highly conserved Zn1-binding domain motif $GHE(X)_2G(X)_5G(X)_2V$, Zn2-binding domain motif $GD(X)_{9,10}C(X)_2C(X)_2C(X)_7C$ and the cofactor (NADPH)-binding motif $GXG(X)_2G$ (Fig. 3), suggesting that LuCADs belong to the zinc-dependent alcohol dehydrogenases. Trp119 and Asp123 are known key residues in an optimal position for substrate binding, whereas Phe299 is a key determinant residue of substrate specificity [25,29]. These residues were found in the sequence of LuCAD3-6. In addition, the amino-acid residues 58HL59 in the LuCAD5 and 6 sequences, believed to be a proton donor in the catalytic mechanism [30], were not found in the other

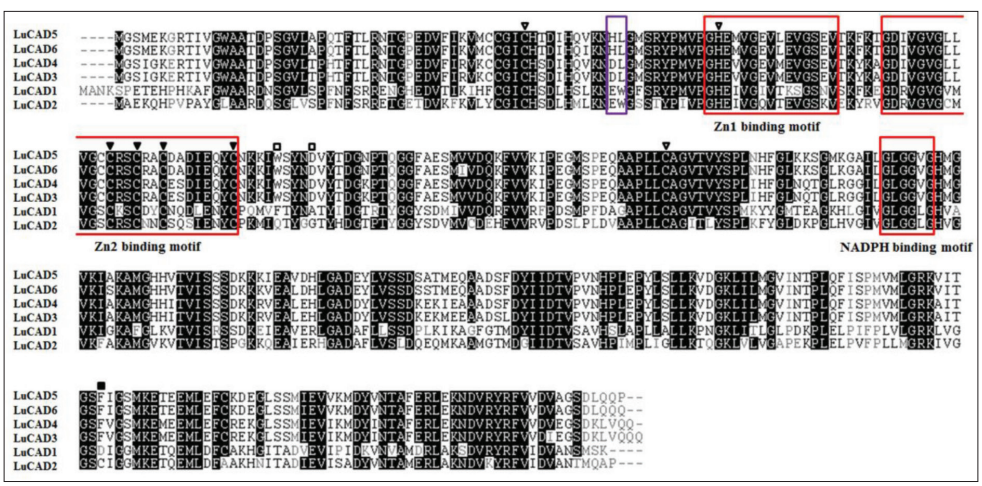

Fig. 3. Multiple sequence alignment of CAD proteins in flax. Red boxes indicate the Zn1-binding motif, Zn2-binding motif and NADPH-binding motif. Location of the amino-acid residues 58HL59 is shown in the violet box. White arrows and black arrows indicate the catalytic Zn ion-coordinating residue and the structural Zn ion-coordinating residue, respectively. The Phe299 residue for substrate specificity is marked with the black square, and Trp199 and Asp123 residues for substrate binding are marked with the white square.

LuCADs that contained either EW (LuCADs 1 and 2) or DL (LuCADs 3 and 4) dipeptide. The difference between key residues in the active site of LuCADs indicates that they require a specific set of key residues for efficient performance against monolignols [29].

Expression pattern of LuCAD genes in various tissues

Tissue-specific expression patterns are helpful in determining whether a gene of interest plays a role in defining the function of given tissues. The expression patterns of LuCAD genes were first examined in different tissues, including the seed, young leaf, cotyledon and cultured cells. *LuCAD1* was expressed predominantly in young leaves and cotyledon more than 25-fold higher than in the seed (Fig. 4). Similar to *LuCAD1*, *LuCAD2* was also expressed highly in young leaves and the cotyledon, but was only approximately two times higher than in seeds. In the case of *LuCAD3*, *4* and *5*, the highest expression level was observed in cultured cells, whereas *LuCAD1* was hardly detected in cultured cells. The expression pattern of two pairs of paralogs (*LuCAD1/2* and *LuCAD3/4*) exhibited a similar expression pattern in different tissues, indicating that the duplication of these genes did not result in the divergence of their expression pattern and function.

