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Full Length Research Paper

Cloning of a novel stearoyl-acyl desaturase gene from white ash (*Fraxinus americana*) and evolution analysis with those from other plants

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Using reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends, a new full-length cDNAs of stearoyl-ACP desturases (SAD) (*FaSAD*) was obtained from white ash. Sequence analysis showed that the deduced amino acid sequence of *FaSAD* has high similarity to that of other reported SAD proteins. They are different from each other by some substitutions, insertions and/or deletions involving single amino acid residues or motifs. The analysis of semi-quantitative RT-PCR showed that the expression of *SAD* gene had the highest level in stem and lowest level in leaves. The tertiary structure prediction indicated that *FaSAD* protein should be a compact globular protein. Based on evolution analysis, it was clear that the genes from the same family were approximately clustered into a group, but all genes from woody plants were not clustered into a separate group. In woody plants, it was indicated that all sequences clustered into two major groups and the *FaSAD* from white ash was closely related to the SAD gene from *Macfadyena unguis-cati*.

Key words: White ash, low temperature; stearoyl-ACP desaturases (SAD), evolution analysis.

INTRODUCTION

The level of cold-hardiness that plant species can attain is one factor limiting their distribution. The chilling-tolerant woody plants show a very fast accumulation in unsaturated acids thereby conferring a more rapid adaptation to cold. This could lead to the conclusion that the chilling-tolerant plants have a better responding mechanism to reduce cold stress membrane damages. From an evolutionary point of view, it is understandable that those environmental conditions, which normally precede cold stress, are the main factors affecting freezing tolerance (Junttila et al., 2002). The best-characterized changes include increases in soluble sugars, proteins, amino acid and organic acids, as well as modification of membrane lipid composition and alterations in gene expression (Guy, 1990; Hiilovaara-Teijo and Palva, 1999).

Stearoyl-ACP desaturases (SAD) are found in all plant cells and are essential for the biosynthesis of unsaturated membrane lipids. SAD catalyzes the desaturation of stearoyl-ACP to oleoyl-ACP and plays a key role in determining the ratio of saturated fatty acids to unsaturated fatty acids in plants. This ratio is closely associated with many functions in plants, particularly of plants acclimation to low temperatures (Kodama et al., 1995; Lindqvist et al., 1996). Many SAD genes have been cloned from different plants, and the structures and functions of several SADs have been studied. Kodama et al. (1995) found that a SAD gene mutant of *A. Thaliana*

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Abbreviations: SAD, Stearoyl-ACP desaturases; **ORF,** open reading frame; **RT-PCR,** reverse transcription polymerase chain reaction.

Table 1. The sequences and use of the primers.

Primer Name	Sequences (5′→3′)	Primer use for
SADF1	GTGTTGGCAACCACAGGA(C/T)TT	middle fragments
SADF2	GAGACATGATCACGGAAGAAGC	middle fragments
SADR1	TCTATCG(A/T)AAATCCA(A/G)CTGAA	middle fragments
SADR2	C(A/G)TACATCAAGTG(A/T)GCAGGCAT	middle fragments
FaSADF1	CTAAACAAATATCTCTATCTATCGGG	3′RACE of FaSAD
FaSADF2	CTCGGACAGAAAACAATCCTTAC	3′RACE of FaSAD
FaSADR1	ATTGTACCACAGATTTGAGCCAG	5′RACE of FaSAD
FaSADR2	TCCAAGGTAAGGATTGTTTTCTG	5′RACE of FaSAD
FaSADR3	AGATATTTGTTTAGAAGGTCTCCAT	5′RACE of FaSAD
FaSADR4	CACCAACTAGAACGACAAAATAAT	5′RACE of FaSAD
ESADF	ACTCTTCGCTCTGGCTCC	expression of FaSAD
ESADF	CAGTCCTCCGTCGCTTTA	expression of FaSAD
actinF	TCCTCTTCCAGCCTTCTTT	control gene
actinR	TTCCTTGCTCATACGGTCA	control gene

with elevated stearic acid levels did not grow as well as the control group at low temperatures. De Palma et al. (2008) suggested that the expression of the SAD gene in plants will increase the cold tolerance in plants due to the increased desaturation of the fatty acids and thus a better membrane control of damage at the membrane level. In woody plants, Luo et al. (2006, 2009) characterized two novel SAD genes from *Cinnamomum longipaniculatum* and *Jatropha curcas*, respectively. The expressions of two SAD genes were analyzed by Southern and Northern blotting. These studies indicated that it is possible to modify the composition of plant fatty acids by manipulating the SAD gene. This modification could probably increase the capability of acclimation to low temperatures in some plants (Davydov et al., 2005; Kodama et al., 1995; Lindqvist et al., 1996).

White ash (*Fraxinus americana*), a member of the *Oleaceae*, is a widely distributed native ash in North America. The wood is economically important for tool handles, sporting equipment, veneer, paneling, and furniture. It is also well suited for landscaping large yards and open areas such as parks and along roadsides (Bates et al., 1992). The low temperature exotherm of white ash was found to be -42°, and visual browning of the living xylem tissue was observed at -46° (George et al., 1974). Results from previous research in Ireland, based upon a single year's data, indicated that the ash was more tolerant of cold storage than sycamore (O'Reilly et al., 2002). These studies indicated that white ash has higher level of cold-hardiness. It allows white ash to extend its range to corresponding isotherm in all world. It was the first time that white ash was introduced from America in the 1950s. After domestication of a number of years, white ash has successfully been inseminated in some areas in Northeast of China (Dong, 2004; Ye et al., 2010).

