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Research Article

Cloning and sequence analysis of a 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*) gene from *Ilex cornuta*

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ABSTRACT

In higher plants, 1-Deoxy-D-xylulose-5-phosphate synthase (*DXS*) catalyzes the first step of the MEP pathway, which is considered a key rate-limiting enzyme related to the synthesis of isoprenoids. In this study, we cloned the cDNA of a DXS gene from *Ilex cornuta.* The cDNA length of *IcDXS* gene is 2349 bp, encoding 732 amino acids. The molecular weight of the deduced IcDXS protein is 78.60 kDa and the isoelectric point is 6.99. Secondary structure predicted that the α-helix, extended strand, β-turn and random coil in IcDXS protein were 37.84%, 18.58%, 11.20% and 32.38% respectively. The IcDXS protein sequence was highly homologous to DXS proteins from other plants. The phylogenetic tree analysis showed that the IcDXS protein was clustered with the DXS proteins of Manihot esculenta and Jatropha curcas, indicating that there was a close relationship between them. This study lays the foundation for the functional identification of *IcDXS* gene.

Keyword: *Ilex cornuta*; 1-deoxy-D-xylulose-5-phosphate synthase; gene clone; sequence analysis

INTRODUCTION

Ilex cornuta Lindl. et Paxt. (Aquifoliaceae), commonly known as Chinese holly or horned holly, is an evergreen shrub or small tree, and is distributed widely in Eastern and Southern China. *Ilex cornuta*, not only has horticultural appreciation value, but important pharmacological value [1]. *Ilex cornuta* leaves are used to make the popular herbal tea, named "Kudingcha" or bitter tea in China, or as an ingredient in dietary supplements [2]. In addition, *Ilex cornuta* can be used to treat headache, bloodshot eyes, toothache and tinnitus [3]. Furthermore, the aqueous decoction that made from its leaves is usually used as a cardiovascular, contraceptive or antibacterial agent [4]. Previous studies have shown that leaves of *Ilex cornuta* are rich in flavonoids, triterpenoids and their corresponding glycosides [5, 6]. In plants, plastid located 2-C-methyl-D-erythritol-4-phosphate (MEP) and the cytoplast-located mevalonic acid (MVA) are two main pathways of biosynthesis of terpenoids [7, 8]. Isopentenyl diphosphate (IPP) and dimethylallyl diphos-phate (DMAPP) are two common precursors of Isoprenoids [9]. 1-Deoxy-D-xylulose-5-phosphate synthase as a key rate-limiting enzyme is involved in the first step of the MEP pathway [10, 11]. Up to now, genes that encode DXS have been cloned and characterized from a variety of plant species, such as *Catharanthus roseus* [12], *Ginkgo biloba*

[13], *tomato* [14] and *Aquilaria sinensis* [15]. In order to clarify the role of DXS in isoprenoids biosynthesis in *Ilex cornuta*, we described the isolation and sequence analysis of the *DXS* gene from *Ilex cornuta* in the study; our results will be an important foundation for a further understanding function of DXS in the regulation of isoprenoids biosynthesis in *Ilex cornuta*.

MATERIALS AND METHODS

Plant material

The *Ilex cornuta* used for cloning of the IcDXS gene was growing in the campus of Yangtze University, in China. The fresh leaves were gathered, immediately frozen in liquid nitrogen and stored at -80°C prior to RNA extraction [16].

cDNA Sequence amplification of *IcDXS*

Ilex cornuta total RNA was extracted from fresh leaves using the MiniBEST Plant RNA extraction kit (TaKaRa, China) [16]. Using the PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, China), the cDNA was reverse transcribed and used to amplify *IcDXS* fragment. A pair of specific primers for *IcDXS*F (5′- GAGAGAGAGAGAGAGAGATGGCTTC-3′) and *IcDXS*R (5′- AACAAGAACTCCTTAGTG AACCCCC-3′) were designed against EST sequences of *Ilex cornuta*, and synthesised by Sangon Biotech (Shanghai, China). PCR reaction was performed with the following conditions: denaturation at 94°C for 4 min; 32 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for the 90 s; extension at 72°C for 10 min. The purified PCR product was ligated into a pMD18-T vector (TaKaRa, China) before transformed into *E. coli* TOP10 competent cells. PCR was carried out with M13 universal primers to identify the clones, and the positive clones were sequenced.

