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Molecular Cloning and Characterization of a New cDNA Encoding Hyoscyamine 6β-hydroxylase from Roots of *Anisodus acutangulus*

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A new full-length cDNA encoding hyoscyamine 6β-hydroxylase (designated as *aah6h*, GenBank Accession No. EF187826), which catalyzes the last committed step in the scopolamine biosynthetic pathway, was isolated from young roots of *Anisodus acutangulus* by rapid amplification of cDNA ends (RACE) for the first time. The full-length cDNA of *aah6h* was 1380 bp and contained a 1035 bp open reading frame (ORF) encoding a deduced protein of 344 amino acid residues. The deduced protein had an isoelectric point (pI) of 5.09 and a calculated molecular mass of about 38.7 kDa. Sequence analyses showed that AaH6H had high homology with other H6Hs isolated from some scopolamine-producing plants such as *Hyoscyamus niger, Datura metel* and *Atropa belladonna* etc. Bioinformatics analyses results indicated AaH6H belongs to 2-oxoglutarate-dependent dioxygenase superfamily. Phylogenetic tree analysis showed that AaH6H had closest relationship with H6H from *A. tanguticus*. Southern hybridization analysis of the genomic DNA revealed that *aah6h* belonged to a multi-copy gene family. Tissue expression pattern analysis firstly founded that *aah6h* expressed in all the tested tissues including roots, stems and leaves and indicated that *aah6h* was a constitutive-expression gene, which was the first reported tissue-independent *h6h* gene compared to other known *h6h* genes.

Keywords: *Anisodus acutangulus*, Bioinformatics analysis, Expression pattern, Hyoscyamine 6β-hydroxylase (H6H), Southern blotting

Abbreviations: *A. acutangulus*: *Anisodus acutangulus,* H6H: hyoscyamine 6β-hydroxylase, ORF: open reading frame, RACE: rapid amplification of cDNA ends, AaH6H: *A. acutangulus* hyoscyamine 6β-hydroxylase, PCR: Polymerase chain reaction, pI: isoelectric point, RT-PCR: reverse transcriptase-polymerase chain reaction.

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Introduction

A few genera of the plant family *Solanaceae* icluding *Anisodus, Atropa*, *Datura*, *Duboisia, Hyoscyamus* and *Scopolia* are able to produce biologically active tropane alkaloids simultaneously (Hsiao *et al*., 1973; Endo *et al*., 1991; Christen *et al*., 1993; Hashimoto and Yamada 1994; Zhang *et al*., 2005). Tropane alkaloids such as hyoscyamine and scopolamine, which are widely used as anticholinergic agents that act on the parasympathetic nervous system, are structurally related and are derived from a common intermediate, the N-methylpyrrolinium cation (Zhang *et al*., 2004). Hyoscyamine and scopolamine are mostly synthesized in young root cells and translocated to the aerial parts of the plant (Hashimoto *et al*., 1992), and hyoscyamine is usually the main alkaloid in many *Solanaceae* plants such as *Hyoscyamus muticus* and *Atropa belladonna* while scopolamine is only produced in small amounts (Suzuki *et al*., 1999; Mateus *et al*., 2000). Being higher physiological activity, fewer side-effects and the low yield of scopolamine, scopolamine is more valuable and is preferred than hyoscyamine in the pharmaceutical market (Häkkinen *et al*., 2005). It is reported there is currently a 10-fold higher commercial demand for scopolamine (in the N-butylbromide form) than for hyoscyamine and atropine combined (Yun *et al*., 1992; Evans, 1996; Zhang *et al*., 2004). So it is of significance and importance to improve tropane alkaloids production especially the much more valuable scopolamine to meet the expansion of clinical need.