Although, it is recognized for the cold-hardiness of white ash, but its molecular mechanism is still not clear yet. To date, there is no literature report on the characterization of the SAD from white ash. In this study, we cloned a SAD gene from white ash and analyzed the expression of this gene by RT-PCR, which described the evolutionary relationships of the SAD genes from woody plants. These could lay the foundation for the transgenic modification of white ash fatty acid composition and enhance of plant stress resistance.

MATERIALS AND METHODS

Plant materials

Seeds, stems, leaves and roots were collected from tissue culture seedlings of white ash (Mississippi provenance), which were immediately frozen in liquid N_2 and stored at −70°C until needed.

RNA extraction and first-strand cDNA synthesis

Seeds, stems, leaves and roots were ground in liquid N_2 with a mortar and pestle. Total RNA was extracted from samples with the plant tissue RNA extraction kit (Promega) following the manufacturer's instructions. The RNA quality was assessed by running 2 µg of the total RNA on a 1.2% agarose gel. The total RNA was stored at −80°C. cDNA synthesis reactions were performed with M-MLV reverse transcriptase (Promega) according to the manufacturer's instructions.

Isolation of the middle fragments of the SAD genes

The homologous primers for middle segments were designed based on conserved sequences of SAD genes from some plants in NCBI GenBank (SADF1, F2, R1 and R2, Table 1). PCR reactions were performed in a total reaction volume of 25 µl containing 50 ng of genomic DNA, 1× *Taq* DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.5 µM each primer, 200 µM each dNTP and 1U *Taq* DNA polymerase (Promega). The program for PCR amplification was as follow: Initial denaturation at 94℃ for 4 min, 35 cycles of 94℃ for 30 s, 56℃ for 1 min, 72℃ for 1 min, and a final extension at 72℃ for 10 min. The PCR products were separated on 1.2% agarose gels, and then the targeted DNA fragments were recovered and cloned into the pGEMT-Easy vector (Promega). The ligated products were transformed into *Escherichia coli* (DH5α) cells and the resulting plasmids were used as a sequencing template.

Amplification of the complete coding sequences of the SAD genes

Primers for 5'- and 3'-end cDNA amplification were designed based on the middle fragment sequences (Table 1). The 3′ and 5′ sequence of cDNA were obtained by RACE with the 3′- and 5′-RACE System for Rapid Amplification of cDNA Ends (Invitrogen) . The PCR products were cloned to pMD18-T vector and sequenced. Based on the nucleotide sequences of the 5′- and 3′-RACE products, primers FaSAD1F, TaSAD1R, TaSAD2F and TaSAD2R (Table 1) were used for the amplification of the complete coding sequences of SAD genes.

Expression analysis of *FaSAD*

Semi-quantitative RT-PCR was performed using gene-specific primers for ESADF and ESADR. As an internal control, a fragment from white ash actin gene was amplified using the actinF and actinR primers (Table 1). The program for PCR amplification was as follow: Initial denaturation at 94℃ for 4 min, 35 cycles of 94℃ for 30 s, 56℃ for 1 min, 72℃ for 1 min, and a final extension at 72℃ for 10 min. Amplified fragments were detected by electrophoresis using 1.5% (w/v) agarose gels.

Bioinformatic analysis and phylogenetic analysis

Primer Premier 5 software (HHUUhttp://www.Premierbiosoft.comUUHH) was used for all the primer designs. Sequences were aligned using softwares DNAman 5.2.2 (HHUUhttp://www.lynnon.comUUHH) and CLUSTAL W 1.81(Thompson et al., 1994). The secondary and tertiary structures of the deduced protein were predicted by PredictProtein (http://www.predictprotein.org) and SWISS-MODEL (http://swissmodel.expasy.org), respectively. The phylogenetic tree was generated based on the NJ (neighbour-joining) sequences distance method (Saitou and Nei, 1987) and depicted and edited by MEGA 3.1 program (Kumar et al., 2004).

RESULTS

Sequence analysis of SAD genes

Initially, a fragment about 600 bp was amplified by RT-PCR. By analysis and comparison of the sequence, the fragment had similar structures with those known SAD genes. This was followed by 3'-and 5'-RACE analysis, and two fragments, 886 and 526 bp in sizes, were obtained. Finally, the full-length cDNA of a SAD gene was obtained by sequence assembly and re-amplification. Sequence analysis revealed that the cDNA fragment was 1,643 bp in length, including an open reading frame (ORF) of 1,188 bp along with 130 bp 5'-and 325 bp 3'-untranslated sequences. This cDNA fragment that could encode 395 amino acids, designated as *FaSAD*, had been deposited in GenBank under the accession number HQ443517 (Figure 1).