Bioinformatics analysis

Online tools blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to verify the amplified cDNA sequence. ORF Finder (NCBI) was used to analyse the nucleic acid structure, DNAMAN 8 was utilized to predict the amino acid sequence of *IcDXS* gene. Molecular mass and theoretical isoelectric point of IcDXS protein was predicted with compute pI/Mw tool (http://web.expasy.org/compute_pi/). The secondary structure of IcDXS protein was analyzed by SOPMA tool. Sequence alignment of DXS proteins from different plants was completed with Blastp and Align X (Vector NTI 11.5). Phylogenetic tree of DXS proteins was constructed via software MEGA 6 with the neighbor-joining (NJ) method [17].

RESULTS

cDNA sequence of *IcDXS*

With the specific primers, a 2349 bp length cDNA fragment was amplified by PCR from the cDNA of *Ilex cornuta*. After blast validation, the amplified sequence was DXS gene (designated as *IcDXS*). The ORF (open reading frame) length of *IcDXS* was 2199 bp with TAA as stop codon, encoding 732 amino acids. The 5'-untranslated region and 3'-untranslated region of *IcDXS* cDNA were 17 bp and 133 bp, respectively (Fig.1).

Characterization of the predicted IcDXS protein

The length of putative IcDXS protein sequence was 732 amino acids. The calculated molecular mass and theoretical isoelectric point (pI) of IcDXS was 78.60 kDa and 6.99, respectively. Secondary structure prediction discovered that α-helix, extended strand, β-turn and random coil in the secondary structure were predicted to be 37.84%, 18.58%, 11.20% and 32.38%, respectively.

Sequence comparison via Blastp (NCBI) search showed that IcDXS belong to thiamine pyrophosphate (TPP) family, DXS subfamily (Fig.2). IcDXS had high homology with DXS proteins from other plants (Fig.3), such as *Coffea canephora* (83% identity, CDP02935), *Stevia rebaudiana* (80% identity, ALJ30087), *Camellia sinensiss* (83% identity, ANB66337), Ziziphus jujube (80% identity, XP_015877067), *Juglans regia* (81% identity, XP_018831018) and *Vitis vinifera* (82% identity, XP_002271585). These results indicated that cloned *IcDXS* was a member of the DXS family.