During the past decades, considerable efforts have been made to develop economically feasible approaches for production of tropane alkaloids, especially scopolamine. Although scopolamine can be synthesized by chemical method, however, the routes are long, expensive, and too low yielding due to the complexity of the chemical structure, and thus is an unrealistic alternative for commercial supply (Huang *et al*., 2005). Conventional interspecific hybridization approach had shortcomings of too long breeding cycle, lack of specific parent materials and environmental limitation (Yun *et al*., 1992). It is clear that in the foreseeable future the supply of tropane alkaloids must depend on biological methods of production. The development of modern biotechnology provides one of promising ways to genetically improve scopolamine production by transferring key genes involved in the scopolamine biosynthetic pathway into scopolamine-producing plants and culturing transgenic cell lines, hairy roots or regenerated plants in a large scale. Therefore, it is very significant to understand in detail the pathway for scopolamine biosynthesis, the enzymes catalyzing the reaction sequence, especially the slow steps, and the genes encoding these proteins, which will be helpful to develop and improve biological processes via genetic manipulation of scopolamine-producing plants.

The biosynthesis of scopolamine is a complex process, requiring many distinct enzymatic steps and several scopolamine biosynthetic genes have now been cloned (Hashimoto *et al*., 1991; Nakajima *et al*., 1993). It has been reported that the last committed step of scopolamine biosynthesis is hydroxylation of hyoscyamine and epoxide formation from 6β-hydroxy-hyoscyamine to generate scopolamine, which was catalyzed by hyoscyamine 6β-hydroxylase (EC1.14.11.11, H6H) (Hashimoto, 1993) (Fig.1). Being a key enzyme involved in biosynthesis of scopolamine, *h6h* gene has recently been isolated from some *Solanaceae* species such as such as *Hyoscyamus niger* (Matsuda *et al*., 1991), *Atropa belladonna* (Suzuki *et al*., 1999) and *A. tanguticus* (Liu *et al*., 2005). It has been reported that an increase in the expression of the *h6h* gene can considerably enhance the production of scopolamine in hairy root cultures of *A. belladonna*, *H. muticus* and *A. baetica* (Yun *et al*., 1992; Jouhikainen *et al*., 1999; Zarate *et al*., 2006), which showed that it was a feasible approach to improve scopolamine production by metabolic engineering.

A. acutangulus is a solanaceous perennial plant that is endemic to China and classified as an endangered species in China. As it contains tropane alkaloids such as hyoscyamine and scopolamine, it has been used as common herbal anaesthetic medicine in Yunnan Province for several hundred of years (Zeng, 1962). *A. acutangulus* is an alternative commercial source of production of tropane alkaloids such as hyoscyamine and scopolamine in China. To our knowledge, until now there is no report on the cloning and characterization of *h6h* gene from *A. acutangulus*. In this work, we report the cloning and characterization of *h6h* gene from *A. acutangulus* for the first time, as an initial step to study underlying molecular mechanisms for tropane alkaloid biosynthesis and improve the production of scopolamine in *A. acutangulus* by metabolic engineering in the future.

Materials and Methods

Materials. *A. acutangulus* plants, collected from Yunnan province of China, were grown in pots in the greenhouse of our laboratory under 25°C with 16h-light period (white fluorescent tubes: irradiance of 350 μmol $m^{-2}s^{-1}$) and relative air humidity of 50%. The pMD18-T vector and one Step RNA PCR Kit was purchased from TaKaRa Biotechnology Co., Ltd. Primers' synthesis and DNA sequencing was performed by Shanghai Sangon Biotechnological Company, China. All the other chemicals used were of analytical grade.

RNA isolation. All tissue materials (1g) including roots, stems and leaves, were excised from *A. acutangulus* plants, pulverised in liquid nitrogen with mortar and pestle, and the total cellular RNA was extracted using the method reported previously (Kai *et al*., 2006a). The quality and concentration of the extracted RNA were checked and stored as described before (Kai *et al*., 2004; 2005; 2006a).

