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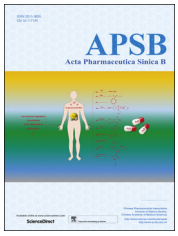

ORIGINAL ARTICLE

Cloning, expression and characterization of *COI1* gene (*AsCOI1*) from *Aquilaria sinensis* (Lour.) Gilg

Yongcui Liao[a], **Jianhe Wei**[a,b,*], **Yanhong Xu**[a], **Zheng Zhang**[a,b]

[a]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China*
[b]*Hainan Branch Institute of Medicinal Plant (Hainan Provincial Key Laboratory of Resources Conservation and Development of Southern Medicine), Chinese Academy of Medical Sciences and Peking Union Medical College, Wanning 571533, China*

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Expression;
Subcellular localization

Abstract *Aquilaria sinensis*, a kind of typically wounding-induced medicinal plant with a great economical value, is widely used in the production of traditional Chinese medicine, perfume and incense. Coronatine-insensitive protein 1 (COI1) acts as a receptor in jasmonate (JA) signaling pathway, and regulates the expression of JA-responsive genes in plant defense. However, little is known about the *COI1* gene in *A. sinensis*. Here, based on the transcriptome data, a full-length cDNA sequence of *COI1* (termed as *AsCOI1*) was firstly cloned by RT–PCR and rapid-amplification of cDNA ends (RACE) strategies. *AsCOI1* is 2330 bp in length (GenBank accession No. KM189194), and contains a complete open frame (ORF) of 1839 bp. The deduced protein was composed of 612 amino acids, with a predicted molecular weight of 68.93 kDa and an isoelectric point of 6.56, and was predicted to possess F-box and LRRs domains. Combining bioinformatics prediction with subcellular localization experiment analysis, AsCOI1 was appeared to locate in nucleus. *AsCOI1* gene was highly expressed in roots and stems, the major organs of agarwood formation. Methyl jasmonate (MeJA), mechanical wounding and heat stress could significantly induce the expression level of *AsCOI1* gene. *AsCOI1* is an early wound-responsive gene, and it likely plays some role in agarwood formation.

*Corresponding author at: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China. Tel.: +86 10 57833358; fax: +86 1057833359.
E-mail address: wjianh@263.net (Jianhe Wei).

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1. Introduction

Agarwood is a non-timber fragrant wood and widely used in perfume, incense and medicine across Asia, Middle East, and Europe[1,2]. *Aquilaria sinensis* (Lour.) Gilg is one of the most important plant resources for agarwood production in China, as well as the only certified source for agarwood products listed in *China Pharmacopoeia*[3]. The formation of agarwood only happens when the tree is wounded, and also as a result of defensive response. The main agarwood compounds are sesquiterpenes and phenylethyl chromone derivatives[4–7]. But so far, the wounding-induced molecular mechanism of agarwood formation remains largely unknown.

In plants, jasmonate (JA) is not only a key endogenous plant hormone but also a long-distance transportation wound signal molecule that participates in plant defense responses[8–14]. As reported, the application of exogenous JA to the suspension cells of *Aquilaria* plants could increase the expression level of *δ*-guaiene synthases gene and the biosynthetic content of sesquiterpenes[4]; *A. sinensis* calli treated with methyl jasmonate (MeJA), an elicitor of plant defensive responses, could cause an increase in sesquiterpene synthase genes (ASSs) expression and four sesquiterpenes production[15]. Up to now, the general and specific components of JA signaling pathway in *Aquilaria* tree are not clear yet. We are interested in the relationship between JA signaling pathway and the regulation mechanism of secondary metabolites biosynthesis-related genes in *A. sinensis*.

Coronatine-insensitive protein 1 (COI1), an F-box protein, interacts with SKP1 and Cullin proteins to form SCF complexes that recruit regulatory proteins targeted for ubiquitination[16]. In *Arabidopsis thaliana*, COI1 mediates JA signalling pathway by promoting hormone-dependent ubiquitylation and degradation of transcriptional repressor JAZ proteins liberating the transcription factors to start the transcription of JA-responsive genes[16–22]. *GmCOI1*, a soybean F-box protein gene, shows ability to interact with *Arabidopsis* ASK1 and Cullin1 proteins to form SCF^{COI1} complex that mediates JA-regulated plant defense and fertility in *Arabidopsis*[23]. Similar roles have been reported for COI1 homologs in several other species, including tomato, tobacco and *Oryza sativa*[24–27], indicating a conserved function of plant COI1. In order to investigate the mechanism of JA signaling pathway in *A. sinensis*, we cloned *A. sinensis COI1* gene, the key factor of JA response pathway. The full-length cDNA sequence of *AsCOI1* from *A. sinensis* was isolated, and its expression patterns responding to MeJA, mechanical wounding and heat were investigated. The results may lay a foundation for further exploring the biological functions of gene and revealing the underlying mechanism of sesquiterpene biosynthesis in *A. sinensis*.

