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Research Article

Molecular Cloning and Expression of a Squalene Epoxidase Gene from *Ilex cornuta*

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Abstract

Background and Objective: *Ilex cornuta* (*I. cornuta*) is a medicinal plant that contains triterpenoid compounds as its pharmacologically active ingredients. Squalene epoxidase (SE) is a key enzyme in the triterpenoid biosynthesis pathway. The current study aimed to characterize a SE gene from *I. cornuta*. **Methodology:** *IcSE2* was isolated from *I. cornuta*. It was predicted the secondary and tertiary structures of the IcSE2 protein, performed multiple sequence alignments and generated a phylogenetic tree. The expression level of *IcSE2* was examined via Quantitative Real Time-PCR (qRT-PCR). **Results:** The cDNA sequence of *IcSE2* was 2058 bp, with an open reading frame of approximately 1605 bp that codes for 534 amino acids. The predicted theoretical isoelectric point and molecular weight are 8.30 and 58.6 kDa, respectively. RxR, Flavin Adenine Dinucleotide (FAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) domains could be found in the deduced IcSE2 protein. Phylogenetic analysis showed that IcSE2 is closely related with the SEs from Araliaceae plants. *IcSE2* expression level was the highest in roots, followed by leaves and male flowers and lowest in stems. **Conclusion:** The *IcSE2* gene from *I. cornuta* was cloned and characterized for the first time. *IcSE2* was strongly expressed in roots and leaves. These results may lay the foundation for studying the molecular regulatory mechanism of triterpenoid saponins in *I. cornuta*.

Key words: *Ilex cornuta*, triterpenoids, squalene epoxidase, molecular cloning, expression analysis, quantitative real time-PCR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ilex cornuta belongs to the family Aquifoliaceae and is a medicinal thorny shrub that is widely distributed in China. Aqueous extracts from *I. cornuta* are used in traditional Chinese medicine for contraception, liver protection, heat clearing[1], dizziness and hypertension reduction and as an antibacterial agent[2,3]. This excellent pharmacological performance is due to the secondary metabolites in *I. cornuta*. At present, 12 compounds have been found in *I. cornuta*, including triterpenoids, flavonoids and triterpenoid saponins[4-7], which are known for their pharmacological benefits. However, data on the genes related to the biosynthesis of these molecules in *I. cornuta* are few.

Plant triterpenoids and triterpenoid saponins are synthesized from the same precursor-squalene. As shown in Fig. 1, squalene is synthesized via two independent pathways: In the cytoplasm of the mevalonate pathway and in the plastids of the 2-C-methyl-D-erythritol-4-phosphate pathway[8]. Squalene is then catalyzed by squalene epoxidase (SE) to 2,3-oxidosqualene, which is the rate-limiting step of inserting an oxygen atom into a linear squalene molecule. Finally, 2,3-oxidosqualene is catalyzed by different enzymes into triterpenoids and triterpenoid saponin backone[9,10].

At present, many *SE* genes have been isolated from various plant species, such as *Panax notoginseng*[11], *Gynostemma pentaphyllum*[12] and *Ginkgo biloba*[13]. In *P. notoginseng*, RNA interference of the *SE* gene completely suppressed *PgSE1* transcription and reduced ginsenoside production[11]. In *G. pentaphyllum*, *GpSE* was expressed as inclusion bodies in *Escherichia coli* cells and the HIS-tagged recombinant protein is successfully renatured and purified *in vitro*[12]. The cDNA sequence of *GbSE* was cloned from *G. biloba* and *GbSE* expression level was analyzed in different tissues[13]. The over expression of *SE* gene alone and in combination with the 3-Hydroxy-3-methylglutaryl coenzyme A gene increases ganoderic acid production in *Ganoderma lingzhi*[14] and the ectopic expression of *Pseudostellaria heterophylla SE* can promote the content of total triterpenes in transgenic plants[15]; furthermore, a study on *Eleutherococcus senticosus SE* gene indicated that the expression of *SE* gene is regulated by many factors, such as plant hormone, light and temperature[16]. However, little information is known about the *SE* in *I. cornuta*.

In the current study, it was cloned and analyzed an *SE* gene by using bioinformatics tools to investigate the key genes that regulate triterpenoid saponin synthesis in *I. cornuta*. Tissue-specific expression was investigated via qRT-PCR methods. This study can lay the foundation for investigating the molecular regulatory mechanism of triterpenoid saponins in *I. cornuta*.