Molecular cloning of the full-length *aah6h* cDNA by RACE. The first strand cDNA was synthesized from 5 μg of total RNA from roots according to the protocol of the 3'RACE System for Rapid Amplification of cDNA Ends (GIBCO BRL, USA) using the adapter primer (AP, 5'-GGCCACGCGTCGACTAGTAC$(T)_{16}$-3') provided within the Kit. For the amplification of 3' end of *aah6h*, a specific primer F2 (5'-CTGGAAAGACACTTTGGCTCATGG-3') was designed according to the conserved region of several known *h6h* gene sequences from certain *Solanaceae* species such as *H. niger*, *D. metel* and *A. belladonna* etc. The 3' RACE was performed using primer F2 as the forward primer and the Abridged Universal Amplification Primer (AUAP, 5'-GGCCACGCGTCGACTAGTAC-3') as the reverse primer in a total volume of 50 μl containing 2 μl cDNA, 10 μM of F2, 10 μM of AUAP, 10 μmol dNTPs, 1 × Ex PCR buffer and 5U Ex Taq polymerase. PCR was performed using the following protocol: the template was denatured at 94°C for 3 min followed by 35 cycles of amplification (94°C for 50 s, 58°C for 50 s, 72°C for 90 s) and by 10 min at 72°C. The PCR product was subcloned into the pMD18-T vector and sequenced.

The first strand cDNA (5'-ready cDNA) synthesis in 5' RACE was performed according to the manual of the SMART™ RACE cDNA Amplification Kit (Clontech) using the 5'-RACE CDS Primer (5'-$(T)_{25}N_{-1}$N-3') provided by the kit. Based on the sequence of the 3' RACE product, the complementary reverse gene specific primer R2 (5'-AGTCGAGCATCCTCATGGTCA-3') was designed to ampify the 5' end of *aah6h*. The 5' RACE-PCR was carried out using primers R2 and Universal Primer A Mix (UPM, Long: 5'-CTAATA CGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT; Short: 5'-CTAATACGACTCACTATAGGGC-3') under the following condition: the template (the 5'-ready cDNA) was denatured at 94°C for 2 min followed by 35 cycles of amplification (94°C for 50 s, 60°C for 50 s and 72°C for 60 s) and by 7 min at 72°C. The PCR

product was purified and cloned into the pMD18-T vector (Takara, Japan) followed by sequencing.

Based on the nucleotide sequence of the 3' and 5' RACE product, the full-length cDNA sequence of *aah6h* was obtained and was subsequently amplified via PCR using a pair of primers F1 (5'-ACAGAAAATTAGAGCAGTGTTCTC-3') and AUAP, which was repeated for three times. The amplified full-length cDNA of *aah6h* was used for molecular characterization such as sequence homology, the presence of conserved motifs and phylogenetic tree reconstruction etc.

Bioinformatics analyses. ORF finder was used to predict coding sequence and BLAST tool was used to find similarity of AaH6H with other H6Hs in the databases online (http://www.ncbi.nlm.nih.gov). SOPMA analyses were performed online (http://www.expasy.org). Clustal X was used for sequence alignment and phylogenetic analysis (Thompson *et al*., 1997). Phylogenic tree was constructed by neighbor-joining method and reliability of each node was established by bootstrap methods using MEGA2 software (Kumar *et al*., 2001).

Extraction of genomic DNA and Southern blotting. Total genomic DNA was isolated from 1 g fresh weight of *A. acutangulus* leaf material according to the standard procedure described before (Sambrook *et al*., 1989). The fragment with the length of about 0.5 kb (the forward part of ORF), which was generated by PCR using the primers KF1 (5'-ATGGCTACTCTTGTCTCAAATTG-3') and R2, was used as a probe. Aliquots of DNA (15 μg/sample) were digested overnight at 37°C with *Bam*HI, *Eco*RV and *Eco*RI, respectively, which did not cut within the full-length cDNA of *aah6h* gene. The digested DNA samples were fractionated by 1.0% agarose gel, and transferred onto a positively charged Hybond-N^+ nylon membrane (Amersham Pharmacia) and hybridized with the probe. The probe was labeled with biotin-dUTP using Gene Images random prime labeling module (Amersham Pharmacia) and Southern blotting were performed according to the manufacturer's instructions. Hybridizing bands were detected using CDP-Star detection system (Amersham Pharmacia) and signals were visualized by exposure to Fuji X-ray film at room temperature for 2 h.