2. Materials and methods

2.1. Plant materials

A. sinensis trees were grown in a field nursery. Leaves, roots, stems and branches were collected from the four-year-old plants and stored in liquid nitrogen for tissue expression analysis. The well-grown *A. sinensis* calli were subcultured to the modified Murashige−Skoog (MS) medium supplemented with 100 μmol/L MeJA, and incubated for 0.5, 1, 2, 4, 6, 8, 12 and 24 h in darkness and then sampled. The same well-grown calli were crushed with a pair of metal forceps and cultured for 0.5, 1, 2, 4, 6, 12 and 24 h in darkness and then sampled as mechanical wounding stress analysis materials. The calli without MeJA treatment or crush wounding treatment were sampled at the same time period and used as control. All samples were quickly poured into liquid nitrogen and stored at −80 °C for analysis. Well-grown *A. sinensis* suspension cells (100 mL) were placed in shaking water bath with 50 °C and 110 rpm holding for 30 min. After heat treatment, *A. sinensis* suspension cells were transferred back to shaker at 25 °C and 110 rpm until harvested. Treated suspension cells were sampled by filtration 0.5, 1, 2, 4, 6, 8, 12, 24 h. The *A. sinensis* suspension cells that without heat treatment were simultaneously sampled at the same time period and used as control. All samples were removed under sterile conditions, rapidly filtered, and shock-frozen in liquid nitrogen and stored at −80 °C.

2.2. RNA isolation and synthesis of cDNA

Total RNA was extracted using a Tiangen RNA extraction kit (RNAprep pure Plant Kit. Tiangen Biotech Beijing Co., Ltd.) according to the manufacturer's instructions. Quality and quantity of each total RNA sample were assessed in agarose gels (1%, *w/v*) and spectrophotometricaly at 260 and 280 nm (Bio-Rad, NanoDrop 2000), respectively.

cDNA was synthesized by reverse transcription (RT) to transcribe poly(A)$^+$ mRNA with oligo-dT primers using a RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, USA) following the manufacturer's instructions. The cDNA was stored at −20 °C for qRT–PCR analysis and gene clone.

2.3. Cloning of AsCOI1 by rapid-amplification of cDNA ends (RACE) method

The primers used in this study are showed in Table 1. The first-strand cDNA was used as the template for *AsCOI1* core fragment amplication based on the unigenes of 454 data[15]. Both 5′ and 3′ untranslatable regions (UTRs) of the *AsCOI1* were obtained by SMARTerTMRACE cDNA Amplification Kit (Clontech, USA), following the manufacturer's instructions. The primer 3′-PA and 5′-PA were used as the primer to synthesize 3′ and 5′ first strand cDNA, respectively. The gene-specific primers of the *AsCOI1* were designed based on previously cloned fragments. Antisense primers *COI1*-5′GSP1 and *COI1*-5′GSP2 were used for synthesizing 5′ rapid amplification of cDNA ends, and sense primers *COI1*-3′GSP1 and *COI1*-3′GSP2 were synthesized for 3′ rapid amplification of cDNA ends. Those primers were all paired with UPM to amplify 5′ and 3′ cDNA ends. The NUP was used as the nested primer. The RACE reaction was performed in a total volume of 50 mL containing 2.5 μL first-strand cDNA, 5 μL Universal Primer Mix (UPM) (10 ×), 1 μL 10 μmol/L 5′ or 3′ specific primer, 5.0 μL 10 × advantage 2 PCR buffer, 2.0 μL 10 μmol/L dNTP mix, and 1.0 μL 50 × advantage 2 polymerase mix. Touchdown-PCR reactions were performed at 94 °C (pre-denaturation) for 4 min, followed by 94 °C for 30 s, 70 °C for 30 s, and 72 °C for 90 s in the first cycle, and the annealing temperature was decreased by 1 °C per cycle. After ten cycles, the conditions were changed to 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s for 20 cycles. The duration of the 72 °C elongation step was 10 min.

The PCR products were then subjected to electrophoresis on a 1% agarose gel for detection and purification. The amplified subjective fragments were cloned into the pGM-T vector

Table 1 Primers for gene cloning and real-time PCR detection.

Primer purpose	Name	Sequence (5′–3′)
5′-RACE primers	*COI1*-5′GSP1	GCAAGCTCATGAAGCCATTGGCCATC
	COI1-5′GSP2	CCTGCAGAACACTCCCTCGCTCCCTAG
3′-RACE primers	*COI1*-3′GSP1	GGAGTACGGGCTCTCCTAAGAGGTTGC
	COI1-3′GSP2	GCTTTGGCTGAGGGCTGCCTTGAGCTTG
Full-length CDS cloing	*COI1*-LF	ATGGAGGAGAGCAGTTACAAG
	COI1-LR	CCAGTTGGATCCTTTACCGTAA
Reference gene primer	*TUA*-f	GCCAAGTGACACAAGCGTAGGT
	TUA-r	TCCTTGCCAGAAATAAGTTGCTC
AsCOI1 RT–qPCR primer	*COI1*-1f	CATCGTCATCGTCTTCTTCAGG
	COI1-1r	GAGTCACATAGCCGCCCCA
Universal primer A Mix (UPM)	UPM-Long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
	UPM-Short	CTAATACGACTCACTATAGGGC
Nested universal primer A	NUP	AAGCAGTGGTATCAACGCAGAGT

(Tiangen). Recombinant plasmids were transformed into *Escherichia coli*, selected by blue/white screening, and verified by PCR. Nucleotide sequencing was performed by Shanghai Sangon Biological Engineering Technology and Service Company, China.