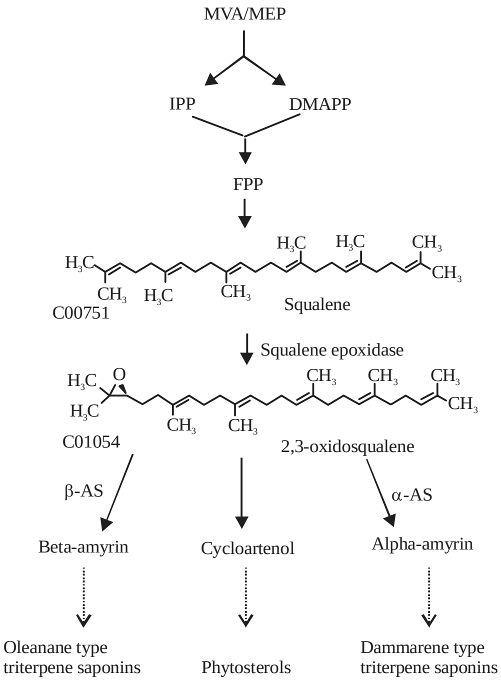

Fig. 1: Biosynthesis of triterpenoids in *I. cornuta*

MVA: Mevalonate pathway, MEP: 2-C-methyl-D-erythritol-4-phosphate pathway, IPP: Isopentenyl diphosphate, DMAPP: Dimethylallyl diphosphate, FPP: Farnesyl pyrophosphate, β-AS: β-amyrin synthase, α-AS: α-amyrin synthase

MATERIALS AND METHODS

Plant materials: All materials were collected from 20-years-old *I. cornuta* plants in the College of Horticulture and Gardening, Yangtze University in May, 2017. All plant materials were harvested and frozen directly into liquid nitrogen and stored at -80°C for RNA extraction.

RNA extraction and cDNA synthesis: Total RNA was isolated from frozen plant tissues using a Plant RNA Extraction Kit (TaKaRa, MiniBEST, Dalian, China) in June, 2017. The quality and concentration of the extracted RNA were verified based on absorbance at $OD_{260/280}$ and 1% agarose gel electrophoresis. Reverse transcription reaction was processed using a PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China).

cDNA cloning of *IcSE2*: According to the transcriptome data of *I. cornuta*, a pair of specific primers, namely, 5-TCACACAAAAATCATTCTAGAGA-3 for IcSE2S and

5-GTTTAAAGTACAAAGTTCAAAGGGA-3 for IcSE2A, was designed and synthesized. PCR was performed according to the following protocol: Initial denaturation at 94°C for 3 min; followed by 30 cycles of 30 sec at 94°C, 65°C for 30 sec and 72°C for 1 min and final extension at 72°C for 10 min. The PCR product was analyzed by 1% agarose gel electrophoresis and then purified using TaKaRaMiniBest Agarose Gel DNA Extraction Kit (TaKaRa, Dalian, China). The purified fragment was cloned into pMD19-T vector and then transferred into the *E. coli* DH5α strain. The inserted fragment was sequenced by Shanghai Sangon Biotechnology Company (Shanghai, China).

Bioinformatics analysis of *IcSE2*: The *IcSE2* nucleotide sequences were spliced using DNAMAN 8.0 and multiple sequence alignment analysis of IcSE2 protein was performed using the BLAST tool (National Center for Biotechnology Information, NCBI) and Program Align X (Vector NTI Suite V 11.5). The molecular weight and theoretical isoelectric point of the deduced IcSE2 protein were estimated by using ProtParam tool (ExPASy). The secondary and tertiary structures were deduced using SOPMA secondary structure prediction and SWISS-MODEL (https://www.swissmodel.expasy.org/), respectively. The phylogenetic tree was constructed by using Clustal X 2.0 and MEGA 6.0 using the neighbor-joining method.