Tissue expression pattern analysis by RT-PCR. In order to investigate the expression pattern of *aah6h* in different tissues including roots, stems and leaves of *A. acutangulus*, semi-quantitative One-step RT-PCR was carried out according to the manufacturer' instruction (Takara, Japan). All RNA templates were digested with DNase I (RNase-free). Aliquots of total RNA (0.5 μg) extracted from roots, stems and leaves of *A. acutangulus*, individually were used as templates in one-step RT-PCR reaction with the forward primer KF1 (5'-ATGGCTACTCTTGTCTCAAATTG-3') and reverse primer KR1 (5'-TAGGCATTGATTTTATATGGC-3') specific to coding sequence of aah6h. Meanwhile the RT-PCR reaction for the house-keeping gene (18S rRNA gene, which is highly conserved in plants) using specific primers 18SF (5'-GTGACAATGGAACTGGAATGG-3') and 18SR (5'-AGACGGAGGATAGCGTGAGG-3') designed according to the conserved regions of plant 18S rRNA genes was performed to estimate if equal amounts of RNA among samples were used as an internal control.

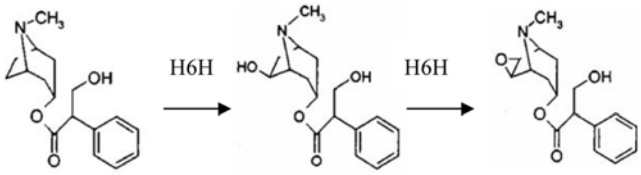

Fig. 1. Biosynthetic pathway from hyoscyamine to scopolamine. Hyoscyamine 6 β-hydroxylase (H6H) catalyzes both the hydroxylation of hyoscyamine and the epoxidation of 6 β-hydroxyhyoscyamine to generate scopolamine.

Amplifications were performed under the following condition: 50°C for 30 min, 94°C for 2 min followed by 25 cycles of amplification (94°C for 50 s, 60°C for 50 s and 72°C for 120 s). RT-PCR images were captured using a UVP transilluminator (UVP).

Results and discussion

Cloning and sequencing of the full-length cDNA of *aah6h*. Based on sequences of the conserved regions of known *h6h* genes from other plant species (Matsuda *et al*., 1991; Suzuki *et al*., 1999; Liu *et al*., 2005), a degenerate primer (F2) was designed and used for the amplification of 3' end of *h6h* from *A. acutangulus*. A single fragment of about 0.9 kb was amplified using primers F2 and AUAP, and a 3' untranslated region (UTR) of 205-bp was found downstream from the stop codon in the amplified sequence. According to the 3' fragment sequence, a specific reverse primer R2 was designed to amplify the 5' end of *aah6h* and then a fragment about 0.65 kb was obtained in which a 5' UTR of 124-bp was found upstream of the first ATG codon. By aligning and assembling the 3' RACE and 5'RACE products, the full-length cDNA of *aah6h* was deduced and confirmed by RT-PCR using primer F1 and AUAP.

The cloned full-length cDNA of *aah6h* gene was 1380 bp, which size was very similar to others reported *h6h* genes (Matsuda *et al*., 1991; Suzuki *et al*., 1999; Liu *et al*., 2005). The cDNA contained a 1035bp open reading frame (ORF) encoding a deduced protein of 344 amino acid residues. The deduced protein had an isoelectric point (pI) of 5.09 and a calculated molecular mass of about 38.7 kDa (Fig. 2). Blast search at NCBI website revealed that *aah6h* showed high homology (more than 90% identity) to some known *h6h* cDNAs from *Solanaceae* species such as *A. tanguticus, S. parviflora* and *H. niger*, suggesting that *aah6h* belongs to plant *h6h* gene superfamily. This indicated scopolamine biosynthetic pathway was highly conserved in some *Solanaceae* species (Hsiao *et al*., 1973; Evans, 1979; Hashimoto *et al*., 1994; Liu *et al*., 2005).