2.4. Isolation and bioinformatics analysis

The sequence encoding AsCOI1 was determined by homology searches in the NCBI databases using the BLAST program, and the homology sequences were downloaded from these databases. The alignment of the AsCOI1 protein with other structurally-related COI1 proteins was performed using the Clustal X program. Some other bioinformatic sequence features of AsCOI1, such as molecular weight (MW), theoretical isoelectric point (p*I*) and stability, were determined as described[28]. The cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) was used to predict the nuclear localization signals[29]. Conserved motifs of COI1s in *A. sinensis* and other species were analyzed using Multiple Expectation Maximization for Motif Elicitation (MEME) version 4.9.1[30] with the following parameters. Optimum motif width was set to ≥ 6 and ≤ 50. The conserved residues were analyzed by aligning amino acid sequences using T-coffee[31] and by searching literature references. SWISS-MODEL was used to analyze the molecular modeling of AsCOI1 protein[32].To determine the relationship between AsCOI1 and other COI1 proteins, phylogenetic analysis was constructed for 16 COI1 proteins of different species using MEGA version 5.05 by the neighbor-joining method with 1000 bootstrap replicates[33].

2.5. Quantitative real-time reverse transcription–PCR (qRT–PCR)

The tissue-specific expression in roots, stems, leaves and branches of four-year-old *A. sinensis* plants as well as the *AsCOI1*expression pattern analysis induced by MeJA, mechanical wounding and heat were analyzed using the qRT–PCR method as described previously[28]. Briefly, gene-specific forward and reverse primers were designed and synthesized (Table 1). About 15 ng cDNA reversely transcribed from total RNA was used as a template in a 25 mL volume. Tubulin (*TUA*) was used as a reference gene[34]. qRT–PCR was carried out in triplicates for each biological sample using the BIORAD iQTM5 system (Bio-Rad). Three fully independent biological replicates were performed. The amplification specificity was assessed by dissociation curve analysis. Gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method, where Ct represents the threshold cycle[35]. Relative amount of transcripts was calculated and normalized as described previously[35]. The average Cts were log transformed, mean centered and autoscaled[36]. Standard deviations of the mean value from three biological replicates were calculated as described previously[36].

2.6. Subcellular localization analysis

A vector pAN580 containing the open reading frame of enhanced green fluorescent protein (EGFP) was used in this study. The whole coding sequence of *AsCOI1* gene was amplified with primers *AsCOI1*-GFP-F and *AsCOI1*-GFP-R (Table 1) using Pfu DNA polymerase (Fermentas, Glen Burnie, USA). The amplification sequence was ligated with *Xho* I- and *Bam*H I-digested pAN580 vector to generate a *AsCOI1*-EGFP fusion construct under the control of cauliflower mosaic virus 35S (CaMV 35S) promoter. The construct was confirmed by sequencing and used for transient transformation of onion epidermis *via* a gene gun (Bio-Rad, PDS-1000, USA). After 24 h of incubation in dark, GFP fluorescence in transformed onion cells was observed under a confocal microscope (OLYMPUS V-TV0.5XC-3, Japan).

3. Resluts

3.1. Molecular cloning of full-length cDNAs and characterization of AsCOI1

Based on the sequences of unigenes from *A. sinensis* transcriptome data, a full-length cDNA clone was obtained using 5′-/3′-RACE extension methods. Two specific primers *COI1*-5′GSP1 and *COI1*-5′GSP2 for 5′-RACE, and *COI1*-3′GSP1 and *COI1*-3′GSP2 for 3′-RACE were designed (Table 1) to yield a 651 bp 5′-cDNA ends sequence and a 819 bp 3′-cDNA ends sequence.

The sequence analysis confirmed that the clone is a *COI1* gene. The full-length *AsCOI1* comprises 2330 bp, containing a 191-bp 5′ untranslated region (5′-UTR), a 300-bp 3′ untranslated region (3′-UTR), and a 30-bp polyA. Its ORF is 1839 bp (Fig. 1), encoding a deduced protein of 612 amino acids with a predicted molecular weight of 68.93 kDa and an isoelectric point of 6.56.