Comparison of amino acid sequences

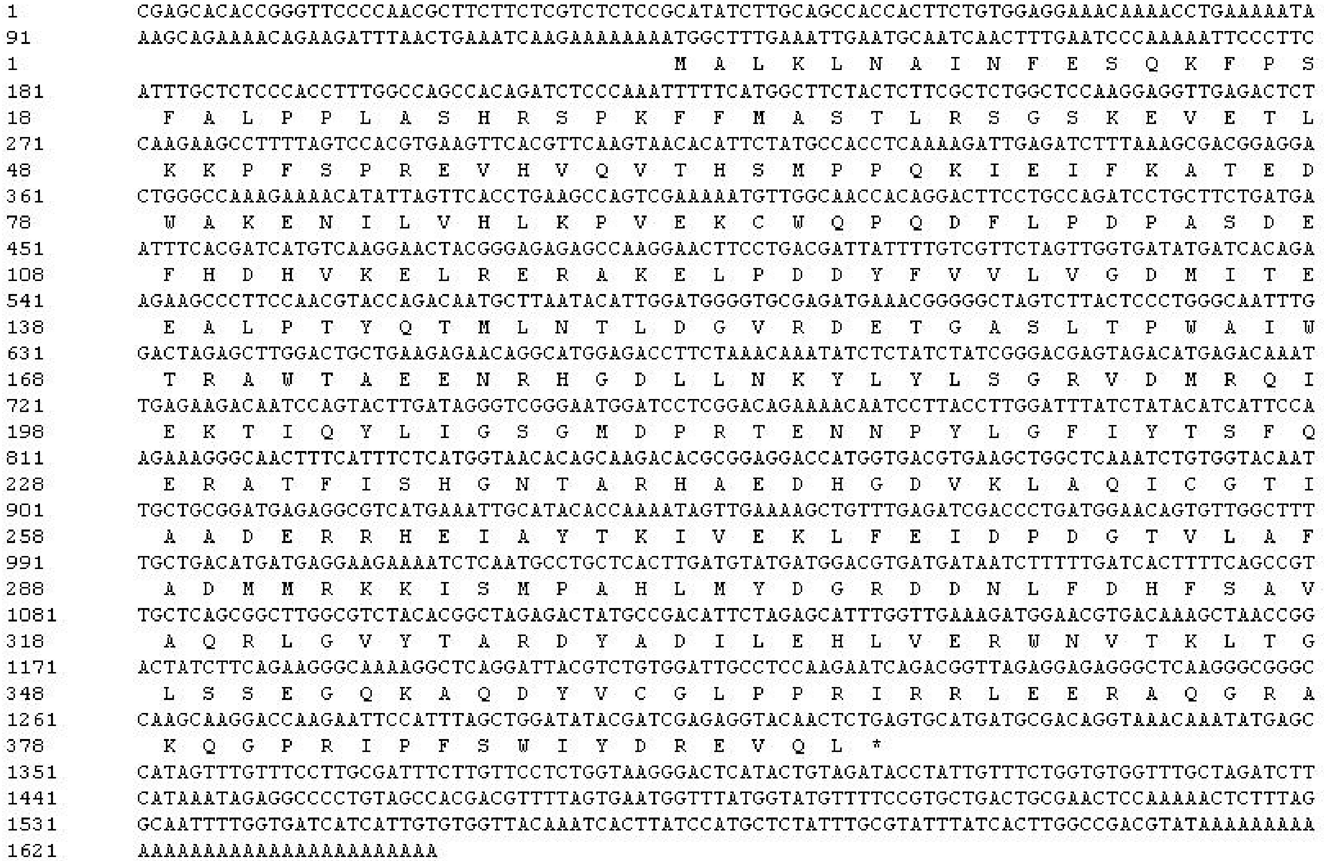
The comparison of amino acid sequences showed that *FaSAD* had the similar typical primary structures to those known SAD genes from woody plants and other plants such as *Arabidops*, *Glycine*, *Triadica*, *Brassica*, *Sesamum* and *Bassia* etc. There are high level of homology in amino acid sequences of all SAD proteins from woody plants and others, and amino acid lengths of all SAD proteins range from 387 to 401 (Table 2). The coding regions begin from the amino acids MALK in all genes from woody plants. The conserved regions of all genes begin from about fortieth amino acid residues (KKPF). All SAD amino acid sequences have two conserved domains belonging to the acyl-ACP desaturase family and the ferritin-like family, respectively. But they were also different from each other by some substitutions, insertions and/or deletions involving single amino acid residues or motifs. The main regions of variability were regions about front 40 amino acid residues of N-terminal domains (Figure 2).

Semi-quantitative RT-PCR analysis

Semi-quantitative RT-PCR was employed to confirm the expression patterns of the SAD gene in four different tissues. It was showed that the expression levels of SAD differed among tissues. It was expressed at significantly higher levels in stem and roots than in leaves and seeds. The expression of SAD gene has the highest level in stem, and lowest level in leaves (Figure 3).

