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Full Length Research Paper

Cloning and characterization of two novel purple pepper genes (*CHS* and *F3H*)

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The complete coding sequences (CDS) of "Yunnan Purple Pepper No.1" (C*apsicum annuum* L.) *CHS* and *F3H* genes were amplified using the reverse transcriptase polymerase chain reaction based on the conserved sequence information of some Solanaceae plants and known highly homologous pepper ESTs. The nucleotide sequences analysis of these two genes revealed that pepper *CHS* gene encodes a protein of 402 amino acids that has high homology with the *CHS-like* protein of six species: *Nicotiana tabacum* (92%), *Rhododendron simsii* (91%), *Petunia × hybrida* (91%), *Solanum tuberosum* (90%), *Vitis vinifera* (90%) and *Camellia chekiangoleosa* (92%). Sequence analysis of the second gene revealed that the pepper *F3H* encodes a protein of 365 amino acids that has high homology with the proteins of eight species: *N. tabacum* (89%), *Petunia x hybrida* (88%), *S. tuberosum* (87%), *Solanum lycopersicum* (88%), *Litchi chinensis* (82%), *Actinidia chinensis* (81%), *Citrus* maxima (81%) and *V. vinifera* (82%). The tissue expression analysis indicated that the pepper *CHS* gene was over-expressed in pericarp, moderately in stem and flower, weakly in leaf and placenta, and hardly expressed in root and seed. The pepper *F3H* gene was over-expressed in pericarp; weakly in leaf, flower and seed, and hardly expressed in root, stem and placenta. Our experiment established the primary foundation for further research on these two pepper genes.

Key words: Pepper, *CHS*, *F3H*, tissue expression analysis.

INTRODUCTION

The chalcone synthase (CHS) is an important enzyme in anthocyanidins biosynthesis. It catalyzes the first committed step of the condensation of one molecule of 4-coumaroyl-COA with three molecules of malonyl-COA to form naringenin chalcone. Naringenin chalcone is the central intermediate to give flavonoids (Holton and Cornish, 1995; Schröder, 1997; Shirley, 2002), which are important for the pigmentation of flowers and other parts of plants. Several *CHS* cDNA and genomic clones have been isolated from a number of plant species, and the structure and reaction mechanism of higher plant *CHSs* proteins have been extensively studied (Ferrer et al., 1999; Suh et al., 2000; Jez and Noel, 2000; Niesbach-Klosgen et al., 1987; Koes et al., 1989).

Flavanone 3-hydroxylase (F3H) is one of the 'key' enzymes acting at the bifurcation of the anthocyanin and flavonols branches, which catalyzes the stereospecific hydroxylation of (2*S*) -eriodictyol and (2*S*)-naringenin to form (2*R*, 3*R*)-dihydroquercetin and (2*R*, 3*R*)-dihydrokaempferol, respectively (Britsch and Grisebach, 1986; Forkmann et al., 1980; Britsch et al., 1981; Heller and Forkmann, 1993). These dihydroflavonols serve as intermediates for the biosynthesis of anthocyanidins (Holton and Cornish, 1995). F3H is classified as a soluble 2-oxoglutarate-dependent dioxygenase based on its requirements for 2-oxoglutarate, molecular oxygen, ferrous iron (Fe^{2+}) and ascorbate. More *F3H* genes have

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been cloned and characterized from a variety of plant species, such as *Arabidopsis thaliana* (Pelletier and Winkel-Shirley, 1996), *Ginkgo biloba* (Shen et al., 2006) *Hordeum vulgare* (Meldgaard, 1992), *Malus* sp. (Davies, 1993), *Medicago sativa* (Charrier et al., 1995), *Perilla frutescenes* (Gong et al., 1997) and *Zea mays* (Deboo et al., 1995).

Due to the involvement of their products in many biological processes (ultraviolet radiation protection, flower coloration, antimicrobial activity, interspecies interactions, plant defense and medicinal properties), we decided to isolate the pepper *CHS* and *F3H* genes. "Yunnan Purple Pepper No.1" is a pepper variety with high content of anthocyanidins, which was selected from a Yunnan local pepper resources, but the pepper *CHS* and *F3H* genes associated with the biosynthesis anthocyanidins in that Chinese species have not been reported yet.