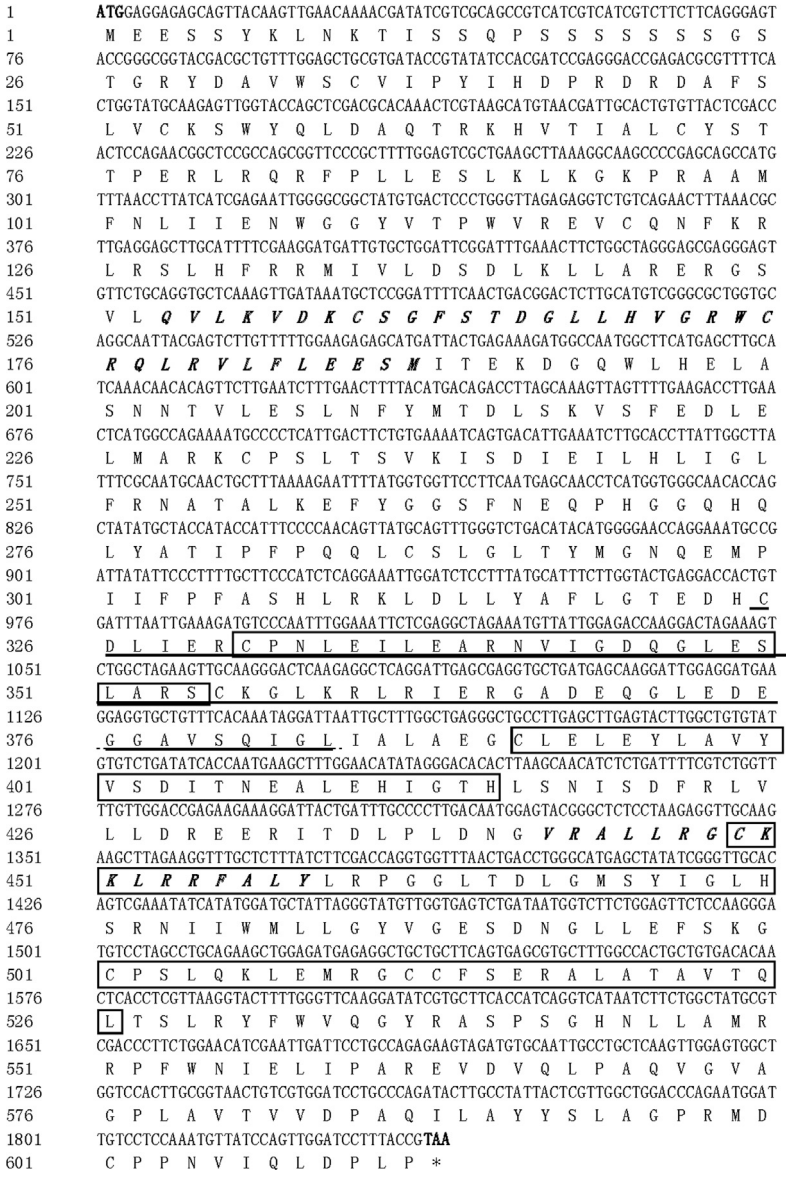

Figure 1 The cDNA sequence and the deduced amino acid sequence of *AsCOI1*. The translation initiation and termination condons are bolded. The characteristic motifs of the AsCOI1 are shown as follows: 4 LRR domains boxed in black; 2 F-box-like domains italic and bold; CGGC domains underlined.

The cloned cDNA has been submitted to GenBank under the accession number KM189194.

3.2. *Bioinformatics analysis of AsCOI1*

AsCOI1 protein contains 29.25% α-helix, 12.25% β-sheet, and 58.50% random coil, and is hydrophilic with a hydropathy value of -0.108 on average. Nuclear localization signals prediction result showed that AsCOI1 has a nuclear localization signal, suggesting that AsCOI1 might be a nuclear protein, and its location needs more empirical evidence. The search for the conserved domains in AsCOI1 protein against the NCBI Conserved Domain Database and SMART online tools showed that AsCOI1 contains the Leucine-rich repeats (LRR) domain, F-box-like domain and CGGC domain. A three-dimensional structural model was also constructed by SWISS-MODEL (Fig. 2). The MEME motif search tool was used to analyze the conserved motifs of AsCOI1 and COI1 in other species (Fig. 3A). The results revealed three motifs conserved in all the seven lipoxygenases (LOXs). These highly conserved motifs might be associated with the gene function of *AsCOI1*. The sequence alignment of COI1 proteins from *A. sinensis* and other species using T-coffee[31] showed that AsCOI1 contains the WMLLGYVGESD and GCPSLQKLE signature, the partial sequence of the third motif (Fig. 3B).

3.3. *Homologous alignment and phylogenetic analysis of AsCOI1*

To determine the evolutionary relationship among COI1 proteins from *A. sinensis* and other species, an unrooted neighbor-joining tree was constructed for further identifying the relationships between the AsCOI1 and COI1 protein sequences of other 14 plants already obtained. As shown in Fig. 4, *A. sinensis*

COI1 lined up with *Solanum lycopersicum* COI1, which indicted that both proteins had similar structures and likely enjoyed some same gene function.

