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- Review -

Regulation of *Cinnamyl Alcohol Dehydrogenase* (*CAD*) Gene Family in Lignin Biosynthesis

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Lignin is a complex phenylpropanoid polymer abundant in the cell walls of vascular plants. It is mainly presented in conducting and supporting tissues, assisting in water transport and mechanical strength. Lignification is also utilized as a defense mechanism against pathogen infection or wounding to protect plant tissues. The monolignol precursors of lignin are synthesized by cinnamyl alcohol dehydrogenase (CAD). CAD catalyzes cinnamaldehydes to cinnamyl alcohols, such as *p*-coumaryl, coniferyl, and sinapyl alcohols. *CAD* exists as a multigenic family in angiosperms, and *CAD* isoforms with different functions have been identified in different plant species. Multiple isoforms of *CAD* genes are differentially expressed during development and upon environmental cues. CAD enzymes having different functions have been found so far, showing that one of its isoforms may be involved in developmental lignification, whereas others may affect the composition of defensive lignins and other wall-bound phenolics. Substrate specificity appears differently depending on the *CAD* isoform, which contributes to revealing the biochemical properties of CAD proteins that regulate lignin synthesis. In this review, details regarding the expression and regulation of the *CAD* family in lignin biosynthesis are discussed. The isoforms of the *CAD* multigenic family have complex genetic regulation, and the signaling pathway and stress responses of plant development are closely linked. The synthesis of monolignol by *CAD* genes is likely to be regulated by development and environmental cues as well.

Key words : Cinnamyl alcohol dehydrogenase, development, lignin, monolignol, stress

Lignin biosynthetic pathway

Lignin is the most abundant organic polymer found in nature after cellulose. It is a complex biopolymer deposited in the cell walls for water transport and mechanical strength. The phenylpropanoids, hydroxycinnamyl alcohol and monolignols (*ρ*-coumaryl, coniferyl, and sinapyl alcohol) account for most of the lignin network [3].

Lignin biosynthesis comprises a highly coordinated and regulated metabolic activity, and many enzymes are involved in this pathway. Phenylalanine ammonia-lyase (PAL) produce cinnamic acid, which acts as a substrate for cinnamate-4-hydroxylase (C4H). C4H produces *p*-coumaric acid and successively caffeic acid by *p*-coumarate-3-hydroxylase (C3H). The caffeic acid is methylated to produce ferulic acid by caffeic acid *O*-methyltransferase (COMT). The CoA esters of *p*-coumaric acid, caffeic acid, ferulic acid, 5-hydroxyferulic acid and sinapic acid are produced by 4-coumarate-CoA ligase (4CL). The *p*-coumaryl CoA, feruloyl CoA, and sinapoyl CoA are considered as substrates for cinnamyl-CoA reductase (CCR), which can produce the corresponding aldehyde. These aldehydes are substrates for cinnamyl alcohol dehydrogenase (CAD) producing the three monolignols (Fig. 1A). The three monolignol precursors are different in their degree of methoxylation, that is, *p*-coumaryl (non-methoxylated), coniferyl (monomethoxylated), and sinapyl (dimethoxylated) alcohols (Fig. 1B). This diversity of subunit substitution indicates that a variety of intermolecular linkages can be made during polymerization [3]. Then, they are oxidized by peroxidase or laccase to form the lignin polymer. The common phenylpropanoid pathway provides the hydroxycinnamoyl-CoAs, which are converted into the monolignols through the lignin specific pathway [44]. Lignin specific pathway involves two enzymes, CCR and CAD, which convert the hydroxycinnamoyl-CoA esters into monolignols [45].

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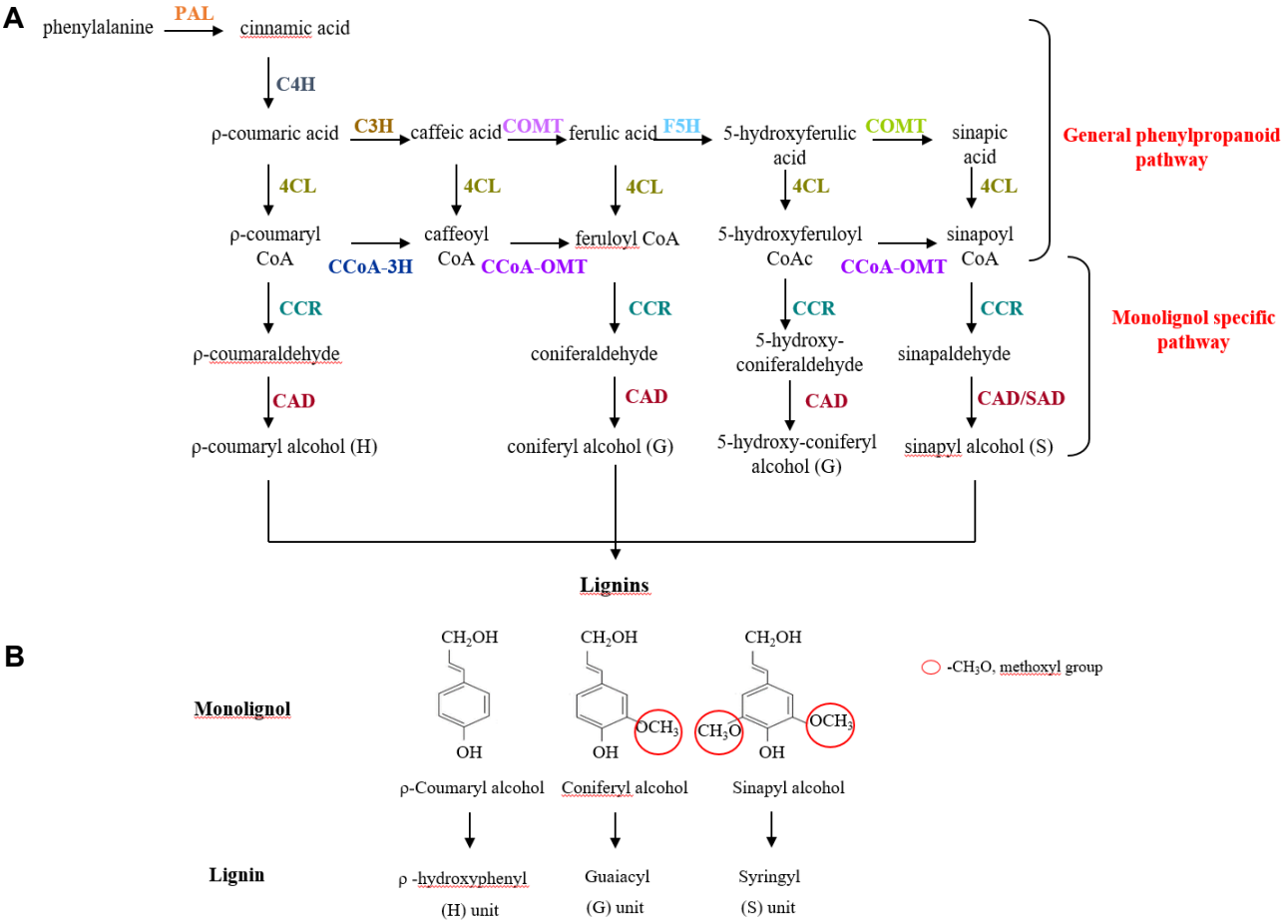