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1      gagagagagagagagagATGGCTTCTTGTGGTGTCTTCAAAAGTAGCTTCCTTCCATCACTCCACATTCAAGATGGGTACTCTTCTTCCA
                          M  A  S  C  G  V  F  K  S  S  F  L  P  S  L  H  I  Q  D  G  Y  S  S  S
91     GCCTTTTAACTGCTGCCAAACCCATTCTTCCCAGTAACAAGCGAAAGTTTACCGGGGTCGTTGCTGCTCAGGACAACAAAGCCAGCGAAG
       S  L  L  T  A  A  K  P  I  L  P  S  N  K  R  K  F  T  G  V  V  A  A  Q  D  N  K  A  S  E
181    AAAACGATGGACTAGTCAAGAGAGACAACTCAACAAAAATCAATGGACAGGCCACAAGAACACTGAATTTCTCAGGAGAGAAGCCATTTA
       E  N  D  G  L  V  K  R  D  N  S  T  K  I  N  G  Q  A  T  R  T  L  N  F  S  G  E  K  P  F
271    CTCCAATCTTGGATACCATTAACCATCCAATTCACATGAATAATCTTTCTCTCCAGGAGCTTGAGGAGTTGGCAGATGAACTCCGGGAAG
       T  P  I  L  D  T  I  N  H  P  I  H  M  N  N  L  S  L  Q  E  L  E  E  L  A  D  E  L  R  E
361    ATATTGTCTACACCGTCTCTAAGACCGGCGGCCACCTAAGCGCTAGCCTTGGAGTGGCTGAGCTAACTGTCGCACTTCACCATGTATTCA
       D  I  V  Y  T  V  S  K  T  G  G  H  L  S  A  S  L  G  V  A  E  L  T  V  A  L  H  H  V  F
451    ACACACCAGAAGACAAGATCATTTGGGACGTTGGGCACCAGGCATACGCACATAAGATTTTGACGGGTAGGAGGTCGAGAATGCATACAA
       N  T  P  E  D  K  I  I  W  D  V  G  H  Q  A  Y  A  H  K  I  L  T  G  R  R  S  R  M  H  T
541    TTCGACAAACTTGTGGTCTAGCAGGCTTCCCCAAGAGGGATGAGAGTATACATGATGCGTTTGGTGCCGGCCACAGCTCTACCAGCATCT
       I  R  Q  T  C  G  L  A  G  F  P  K  R  D  E  S  I  H  D  A  F  G  A  G  H  S  S  T  S  I
631    CTGCTGGCTTAGGGATGGCTGTTGGAAGAGACTTGTTAGGGAAGAACAATCACGTAATTTCGGTAATAGGCGATGGAGCCATGACCGCCG
       S  A  G  L  G  M  A  V  G  R  D  L  L  G  K  N  N  H  V  I  S  V  I  G  D  G  A  M  T  A
721    GGCAGGCATACGAAGCAATGAACAATGCAGGCTACCTCGATTCGAATCTTATTGTCATCTTGAATGACAACAAACAAGTCTCATTGCCAA
       G  Q  A  Y  E  A  M  N  N  A  G  Y  L  D  S  N  L  I  V  I  L  N  D  N  K  Q  V  S  L  P
811    CCGCCACTGTCGACGGCCCAGCTCCTCCAGTCGGAGCTCTAAGCAGAGCCCTAACAAAGTTGCACTCAAGCCGAAAGTTTCGTCAACTTC
       T  A  T  V  D  G  P  A  P  P  V  G  A  L  S  R  A  L  T  K  L  H  S  S  R  K  F  R  Q  L
901    GTGAAGCTGCAAAAGGTGTGACCAAACAAATTGGAAGCCAAGCACACGAAATCGCGGCCAAAGTTGACACGTGCGTGAGAGGGATGTCTG
       R  E  A  A  K  G  V  T  K  Q  I  G  S  Q  A  H  E  I  A  A  K  V  D  T  C  V  R  G  M  S
991    CTGGCAATGGGGCTTCACTGTTTGAAGAGCTAGGGCTATACTATATTGGTCCAGTGGATGGTCACGATGTAGAAGACCTTGTGTATATTT
       A  G  N  G  A  S  L  F  E  E  L  G  L  Y  Y  I  G  P  V  D  G  H  D  V  E  D  L  V  Y  I
1081   TGAAAAAGGTGAAGGCCATGCCAGCCCCTGGACCTGTTCTCCTCCACGTTATTACAGAGAAAGGAAGAGGCTATCCTCCGGCCGAAGCTG
       L  K  K  V  K  A  M  P  A  P  G  P  V  L  L  H  V  I  T  E  K  G  R  G  Y  P  P  A  E  A