Evolutionary relationship analysis

Because there are high level of homology in amino acid sequences of all SAD proteins from woody plants and others, we could align all the known SAD genes from different families so as to research the phylogenetic relationships among SAD genes. The dendrogram was constructed based on the alignment of 32 amino acid sequences from different families of woody plants such as *Oleaceae*, *Bignoniaceae*, *Lauraceae*, *Euphorbiaceae*, *Lauraceae*, *Simmondsiaceae*, *Vitaceae* and *Saliceae* and other families such as *Poaceae*, *Cucurbitaceae*, *Pedaliaceae*, *Compositae*, *Linaceae*, *Leguminosae*, *Brassicaceae*, *Chenopodiaceae*, *Amaranthaceae* and *Acanthaceae* (Table 2). It was clear that the genes from the same family approximately were clustered into a



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1         CGAGCACACCGGGTTCCCCAACGCTTCTTCTCGTCTCTCCGCATATCTTGCAGCCACCACTTCTGTGGAGGAAACAAAACCTGAAAAATA
91        AAGCAGAAAACAGAAGATTTAACTGAAATCAAGAAAAAAAAATGGCTTTGAAATTGAATGCAATCAACTTTGAATCCCAAAAATTCCCTTC
1                                                   M  A  L  K  L  N  A  I  N  F  E  S  Q  K  F  P  S
181       ATTTGCTCTCCCACCTTTGGCCAGCCACAGATCTCCCAAATTTTTCATGGCTTCTACTCTTCGCTCTGGCTCCAAGGAGGTTGAGACTCT
18          F  A  L  P  P  L  A  S  H  R  S  P  K  F  F  M  A  S  T  L  R  S  G  S  K  E  V  E  T  L
271       CAAGAAGCCTTTTAGTCCACGTGAAGTTCACGTTCAAGTAACACATTCTATGCCACCTCAAAAGATTGAGATCTTTAAAGCGACGGAGGA
48          K  K  P  F  S  P  R  E  V  H  V  Q  V  T  H  S  M  P  P  Q  K  I  E  I  F  K  A  T  E  D
361       CTGGGCCAAAGAAAACATATTAGTTCACCTGAAGCCAGTCGAAAAATGTTGGCAACCACAGGACTTCCTGCCAGATCCTGCTTCTGATGA
78          W  A  K  E  N  I  L  V  H  L  K  P  V  E  K  C  W  Q  P  Q  D  F  L  P  D  P  A  S  D  E
451       ATTTCACGATCATGTCAAGGAACTACGGGAGAGAGCCAAGGAACTTCCTGACGATTATTTTGTCGTTCTAGTTGGTGATATGATCACAGA
108         F  H  D  H  V  K  E  L  R  E  R  A  K  E  L  P  D  D  Y  F  V  V  L  V  G  D  M  I  T  E
541       AGAAGCCCTTCCAACGTACCAGACAATGCTTAATACATTGGATGGGGTGCGAGATGAAACGGGGGCTAGTCTTACTCCCTGGGCAATTTG
138         E  A  L  P  T  Y  Q  T  M  L  N  T  L  D  G  V  R  D  E  T  G  A  S  L  T  P  W  A  I  W
631       GACTAGAGCTTGGACTGCTGAAGAGAACAGGCATGGAGACCTTCTAAACAAATATCTCTATCTATCGGGACGAGTAGACATGAGACAAAT
168         T  R  A  W  T  A  E  E  N  R  H  G  D  L  L  N  K  Y  L  Y  L  S  G  R  V  D  M  R  Q  I
721       TGAGAAGACAATCCAGTACTTGATAGGGTCGGGAATGGATCCTCGGACAGAAAACAATCCTTACCTTGGATTTATCTATACATCATTCCA
198         E  K  T  I  Q  Y  L  I  G  S  G  M  D  P  R  T  E  N  N  P  Y  L  G  F  I  Y  T  S  F  Q
811       AGAAAGGGCAACTTTCATTTCTCATGGTAACACAGCAAGACACGCGGAGGACCATGGTGACGTGAAGCTGGCTCAAATCTGTGGTACAAT
228         E  R  A  T  F  I  S  H  G  N  T  A  R  H  A  E  D  H  G  D  V  K  L  A  Q  I  C  G  T  I
901       TGCTGCGGATGAGAGGCGTCATGAAATTGCATACACCAAAATAGTTGAAAAGCTGTTTGAGATCGACCCTGATGGAACAGTGTTGGCTTT
258         A  A  D  E  R  R  H  E  I  A  Y  T  K  I  V  E  K  L  F  E  I  D  P  D  G  T  V  L  A  F
991       TGCTGACATGATGAGGAAGAAAATCTCAATGCCTGCTCACTTGATGTATGATGGACGTGATGATAATCTTTTTGATCACTTTTCAGCCGT
288         A  D  M  M  R  K  K  I  S  M  P  A  H  L  M  Y  D  G  R  D  D  N  L  F  D  H  F  S  A  V
1081      TGCTCAGCGGCTTGGCGTCTACACGGCTAGAGACTATGCCGACATTCTAGAGCATTTGGTTGAAAGATGGAACGTGACAAAGCTAACCGG
318         A  Q  R  L  G  V  Y  T  A  R  D  Y  A  D  I  L  E  H  L  V  E  R  W  N  V  T  K  L  T  G
1171      ACTATCTTCAGAAGGGCAAAAGGCTCAGGATTACGTCTGTGGATTGCCTCCAAGAATCAGACGGTTAGAGGAGAGGGCTCAAGGGCGGGC
348         L  S  S  E  G  Q  K  A  Q  D  Y  V  C  G  L  P  P  R  I  R  R  L  E  E  R  A  Q  G  R  A
1261      CAAGCAAGGACCAAGAATTCCATTTAGCTGGATATACGATCGAGAGGTACAACTCTGAGTGCATGATGCGACAGGTAAACAAATATGAGC
378         K  Q  G  P  R  I  P  F  S  W  I  Y  D  R  E  V  Q  L  *
1351      CATAGTTTGTTTCCTTGCGATTTCTTGTTCCTCTGGTAAGGGACTCATACTGTAGATACCTATTGTTTCTGGTGTGGTTTGCTAGATCTT
1441      CATAAATAGAGGCCCCTGTAGCCACGACGTTTTAGTGAATGGTTTATGGTATGTTTTCCGTGCTGACTGCGAACTCCAAAAACTCTTTAG
1531      GCAATTTTGGTGATCATCATTGTGTGGTTACAAATCACTTATCCATGCTCTATTTGCGTATTTATCACTTGGCCGACGTATAAAAAAAAA
1621      AAAAAAAAAAAAAAAAAAAAAAA
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Figure 1. Nucleotide and deduced amino-acid sequences of *FaSAD*.