Fig. 1. A. The lignin biosynthetic pathway. B. Structure of most common lignin monomers. PAL, phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; C3H, *p*-coumarate-3-hydroxylase; COMT, caffeic acid 3-*O*-methyltransferase; CCoAOMT, caffeoyl-CoA 3-*O*-methyltransferase; F5H, ferulate-5-hydroxylase; 4CL, 4-coumarate CoA-ligase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase.

Lignin biosynthetic *CCR* gene

The CCR is the first step in the monolignol specific pathway, by converting hydroxycinnamoyl-CoA esters to their corresponding aldehydes. CCR is encoded by a single gene in *Eucalyptus gunnii* (*EgCCR*) and perennial ryegrass *Lolium perenne* (*LpCCR*). *EgCCR* mRNA was expressed in xylem, supporting the role of the enzyme in lignification. *EgCCR* was also strongly expressed in less lignified leaves [23]. *LpCCR* mRNA was expressed in all organs, but was most abundant in lignified organs. *LpCCR* was stimulated by mechanical wounding [35]. Two *CCR* isoforms have been characterized in *Zea mays* [36] and *Arabidopsis thaliana* [25]. In different tissues of maize, *ZmCCR1* was involved in developmental lignification, while the *ZmCCR2* was expressed only in roots and involved in stress responses. Arabidopsis *AtCCR1* was mostly expressed in tissues undergoing lignification, which was involved in constitutive lignification. But, *AtCCR2* was nearly expressed during development, but strongly induced by pathogen treatment. It contributed to biotic stress resistance by producing phenolics. There are differences in function between members of *CCR* gene in angiosperm species.

Lignin biosynthetic *CAD* gene

At the end of the monolignol biosynthetic pathway, CAD catalyzed the reduction of hydroxycinnamyl aldehydes (*p*-coumaraldehyde, coniferaldehyde, and sinapaldehyde). It was an important enzyme in lignin biosynthesis and has been studied in many plant species. *CAD* gene was abundantly expressed in developing xylem. It can be explained that secondary walls of xylem cell are the important site of lignin synthesis during plant development. On the other

hand, *CAD* genes are involved in defense response or metabolic processes not related to the developmental lignification of the vascular tissue. *CAD* is responded to stress by hardening the cell wall. When wheat leaves were treated with fungi elicitor, CAD activity was highly increased and sinapyl alcohol was oxidized, specifically. It was enriched in syringyl (S) units in response to pathogens, and CAD could play a role in the control of defense lignin synthesis [34]. In pine (*Pinus banksiana*) cell culture, fungi elicitor was induced lignification. Following elicitation, it accumulated guaiacyl (G) lignin with change in monolignol biosynthetic enzyme activity [6]. Therefore, CAD is an important indicator for lignification during development and plant stress responses [24].

Knockout and overexpression of *CAD* gene

Transgenic plants and natural mutants of lignin biosynthetic genes can provide a good source to understand the lignification process. The role of CAD in lignin biosynthesis has been addressed by studies of natural mutants and genetically engineered antisense CAD mutants in various plants [2, 7, 12]. In case of down-regulating CAD activity, transgenic tobacco plants were strongly decreased in CAD activity and insignificant change of lignin content [12]. The composition of lignin subunit was specifically altered, and the proportion of cinnamaldehyde was significantly increased. In pine (*Pinus taeda* L.) CAD mutant (*cad-n1*), CAD activity was severely reduced and decreased lignin content [33]. Double mutants of developmental CADs (AtCAD4 and AtCAD5) were characterized in Arabidopsis. CAD activities were greatly reduced and lignin composition was also modified [42]. Natural CAD-deficient mutant *bm1* of maize showed a lower lignin content than normal genotype and better digestibility [13]. *gold hull and internode2* (*gh2*) mutant is a *brown midrib1* (*bm1*) orthologue in rice and identified to a lignin-deficient mutant. It exhibits a reddish-brown pigmentation in the hull and internode, instead of the midrib. *flexible culm1* (*fc1*) mutant in rice has deficiency of the OsCAD7 and caused a reduction in cell wall thickness and lignin content. CAD enzyme activity was also reduced [28].