In the present work, we isolated the purple pepper genes encoding *CHS* and *F3H* owing to their high homology among Solanaceae plant species. We then analyzed the resulting sequences and established their tissue expression distribution.

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis

Yunnan Purple Pepper No.1 was grown in experimental fields of Yunnan Agricultural University. Fresh tissues (root, stem, leaf, flower, pericarp, placenta and seed) for RNA isolations were frozen in liquid nitrogen and stored at -80°C until further use. Total RNA was extracted using the RNAiso Plus (TaKaRa, Dalian) according to the manufacturer's instructions. To remove genomic DNA contamination, total RNA was digested with RNase-free DNase I (TaKaRa, Dalian). Three micrograms of RNA were reverse transcribed with oligo $(dT)_{18}$ primer and M-MLV reverse transcriptase (Invitrogen, USA). The efficiency of reverse transcription was checked on 2% agarose gels stained with ethidium bromide.

Isolation of the pepper *CHS* and *F3H* genes

The real-time polymerase chain reaction (RT-PCR) was performed to isolate these two pepper genes using the cDNAs from different tissues above. The primers for pepper *CHS* and *F3H* gene isolation were designed based on the conserved coding sequences information from some Solanaceae *CHS* and *F3H* genes and their highly homologous pepper ESTs sequences. The primers for pepper *CHS* were: 5'-CCAGCTAGTTGGTATTTCT-3' and 5'-TAGTCACCCAGTTTATTCG-3' and the primers for pepper *F3H* gene were: 5'-ATAGAAATGCCACCTTCAT-3' and 5'-TTAAGCAAGAATTTCCTCAAT-3'. RT-PCR was carried out as previously described (deng et al., 2011). After the PCR, the gene product was cloned into pMD18-T vector (TaKaRa, Dalian) and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced.

Bioinformatics analysis

Sequence analysis of pepper *CHS* and *F3H* genes were performed using softwares in NCBI (http://www.ncbi.nlm.nih.gov) and ExPaSy (http://www.expasy.org). The cDNA sequences were predicted using the GenScan software (http://genes.mit.edu/GENSCAN.html). Putative protein theoretical molecular weight (Mw) and isoelectric point (pI) prediction, signal peptide prediction, subcellular localization prediction and transmembrane topology prediction were performed using the Compute pI/Mw Tool (http://us.expasy.org/tools/pi_tool.html), SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/), PSort II (http://psort.hgc.jp/), TMHMM-2.0 server (http://www.cbs.dtu.dk/ services/TMHMM-2.0/), respectively. The Blastp program and Conserved Domain Architecture Retrieval Tool were used to search for similar proteins and conserved domain, respectively (http://www.ncbi.nlm.nih.gov/Blast). The alignment of the nucleotide sequences and deduced amino acid sequences were computed using ClusterX, and the phylogenetic trees were computed using the ClustalX and Mega 4.0 softwares with standard parameters. Secondary structures of deduced amino acid sequences were predicted with SOPMA (http://npsa-pbil.ibcp.fr/). The 3D structures were predicted based on the existing 3D structures by the amino acids homology modeling on swiss server (http://swissmodel.expasy.org/).

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously reported (Deng et al., 2011). The housekeeping gene *Actin* was selected as a positive control. Control primers used were 5'-TGCAGGAATCCACGAGACTAC-3' and 5'-TACCACCACTGAGCACAATGTT-3'. The primers used were the same as those used for isolation RT-PCR above.