1171   CAGCTGATAAAATGCATGGGGTGGTGAAATTCGATCCCAAGACGGGAAAACAAATGAAGACCAAATCGAAGACACTTTCGTACACCCAAT
       A  A  D  K  M  H  G  V  V  K  F  D  P  K  T  G  K  Q  M  K  T  K  S  K  T  L  S  Y  T  Q
1261   ACTTCGCTGAGTCTTTAATTGCTGAAGCTGAGAGAGATGACAAGATAGTAGCAGTCCATGCTGCAATGGGAGGAGGTACAGGACTCAACG
       Y  F  A  E  S  L  I  A  E  A  E  R  D  D  K  I  V  A  V  H  A  A  M  G  G  G  T  G  L  N
1351   CATTCCAGAAACGCTTCCCCGACCGGTGTTTCGATGTTGGGATCGCCGAGCAACATGCCGTCACCTTTTCTGCTGGTCTTGCCACTGAAG
       A  F  Q  K  R  F  P  D  R  C  F  D  V  G  I  A  E  Q  H  A  V  T  F  S  A  G  L  A  T  E
1441   GGATCAAGCCCTTTTGTACCATTTACTCATCTTTTCTGCAGAGAGGTTATGATCAGGTGGTTCACGATGTTGATCTTCAAAAGATTCCGG
       G  I  K  P  F  C  T  I  Y  S  S  F  L  Q  R  G  Y  D  Q  V  V  H  D  V  D  L  Q  K  I  P
1531   TGAGATTCGCGATGGACCGGGCTGGGCTAGTTGGTGCAGATGGACCAACCCATTGTGGGGCATTTGACACTACTTACATGGCCTGTTTGC
       V  R  F  A  M  D  R  A  G  L  V  G  A  D  G  P  T  H  C  G  A  F  D  T  T  Y  M  A  C  L
1621   CTAACATGGTGGTTATGGCCCCTTCTTGTGAAAGCGAGCTTATGCACATGGTTGCCACAGCCGCAGCCATTGATGATCGGCCTAGTTGCT
       P  N  M  V  V  M  A  P  S  C  E  S  E  L  M  H  M  V  A  T  A  A  A  I  D  D  R  P  S  C
1711   TTAGGTACCCTAGAGGAAATGGCATTGGTTCAGTTCTTCCTCCAAACAACAAGGGAATCCCTCTAGAGGTTGGAAGGGGAAGGGTGCTGA
       F  R  Y  P  R  G  N  G  I  G  S  V  L  P  P  N  N  K  G  I  P  L  E  V  G  R  G  R  V  L
1801   GAGAAGGAAGTAGAGTGGCGATTTTAGGGTATGGAACAATAGTACAGAACTGTCTAGCATCAGCACAACTTCTTCAAGTGCTTGGAATTT
       R  E  G  S  R  V  A  I  L  G  Y  G  T  I  V  Q  N  C  L  A  S  A  Q  L  L  Q  V  L  G  I
1891   CAGCAACTGTGGTGGATGCAAGATTCTGCAAACCTCTTGATGGGCCTCTAATTAGAAGGTTAGCTCAAGAACACGAGGTCCTTATCACTG
       S  A  T  V  V  D  A  R  F  C  K  P  L  D  G  P  L  I  R  R  L  A  Q  E  H  E  V  L  I  T
1981   TTGAAGAAGGATCTATAGGTGGATTTGGCTCCCATGTTTCCCATTTCTTGGGCTTGAATGGATTGCTTGATGGAAACCTTAAGTGGAGGG
       V  E  E  G  S  I  G  G  F  G  S  H  V  S  H  F  L  G  L  N  G  L  L  D  G  N  L  K  W  R
2071   CTATGATGCTACCTGACAGATACATAGATCATGGAGCTCAAAAGGATCAGCTTGAAGAGGCTGGGCTAACTCCAAAACATATTGCGGCCA
       A  M  M  L  P  D  R  Y  I  D  H  G  A  Q  K  D  Q  L  E  E  A  G  L  T  P  K  H  I  A  A
2161   CTGTTTTGTCGCTAATTGGAGAAAACAAGGACAGCCTTCACCTTGTTAACCTGTAAtattggagttctttcccaacactagagctgtaga
       T  V  L  S  L  I  G  E  N  K  D  S  L  H  L  V  N  L  *
2251   acttgtaaccctccacagcaatgtagggccaaatctaaaagcaatacttcctttctcctcttgtaaatagctaagggggttcactaagga
2341   gttcttgtt
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Fig. 1: The cDNA sequence and deduced amino acid sequence of *IcDXS*.
5'-untranslated region and 3'-untranslated region are indicated in lowercase.