There have been a few reports on overexpression of the *CAD* gene in transgenic plants. In the case of CAD associated with developmental lignification, rice *FC1* gene and sweet wormwood (*Artemisia annua*) *AaCAD* gene have been overexpressed in planta [28, 31]. Overexpression of *FC1* did not affect the lignin content of cell walls, while *AaCAD* showed remarkably higher lignin content in transgenic plants. Meanwhile, in case of stress-related CAD, overexpressing wheat *TaCAD12* related to a defense lignin was contributed to host resistance against fungi [38]. Overexpression of *IbCAD1* from sweetpotato in Arabidopsis plants, which is responded in environmental stresses and involved in developmental lignification, clearly affected lignin content and composition by controlling lignin biosynthesis. The IbCAD1 activity was increased in transgenic plants. It was shown that enhanced tolerance to ROS stresses [22].

Analysis of expression pattern for *CAD* gene family

CAD gene family has been isolated from various higher plants. The expression of *CAD* family is diverse with tissue types and developmental stages of the plant. *CAD* genes showed high sequence homology (~70%) between gymnosperm and angiosperm, suggesting that those are very well conserved during evolution [4]. In gymnosperm, *CAD* is a single gene and coniferaldehyde-specific [32]. In contrast, multiple *CAD* isoforms have been isolated from many angiosperms, and catalyze the reduction of both coniferaldehyde and sinapaldehyde. The expression of *CAD* family genes varied according to tissue types and developmental stages of the plant. The expression characteristics of the *CAD* genes are summarized in Table 1.

Kim *et al*. [18] classified the Arabidopsis *CAD* multigene family (*AtCAD1-AtCAD9*) through the kinetic properties and substrate specificities to establish physiological functions. AtCAD4 and AtCAD5 are involved in developmental lignification. AtCAD7 and AtCAD8 are induced in response to pathogen infection. Detectable CAD catalytic activities of AtCAD1, AtCAD6, and AtCAD9 are not presented [17].

There are 12 *OsCADs* genes in rice (*Oryza sativa*) genome, of which *OsCAD2* is similar to *CAD* gene present at the *bm1* locus of maize. *CAD*-deficient mutant *bm1* was associated with developmental lignification showing lower lignin content [43]. *gh2* mutant was also identified as lignin-deficient in rice [46]. *GH2* gene, which encodes a CAD, was defined as *OsCAD2*. *OsCAD2* is constitutively expressed throughout all developmental stages, being most highly expressed in actively lignifying tissues. The expression of *OsCAD2* was also induced by biotic and abiotic stresses, suggesting that it plays a role in the defense response [36]. *fc1* mutant caused

Table 1. Characterization of *CAD* family in several plant species

Species	Class/ Group	Gene	Seedling		Mature plant				Substrate specificity	Ref.
			Shoot	Root	Leaf	Stem	Root	Flower		
Arabidopsis (*Arabidopsis thaliana*)	Class I	AtCAD4	++	+++	+	++	+++	-/+	coumaldehyde	19, 42
		AtCAD5	+	++	+	++	++	+++	coumaraldehyde, coniferaldehyde. sinapaldehyde	
	Class II	AtCAD6	+++	+		+				19
		AtCAD7	+++	++++		-/+		++	coumaraldehyde	
		AtCAD8	+++	++		-/+		++	coumaraldehyde, benzaldehyde	19, 41
	Class III	AtCAD2	N/D	N/D		+			caffeyl aldehyde	19
		AtCAD3	N/D	N/D		+			coumaraldehyde	
		AtCAD9	+++	+						
	Class IV	AtCAD1	++	+						
Rice (*Oryza sativa*)	Class I	OsCAD2 (*bm1*,GH2)	+	+++	++	+++	+++		coniferaldehyde sinapylaldehyde	28. 36. 46
	Class II	OSCAD7 (FC1)	++	++	+	+	++			
		OsCAD6	+	-/+	+	+				
		*OsCAD3, OsCAD5, OsCAD8A, OsCAD8B, OsCAD8C, OsCAD8D, OsCAD9								
	Class III	OsCAD1	++	+	++	+++				
		*OsCAD4								
Sorghum (*Sorghum bicolor*)	Group I	SbCAD2 (*bm6*)	++	+++	++++	++++	+++			39
	Group V	SbCAD4-2	- /+	+	-/+	-/+	-/+			
		SbCAD4-3	+	++	++	++	++			
		*SbCAD4-1SbCAD4-4, SbCAD4-5, SbCAD10								
	Group III	SbCAD5	variable	-/+	+	+	N/D			
		SbCAD7	++	N/D	+	+	N/D			
		SbCAD8-1								
		SbCAD8-2	+++	+	++	++	N/D			
		*SbCAD8-3, SbCAD8-4								
	Group IV	SbCAD6	+	+	N/D	N/D	++			
Ryegrass (*Lolium perenne* L.)		LpCAD1	++	++++	+++	++	++++			29
		LpCAD2	++	N/D	+	+++	N/D			
Eucalyptus (*Eucalyptus gunnii*)		EgCAD1			++	++	N/D		coniferaldehyde, coumaraldehyde. benzaldehyde	9, 10
		EgCAD2			+++	++	N/D		coniferaldehyde, coumaraldehyde, sinapaldehyde	
Alfafa (*Medicago sativa* L.)		MsaCAD1	-/+	-/+	-/+	++	-/+	++	cinnamaldehyde, benzaldehyde, aliphatic aldehyde	5
		MsaCAD2	++	+++	-/+	+++	+++		coniferaldehyde. sinapaldehyde	
Poplar (*Populus tremuloides*)		PtCAD			N/D	++++			coniferaldehyde	28
		PtSAD			N/D	+++			sinapaldehyde	
Melon (*Cucumis melo L.*)	Group I	CmCAD1			+/-	++	+	++		16
		CmCAD2			+/-	++	++	++		
	Group VII	CmCAD3			+/-	+++	++	+/-		
	Group IV	CmCAD4			+/-	+/-	+/-	+/-		
	Group II	CmCAD5			+/-	+++	+++	+++		