3.4. Tissue-specific expression of AsCOI1 gene

To preliminarily elucidate the function of *AsCOI1* gene, we analyzed the expression patterns of the *AsCOI1* in roots, stems, leaves and branches of four-year-old and field nursery-grown *A. sinensis* tree using the quantitative RT–PCR technique. The results showed that *AsCOI1* was constitutively expressed in all tested tissues, but at very different levels. The transcription of *AsCOI1* gene was the highest in roots, moderate in stems and the weakest in leaves (Fig. 5). The highest transcript of *AsCOI1* tested in roots was more than 10 times higher than in leaves.

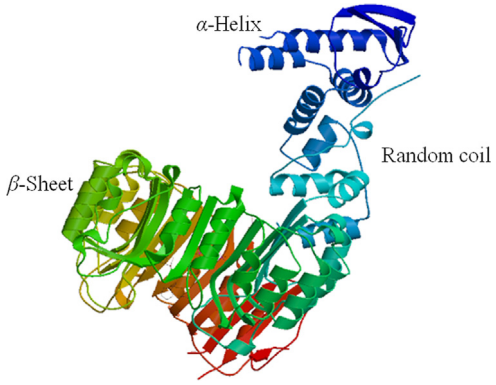

Figure 2 Predicted AsCOI1 3-D mode.

3.5. The response of AsCOI1 to MeJA, mechanical wounding, and heat treatment

To examine the response of *AsCOI1* to different stresses, mechanical wounding, MeJA and heat, the level of *AsCOI1* transcripts was analyzed using the quantitative RT–PCR method in *A. sinensis* calli and suspension cells. Results showed that *AsCOI1* were positively and significantly induced in all the test stresses (Fig. 6A–C). Generally speaking, the expression level of the *AsCOI1* transcripts increased firstly, then decreased, and finally went back to the normal. In mechanical wounding treatment, there appeared a relative higher increase at 1 h about 20 times, and the highest expression level represented at 6 h about 33 times (Fig. 6A). The above results indicated that *AsCOI1* might be involved in wound defense in *A. sinensis*. In MeJA treatment, the relative higher increase was at 0.5 h nearly 40 times of control, and the highest point was at 4 h about more than 80 times compared to the control and declined rapidly to the normal (Fig. 6B). In heat treatment group, there presented a more dramatic rise. The highest increase peak appeared at 4 h about 100 times compared to the control, and decreased back to the normal at 24 h after heat treatment (Fig. 6C).

3.6. Localization of AsCOI1

To examine the subcellular localization of AsCOI1, the ORF of *AsCOI1* gene was fused to the N-terminal of the GFP reporter gene under the control of the CaMV 35S promoter. The recombinant constructs of the *AsCOI1-GFP* fusion gene and GFP alone were introduced into onion epidermal cells by gold particle bombardment, respectively. As showed in Fig. 7, the *AsCOI1-GFP* fusion gene was specifically localized in the nucleus, whereas GFP alone showed ubiquitous distribution in the whole cell. This result