Effects of elicitation on CAD enzymatic activity in suspension-cultured cells

The enzymatic activity of CADs was previously shown to be induced by abiotic and biotic stresses [6,31-34], indicating that lignin deposition is a part of stress resistance in higher plants. To assess the activation pattern of LuCADs during response to various stimuli, flax suspension-cultured calls were treated with chitosan, pectin, SA and MeJA. A rapid and strong (up to threefold) increase in CAD activity was observed in MeJA-treated cell cultures at 5 h post-elicitation (Fig. 5). The maximum activity of LuCADs was observed at 48 h after MeJA treatment. The treatment of pectin also resulted in the induction of LuCADs (up to twofold), whereas the enzymatic activity was not

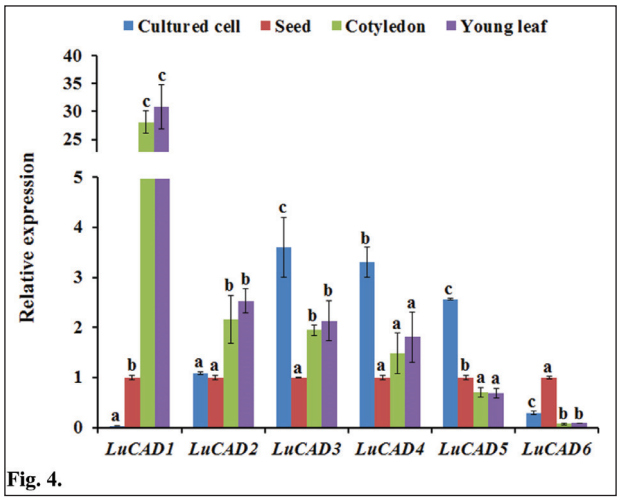

Fig. 4. Transcript levels of *LuCADs* in different flax tissues. The expression levels for each gene in different tissue samples were calculated relative to its expression in seeds. Data are expressed as the means±SD of three independent experiments. Different letters correspond to means that are statistically different ($P < 0.05$).

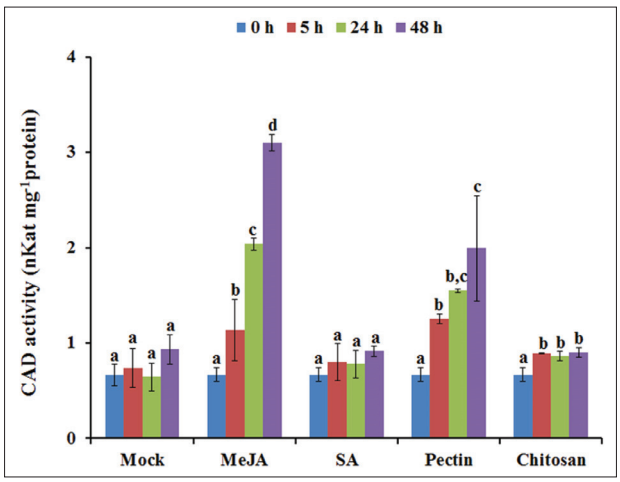

Fig. 5. Variation in LuCAD enzymatic activity in elicitor-treated flax cell cultures. Flax suspension-cultured cells were treated with MeJA, SA, pectin or chitosan, and CAD activity of the total proteins was analyzed using coniferaldehyde as substrate. The SD bars were obtained from independent experiments performed in triplicate.

changed or slightly changed by treatment with SA or chitosan (Fig. 5). These results indicate that LuCADs are likely to be involved in the MeJA-mediated plant defense response.