RESULTS

Cloning and identification of pepper *CHS* and *F3H* cDNA

Using different tissue cDNAs, the RT-PCR products for the pepper *CHS* and *F3H* genes were 1296 and 1104 bp (Figure 1). These cDNA nucleotide sequences analysis using the BLAST software at NCBI server revealed that pepper *CHS* and *F3H* genes were not homologous to any of the known pepper genes and they were then deposited into the GenBank database (Accession No. JN808444 and JN808445). The sequences prediction were carried out using the GenScan software and results showed that the 1209 and 1098 bp cDNA sequence represent two single genes which encoded 402 and 365 amino acids, respectively. The complete coding sequences (CDS) and the encoded amino acids are presented in Figures 2 and 3.

Physical and chemical characteristics of pepper *CHS* and *F3H*

The theoretical pI and Mw of pepper *CHS* and *F3H*, computed using the Compute pI/Mw Tool, were 5.81, 5.31, 44317 and 41108, respectively. The signal peptide prediction performed by SignalP 3.0 on the basis of a combination of several artificial neural networks and

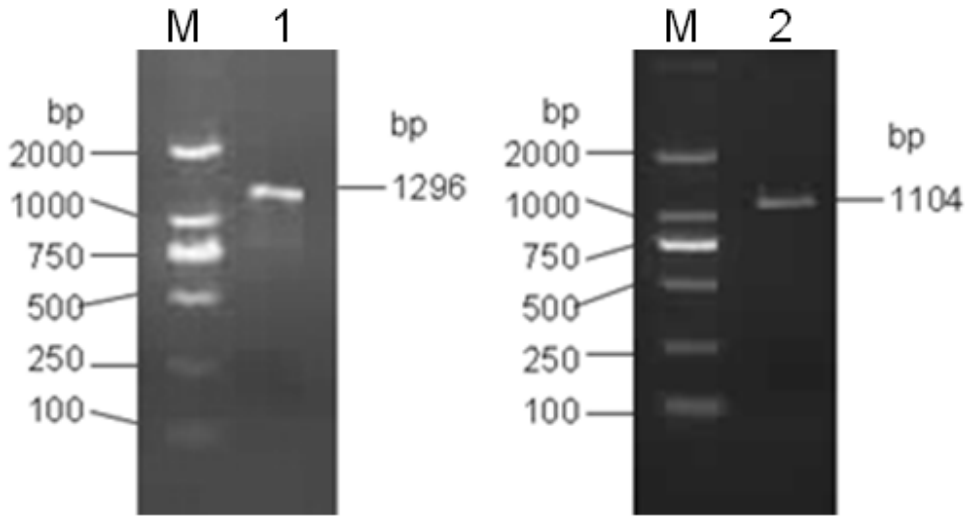

Figure 1. RT-PCR result for pepper *CHS* and *F3H* genes. M, DL2000 DNA marker; 1, PCR product for pepper *CHS* gene; 2, PCR product for pepper *F3H* gene.