group, but all genes from woody plants could not be clustered into a separate group. In twelve genes from woody plants, eight genes from *Vernicia Montana*, *Vernicia fordii*, *Triadica sebifera*, *Cinnamomum longipaniculatum*, *Jatropha curcas*, *Persea Americana*, *Macfadyena unguis-cati* and *Fraxinus americana* could be clustered together with two genes from other species (*Cucumis sativus* and *Sesamum indicum*) by an interior paralleled branch (Real line box in Figure 4). Three genes from *Populus trichocarpa*, *Simmondsia chinensis* and *Olea europaea* were clustered into a separate group with two genes from other species (*Thunbergia alata* and *Bassia scoparia*). Although, these genes all gained from the woody plants, but this group was not closely related to the above-mentioned group of the eight genes. A gene from *Vitis vinifera* was closer with the group of the eight genes from woody plants (Broken line box in Figure 4). In addition, all genes from *Poaceae* were clustered into a separate group (Dot box in Figure 4).

To better understand the evolutionary relationships among SAD genes in woody plants, these genes were aligned and constructed the dendrogram. It was indicated that all sequences were clustered into two major groups and the *FaSAD* from white ash was closely related to the SAD gene from *Macfadyena unguis-cati*. The genes from *Populus trichocarpa*, *Simmondsia chinensis* and *Olea europaea* were clustered into a group and separated with the other one group included nine genes (Figure 5). This result corresponded with the above-mentioned dendrogram constructed by alignment from SAD genes from all species.

The secondary and tertiary structures prediction

Secondary structure prediction by PredictProtein showed that *Fa*SAD protein contained 55.1% α-helix, 9.7% extended strand and 35.2% random coil. The tertiary structure prediction indicated that *Fa*SAD protein was a compact globular protein (Figure 6). The result from 3D model pictures corresponded with the results of secondary structure prediction. The predictions were carried out based on the sequences from 53 to 392 amino acid residues.

DISCUSSION

The low temperature injury is often associated with changes in the membrane lipid bilayer of one or more

Table 2. Information of the sequences used in the evolutionary relationship analysis.

Genbank number	Family	Originism	Numbers of amino acid	Reference
HQ443517	Oleaceae	*Fraxinus americana*	395	This study
AF051134	Bignoniaceae	*Macfadyena unguis-cati*	396	Cahoon et al.,1998
AF116861	Lauraceae	*Persea americana*	396	Madi and Prusky, 1999
DQ084491	Euphorbiaceae	*Jatropha curcas*	396	Luo et al., 2006
EF079655	Euphorbiaceae	*Triadica sebifera*	396	Niu et al., 2008 submitted
EU072353	Euphorbiaceae	*Vernicia montana*	396	Li et al., 2007 submitted
EU131523	Lauraceae	*Cinnamomum longipaniculatum*	396	Luo et al., 2009
GU363502	Euphorbiaceae	*Vernicia fordii*	392	Tan et al., 2009 submitted
M83199	Simmondsiaceae	*Simmondsia chinensis*	398	Sato et al.,1992
U58141	Oleaceae	*Olea europaea*	390	Baldoni et al.,1996
XM_002265150	Vitaceae	*Vitis vinifera*	393	Jaillon et al., 2007
XM_002316135	Saliceae	*Populus trichocarpa*	387	Tuskan et al., 2006
XM_002446233	Poaceae	*Sorghum bicolor*	396	Paterson et al., 2009
HQ589252	Poaceae	*Triticum aestivum*	392	Guo et al., 2010 submitted
AK250596	Poaceae	*Hordeum vulgare*	395	Sato et al., 2009
AK058979	Poaceae	*Oryza sativa*	400	Kikuchi et al., 2003
BT039581	Poaceae	*Zea mays*	396	Yu et al., 2008 submitted
M59858	Cucurbitaceae	*Cucumis sativus*	396	Shanklin et al.,1991
D42086	Pedaliaceae	*Sesamum indicum*	396	Yukawa et al.,1996
AJ242631	Compositae	*Helianthus annuus*	396	Martinez-Force et al., 2005 submitted
DQ516384	Compositae	*Saussurea involucrata*	396	Zhu et al., 2006 Submitted
M61109	Compositae	*Carthamus tinctorius*	396	Thompson et al.,1991
AJ006957	Linaceae	*Linum usitatissimum*	396	Jain et al., 2006 submitted
DQ007889	Leguminosae	*Medicago truncatula*	393	Hu et al., 2005 submitted
FJ393221	Leguminosae	*Glycine max*	391	Li et al., 2008 submitted
FJ230310	Leguminosae	*Arachis hypogaea*	396	Yu et al., 2008 submitted
X97325	Brassicaceae	*Brassica.napus*	398	Piffanelli and Murphy, 2005 submitted
EF524186	Brassicaceae	*Descurainia sophia*	400	Ye et al., 2008 submitted
NM_129933	Brassicaceae	*Arabidopsis.thaliana*	401	Town et al., 2002 submitted
FJ418167	Chenopodiaceae	*Spinacia oleracea.*	399	Ma et al., 1996
AF315600	Amaranthaceae	*Bassia scoparia*	399	Whitney et al., 2004
U07597	Acanthaceae	*Thunbergia alata*	390	Cahoon et al.,1994