Table 1. Continued

Species	Class/ Group	Gene	Seedling		Mature plant				Substrate specificity	Reference
			Shoot	Root	Leaf	Stem	Root	Flower		
Poplar (*Populus trichocarpa*)	Group1	PoptrCAD4			N/D	++++				1
		PoptrCAD10			N/D	+++				
	Group 2	PoptrCAD7			+/-	++++				
		PoptrCAD12			+/-	++++				
		PoptrCAD13			++	+/-				
	Group 3	PoptrCAD9			++	+				
	Group 4	PoptrCAD1			++	+				
		PoptrCAD2			++	++				
		PoptrCAD3								
		PoptrCAD5			++	++				
		PoptrCAD6			++	+				
		PoptrCAD8								
		PoptrCAD11			+++	++				
		PoptrCAD14								
		PoptrCAD15			+++	++				
		PoptrCAD16			++	+				
Maize (*Zea mays*)		Y13733 (*bm1*,ZmCAD2)			+	+	++++			11
		2405118.2.1				+	+++			
		3071507.2.1			+	+				
		4424417.2.1			+++	++	+++			
		2485944.3.1			+	+				
		*2949673.2.1, 3203838.2.1								
Wheat (*Triticum aestivum* L.)	Group I	TaCAD1			+/-	+++	-	N/D	coniferaldehyde, coumaraldehyde, sinapaldehyde	30
		*TaCAD2, TaCAD4								
	Group III	*TaCAD5, TaCAD6, TaCAD11								
	Group IV	TaCAD12							coniferaldehyde, sinapaldehyde	38
		*TaCAD3								30
	Group V	*TaCAD10								
	GroupVI	*TaCAD7, TaCAD8, TaCAD9								
Sweetpotato (*Ipomoea batatas*)	Group I	IbCAD1	N/D	N/D	N/D	+	+++	+	sinapaldehyde	21
		IbCAD3			N/D	+	+			
		*IbCAD2, IbCAD4, IbCAD5, IbCAD6								
	Group II	IbCAD7			-/+	+	+			
		*IbCAD8, IbCAD9, IbCAD10, IbCAD11								
	Group III	IbCAD12			+	+	++			
	Group IV	IbCAD13			++	+	+			

-/+, very weak; +, weak; ++, medium; +++, strong; ++++, very strong; N/D, not detected.
*, not determined.

by T-DNA insertion into *OsCAD7* gene decreased secondary cell wall thickness and mechanical strength. FC1 showed strong CAD activity that affects the mechanical strength of rice culms [28]. The expression of *OsCAD6* was stimulated by pathogen infection and UV-irradiation, which was involved in the defense response of rice against biotic and abiotic stresses [36].

Fourteen *SbCAD* genes were characterized from the analysis of sorghum (*Sorghum bicolor*) genome. Based on the phylogenetic relationship with other species CADs, SbCAD2

was involved in lignification, such as developmental CADs including AtCAD4, AtCAD5, and OsCAD2 [39]. In perennial grass (*Lolium perenne*), three *CAD* genes (*LpCAD1*, *LpCAD2,* and *LpCAD3*) were identified. *LpCAD3* was involved in developmental lignin biosynthesis, but *LpCAD1* and *LpCAD2* were more closely to other CADs related defense lignification or other functions [29]. Two enzymes, EgCAD1 and EgCAD2, were identified in *Eucalyptus gunnii* [9]. EgCAD1 has substrate specificity with coniferaldehyde and involved in G lignin synthesis. On the other hand, EgCAD2 can synthesize all three monolignols. *MsaCAD1* and *Msa CAD2* were isolated from alfafa (*Medicago sativa*). *MsaCAD1* encoded a benzaldehyde dehydrogenase, which was associated with defense response to pathogen. MsaCAD2 catalyzed the reduction of coniferaldehyde and sinapaldehyde [5]. *PtSAD* is a gene encoding sinapyl alcohol dehydrogenase from poplar (*Populus tremuloides*). It was phylogenetically distinct from PtCAD. Two genes were synthesized the S and G lignin differently. Specifically, PtSAD was required for the biosynthesis of S lignin in plants [27].

Five *CAD* genes, *CmCAD1* to *CmCAD5*, were identified in the genome of melons (*Cucumis melo* L.). *CmCAD1* and *CmCAD2* belonged to the bona fide CAD group, involved in developmental lignin synthesis. Except *CmCAD4*, the other *CmCADs* were highly expressed in young tissues. *CmCAD4* was not expressed or expressed at very low levels in these tissues [16]. The *PoptrCADs* gene family in poplar (*Poplus trichocarpa*) were sorted into four groups by expression pattern. *PoptrCAD4* and *PoptrCAD10* in Group I were strongly expressed in xylem and significantly different from the other three groups. *PoptrCAD7*, *PoptrCAD12* and *PoptrCAD13* in Group II were expressed in all tissues, particularly high in leaves. *PoptrCAD9* in Group III was preferentially expressed in leaves and xylem. *PoptrCAD2*, *PoptrCAD3*, *PoptrCAD5*, *PoptrCAD6*, *PoptrCAD11*, *PoptrCAD14,* and *PoptrCAD15* in Group IV showed a similar expression pattern in all tissues [1].