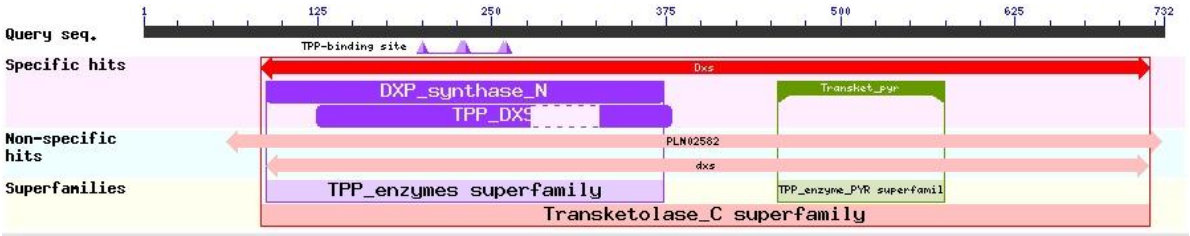

Fig. 2: Conserved domains of IcDXS

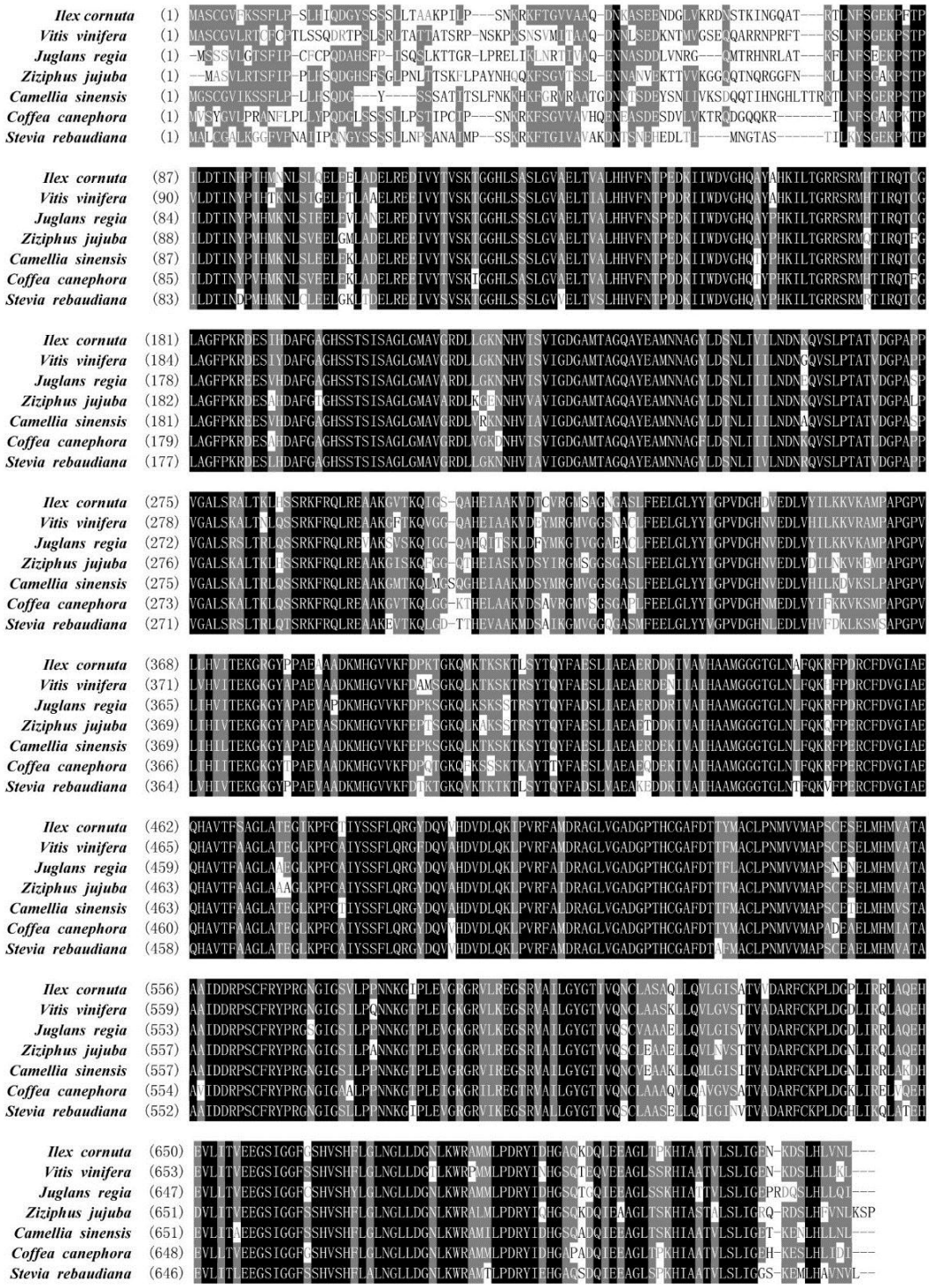

Fig.3: Sequence alignment of the DXS proteins from different plants
The completely identical amino acids are indicated with a black background and white foreground; Non-similar amino acids are indicated with a white background and black foreground.