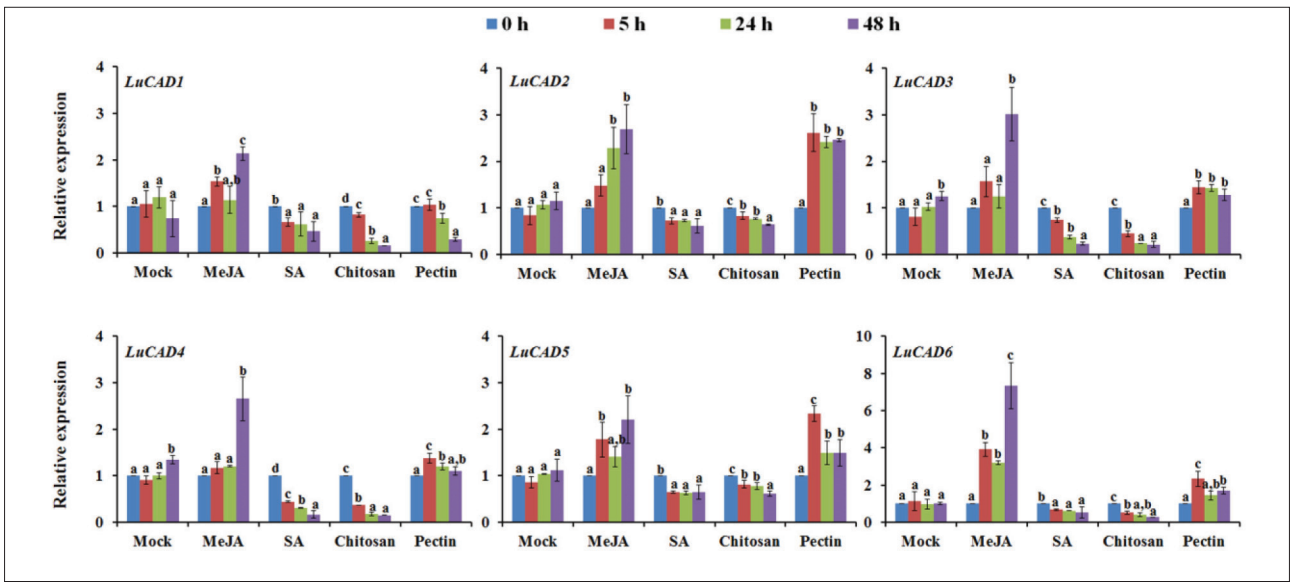

Fig. 6. The expression profiling of LuCAD genes in elicitor-treated flax cell cultures. Transcript levels of *LuCAD1-6* were normalized to the constitutive expression level of flax actin and were expressed relative to the values at 0 h. Data represent the means±SD of three independent experiments.

Expression profiling of LuCAD genes in elicitor-treated cultured cells

To investigate whether the variation of LuCAD enzymatic activity by elicitors was reflected by transcription of *LuCADs*, the expression pattern of each *LuCAD* in elicitor-treated cell cultures was analyzed by qRT-PCR. When flax-cultured cells were treated with MeJA, we also observed the increased expression levels of all *LuCADs*. However, T/GBOXATPIN2 for jasmonate signaling was only found in *LuCAD5* (Supplementary Table 3), although all *LuCADs* were induced by MeJA (Fig. 6). This indicates the presence of a novel jasmonate-responsive element in the *LuCAD* promoters. In addition, during the response to pectin, five of the six *LuCADs* were induced, whereas the expression of all *CADs* decreased following treatment with SA or chitosan in flax-cultured cells (Fig. 6). These findings suggest that the variation of LuCAD enzymatic activity by elicitor treatments is mediated by *LuCAD* transcripts. *LuCAD3/4* and *LuCAD5/6* exhibited similar expression patterns during responses to all elicitors tested, implying redundant functions. Furthermore, several stress-responsive elements, together with phytohormone-responsive elements, were found in the LuCAD gene promoters, including W box (WBOXATNPR1, WBOXATNPR3 and WBOXNTCHN48), ELRECOREPCRP1 motif (elicitor responsive element), MYB1LEPR (defense-related gene expression), ARR1AT (cytokinin-responsive element) and ERELEE4 (ethylene-responsive element) (Supplementary Table 3). The presence of the aforementioned putative cis-elements in *LuCAD* promoters suggests that LuCADs might respond to environmental stresses.

DISCUSSION

CAD is a major rate-limiting enzyme in monolignol biosynthesis, and catalyzes the NADPH-dependent reduction in the conversion of cinnamaldehydes into monolignol building blocks for lignin and lignan biosynthesis. In angiosperms, CAD genes usually exist as a multigene family consisting of members thought to have distinct roles in wood development and plant defense [35,36].

In this study, we identified six CAD genes in flax from available flax genome sequences, classified by

the presence of the highly conserved ADH_N and ADH_zinc_N domains (Table 1). Phylogenetic analysis of LuCAD genes indicates that there are two classes (Supplementary Fig. 1), whereas three main classes are represented by CAD sequences from eudicots, monocots and basal angiosperms [37]. Classes II and III include CADs from monocots and eudicots, whereas class I included sequences from monocots, eudicots and gymnosperm clades [13]. LuCAD3-6 were clustered into class I containing all the *bona fide* CAD genes (*AtCAD4* and *AtCAD5*), suggesting that these proteins might be associated with lignin biosynthesis in flax. In addition, OsCAD7 in class II plays an important role in lignin biosynthesis and the control of culm strength in rice [38]. Similarly, AtCAD7 and 8 had been preliminarily shown to be involved in lignin biosynthesis [39]. This indicates the function of class II members in lignin biosynthesis, although the functions of class III members are poorly understood [37].