The richest amino acid in the deduced H6H was Leu (11.7% by frequency), followed by Glu (7.8%), Lys (7.5%),

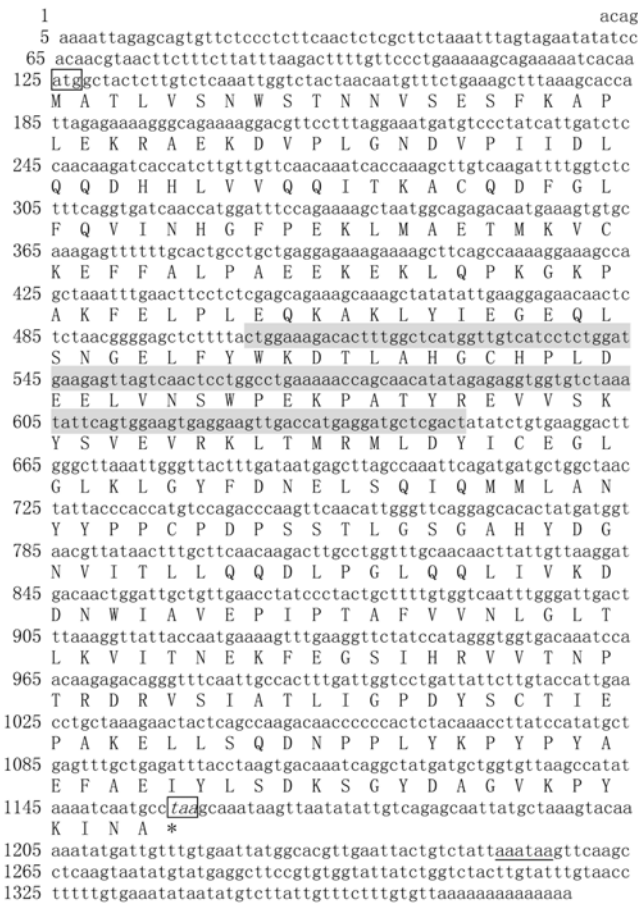

Fig. 2. The full-length cDNA sequence and deduced amnio acid sequence of *aah6h*. The start codon (ATG) is boxed and the stop conon (TAA) is underlined italically.

Pro (7.5%), Val (6.7%) and Ala (6.3%). Acidic and basic amino acids constituted 14% and 10% of the polypeptide, respectively. 32% of the total amino acids were charged and the percentages of polar and hydrophobic amino acids were 26% and 34%, respectively.

Bioinformatics analysis of AaH6H. Sequence comparison by performing Blast Search in GenBank database (http://www.ncbi.nih.gov) revealed that AaH6H had high homology with many other H6Hs such as *A. tanguticus* H6H (AtH6H); *A. belladonna* H6H (AbH6H); *S. parviflora* H6H (SpH6H), *H. niger* H6H (HnH6H) and *D. metel* H6H (DmH6H) (Matsuda *et al*., 1991; Suzuki *et al*., 1999; Liu *et al*., 2005), suggesting that AaH6H belongs to the plant H6H superfamily. On the amino acid level, AaH6H was 94, 92, 91, 89 and 86% identical to AtH6H, AbH6H, SpH6H, HnH6H and DmH6H, respectively, and was 97, 95, 94, 95 and 93% similar to AtH6H, AbH6H, SpH6H, HnH6H and DmH6H, respectively (Fig. 3). AaH6H even showed high similarity (about 61%) to other 2-oxoglutarate-dependent dioxygenases from some solanaceous species including *Solanum demissum, S. tuberosum, S. melongena, S. lycopersicum* and *S. chacoense*, respectively, indicating that there is high conservation among plant dioxygenases. The highest conservation with *A. tanguticus* is due to both plants belonging to the same species *Anisodus*.

Multiple sequence alignment of AaH6H with some homologous H6Hs from family *Solanaceae* plant revealed that very high conservation of H6H exited among various species (Fig. 3). The deduced AaH6H polypeptide shows typical motifs of 2-oxoglutarate-dependent dioxygenase and contained the similar active sites for dioxygenase activity, such as the two iron-binding regions corresponding to amino acid residues Gly^{59}-Gly^{67} and Val^{253}-Val^{276}, and the 2-oxoglutarate co-substrate binding region corresponding to his^{217}-Asp^{229} (Matsuda *et al.*, 1991; Suzuki *et al.*, 1999), suggesting that AaH6H belongs to 2-oxoglutarate-dependent dioxygenase superfamily (Fig. 3, marked in box). The above three conserved regions might be important for the enzyme function specific to the dioxygenase (*e.g.* for binding 2-oxoglutarate co-substrate) and thus are preserved in evolution.