Phylogenetic tree of DXS proteins

Using the neighbor-joining method, the phylogenetic tree was constructed to analyze the relationship between the IcDXS protein and DXS protein sequences from other plants. As showed in Figure 4, the IcDXS protein was clustered with the DXS proteins of *Manihot esculenta* and Jatropha curcas, indicating it had a close relationship to them. DXS proteins from the same family having the closest relationship.

DXS proteins of *Glycine max*, *Cajanus cajan*, *Vigna angularis, Arachis ipaensis and Arachis duranensis* that from Leguminosae family were clustered in the same branch. DXS proteins from Rosaceae plants such as *Prunus mume*, *Prunus avium* and *Prunus persica* were grouped into the same cluster. In addition, DXS proteins from Solanaceae including *Nicotiana attenuata*, *Lycium ruthenicum* and *Nicotiana tomentosiformis* were clustered in another branch. The results indicated that DXS proteins from different plants shared a common evolutionary origin.

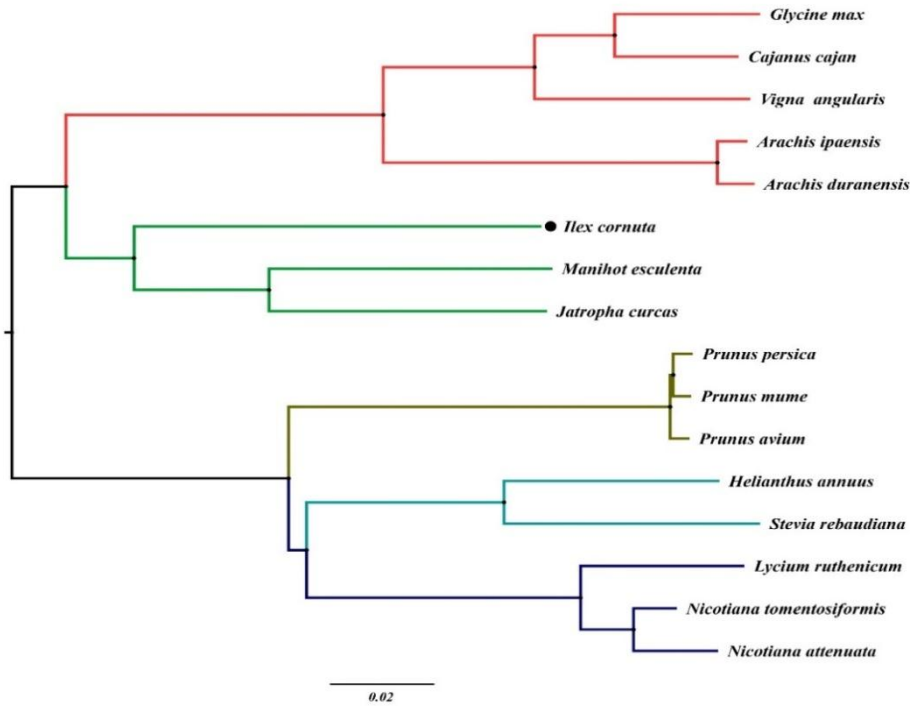

Fig. 4: Phylogenetic tree of DXS proteins from different plant species

GenBank accession numbers of DXS protein sequences are as follows: Manihot esculenta (XP_021616770), Jatropha curcas (XP_012065282), Glycine max (KRH02914), Cajanus cajan (XP_020228516), Vigna angularis (XP_017409677), Arachis ipaensis (XP_016200847), Arachis duranensis (XP_015933619), Nicotiana tomentosiformis (XP_009590491), Prunus mume (XP_008236351), Prunus avium (XP_021802169), Helianthus annuus (OTG05024), Prunus persica (XP_007208040), Lycium ruthenicum (AIX87516), Nicotiana attenuata (OIT38069), Stevia rebaudiana (CAD22155).