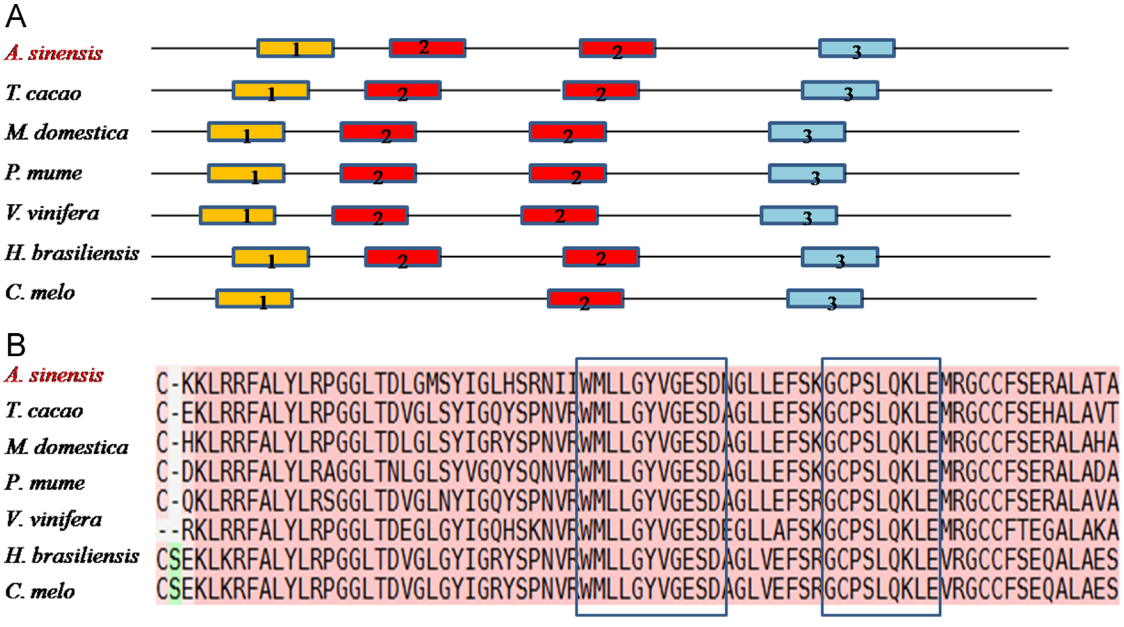

Figure 3 Conserved motifs and sequences of COI1 proteins in *A. sinensis* and other species. (A) Conserved motifs identified with the MEME search tool. Motifs are represented by boxes. The numbers (1–3) and different colors in boxes represent motif 1–3, respectively. Box size indicates the length of motifs. Abbreviation: *Aquilaria sinensis* (*A. sinensis*), *Theobroma cacao* (*T. cacao*), *Malus domestica* (*M. domestica*), *Prunus mume* (*P. mume*), *Vitis vinifera* (*V. vinifera*), *Hevea brasiliensis* (*H. brasiliensis*), *Cucumis melo* (*C. melo*). (B) Alignment of partial sequences using T-coffee. Consistent sequences are boxed. Different colors represent different alignment qualities: red for the highest, and green for the worst.

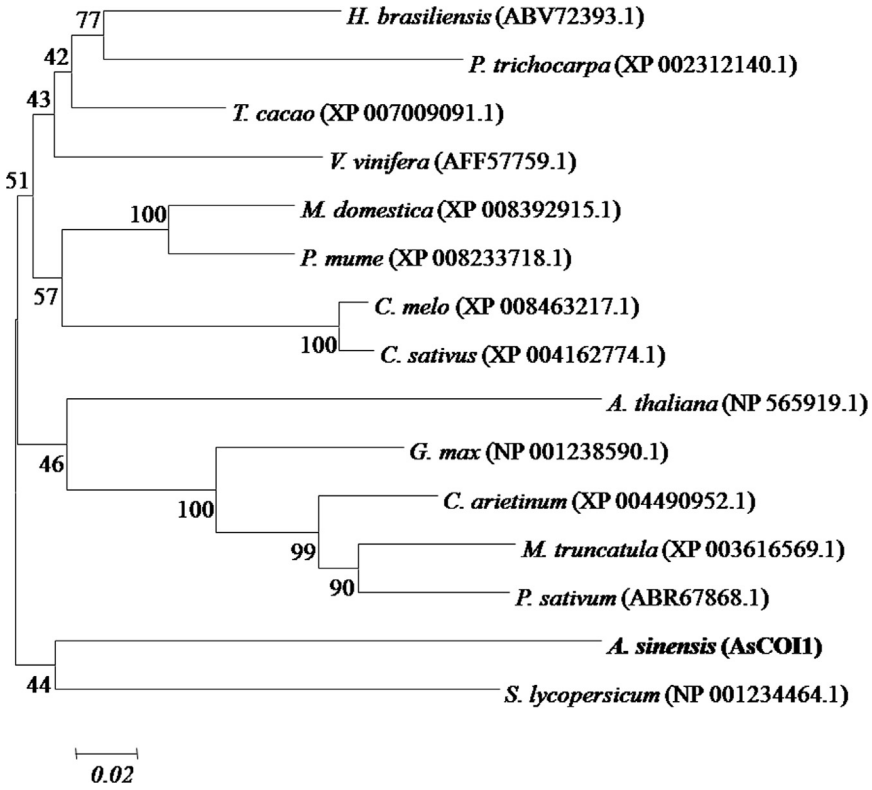

Figure 4 Phylogenetic tree based on the amino acid sequence of AsCOI1 and other homologues sequences. The relationships were analyzed for deduced full-length amino acid sequences using MEGA 5.05 by the neighbor-joining (NJ) method with 1000 bootstrap replicates. Bootstrap values are shown near the nodes. Abbreviation: *Populus trichocarpa* (*P. trichocarpa*), *Cucumis sativus* (*C. sativus*), *Arabidopsis thaliana* (*A. thaliana*), *Glycine max* (*G. max*), *Cicer arietinum* (*C. arietinum*), *Medicago truncatula* (*M. truncatula*), *Solanum lycopersicum* (*S. lycopersicum*).

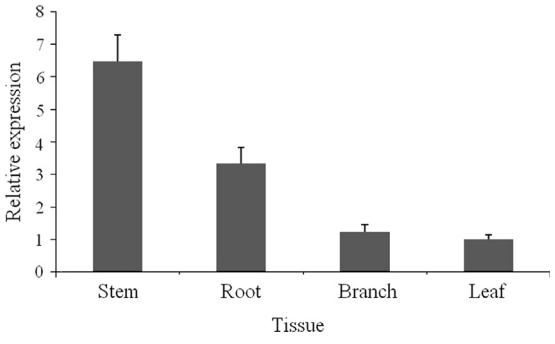

Figure 5 The relative expression of *AsCOI1* in roots, stems, leaves and branches of *A. sinensis*. The expression patterns were analyzed by the quantitative RT–PCR method. PCR was carried out in triplicates for each biological sample. Three independent biological replicates were performed. Tubulin (*TUA*) was used as a reference. Fold changes of *AsCOI1* expression were measured. Error bars represent the standard deviations of the mean value of three biological replicates.

indicated that the AsCOI1 protein was localized in the nucleus and may act as binding protein in gene transcriptional regulating.