cellular membrane systems. Several mechanisms have been put forward as possible adaptive changes in membrane lipids in response to low temperature (Thompson, 1992; Uemura and Steponkus, 1997). Tasseva et al. (2004) suggested that the expression of the SAD genes in plants will increase the cold tolerance in plants due to the increased desaturation of the fatty acids and thus a better membrane control of damage at the membrane level. A comparative study on several deciduous and evergreen tree species revealed that the relative levels of saturated and monounsaturated phosphatidyl glycerol were positively correlated with the sensitivity of the leaves to chilling (Tasaka et al., 1990). Homology analysis and multiple sequence alignment analysis showed the SAD genes from different plants had high level of identity in amino acid sequences. In this study, two novel SAD genes were cloned and analyzed from white ash by RT-PCR and RACE analysis. By sequence analysis, the deduced peptide sequence of *FaSAD* was highly homologous to those SAD genes from other plants. Luo et al. (2009) indicated that the SAD polypeptide from *C. longepaniculatum* has two conserved domains, one belongs to acyl-ACP desaturase family with considerable homology in a number of highly conserved blocks and another belongs to ferritin-like family. These conserved domains were also found in the *FaSAD* polypeptide. This suggests that *FaSAD* protein belong to the acyl-ACP desaturase family and to the ferritin-like family. The six ligands of the diiron center along with several amino acid residues closely related to the homodimer interface and the substrate-binding pocket of acyl-ACP-desaturase are located in the conserved domains (Figure 2; Fox et al., 1994; Lindqvist et al., 1996). Moreover, the high amino acid sequence identity