DISCUSSION

Many secondary metabolites have important medicinal and commercial significance, the isoprenoid precursors for their biosynthesis are often provided by the MEP pathway [18]. It is believed that the enzymes of the MEP pathway play an important role in providing biosynthetic precursors of higher complex terpenoid alkaloids. DXS is one of a key enzymes involved in isoprenoid biosynthesis and it has been proved by some studies [19, 20]. In suspension cells of *Tripterygium wilfordii*, when the mRNA expression levels of *TwDXS1*, *TwDXS2* were increased, the triptophenolide was rapidly accumulated [21]. In *Catharanthus roseus* hairy roots, terpenoid indole alkaloid contents were increased after overexpression of *DXS* and *G10H* genes [22]. A higher accumulation of carotenoids and chlorophyll was found in Arabidopsis after overexpression *Solanum tuberosum StDXS1* gene [10]. According to previous studies, different components have been isolated from *Ilex cornuta*, including saponins, triterpenoids and flavonoids, and some of these compounds reported exhibit promising anti-fertility, cardiovascular system protection and immune

inhibition activities [23, 24]. In this study, a *DXS* gene was successfully isolated from *Ilex cornuta,* which maybe participate in terpenoid biosynthesis. The length of IcDXS protein was 732 aa, and it is agree with a previous study suggesting that plant DXS protein is approximately 691 to 738 aa long [25]. Homology analysis found that the deduced IcDXS was high homologous with DXS proteins from other plants, and they all belong to DXS subfamily. The cDNA and protein sequence of DXS from different species had certain levels of similarity. Phylogenetic tree analysis suggested that DXS proteins from different plants had a common evolutionary origin, and IcDXS was closely related to DXS from Euphorbiaceae plants. This study laid a foundation for further validation of the function of DXS gene in *Ilex cornuta.*

CONCLUSION

One DXS gene involved in terpenoid biosynthesis was isolated from *Ilex cornuta*, and bioinformatics analysis of DXS protein was carried out. The isolated *DXS* gene belongs to the DXS subfamily and has a high similarity to the *DXS* from other plants. In addition, DXS protein is closely related to Euphorbiaceae plants in the evolutionary relationship.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

REFERENCES

1. Tao W, Yan L, Tang Q, *et al*. Two unusual minor 18,19-seco-ursane glycosides from leaves of *Ilex cornuta*. Food Chem 2008; 111(1):78-82.
2. Chau C F, Wu S H. The development of regulations of Chinese herbal medicines for both medicinal and food uses. Trends Food Sci Tech 2006; 17(6): 313-323.
3. Zhang A L, Ye Q, Li B G, *et al*. Phenolic and Triterpene Glycosides from the Stems of Ilex l itseaefolia. J Nat Prod 2005; 68(10): 1531-1535.
4. Zhang J, Lin C, Cen Y Z, *et al*. Antibacterial effect of leaves of *Ilex cornuta*. Chinese J Pathophysiol 2003; 19:1562-1563.
5. Wu T, Cheng Z, Liu H. Liposoluble components from leaves of *Ilex cornuta*. Chinese Pharmaceutical J 2005; 40(19): 1460.
6. Li Y, Wu T, Chen Z H, *et al*. New triterpene saponins and flavonol glycosides from the leaves of *Ilex cornuta*. Chinese J Chem 2006; 24(4): 577-579.
7. Rohmer M. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat Prod Rep 1999; 16(5): 565-574.
8. Eisenreich W, Rohdich F, Bacher A. Deoxyxylulose phosphate pathway to terpenoids. Trends Plant Sci 2001; 6(2): 78-84.
9. McGarvey D J, Croteau R. Terpenoid metabolism. Plant Cell 1995; 7(7): 1015.
10. Henriquez M A, Soliman A, Li G, *et al*. Molecular cloning, functional characterization and expression of potato (*Solanum tuberosum*) 1-deoxy-d-xylulose 5-phosphate synthase 1 (*StDXS1*) in response to Phytophthora infestans. Plant Sci 2016; 243: 71-83.
11. Wu S J, Shi M, Wu J Y. Cloning and characterization of the 1-deoxy-d-xylulose 5-phosphate reductoisomerase gene for diterpenoid tanshinone biosynthesis in *Salvia miltiorrhiza* (Chinese sage) hairy roots. Biotechnol Appl Biochem 2009; 52(1): 89-95.
12. Chahed K, Oudin A, Guivarc'h N, *et al*. 1-Deoxy-D-xylulose 5-phosphate synthase from periwinkle: cDNA identification and induced gene expression in terpenoid indole alkaloid-producing cells. Plant Physiol Bioch 2000; 38(7): 559-566.
13. Gong Y, Liao Z, Guo B, *et al*. Molecular cloning and expression profile analysis of *Ginkgo biloba DXS* gene encoding 1-deoxy-D-xylulose 5-phosphate synthase, the first committed enzyme of the 2-C-methyl-D-erythritol 4-phosphate pathway. Planta Med 2006; 72(04): 329-335.
14. Lois L M, Rodríguez-Concepción M, Gallego F, *et al*. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-d-xylulose 5-phosphate synthase. Plant J 2000; 22(6): 503-513.
15. Xu Y, Liu J, Liang L, *et al*. Molecular cloning