4. Discussion

In plants, *COI1* gene has been cloned and characterized from *Arabidopsis*, soybean, tobacco, rubber, and a few other species[18,25–27,37], but not from *Aquilaria sp.* trees. Here, we firstly report on the *COI1* gene cloning and characterization. The deduced amino acid sequence of AsCOI1 showed extensive similarity to its counterparts in other species. COI1, the first identified F-box protein, is one of the three components of the SCF complex, which mediates ubiquitination of the proteins targeted for degradation by the proteasome[38]. In *A. thaliana*, *COI1* is a gene required for JA-regulated defense[17]. In *Nicotiana* plants[26,27], the transgenic suppression of *Nicotiana* COI1 (NtCOI1) homologs results in JA insensitivity of root growth, impaired anther dehiscence, and down-regulated JA-responsive genes; Furthermore, NtCOI1 functions upstream of NtMYB305 and plays a fundamental role in coordinating plant primary carbohydrate metabolism and correlative physiological processes[39]. In *Arabidopsis*, the mutation of thylakoid formation 1 (*THF1*) lead to basal and wound-induced levels of oxylipins increase that stimulate anthocyanin biosynthesis *via* COI1 signaling[40]. These results suggest that the COI1-related F-box protein is an essential conserved component of JA signaling pathway in plants secondary metabolism.

In this study, based on the unigene sequence of *COI1*, we designed specific primers and firstly cloned the full-length cDNA sequence from *A. sinensis*, named *AsCOI1*. The deduced AsCOI1 protein was observed to contain 2F-box domains and 4 LRR domains, indicating that this predicted protein belonged to the plant COI1 protein family. Besides that, it also contained a CGGC domain, which was rich in many conserved cysteines and histidines, suggesting that it might has a zinc-binding function.