The expression profiles reveal spatial variations in the expression of *LuCADs* in different organs (Fig. 4). *LuCAD1* and *2* are highly expressed in the cotyledon and young leaves, whereas the expression of *LuCAD3* and *4* was relatively higher in cultured cells. During seed development, the high expression level of the soybean CAD gene has been detected in the final phases of seed development [40]. The high levels of lignin polymers in the seed coat very likely play an important role in defense against biotic and abiotic stresses, as well as mechanical damage [41]. This indicates that *LuCAD6*, highly expressed in the seeds, plays an important role in the lignification of seed coat. In higher plants, the expression analysis of studied *CADs* suggested that they had different tissue-specific expression profiles [13,29,30], indicating that CADs might have divergent functions in different tissues. *CADs* are known as stress-induced genes. In poplar, CAD genes were significantly induced in stem tissue infected with *Rhizoctonia solani*, *Fusarium oxysporum* or *Cytospora* sp. [42]. In addition, the expression levels of *CADs* in melon were changed by phytohormones including ABA, auxin and ethylene [27]. In the promoter region of *LuCADs*, we also identified several phytohormone-responsive cis-elements, together with stress-responsive elements (Supplementary Table 3). In flax suspension-cultured cells, treatment with MeJA and pectin induced *LuCAD* expression (Fig. 6), and resulted in increased CAD enzymatic activity (Fig. 5). MeJA is a well-known plant defense elicitor that induces the production of defensive compounds against herbivores. In cultured Arabidopsis cells, MeJA elicitation significantly induced *de novo* transcription of genes involved in phenylpropanoid and monolignol biosynthesis [43]. Similarly, the expression of ethylene and lignin biosynthesis genes was induced in MeJA-treated *Fragaria chiloensis* fruit [44]. In addition, exogenously applied MeJA induced *CAD* transcription and CAD enzymatic activity in higher plants [32,45,46], indicating the active role of MeJA in monolignol biosynthesis. Although SA and chitosan have been used for the induction of monolignol biosynthesis in eggplant [32], in Ginkgo biloba [47] and tomato [45] the expression of LuCADs was reduced after exogenous application of SA and chitosan (Fig. 6). The different responses among plants might be due to their different tolerance against various stimuli. To support this hypothesis, further analysis with different concentrations of elicitors will be required.

In conclusion, using genome-wide analysis, we identified six flax CADs (LuCADs) that belong to two classes. Comparison between the LuCAD enzymatic activity and *LuCAD* transcripts suggest that elicitor-induced LuCAD enzymatic activity is mediated by *de novo* transcription of *LuCADs*. Expression profiling of *LuCADs* under different conditions provides an important starting point for future efforts to understand the physiological function of CADs in flax, and to provide insights into the physiological function of monolignols associated with plant defense.

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Authors' contributions: S.H. Eom and T.K. Hyun contributed to the experimental design and management, data analysis and manuscript preparation. H. Kim co-designed experiments and discussed analyses and the manuscript. All authors have read and approved the final manuscript.

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Supplementary Data

Supplementary Table 1. Primer sequences for qRT-PCR analysis.
Table S1 is available on: http://www.serbiosoc.org.rs/sup/2/SupplementaryTable1.doc

Supplementary Table 2. List of plant genes used in CAD gene phylogenetic analyses.
Table S2 is available on: http://www.serbiosoc.org.rs/sup/2/SupplementaryTable2.xls

Supplementary Table 3. Regulatory sequences and *cis*-elements in the *LuCAD* promoter regions.
Table S3 is available on: http://www.serbiosoc.org.rs/sup/2/SupplementaryTable3.doc

Supplementary Fig. 1. Phylogenetic analysis of CAD family in flax and other plants. The gene names used in this study, the accession number and species are indicated in Supplementary Table 2.
Fig. S1 is available on: http://www.serbiosoc.org.rs/sup/2/SupplementaryFig1.tif