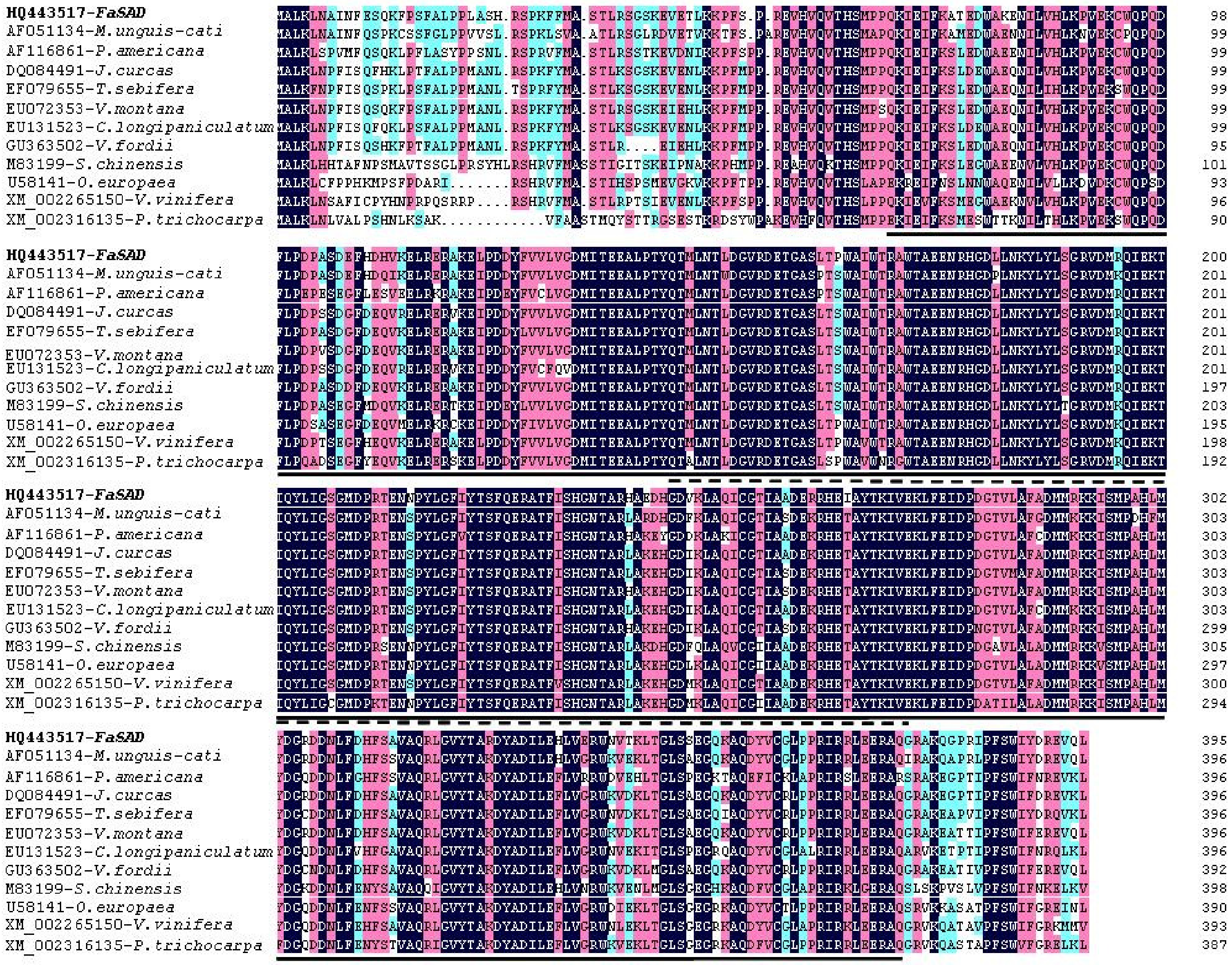

Figure 2. Comparison of the deduced amino acid sequences of *FaSAD* with other known SAD genes from other species.The domain of acyl-ACP desaturase and the domain of ferritin-like family family are underlined by real line and broken line, respectively.

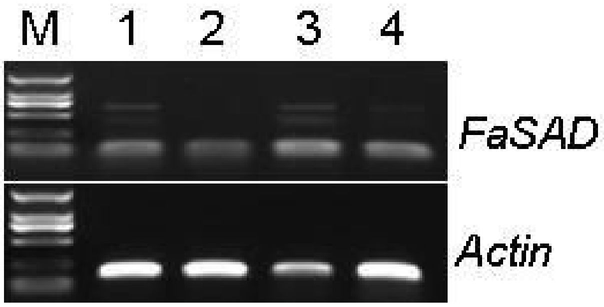

Figure 3. Expression analysis of FaSAD M: Marker; lane 1: seed; lane 2: leaves; lane 3: stem; lane 4: roots.

of all SAD genes suggested that SAD proteins have been highly conserved during evolution and further demonstrating their critical enzymatic roles in fatty acid synthesis in plants.

Bioinformatics plays more and more important roles in gene and protein studies, such as gene identification, prediction of protein function and functional sites, estimation of protein physicochemical properties and protein structure forecast, among others (Bartlett et al., 2003). Lindqvist et al. (1996) analyzed the crystal structure of SAD from castor and found that except for a hairpin loop at the C-terminus, the secondary structure was primarily a helix domain composed of 11 smaller α-helices. In this study, the secondary and tertiary structure of *Fa*SAD protein was predicted by bioinformatics methods. The predicted structures of *Fa*SAD are similar to Lindqvist's rensults. The predictions were carried out