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ATGGTGACCGTGGAGGAGGTTCGAAGGGAACAACGCGCAAAGGGACCAGCCACCATCATGGCCATTGGCACGGCGACTCCTTCAAACTGT
 M  V  T  V  E  E  V  R  R  E  Q  R  A  K  G  P  A  T  I  M  A  I  G  T  A  T  P  S  N  C
GTTGATCAAGCCACCTATCCTGATTATTACTTTCGAATCACTAATAGCGAACATATGACTGAGCTTAAGGAAAAATTTCAACGCATGTGT
 V  D  Q  A  T  Y  P  D  Y  Y  F  R  I  T  N  S  E  H  M  T  E  L  K  E  K  F  Q  R  M  C
GATAAATCAATGATTAAGAAGAGGTATATGCATTTAACAGAAGAAATTCTTAAAGAAAATCCTAATATTTGTGAATATATGGCTCCTTCT
 D  K  S  M  I  K  K  R  Y  M  H  L  T  E  E  I  L  K  E  N  P  N  I  C  E  Y  M  A  P  S
CTTGATGCTAGGCAAGATATAGTGGTGGTTGAAATTCCAAAACTTGGCAAAGAAGCAGCCAAAAAGGCTATTAAAGAATGGGGCCAGCCC
 L  D  A  R  Q  D  I  V  V  V  E  I  P  K  L  G  K  E  A  A  K  K  A  I  K  E  W  G  Q  P
AAATCGAAGATTACCCATTTGGTGTTTTGCACTACTAGTGGTGTGGACATGCCCGGGGCTGACTACCAGCTCACTAAGCTTCTTGGGCTT
 K  S  K  I  T  H  L  V  F  C  T  T  S  G  V  D  M  P  G  A  D  Y  Q  L  T  K  L  L  G  L
CGACCCTCAGTCAAACGACTCATGATGTACCAACAAGGTTGCTTTGCTGGTGGAACCGTTATCCGACTAGCAAAAGACTTGGCTGAAAAC
 R  P  S  V  K  R  L  M  M  Y  Q  Q  G  C  F  A  G  G  T  V  I  R  L  A  K  D  L  A  E  N
AACAAGGGAGCTCGAGTCCTTGTTGTTTGCTCTGAAATAACTGCAGTTACTTTCCGTGGCCCAAGTGATACACACTTAGATAGTATGGTT
 N  K  G  A  R  V  L  V  V  C  S  E  I  T  A  V  T  F  R  G  P  S  D  T  H  L  D  S  M  V
GGACAAGCACTCTTTGGTGATGGGGCAGCCGCACTCATTGTAGGTTCAGATCCATTACCAGAGGTTGAAAgGCCTTTGTTTGAGCTTGTT
 G  Q  A  L  F  G  D  G  A  A  A  L  I  V  G  S  D  P  L  P  E  V  E  R  P  L  F  E  L  V
TCTGCGGCCCAAACTCTTCTCCCTGATAGCGAAGGCGCTATAGATGGTCACCTTCGTGAAGTTGGGCTAACATTTCACTTACTCAAAGAT
 S  A  A  Q  T  L  L  P  D  S  E  G  A  I  D  G  H  L  R  E  V  G  L  T  F  H  L  L  K  D
GTTCCTGGATTGATCTCAAAGAATATCGAGAAGAGTTTGATAGAAGCATTCCAACCATTGGGGATTTCTGATTGGAACTCTATCTTCTgG
 V  P  G  L  I  S  K  N  I  E  K  S  L  I  E  A  F  Q  P  L  G  I  S  D  W  N  S  I  F  W
ATCGCTCACCCTGGCGGGCCAGCAATCCTCGACCAAGTTGAATTAAAGTTGGGCCTAAAGCTCGAAAAACTTCGAGCTACTAGGCAAGTC
 I  A  H  P  G  G  P  A  I  L  D  Q  V  E  L  K  L  G  L  K  L  E  K  L  R  A  T  R  Q  V
TTGAGTGACTATGGAAACATGTCTAGTGCTTGTGTTCTATTCATTTTGGATGAAATGAGAAAGGCCTCAGCCAAAGAAGGACTTAATACT
 L  S  D  Y  G  N  M  S  S  A  C  V  L  F  I  L  D  E  M  R  K  A  S  A  K  E  G  L  N  T
ACTGGTGAAGGCCTTGATTGGGGTGTGCTTTTCGGATTTGGGCCTGGGCTTACAGTTGAGACTGTTGTACTCCATAGTGTCTCTACTAAT
 T  G  E  G  L  D  W  G  V  L  F  G  F  G  P  G  L  T  V  E  T  V  V  L  H  S  V  S  T  N
CTCGAGCACCCTAGGGTGCAAGAGAAAGAGTTCGAATAA
 L  E  H  P  R  V  Q  E  K  E  F  E  .
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Figure 2. The complete cDNA sequence and amino acid sequence of the protein encoded by *CHS* (GenBank accession number: JN808444).