and characterization of three cDNAs encoding 1-deoxy-d-xylulose-5-phosphate synthase in *Aquilaria sinensis* (Lour.) Gilg. Plant Physiol Biochem 2014; 82: 133-141.

16. Ma L, Jiang D, Zhang W, *et al*. Cloning and Sequence Analysis of an Anthocyanidin reductase (*ANR*) Gene from *Ginkgo biloba*. J Pharm Chem Biol Sci 2017; 5(1):10-16.
17. Tamura K, Stecher G, Peterson D, *et al*. MEGA 6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013; 30(12): 2725-2729.
18. Yang J, Adhikari M N, Liu H, *et al*. Characterization and functional analysis of the genes encoding 1-deoxy-D-xylulose-5-phosphate reductoisomerase and 1-deoxy-D-xylulose-5-phosphate synthase, the two enzymes in the MEP pathway, from *Amomum villosum* Lour. Mol Biol Rep 2012; 39(8): 8287-8296.
19. Harker M, Bramley P M. Expression of prokaryotic 1-deoxy-Dxylulose 5-phosphate in Escherichia coli increases carotenoid and ubiquinone biosynthesis. FEBS Lett 1999; 448(1): 115-119.
20. Miller B, Heuser T, Zimmer W. Functional involvement of a deoxy-D-xylulose 5-phosphate reductoisomerase gene harboring locus of Synechococcus leopoliensis in isoprenoid biosynthesis. FEBS Lett 2000; 481(3): 221-226.
21. Tong Y, Su P, Zhao Y, *et al*. Molecular cloning and characterization of *DXS* and *DXR* genes in the terpenoid biosynthetic pathway of *Tripterygium wilfordii*. Int J Mol Sci 2015; 16(10): 25516-25535.
22. Peebles C A M, Sander G W, Hughes E H, *et al*. The expression of 1-deoxy-D-xylulose synthase and geraniol-10-hydroxylase or anthranilate synthase increases terpenoid indole alkaloid accumulation in Catharanthus roseus hairy roots. Metab Eng 2011; 13(2): 234-240.
23. Si-Xiang Z, Zhi-Rong Y, Jun L I, *et al*. Flavonoids from the leaves of *Ilex cornuta*. Chinese J Nat Med 2012; 10(2): 84-87.
24. Li W, Wu J, Ren B, *et al*. The pharmacological action of 3 compounds isolated from *Ilex cornuta* Lindl. ex Paxt. for angiocardiopathy. J Plant Resour Environ 2003; 12(3): 6-10.
25. Jin R , Zhu C Q, Xu C J. 1-Deoxy-D-Xylulose 5-Phosphate Synthase (*DXS*) and Its Encoding Genes. Chinese J Cell Biol 2007; 29: 706-712.

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