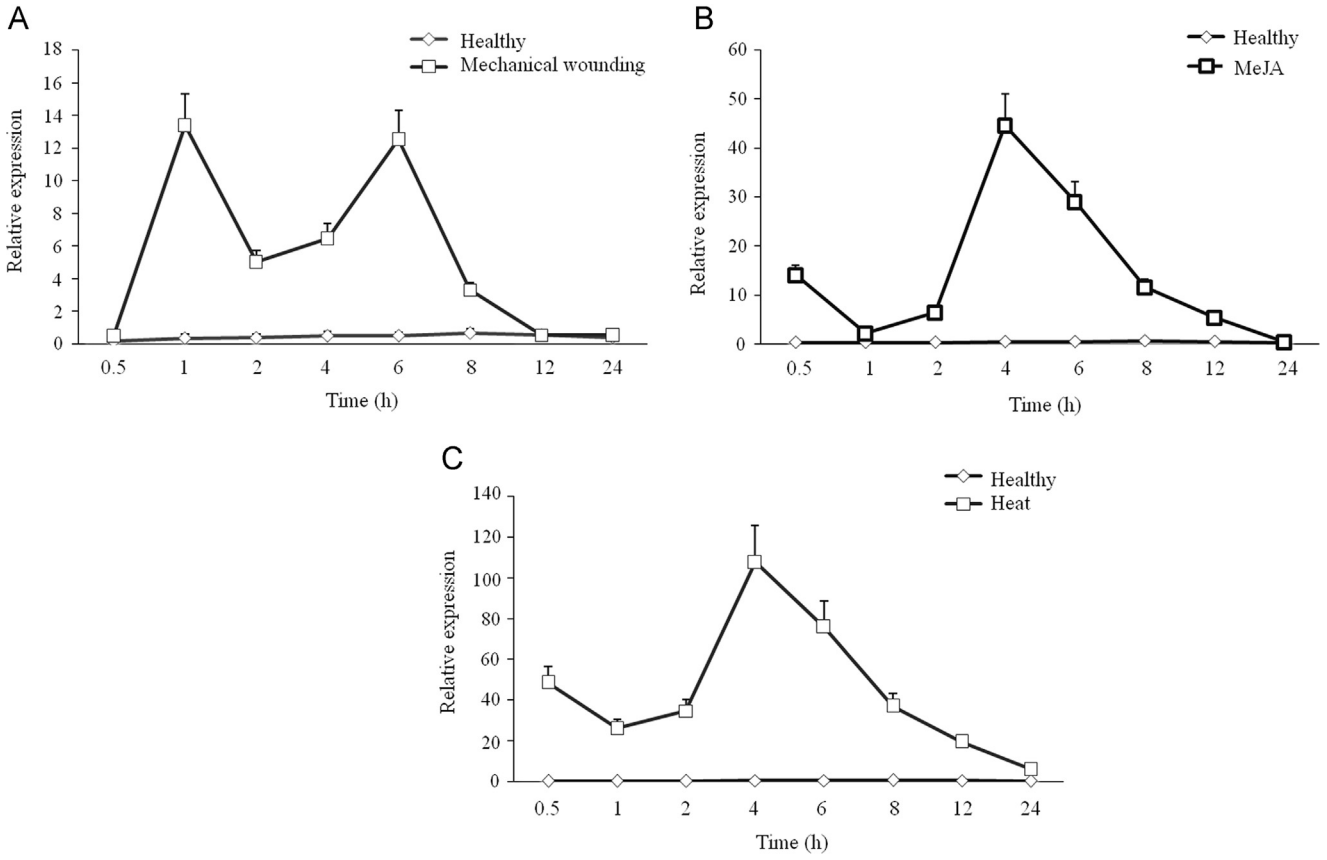

Figure 6 The expression analysis of *AsCOI1* gene responding to stresses. (A) Mechanical wounding. (B) MeJA treatment. (C) Heat stress. The expression patterns were analyzed using the quantitative RT–PCR method. PCR was carried out in triplicates for each biological sample. Three independent biological replicates were performed. Tubulin (*TUA*) was used as a reference gene.

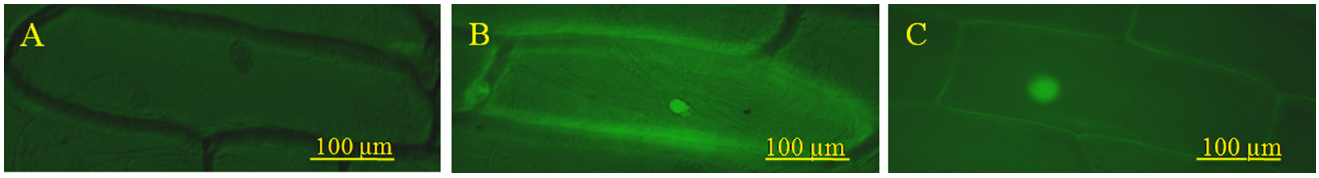

Figure 7 Nuclear localization of AsCOI1. (A) Onion epidermis cells. (B) Onion epidermis cells transformed with pGEX-4 T-1 plasmid. (C) Nuclear localization of AsCOI1-GFP. Confocal images of onion epidermis cells under the GFP channel show the constitutive localization of GFP and nuclear localization of AsCOI1-GFP.

Multiple alignments analysis showed that AsCOI1 had more than 70% sequence identity with the COI1 proteins of several other species, which suggested that COI1 proteins were highly conserved. COI1 proteins were observed to be highly conserved, confirming the high degree of COI1 conservation during the evolution, which reflects the selective pressure imposed by the essential functions of COI1 in plants.

Previous studies have demonstrated that the *COI1* gene expression patterns in plants are not identical: some are constitutive type, and some are inducible type. In our experiment, *AsCOI1* was expressed mainly in roots and stems, the major organs for agarwood accumulation in *Aquilaria* plants. This result suggested that *AsCOI1* might play an important role in agarwood accumulation. Rice *OsCOI1* expression was induced by MeJA and abscisic acid (ABA)[41], and the *Hevea brasiliensis HbCOI1* was induced by JA and tapping wound[42]. In this study, the expression of *AsCOI1* gene was significantly induced by MeJA and mechanical wounding in *A. sinensis* calli, and by heat in *A. sinensis* suspension cells. *AsCOI1* more dramatically responded to heat, moderately to MeJA and relative weaker to mechanical wounding. The highest peak pointed at 4–6 h after been treated, and went back to the normal at 12–24 h after been treated. All the above results suggested that *COI1* gene probably worked in different way in the JA signal transduction pathway responding to different stresses in plants. Proper responses to JA were dependent on COI1 dosage, and most COI1-dependent JA-responsive genes require COI1 in dose-dependent manner and specific JA responses have different sensitivities to COI1 abundance[40]. Although JA responses molecular mechanism is already mostly clear in plants, it is completely unclear in *A.*sp. plants. Consequently, our study on *AsCOI1* would help to reveal the relation between the JA signal transduction and the regulated natural agarwood accumulation in *Aquilaria* defense responses.

5. Conclusions

Here we cloned a lipoxygenase gene (*AsCOI1*) from *A. sinensis* trees for the first time. According to the experimental results, the full-length ORF of *AsCOI1* is 2330 bp, encoding 612 amino acids with a predicted molecular weight (MW) of 68.93 kDa and an isoelectric point (PI) of 6.56. AsCOI1 belongs to a kind of conservative protein, F-box and LRRs domains. *AsCOI1* gene is mainly expressed in roots and stems, but lowest in leaves. AsCOI1 locates in nucleus. The expression of *AsCOI1* could be significantly induced by MeJA, mechanical wounding and heat stress in *A. sinensis* callus. This work may lay a theoretical and experimental foundation for the future research on gene functions, and the transgenic *A. sinensis* trees with varied *AsCOI1* expression will give deeper insight into the *AsCOI1* role in *A. sinensis*.

Acknowledgments

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