hidden Markov models revealed that pepper *CHS* and *F3H* did not contain a potential signal peptide with 0.7 and 0.3% probability, respectively (Bendtsen et al., 2004). Using a hidden Markov model algorithm, a transmembrane topology prediction made by the TMHMM program showed that pepper *CHS* and *F3H* were not potential membrane proteins (Moller et al., 2001). For subcellular localization analysis, the amino acid

```
ATGCCACCTTCATCCCTAACGTCTCTGGCGAACGAAACTACCGTTCCAACAAGTTTCATTAGGGATGAACAAGAGCGTTCTAAAGTGGCT
 M  P  P  S  S  L  T  S  L  A  N  E  T  T  V  P  T  S  F  I  R  D  E  Q  E  R  S  K  V  A
TACAACAACTTCAGTGATGACATTCCAGTCATATCGTTGAAGGATATTGATGAGATTGGAACAAGAGGTGAAATATGTGAAAAAATTGTA
 Y  N  N  F  S  D  D  I  P  V  I  S  L  K  D  I  D  E  I  G  T  R  G  E  I  C  E  K  I  V
GAGGCATGCGAAGATTGGGGCATTTTCCAGGTAGTTGATCATGGGGTAGACCCACAATTAATCTCACAAATGACAAAATTTGCTAAAGAA
 E  A  C  E  D  W  G  I  F  Q  V  V  D  H  G  V  D  P  Q  L  I  S  Q  M  T  K  F  A  K  E
TTCTTCGCGTTGCCCTCTGAGGAAAAGCTTCGATTTGACATGTCCGGCGGTAAGAAAGGTGGTTTCATAGTCTCTAGCCATCTTCAGGGT
 F  F  A  L  P  S  E  E  K  L  R  F  D  M  S  G  G  K  K  G  G  F  I  V  S  S  H  L  Q  G
GAAGTGGTCCAAGATTGGCGTGAAATAGTGACCTATTTCTCATACCCAATTCGAGCTAGAGATTACTCTAGATGGCCAGACAAACCGCAC
 E  V  V  Q  D  W  R  E  I  V  T  Y  F  S  Y  P  I  R  A  R  D  Y  S  R  W  P  D  K  P  H
GGATGGATAGCTGTAACTGAGAAGTACAGTGAAAAGTTAATGGAGTTGGCTTGCAAATTATTAGAAGTACTATCAGAAGCAATGGGTTTG
 G  W  I  A  V  T  E  K  Y  S  E  K  L  M  E  L  A  C  K  L  L  E  V  L  S  E  A  M  G  L
GAGAAGGAGGCCTTAACCAAGGCATGTGTGGATATGGACCAAAAAGTTGTTGTCAATTTTTACCCAAAGTGTCCACAGCCTGATCTTACC
 E  K  E  A  L  T  K  A  C  V  D  M  D  Q  K  V  V  V  N  F  Y  P  K  C  P  Q  P  D  L  T
CTTGGGTTGAAAAGGCACACTGATCCTGGAACCATCACCCTCTTGTTACAAGACCAAGTTGGTGGTCTTCAAGCCACTAAAGATAATGGC
 L  G  L  K  R  H  T  D  P  G  T  I  T  L  L  L  Q  D  Q  V  G  G  L  Q  A  T  K  D  N  G
AAAACTTGGATCACGGTTAAGCCCATTGAAGGCGCTTTTGTTGTTAATCTTGGTGATCATGGTCATTATTTGAGCAACGGGAGGTTCAAG
 K  T  W  I  T  V  K  P  I  E  G  A  F  V  V  N  L  G  D  H  G  H  Y  L  S  N  G  R  F  K
AACGCTGATCATCAAGCAGTGGTGAACTCGAATAGCAGCAGATTATCGATAGCCACATTTCAGAATCCAGCACCGGAGGCAATAGTGTAT
 N  A  D  H  Q  A  V  V  N  S  N  S  S  R  L  S  I  A  T  F  Q  N  P  A  P  E  A  I  V  Y
CCATTAAAAATACGAGAAGGAGAGAAGGCAGTAATGGATGAGCCCATTACATTTGCAGAAATGTATAGGAGGAAAATGAGTAAGGATCTT
 P  L  K  I  R  E  G  E  K  A  V  M  D  E  P  I  T  F  A  E  M  Y  R  R  K  M  S  K  D  L
GAGGCTGCTAGATTCAAAAAGCTGGCCAAGGAGCAGCAGATACAAGCTGAAGAGGTTGCCGAAAAGGCCAAGTTGGAATCCATGCCCATT
 E  A  A  R  F  K  K  L  A  K  E  Q  Q  I  Q  A  E  E  V  A  E  K  A  K  L  E  S  M  P  I
GAGGAATTCCTTGCTTAA
 E  E  F  L  A  .
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Figure 3. The complete cDNA sequence and amino acid sequence of the protein encoded by *F3H* (GenBank accession number: JN808445).

sequences were submitted to the PSORT program, and Reinhardt's method showed that pepper *CHS* and *F3H* were probably located in the cytoplasm with upto 45 and 73.9% probability, respectively (Nakai et al., 1999).

Prediction and analysis of structures and conserved domains of pepper *CHS* and *F3H*