The secondary structure of AaH6H was analyzed by SOPMA (Geourjon and Deléage 1995) and the result showed that the putative AaH6H polypeptide contained 33% of alpha helices, 19% of extended strands, 3% of β turns and 44% of random coils. The alpha helices and random coils constituted interlaced domain of the main part of the secondary structure.

Molecular evolution analysis. H6H is part of the scopolamine biosynthetic pathway in certain species of the *Solanaceae* (Kanegae *et al.*, 1994). To investigate the evolutionary relationship between AaH6H and other highly homologous hydroxylases from solanaceous plants, a phylogenetic tree was constructed as described above. The result showed that AaH6H, AtH6H, AbH6H, SpH6H, HnH6H and DmH6H were expectedly grouped into the one cluster (Group I), while other 2-oxoglutarate-dependent dioxygenases from some solanaceous species including *S. demissum, S. tuberosum, S. melongena, S. lycopersicum* and *S. chacoense* were naturally classified into the other cluster (Group II). This result agrees well with that *A. acutangulus, A. belladonna*, *D. metel*, *H. niger*, *S. parviflora* and *A. tanguticus* can produce scopolamine (Hsiao *et al.*, 1973; Zhang *et al.*, 2004; 2005), whereas *S. demissum, S. tuberosum, S. melongena, S. lycopersicum* and *S. chacoense* can not. Thus, H6Hs seem to occur only in scopolamine-producing plants (Matsuda *et al.*, 1991; Suzuki *et al.*, 1999; Liu *et al.*, 2005).

As shown in dendrogram (Fig. 4), all the solanaceous hydroxylases evolved from a common ancestor based on their similar roles and conserved structural and sequence characteristics such as amino acid homologies and conserved domain motifs. This implies that the conserved motifs may play an important role in the biological functions and thus are preserved in evolution, while some variations on un-conserved domain can form the molecular foundation for the diversity of hydroxylases' structures and functions (Kai *et al*., 2006b).