Real-time quantitative PCR (qRT-PCR) analysis of *IcSE2*: The relative expression profiles of *IcSE2* were investigated from five different tissues, namely, root, stem, leaf, male flower and female flower. Total RNA was extracted from each tissue as described above and first-strand cDNA was synthesized using PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China). qRT-PCR was performed using the qRT-PCR system (LineGene 9600 plus) with SYBR Premix Ex Taq™ II Kit (TaKaRa, Dalian, China). Gene-specific primers *IcSE2*-RTS 5-TTACCTAAGTCTTGGAGGGATTTG-3 and *IcSE2*-RTA 5-GCGATGGAAATGGAATCAACAA-3 were designed for qRT-PCR amplification. *IcGAPDH* was selected as an internal control to normalize the relative quantification and the primer sequences used were *IcGAPDH*-S: 5-TATCAACGGCTTCGGTCGCA-3 and *IcGAPDH*-A: 5-GGACGGAGTCGTACTTGAGCAT-3. The qRT-PCR amplification parameters were as follows: 95°C for 5 min; 40 cycles of 95°C for 10 sec and 60°C for 30 sec; 95°C for 15 sec; 60°C for 1 min; and 95°C for 15 sec. The relative expression levels of *IcSE2* gene was calculated using the $2^{-\Delta\Delta Ct}$ method[17] and three replicates were performed per sample.

RESULTS

Cloning and sequence analysis of *SE2* gene from *I. cornuta*: The cDNA sequence of the *SE* gene was amplified from *I. cornuta* by PCR, yielding a product length of 2058 bp. The open reading frame of *IcSE2* was 1605 bp and encodes 534 amino acids (Fig. 2).

Characterization of the IcSE2 protein: Online analysis using ProtParam tool showed that the predicted theoretical isoelectric point and molecular weight of the deduced IcSE2 protein were 8.30 and 58.6 kDa, respectively. The secondary structure of IcSE2 protein contained 34.08% alpha helixes, 22.28% extended strands, 11.99% beta turns and 31.65% random coils. The predicted IcSE2 amino acid sequence was compared to SEs from *Aralia elata* (ADC32655.1), *Morus alba* (AOV62783.1), *Hedera helix* (APV45531.1), *Jatropha curcas* (NP_001295656.1), *Manihot esculenta* (XP_021613973.1), *Herrania umbratica* (XP_021285374.1) and *Eleutherococcus senticosus* (AEJ79818.1), which show sequence similarities of 79, 79, 78, 79, 77, 80 and 79%, respectively (Fig. 3). Moreover, conserved NAD(P)-binding domain and substrate binding domain were found in the IcSE2 protein (Fig. 3). A dinucleotide-binding fold of NAD(P)-binding site, which was known as Rossmann-fold, was involved in the catalytic function of SE together with a substrate-binding site. The FAD conservative domain was highly similar with the NAD(P)-binding site. The PDRIVGELLQPGG domain, substrate-binding site and the highly similar region were predicted FAD binding sites[18,19]. This result was consistent with the reports on the SE proteins in *Arabidopsis thaliana*[20], *Panax ginus*[21] and *Withania somnifera*[22]. In addition, the RxR domain was related to the diphosphate in SE protein after separation from the substrate.

3D structure analysis of IcSE2 protein: The 3D structure of IcSE2 protein was generated by using the homology modeling tool SWISS-MODEL (Fig. 4) and the FAD-dependent monooxygenase (2×3n.1.A) was used as a template based on the highest query coverage.

Three different perspectives of IcSE2 protein for observation are shown in Fig. 4. The NAD(P)-binding and C-terminal domains occurred in the alpha helix structure region, whereas the N-terminal domain was found in the beta turn structure region (Fig. 4a). The section of the NAD(P)-binding conserved domain shared with the FAD was located in the N-terminal region. In IcSE2, the FAD2-binding domain consisted of a consensus sequence