The expression of *ZmCAD2* in maize (*Zea mays*) was shown to be excessively affected in the *bm1* mutant, resulting in modified lignin content and structure [13]. Six other *CAD* family genes were identified according to maize cell wall database [11]. Eleven wheat (*Triticum aestivum*) *CAD* isoforms were obtained from wheat EST database. TaCAD1 belonged to the developmental CAD group involved in lignin synthesis. It was highly expressed in stem, with quite low expression in leaf and undetectable in root [30]. In addition, *TaCAD12* in response to *R. cerealis* infection through comparative transcriptomics was isolated. *TaCAD12* is a gene to improve wheat resistance to sharp eyespot representing the roles in plant defense responses [38].

Thirteen *IbCADs* were isolated from EST library of the sweetpotato (*Ipomoea batatas*) suspension culture. They were classified Group I to IV, according to sequence homology. Group I (*IbCAD1-IbCAD6*), group II (*IbCAD7-IbCAD11*), group III (*IbCAD12*), and group IV (*IbCAD13*) showed different structural characteristics and different expression patterns. *IbCAD* gene family under environmental stresses represented diverse expression, which could be useful for tolerance to stresses [21]. It may be involved in the lignin biosynthesis induced by both abiotic and biotic stresses and in tissue-specific developmental lignification through a *CAD* gene family network.

Multiple *CAD* isoforms described above were differentially expressed during plant development and environmental stresses. They may have substrate preferences, and this makes it possible to determine the lignin type to synthesize. According to several reports, chemical composition of defense lignin and developmental lignin was different. Substrate-specific induction of CAD was affected to its function in regulating the composition of lignin types. It was shown that G lignin was apparently presented to defense response in wounded poplar and almond tree, while G-S developmental lignin was found in these species [14]. In wounded wheat tissues, the content of S lignin was increased and sinapyl alcohol dehydrogenase activity was induced [27]. These inconsistent results in relation to the monomer composition of between developmental and defense lignin assumed a complex control of monolignol biosynthesis under external stimulation.

CAD gene family have different functions, such as, one isoform could be associated with the developmental lignification, whereas others are related defense lignins and wall-bound phenolics. The *CAD* isoforms indicated comparable catalytic activities with coniferaldehyde and sinapaldehyde. Different functional and biochemical characteristics of CAD proteins have been considered to the different isoforms identified in various plants [21, 40].

Transcriptional regulation by *CAD* promoter

To identify potential mechanisms controlling *CAD* gene expression, expression of *CAD* promoter was represented.

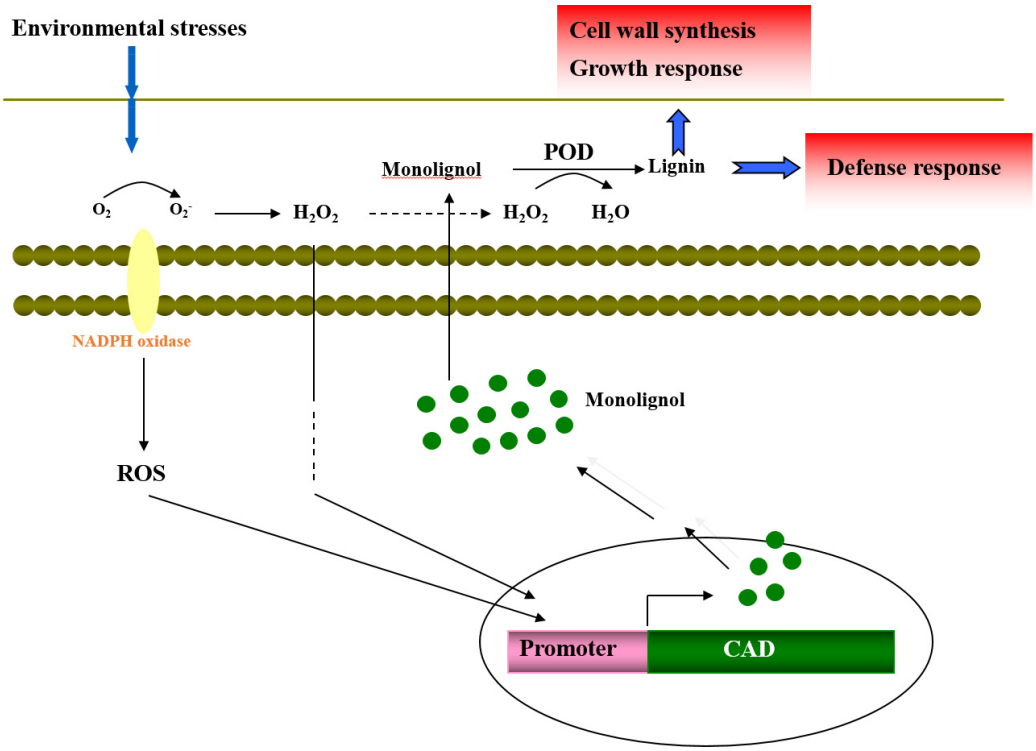

Fig. 2. Overlapping signal pathway for development and defense-related regulation of *CAD* gene.