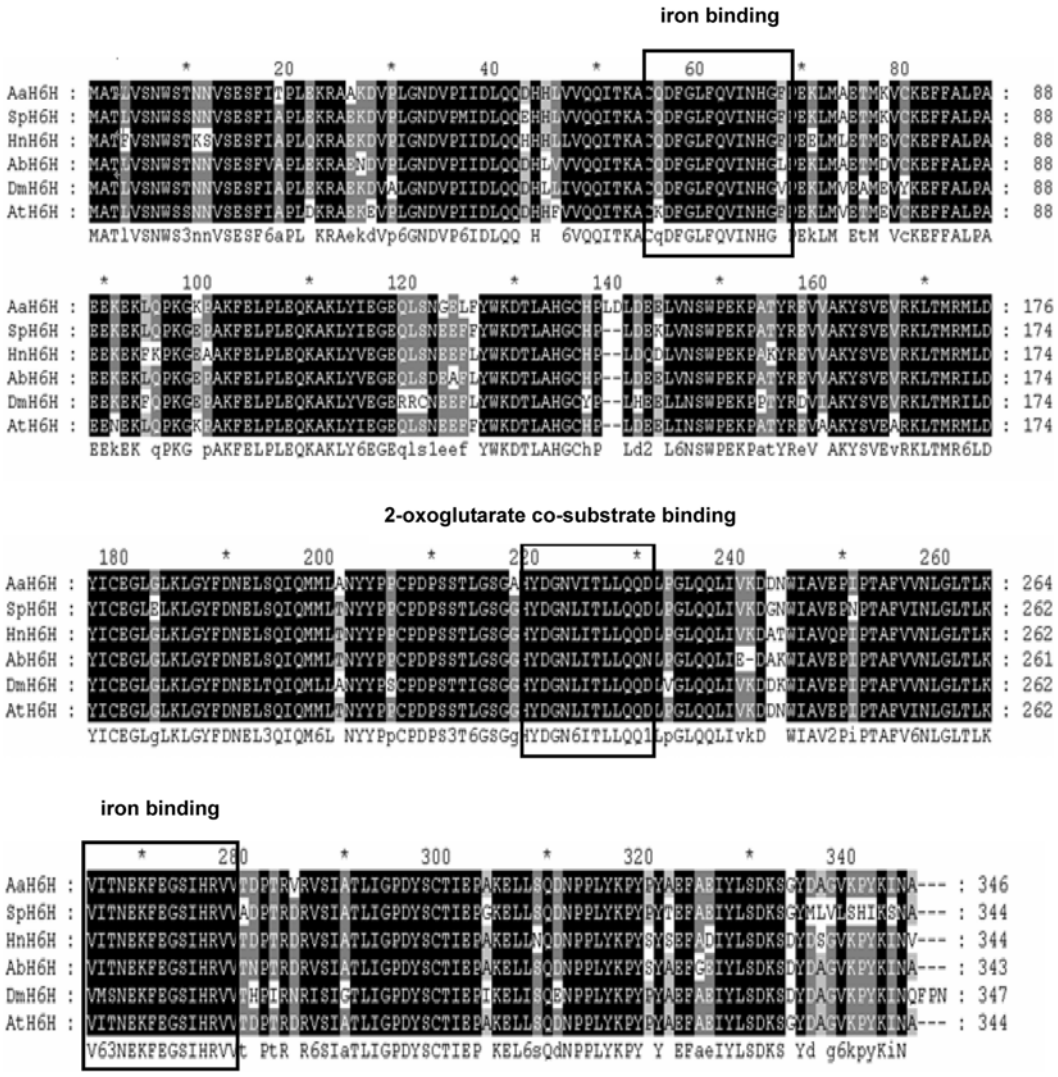

Fig. 3. Multiple sequence alignments of AaH6H with other known plant H6Hs including AbH6H (*A. belladonna* H6H, BAA78340), AtH6H (*A. tanguticus* H6H, AAQ75700), DmH6H (*D. metel* H6H, AAQ04302), HnH6H (*H. niger* H6H, ABG89397), SpH6H (*S. parviflora* H6H, AAY53932). Black boxes and grey boxes indicate the completely identical residues and the conserved residues among the aligned sequences, respectively. Two iron binding regions and a 2-oxoglutrate co-substrate binding site are boxed, respectively. The different numbers (1-6) represent various combinations of amino acid residues as follows, 1:DN; 2:EQ; 3:ST; 4:KR; 5:FYW and 6:LIVM.

Southern blotting analysis. To examine the copy number of the *aah6h* gene, 15 μg genomic DNA of *A. acutangulus*, was digested with *Bam*HI, *Eco*RI and *Eco*RV, respectively. Southern blotting was performed using the probe described above. The result showed that there were several hybridization bands on the three lanes (Fig. 5), suggesting that the *aah6h* gene belongs to a multi-copy gene family. This result was not consistent with the earlier studies that *h6h* genes from *H. niger, H. muticus* and *A. belladonna* belong to low-copy gene family (Yun et *et al.*, 1992; Kanegae *et al.*, 1994).

Tissue expression pattern analysis. The underground parts (roots) of tropane alkaloid-producing plants such as *H. niger* etc. are usually used as main source for tropane alkaloid extraction, as tropane alkaloid content in underground parts is higher than that in aerial parts of plants (Hsiao *et al.*, 1973; Matsuda *et al*., 1991; Hashimoto *et al*., 1991). Hence, it is interesting to know if *aah6h* expression is positively correlated with the tropane alkaloid content in different parts of the *A. acutangulus* plant. Total RNA was isolated from different tissues including root, stem and leaf, respectively, and subjected to one-step RT-PCR analysis using the primers KF1 and KR1. As shown in Fig. 6, *aah6h* gene transcripts could be detected in all the tested tissues including roots, stems and leaves, so *aah6h* was considered to be a constitutive-expression gene, which seemed to be not contrary to the fact that tropane alkaloids existed in the above tissues (Zeng, 1962; Hsiao *et al.*, 1973; Zheng and Liang, 1976). Interestingly, *aah6h*