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1       TCACACAAAAATCATTCTAGAGAgagaacgagaaagttagggagagataccgtagcaatcATGCCGAATTCATCA
1                                                                    M  P  N  S  S
76      TCACCATCATCTTCCTATTCTTTCACAGAACTAAAGAACATGATGATAATAGATCAGTACTTTCTTGGAGGACTC
6        S  P  S  S  S  Y  S  F  T  E  L  K  N  M  M  I  I  D  Q  Y  F  L  G  G  L
151     ATTGCCTTTGTGTTGGGGTTCGTTTTGTTTTACAGTTCAAGAATGATGAAAAAGAAGAGTAGAGCTTCTTCATTG
31       I  A  F  V  L  G  F  V  L  F  Y  S  S  R  M  M  K  K  K  S  R  A  S  S  L
226     GAGGCGCGAAGGGATGGACAGGTTGAGAGCTCCGACTACGGTCATTGCCGGCCGGAGAATAACGGAAGTACGGAC
56       E  A  R  R  D  G  Q  V  E  S  S  D  Y  G  H  C  R  P  E  N  N  G  S  T  D
301     ATCATCATCGTCGGTGCTGGTGTTGCCGGTTCTGCTCTCGCTTACACTCTTGGCAAGGAAGGGCGGCGAGTACAA
81       I  I  I  V  G  A  G  V  A  G  S  A  L  A  Y  T  L  G  K  E  G  R  R  V  Q
376     GTAATTGAACGAGACTTGACTGAACCGGATAGAATTGTGGGTGAACTTTTACAACCAGGAGGCTATTTGAAGTTA
106      V  I  E  R  D  L  T  E  P  D  R  I  V  G  E  L  L  Q  P  G  G  Y  L  K  L
451     ATTGAGTTGGGTCTTGAGGATTGTGTAAGAGGGATTGATGCTCAAAAAGTGTTTGGATATGCCCTCTATAAGGAT
131      I  E  L  G  L  E  D  C  V  R  G  I  D  A  Q  K  V  F  G  Y  A  L  Y  K  D
526     GGTAAAGATACCAAATTGTCTTATCCCTTAGAGAAATTTGATGCTGATGTCTCAGGAAGGAGCTTTCACAATGGG
156      G  K  D  T  K  L  S  Y  P  L  E  K  F  D  A  D  V  S  G  R  S  F  H  N  G
601     CGTTTTATACAGCGTATGCGAGAGAAAGCTGCGACTCTTCCAAACATAAGATTGGAACAAGGAACGGTAACATCT
181      R  F  I  Q  R  M  R  E  K  A  A  T  L  P  N  I  R  L  E  Q  G  T  V  T  S
676     TTGCTTGAAGAAAAGGGAACTATCAAAGGGGTGCAGTACAAAACCAAGGATGGGGAAGAAATGACAGCATATGCT
206      L  L  E  E  K  G  T  I  K  G  V  Q  Y  K  T  K  D  G  E  E  M  T  A  Y  A
751     CCTCTCACAATTGTGTGCGATGGTTGCTTTTCAAATTTGCGACGCACTCTGTGCAAACCCCAGGTGGAAGTGCCT
231      P  L  T  I  V  C  D  G  C  F  S  N  L  R  R  T  L  C  K  P  Q  V  E  V  P
826     TCTTGTTTCGTTGGCTTGATCTTGGAAAATTGTAATCTTCCATACATAGATCATGGGCACGTTATTCTGGCAGAC
256      S  C  F  V  G  L  I  L  E  N  C  N  L  P  Y  I  D  H  G  H  V  I  L  A  D
901     CCTTCACCCATCTTGTTTTACAAAATCAGTAGTACTGAGATTCGCTGCTTGGTTGATGTACCCGGGCAAAAGGTC