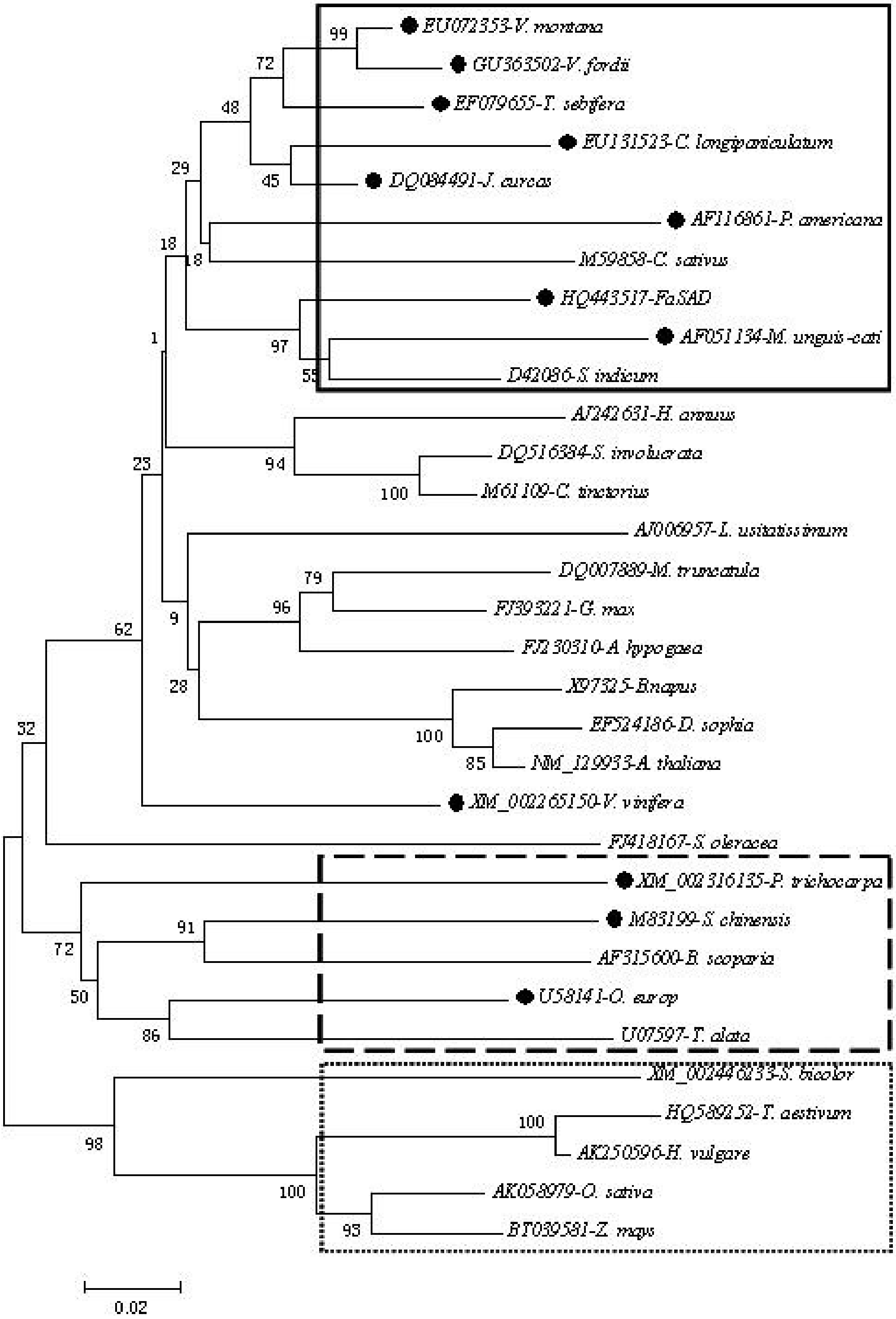

Figure 4. The evolutionary relationships based on the alignments of the amino acid sequences from all species.

based on the sequences from 53 to 392 amino acid residues. It is possible that this is caused by the nonconservation of the region from start codon to about fortieth amino acid residues.

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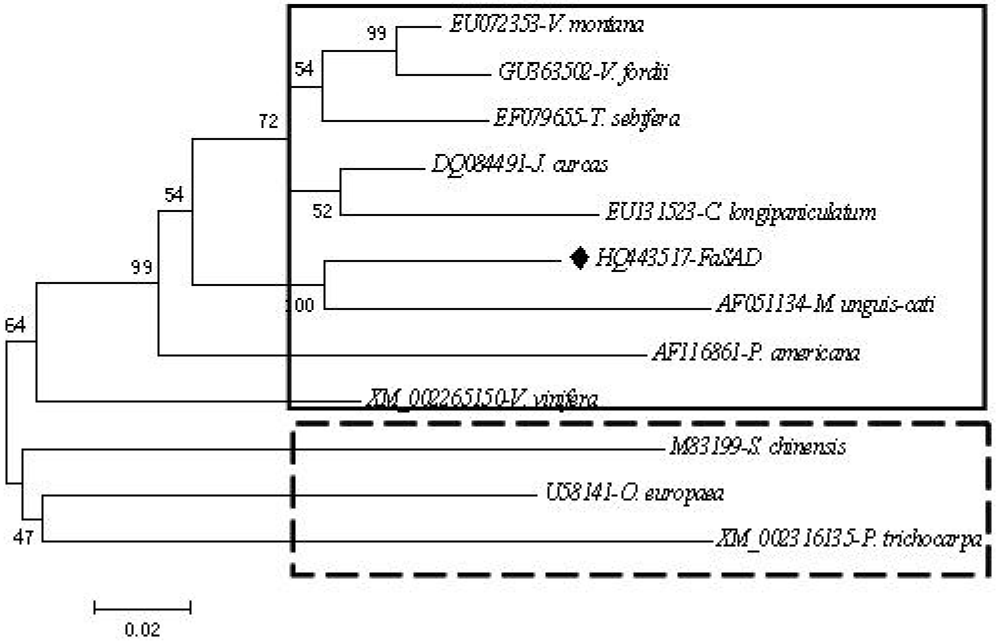

Figure 5. The evolutionary relationships based on the alignments of the amino acid sequences from woody.

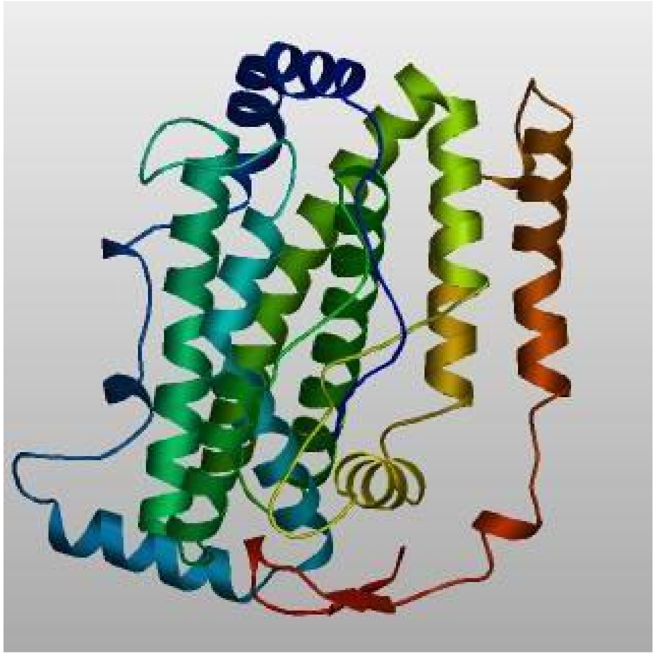

Figure 6. Tertiary structure prediction of *FaSAD* protein.

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