Fig. 4. Phylogenetic tree analysis of AaH6H with H6H from *A. tanguticus* (AAQ75700); *A. belladonna* (BAA78340); *S. parviflora* (AAY53932), *H. niger* (ABG89397) and *D. metel* (AAQ04302), and dioxygenase from *S. demissum* (AAT40509), *S. tuberosum* (BAC23050), *S. melongena* (CAA54557), *S. lycopersicum* (AAA80501) and *S. chacoense* (AAC95363).

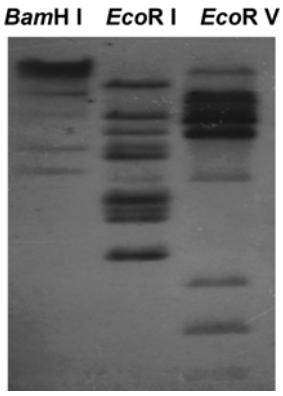

Fig. 5. Southern blotting analysis to determine the copy number of *aah6h* gene in the genome of *A. acutangulus*. Lane 1~3: Genomic DNA of *A. acutangulus* was digested with *Bam*HI, *Eco*RI and *Eco*RV, respectively.

expression in this plant is almost ubiquitous as revealed by RT-PCR analysis, however, this result is not in good accordance with earlier findings that expression of *h6h* gene was root-specific in other *solanaceae* species such as *H. niger* (Matsuda *et al*., 1991; Kanegae *et al*., 1994) and *A. belladonna* (Suzuki *et al*., 1999), reflecting that the complexity of *h6h* gene expression may diverge significantly in different plant species.

Though *aah6h* expression is detected in roots and aerial parts of this plant, this species doesn't accumulate more scopolamine than hyoscyamine reported before (Wu *et al*., 1962). Total alkaloid content in *A. acutangulus* is over 1% and much more than those in *H. niger*, *D. stramonium* and *A. belladonna* etc., and most of alkaloid is hyoscyamine (about

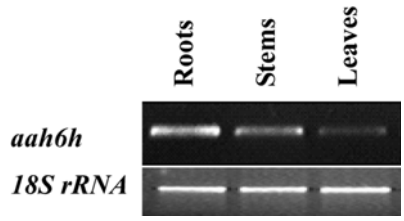

Fig. 6. Expression pattern of *aah6h* in different tissues of *A. acutangulus*. Total RNA (0.5 μg) was isolated from roots, stems and leaves, respectively, and subjected to one-step RT-PCR amplification (upper panel). 18S rRNA gene was used as the control to show the normalization of the templates in PCR reactions (lower panel).

0.85%) while scopolamine is 0.018% (Wu *et al*., 1962), the alkaloid profile of this species is similar to other related species. Tropane alkaloids including hyoscyamine, are synthesized mainly in the root, after which they are translocated to the leaf (Yun *et al*., 1992). It was reported that the scopolamine content of *A. belladonna* was relatively high in the seedling and vegetative stages but progressively decreased toward the flowering stage, at which time total alkaloid content had considerably increased and hyoscyamine was the predominant alkaloid (Yun *et al*., 1992). This phenomenon was also observed and testified in *A. acutangulus* at flowering stage (Hsiao *et al*., 1973). Therefore, the content and composition of tropane alkaloids may vary considerably based on plant developmental stages as well as producing area and growing environment etc.

In conclusion, molecular cloning and characterization of AaH6H showed that AaH6H was very similar to other H6Hs from scopolamine-producing plants with typical motifs of 2-oxoglutarate-dependent dioxygenase, indicating that AaH6H belonged to plant H6H superfamily. Based on the cloning and characterization of *aah6h*, plant expression vector containing the *aah6h* has been constructed and genetic transformation of *A. acutangulus,* which is one of endemic sources currently for commercial production of hyoscyamine and scopolamine in China, is undergoing in order to test its potential role in improving scopolamine production by genetic engineering in the future.

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