281      P  S  P  I  L  F  Y  K  I  S  S  T  E  I  R  C  L  V  D  V  P  G  Q  K  V
976     CCCTCCATTTCTAATGGTGAAATGGCTCACTATCTGAAGACTGTGGTGGCTCCTCAGATCCCTCCTGAGCTATAC
306      P  S  I  S  N  G  E  M  A  H  Y  L  K  T  V  V  A  P  Q  I  P  P  E  L  Y
1051    AATGCCTTCATAGCAGCAATTGAAAAAGGAAGCATAAAAACAATGCCAAACAGAAGCATGCCTGCAGCTCCCCAT
331      N  A  F  I  A  A  I  E  K  G  S  I  K  T  M  P  N  R  S  M  P  A  A  P  H
1126    CCCACTCCTGGTGCACTTCTCTTGGGGGATGCTTTCAACATGCGACATCCTTTAACTGGAGGAGGAATGACAGTG
356      P  T  P  G  A  L  L  L  G  D  A  F  N  M  R  H  P  L  T  G  G  G  M  T  V
1201    GCTCTGTCGGATATTGTTCTCCTCCGTGATCTTCTCAGACCTCTACGTGATCTGAATGATGCACCTTCCCTATGC
381      A  L  S  D  I  V  L  L  R  D  L  L  R  P  L  R  D  L  N  D  A  P  S  L  C
1276    AAATATCTCGAATCCTTTTACACTCTTCGTAAGCCCGTGGCATCTACAATAAACACATTGGCAGGTGCCCTTTAC
406      K  Y  L  E  S  F  Y  T  L  R  K  P  V  A  S  T  I  N  T  L  A  G  A  L  Y
1351    AAGGTTTTTTGTGCATCACCTGACCAAGCAAGAAAAGAAATGCGCGAAGCGTGTTTCAATTACCTAAGTCTTGGA
431      K  V  F  C  A  S  P  D  Q  A  R  K  E  M  R  E  A  C  F  N  Y  L  S  L  G
1426    GGGATTTGTTCAACTGGACCAGTGGCCTTACTTTCTGGTCTTAACCCGCGCCCCCTTAGCCTGGTCTGCCATTTC
456      G  I  C  S  T  G  P  V  A  L  L  S  G  L  N  P  R  P  L  S  L  V  C  H  F
1501    TTTGCTGTTGCTGTCTATGGTGTTGGGCGCTTGTTGATTCCATTTCCATCGCCCCAGCGAATTTGGCTGGGGGCA
481      F  A  V  A  V  Y  G  V  G  R  L  L  I  P  F  P  S  P  Q  R  I  W  L  G  A
1576    AGATTGATATCAGTAACTGTCTCAATCTTTTCGCAAGGAATTGAATTCACAGAACTCAGTTTCGAACATTTTCTA
506      R  L  I  S  V  T  V  S  I  F  S  Q  G  I  E  F  T  E  L  S  F  E  H  F  L
1651    CTGATTTCTGTTTGAttttacattgcacaagacttaacctctgattaattatttgtttcagggtgcatctggaa
531      L  I  S  V  *
1726    tcattttcccaatcatcaaggctgaaggagttaggcaaatgttcttccctctgactgttccagcatactacagag
1801    ctggcactcttggttaggacagtgaataaacattatttttctcaattaataagaatagtggcatgaataatttttt
1876    tttttcctttaaccaagagagagaaaagaaggtttgtatcagcagctgacctcccctggtaaagaattggggtgg
1951    gttataattaaagagagaatgcggtgggggcataaattgtaattaatattgacagatttttgctcgtaaagtggg
2026    aattgcttTCCCTTTGAACTTTGTACTTTAAAC
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Fig. 2: cDNA and deduced amino acid sequence of *IcSE2*