CAD has been purified from the xylem tissues in Eucalyptus, and the corresponding mRNA was highly expressed. The activity of *EgCAD2* promoter was high in cells undergoing lignification, such as vasculature, root tip and emerging lateral root [26]. Whereas, *EgCAD1* promoter was active in other cells and tissues which were not lignified. In such cells, CAD activity is likely to be associated with monolignol production for non-lignin. *OsCAD2* promoter in rice was expressed in vascular tissues in aerial parts of the plant, which is correlated with lignification [15]. The GUS expression of each *AtCAD* promoter revealed complex patterns, including both temporal and spatial regulation during growth and development. *AtCAD4* and *AtCAD5* were primarily involved in the lignin formation of the xylem tissues. The expression pattern of *AtCAD1*, *AtCAD6*, and *AtCAD9* showed similar to *AtCAD4* and *AtCAD5*. Especially, *AtCAD9* is involved in developmental lignification in the primary xylem of stems. On the other hands, *AtCAD7* and *AtCAD8* might not be associated with developmental lignification. *AtCAD2* and *AtCAD3* also are involved in other biosynthetic pathway because they had no GUS expression pattern specific to the vascular system [8, 19]. Sweetpotato *IbCAD1* promoter expression was strong in the roots. Weak GUS expression was observed in lignified tissues of vascular system of mature leaves and stems. *IbCAD1* promoter activity was strongly induced in response to the biotic and abiotic stresses [20].

In conclusion, genetic regulation of *CAD* multigene family is complex and the signal pathway is overlapping. Synthesis of monolignol by *CAD* genes is likely to be regulated by development and environmental cues as well (Fig. 2).

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Barakat, A., Bagniewska-Zadmorna, A., Choi, A., Plakkat, U., Diloreto, D. S., Yellanki, P. and Carlson, J. E. 2009. The cinnamyl alcohol dehydrogenase gene family in *Populus*: phylogeny, organization and expression. *BMC Plant Biol.* **9**, 26.
2. Baucher, M., Bernard-Vailhe, M. A., Chabbert, B., Besle, J. M., Opsomer, C., Montagu, M. V. and Botterman, J. 1999. Down-regulation of cinnamyl alcohol dehydrogenase in transgenic alfafa (*Medicago sativa* L.) and the effect on lignin composition and digestibility. *Plant Mol. Biol.* **39**, 437-447.
3. Boerjan, W., Ralph, J. and Bouncher, M. 2003. Lignin biosynthesis. *Annu. Rev. Plant Biol.* **54**, 519-546.