The initial codon and termination codon are highlighted, letters with underlined indicate the primers sequence

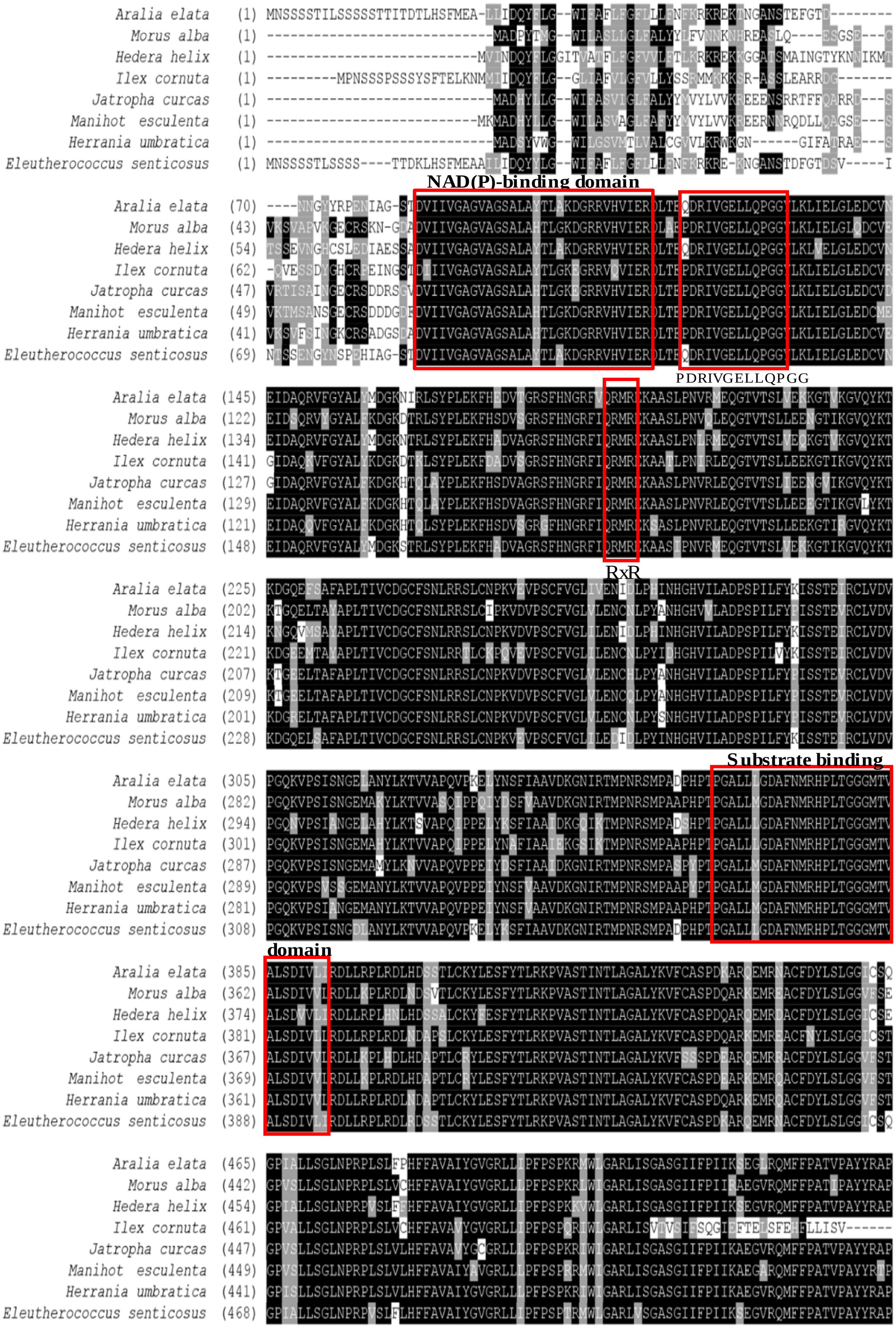

Fig. 3: Multiple alignments of IcSE2 amino acid sequence and squalene epoxidase from other species

Completely identical and conserved amino acids are shaded in black and grey, respectively. Conserved regions of NAD(P)-binding domain and a substrate-binding domain, RxR and PDRIVGELLQPGG are boxed in red

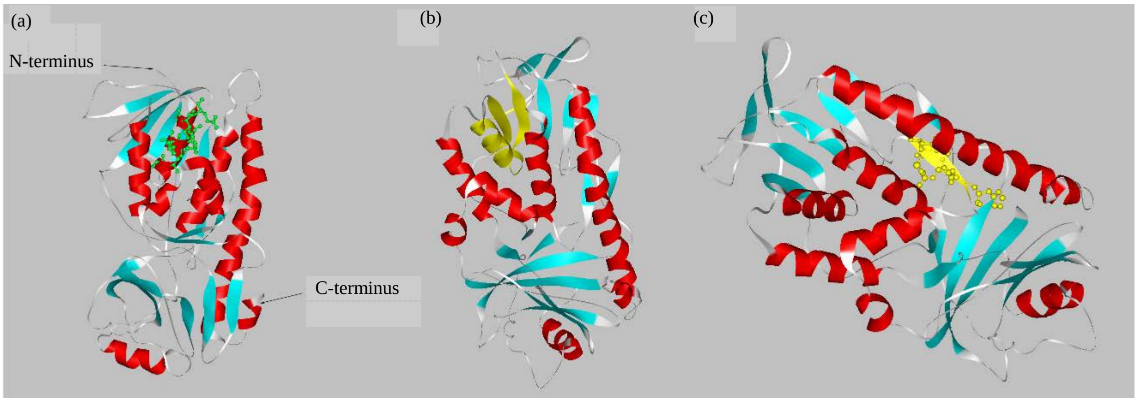

Fig. 4(a-c): Three-dimensional model of IcSE2 protein

a, b, c are three different perspectives of IcSE2 constructed with FAD-dependent monooxygenase (2×3n.1.A) as a template. The alpha helix structure region is in red, beta turn structure region is in blue, extended strand and random coil structure regions are in silver. (a) Conserved motif of NAD(P)-binding domain is in green. (b) The typical βαβ sandwich of the FAD2 binding domain is in yellow and (c) FAD3 motif is in yellow

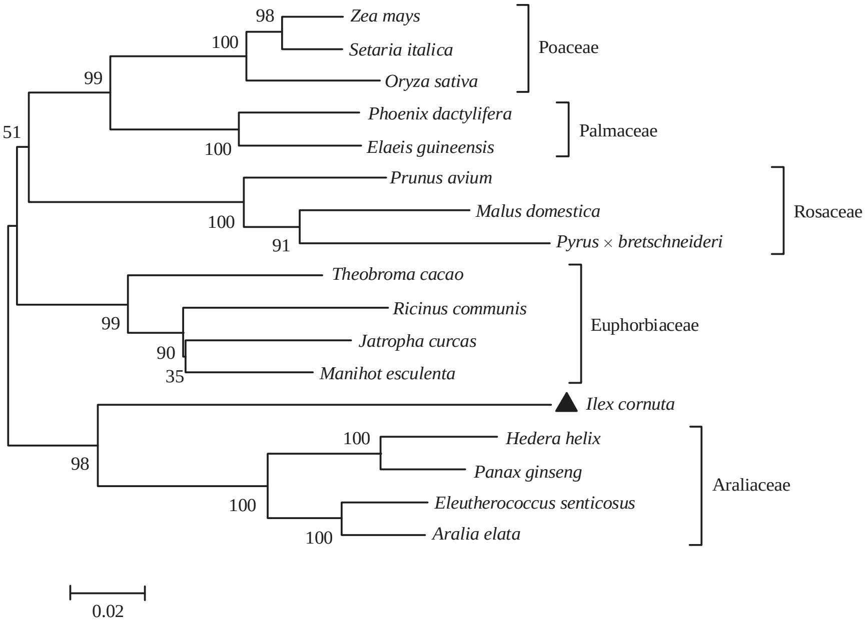

Fig. 5: Phylogenetic tree of SE proteins from different species. The reliability of the tree is measured by bootstrap analysis with 1000 replications. The sequences and their accession numbers are as follows: *Zea mays*, ONL95392.1; *Setaria italic*, XP_004985122.1; *Oryza sativa*, XP_015630703.1; *Phoenix dactylifera*, XP_008790270.1; *Elaeis guineensis*, XP_010936193.1; *Prunus avium*, XP_021801436.1; *Malus domestica*, XP_008342186.1; *Pyrus × bretschneideri*, XP_009339163.1; *Theobroma cacao*, XP_007018417.1; *Ricinus communis*, XP_002510043.2; *Manihot esculenta*, XP_021613973.1; *Jatropha curcas*, AGW81843.1; *Hedera helix*, APV45531.1; *Panax ginseng*, ACJ24907.2; *Eleutherococcus senticosus*, AEJ79818.1; *Aralia elata*, ADC32655.1

comprising "DVIVGAGVAGSALAYTLGKDGRRVHVIER" and "PDRIVGELLQPGG", which exhibited the typical βαβ sandwich, and was highly conserved in the flavoprotein for binding with the FAD cofactor (Fig.4b). The substrate-binding site of FAD3 presented in Fig.4c was involved in the catalytic function of SE.

Phylogenetic analysis of IcSE2: A phylogenetic tree of the *SE* gene was constructed using SE proteins from different species. The phylogenetic relationship between IcSE2 and other SE proteins is shown in Fig. 5. The SE proteins from 17 species shared a common ancestor and the SEs from the same family

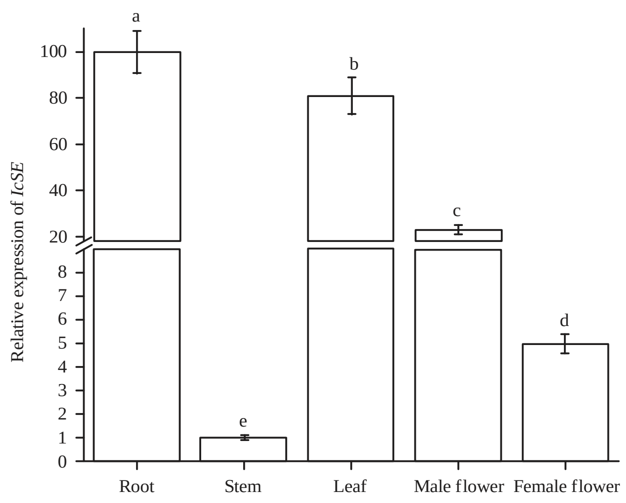

Fig. 6: Spatial expression analysis of *IcSE2* in different organs of *I. cornuta*

Data are Mean±SD from triplicate experiments. Means with different letters are significantly different at $p<0.05$

of plants clustered in the same branch, indicating that they possess a close genetic relationship. For example, the SEs of the Poaceae *Zea mays*, *Setaria italic* and *Oryza sativa* and the Palmaceae *Phoenix dactylifera* and *Elaeis guineensis* clustered into different branches, whereas the relationship of species among each family was closer than SEs from other dicotyledons. The SEs of dicots can be divided into three clades, including Rosaceae, Euphorbiaceae and Araliaceae. IcSE2 exhibited a closer relationship with SEs from Araliaceae than from other dicotyledons. *Hedera helix*[23], *Panax ginseng*[9], *Eleutherococcus senticosus*[24] and *Aralia elata*[25] are medicinal plants and their pharmaceutical active ingredients are triterpenoids that are regulated by SEs. In conclusion, the IcSE2 and SEs of Araliaceae were grouped together in the same branch, possibly due to their similar functions.