4. Boudet, A. M., Lapierre, C. and Grima-Pettenati, J. 1995. Biochemistry and molecular biology of lignification. *New Phytol.* **129**, 203-236.
5. Brill, E. M., Abrahams, S., Hayes, C. M., Jenkins, C. L. D. and Watson, J. M. 1999. Molecular characterization and expression of a wound-inducible cDNA encoding a novel cinnamyl alcohol dehydrogenase enzyme in lucerne (*Medicago sativa* L.). *Plant Mol. Biol.* **41**, 279-291.
6. Campbell, M. M. and Ellis, B. E. 1992. Fungal elicitor-mediated responses in pine cell cultures. *Planta* **186**, 409-417.
7. Chen, L., Auh, C. K., Dowling, P., Bell, J., Chen, F., Hopkins, A., Dixon, R. A. and Wang, Z. Y. 2003. Improved forage digestibility of tall fescue (*Festuca arundinacea*) by transgenic down-regulation of cinnamyl alcohol dehydrogenase. *Plant Biotechnol. J.* **1**, 437-449.
8. Eudes, A., Pollet, B., Sibout, R., Do, C. T., Seguin, A., Lapierre, C. and Jouanin, L. 2006. Evidence for a role of AtCAD1 in lignification of elongating stems of *Arabidopsis thaliana*. *Planta* **225**, 23-39.
9. Goffner, D., Joffroy, I., Grima-Pettenati, J., Halpin, C., Knight, M. E., Schuch, W. and Boudet, A. M. 1992. Purification and characterization of isoforms of cinnamyl alcohol dehydrogenase from *Eucalyptus* xylem. *Planta* **188**, 48-53.
10. Grima-Petterniati, J., Feuillet, C., Goffner, D., Borderies, G. and Boudet, A. M. 1993. Molecular cloning and expression of a *Eucalpytus gunnii* cDNA clone encoding cinnamyl alcohol dehydrogenase. *Plant Mol. Biol.* **221**, 1085-1095.
11. Guillaumie, S., San-Clemente, H., Deswarte, C., Martinez, Y., Lapierre, C., Murigneux, A., Barriere, Y., Pichon, M. and Goffner, D. 2007. MAIZEWALL. Database and developmental gene expression profiling of cell wall biosynthesis and assembly in maize. *Plant Physiol.* **143**, 339-363.
12. Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., Chabbert, B., Tollier, M. and Schuch, W. 1994. Manipulation of lignin quality by down-regulation of cinnamyl alcohol dehydrogenase. *Plant J.* **6**, 339-350.
13. Halpin, C., Holt, K., Chojecki, J., Oliver, D., Chabbert, B., Monties, B., Edewards, K., Barakate, A. and Foxon, G. A. 1998. Brown-midrib maize (*bm1*): a mutation affecting the cinnamyl alcohol dehydrogenase gene. *Plant J.* **14**, 545-553.
14. Hawkins, S. W. and Boudet, A. M. 2003. 'Defense lignin' and hydroxycinnamyl alcohol dehydrogenase activities in wounded *Eucalyptus gunnii*. *For. Path.* **33**, 91-104.
15. Hirano, K., Aya, K., Kondo, M., Okuno, A., Morinaka, Y. and Matsuoka, M. 2012. OsCAD2 is the major CAD gene responsible for monolignol biosynthesis in rice culm. *Plant Cell Rep.* **31**, 91-101.
16. Jin, Y., Zhang, C., Liu, W. and Qi, H. 2014. The cinnamyl alcohol dehydrogenase gene family in melon (*Cucumis melo* L.): bioinformatic analysis and expression patterns. *PLoS One* **9**, 7.
17. Kiedrowski, S., Kawalleck, P., Hahlbrock, K., Somssich, I. E. and Dangl, E. 1992. Rapid activation of a novel plant defense gene is strictly dependent on the Arabidopsis RPM1 disease resistance locus. *EMBO J.* **11**, 4677-4684.
18. Kim, S. J., Kim, M. R., Bedgar, K. L., Moinuddin, S. G. A., Cardenas, C. L., Davin, L. B., Kang, C. and Lewis, N. G. 2004. Functional reclassification of the putative cinnamyl alcohol dehydrogenase multigene family in Arabidopsis. *Proc. Natl. Acad. Sci. USA.* **101**, 1455-1460.
19. Kim, S. J., Kim, K. W., Cho, M. H., Franceschi, V. R., Davin, L. B. and Lewis, N. G. 2007. Expression of cinnamyl alcohol dehydrogenases and their putative homologues during *Arabidopsis thaliana* growth and development: Lessons for database annotations? *Phytochemistry* **68**, 1957-1974.
20. Kim, Y. H., Bae, J. M. and Huh, G. H. 2010. Transcriptional regulation of the cinnamyl alcohol dehydrogenase gene from sweetpotato in response to plant developmental stage and environmental stress. *Plant Cell Rep.* **29**, 779-791.
21. Kim, Y. H. and Huh, G. H. 2019a. Molecular characterization and expression of the cinnamyl alcohol dehydrogenase gene family in sweetpotato (*Ipomoea batatas*) under environmental stresses. *Hortic. Sci. Technol.* **37**, 279-289.
22. Kim, Y. H. and Huh, G. H. 2019b. Overexpression of cinnamyl alcohol dehydrogenase gene from sweetpotato enhances oxidative stress tolerance in transgenic Arabidopsis. *In Vitro Cell Dev. Biol. Plant* **55**, 172-179.
23. Lacombe, E., Van Doorsselaere, J., Boerjan, W., Boudet, A. M. and Grima-Pettenati, J. 2000. Characterization of *cis*-elements required for vascular expression of the cinnamoyl-CoA reductase gene and for protein-DNA complex formation. *Plant J.* **23**, 663-676.
24. Lange, B. M., Lapierre, C. and Sandermann, H. Jr. 1995. Elicitor-induced spruce stress lignin: Structural similarity to early developmental lignins. *Plant Physiol.* **108**, 1277-1287.
25. Lauvergeat, V., Lacomme, C., Lacombe, E., Lasserre, E., Roby, D. and Grima-Petterniati, J. 2001. Two cinnamoyl-CoA reductase (CCR) genes from Arabidopsis thaliana are differentially expressed during development and in response to infection with pathogenic bacteria. *Phytochemistry* **57**, 1187-1195.
26. Lauvergeat, V., Rech, P., Jauneau, A., Guez, C., Coutos-Thevenot, P. and Grima-Pettenati, J. 2002. The vascular expression pattern directed by the *Eucalyptus gunnii* cinnamyl alcohol dehydrogenase EgCAD2 promoter is conserved among woody and herbaceous plant species. *Plant Mol. Biol.* **50**, 497-509.
27. Li, L., Cheng, X. F., Leshkevich, J., Umezawa, T., Harding, S. A. and Chiang, V. L. 2001. The last step of syringyl monolignol biosynthesis in angiosperm is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. *Plant Cell* **13**, 1567-1585.
28. Li, X., Yang, Y., Yao, J., Chen, G., Li, X., Zhang, Q. and Wu, C. 2009. FLEXIBLE CULM1 encoding a cinnamyl alcohol dehydrogenase controls culm mechanical strength in rice. *Plant Mol. Biol.* **69**, 685-697.
29. Lynch, D., Lidgett, A., Mclnnes, R., Huxley, H., Jones, E., Mahoney, N. and Spangenberg, G. 2002. Isolation and characterization of three cinnamyl alcohol dehydrogenase homologue cDNAs from perennial ryegrass (*Lolium perenne* L.). *J. Plant Physiol.* **159**, 653-660.