Expression analysis of *IcSE2*: The expression profile of *IcSE2* was assessed by qRT-PCR in five organs of *I. cornuta* (Fig. 6). Present study results showed that the *IcSE2* transcripts accumulated preferentially in the roots. Moreover, *IcSE2* was expressed at a higher level in young leaves and male flowers, followed by female flowers and lowest in stems.

DISCUSSION

Numerous pharmacological properties have been extensively studied in *Ilex* species in recent years, such as triterpenoid glycosides in *I. brevicuspis*[26], triterpenoid saponins in *I. latifolia*[27], saponins in *I. dumosa*[28], hemiterpene glycosides in *I. macropoda*[29] and flavonoid aglycones in *Ilex* species[30]. *Ilex* species demonstrate pharmacological activities, such as increased antihematoblastic coagulation activity and coronary blood flow. SE, a key gene involved in the skeleton biosynthesis of triterpenoids, has been identified in many plants. However, no studies have been reported on the gene in *I. cornuta*.

In the current study, an *SE* gene was isolated from *I. cornuta*. Multiple sequence alignment showed that IcSE2 contains FAD and NADP(H)-binding sites, which may be closely related to the function of the protein. The RxR domain has been found in sesquiterpene synthase in *Chamaemelum* and interestingly performs the same function in both enzymes, diphosphate after separation from the substrate[31,32]. SE is a flavoprotein monooxygenase that uses NADPH and molecular oxygen to oxidize squalene. SE obtains electrons from NADPH-cytochrome P450 reductase and contains a loosely bound FAD flavin rather than directly binding with nicotinamide cofactor NADPH. In the current study, phylogenetic tree analysis showed that IcSE2 possessed a closer relationship with SEs from Araliaceae plants than from other families, suggesting that SE proteins from different plants may demonstrate similar functions.

qRT-PCR analysis showed that *IcSE2* has distinct tissue-specific expression. *IcSE2* expression is highest in roots and leaves, followed by male flowers and female flowers and lowest in stems. *SE* expression level was consistent with triterpenoid concentration in various tissues of plants. The *SE* expression level in roots is higher than that in leaves and other tissues in both *Achyranthus bidentata*[33] and *Dioscorea zingiberensis*[34], which are consistent with present study results. However, in *Chlorophytum borivilianum*, CbSE expression is 17.6% higher in leaves compared with that in roots[10]; furthermore, in *Euphorbia tirucalli*, the *EtSE* expression level in leaves and stem in ternodes are almost equal and more abundant than that in roots[35]. The expression pattern of *IcSE2* in different organs may be related to triterpenoid biosynthesis, post-processing, function and storage sites. The initial reactions of triterpenoid biosynthesis in *I. cornuta* may occur in roots and modification and storage may proceed in other tissues.

CONCLUSION

In summary, it was cloned the *SE2* gene from *I. cornuta* and found that it contains a 1605 bp open reading frame that codes 534 amino acids. The predicted theoretical isoelectric point and molecular weight are 8.30 and 58.6 kDa, respectively. The deduced IcSE2 protein contains RxR, FAD and

NADP(H) domains and is highly similar to the SEs from other plants. IcSE2 is closely related with the SEs of Araliaceae plants. *IcSE2* is abundantly expressed in roots of *I. cornuta*. These findings can lay foundation for the molecular study on the triterpenoid regulatory mechanism in *I. cornuta*.

SIGNIFICANCE STATEMENT

SE is a key enzyme that regulates the synthesis of triterpenoids, which is a class of pharmacologically active compounds that are beneficial to humans, animals and plants. In the current study, *IcSE2* was isolated and expressed to provide important genetic resources for researchers to study the functions of the SE enzyme. The study may be helpful for increasing triterpenoid content via genetic improvements in *I. cornuta*.

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