30. Ma, Q. H. 2010. Functional analysis of a cinnamyl alcohol dehydrogenase involved in lignin biosynthesis in wheat. *J. Exp. Bot.* **61**, 2735-2744.
31. Ma, D., Xu, C., Alejos-Gonzalez, F., Wang, H., Yang, J., Judd, R. and Xie, D. Y. 2018. Overexpression of *Artemisia annua* cinnamyl alcohol dehydrogenase increases lignin and coumarin and reduces artemisinin and other sesquiterpenes. *Front. Plant Sci*. **9**, 828.
32. MacKay, J. J., Liu, W., Whetten, R., Sederoff, R. R. and O'Malley, D. M. 1995. Genetic analysis of cinnamyl alcohol dehydrogenase in loblolly pine: Single gene inheritance, molecular characterization and evolution. *Mol. Gen. Genet.* **247**, 537-545.
33. MacKay, J. J., O'Malley, D. M., Presnell, T., Booker, F. L., Campbell, M. M., Whetten, R. W. and Sederoff, R. R. 1997. Inheritance, gene expression, and lignin characterization in a mutant pine deficient in cinnamyl alcohol dehydrogenase. *Proc. Natl. Acad. Sci. USA*. **94**, 8255-8260.
34. Mitchell, H. J., Hall, J. L. and Barber, M. S. 1994. Elicitor-induced cinnamyl alcohol dehydrogenase activity in lignifying wheat (*Triticum aestivum* L.) leaves. *Plant Physiol*. **104**, 551-556.
35. Mclnnes, R., Lidgett, A., Lynch, D., Huxley, H., Jones, E., Mahoney, N. and Spangenberg, G. 2002. Isolation and characterization of a cinnamoyl-CoA reductase gene from perennial ryegrass (*Lolium perenne*). *J. Plant Physiol.* **159**, 415-422.
36. Park, H. L., Kim, T. L., Bhoo, S. H., Lee, T. H., Lee, S. W. and Cho, M. H. 2018. Biochemical characterization of the rice cinnamyl alcohol dehydrogenase gene family. *Molecules* **23**, 2659.
37. Pichon, M., Courbou, I., Bechert, M., Boudet, A. M. and Grima-Pettenati, J. 1998. Cloning and characterization of two maize cDNAs encoding cinnamoyl CoA reductase (CCR) and differential expression of the corresponding genes. *Plant Mol. Bol*. **38**, 671-676.
38. Rong, W., Luo, M., Shan, T., Wei, X., Du, L., Xu, H. and Zhang, Z. 2016. A wheat cinnamyl alcohol dehydrogenase TaCAD12 contributes to host resistance to the sharp eyespot disease. *Front. Plant Sci*. **7**, 1723.
39. Saballos, A., Ejeta, G., Sanchez, E., Kang, C. and Vermerris, W. 2009. A genome-wide analysis of the cinnamyl alcohol dehydrogenase family in sorghum (*Sorghum bicolor* (L.) Moench) identities *SbCAD2* as the *Brown midrib6* gene. *Genetics* **181**, 783-795.
40. Somers, D. A., Nourse, J. P., Manners, J. M., Abrahams, S. and Watson, J. M. 1995. A gene encoding a cinnamyl alcohol dehydrogenase homolog in *Arabidopsis thaliana*. *Plant Physiol.* **108**, 1309-1310.
41. Somssich, I. E., Wernert, P., Kiedrowski, S. and Hahlbrock, K. 1996. Arabidopsis thaliana defense-related ELI3 is an aromatic alcohol:$NADP^+$ oxidoreductase. *Proc. Natl. Acad. Sci. USA.* **93**, 14199-14203.
42. Sibout, R., Eudes, A., Mouille, G., Pollet, B., Lapierre, C., Jouanin, L. and Seguin, A. 2005. Cinnamyl alcohol dehydrogenase-C and -D are the primary genes involved in lignin biosynthesis in the floral stem of Arabidopsis. *Plant Cell* **17**, 2059-2076.
43. Tobias, C. M. and Chow, E. K. 2005. Structure of the cinnamyl alcohol dehydrogenase gene family in rice and promoter activity of a member associated with lignification. *Planta* **220**, 678-688.
44. Whetten, R. and Sederoff, R. 1995. Lignin biosynthesis. *Plant Cell* **7**, 1001-1013.
45. Yoon, J., Choi, H. and An, G. 2015. Roles of lignin biosynthesis and regulatory genes in plant development. *J. Integr. Plant Biol.* **57**, 902-912.
46. Zhang, K., Qian, Q., Huang, Z., Wang, Y., Li, M., Hong, L., Zeng, D., Gu, M., Chu, C. and Cheng, Z. 2006. GOLD HULL AND INTERNODE2 encodes a primarily multifunctional cinnamyl alcohol dehydrogenase in rice. *Plant Physiol*. **140**, 972-983.

초록 : 리그닌 생합성에서 *cinnamyl alcohol dehydrogenase* (*CAD*) 유전자 family의 조절

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리그닌은 식물의 세포벽에 풍부하게 존재하는 복잡한 phenylpropanoid 중합체이다. 주로 물 수송과 기계적 강도를 유지하는 조직에 존재하며 수분을 운반하거나, 기계적인 지지를 담당한다. 또한, 리그닌은 병원균의 감염이나 상처에 대한 물리적인 장벽으로 작용함으로써 방어 기작에 관여한다. 리그닌을 생성하는 모노리그놀 전구체는 *cinnamyl alcohol dehydrogenase* (*CAD*) 유전자에 의해 합성된다. *CAD*는 cinnamaldehyde를 cinnamyl alcohol (*p*-coumaryl, coniferyl, sinapyl alcohol)로 전환하는 효소이다. *CAD*는 속씨식물에서 multigenic family로 존재하며 여러 식물 종에서 다른 기능을 가진 *CAD* isoform이 밝혀졌다. *CAD* 유전자의 여러 isoform은 식물의 발달 및 환경 신호에 따라 다르게 발현되었다. 하나의 isoform이 발달 리그닌화에 관여하는 반면, 다른 isoform은 방어 리그닌 및 기타 세포벽에 결합된 페놀의 구성에 영향을 미칠 수 있음을 보여주었다. *CAD* isoform에 따라 기질 특이성이 다르게 나타나고, 이는 리그닌 합성을 조절하는 CAD 단백질의 생화학적 특성을 나타내는데 기여한다. 본 논문에서는 리그닌 생합성에서 *CAD* multigenic family 유전자의 발현과 조절에 대하여 설명하였다. *CAD* multigenic family의 isoform들은 유전적 조절이 복잡하고, 식물 발달 과정의 신호 경로와 스트레스 반응이 밀접하게 연동되어 있다. *CAD* 유전자에 의한 모노리그놀 합성은 발달 및 환경 신호에 의해 조절될 가능성이 높다.