Proteins often contained several domains, each with their own evolutionary origins and functions. Examination using the Conserved Domain Architecture Retrieval Tool of BLAST at the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) indicated that pepper *CHS* and *F3H* contained one separated conserved domain-*CHS-like* and PLN03176 superfamily, respectively.

The prediction of secondary structure by SOPMA indicates that the deduced pepper *CHS* protein consisted of 45.02% alpha helix, 15.67% extended strands, 6.72% beta turn and 32.59% random coils and pepper *F3H* protein consisted of 37.81% alpha helix, 15.07% extended strands, 5.48% beta turn and 41.64% random coils.

Homology modeling

To better understand the detailed structures of pepper *CHS* and *F3H*, homology modeling of *CHS* and *F3H* were performed to estimate their 3D structures, which were similar to that of the alfalfa *CHS* (1bi5A, Single chain) and *A. thaliana* PLN03176 superfamily (1gp6A, single chain). The 3D structures analysis may provide the basis for further study of the relationship between structure and function of these genes from pepper and other species.

Analysis of sequence identity and evolutionary relationships of pepper *CHS* and *F3H*

The deduced protein sequences of pepper *CHS* and *F3H* were submitted to generate BLAST reciprocal best hits, and similarity comparison revealed that pepper *CHS* protein has high homology with the *CHS* proteins of six other species: *Nicotiana tabacum* (92%), *Rhododendron simsii* (91%), *Petunia* × *hybrida* (91%), *Solanum tuberosum* (90%), *Vitis vinifera* (90%) and *Camellia chekiangoleosa* (92%) (Figure 4). The pepper *F3H* has high homology with the proteins of other eight species: *N. tabacum* (89%), *Petunia* × *hybrida* (88%), *S. tuberosum* (87%), *Solanum lycopersicum* (88%), *Litchi chinensis* (82%), *Actinidia chinensis* (81%), *Citrus* maxima (81%) and *V. vinifera* (82%) (Figure 5).

To evaluate the evolutionary relationships of pepper *CHS* and *F3H* with other species, we constructed a phylogenetic tree using DNAstar, Cluster, Mega and

Figure 4. Alignment of the protein encoded by the pepper *CHS* and six other types of *CHS* from *N. tabacum* (AAK49457), *C. chekiangoleosa* (ADW11243), *S. tuberosum* (AEN83501), *Petunia x hybrida* (CAA32731), *R. simsii* (CAC88858) and *V. vinifera* (XP_002264019).

DNAman softwares on the basis of the *CHS* and *F3H* amino acid sequences, respectively. This analysis revealed a closer genetic relationship between the pepper *CHS* gene and that of *N. tabacum*, *C. chekiangoleosa* than with those of *R. simsii*, *V. vinifera S. tuberosum* and *Petunia* × *hybrida* (Figure 6). The pepper *F3H* gene has a closer genetic relationship with that of *N. tabacum* than with those of *Petunia* × *hybrida, S. lycopersicum*, *S.*

Figure 5. Alignment of the protein encoded by the pepper *F3H* and eight other types of *F3H* from *Petunia x hybrida* (AAC49929), *S. tuberosum* (AAM48289), *A. chinensis* (ACL54955), *C. maxima* (ADB92595), *L. chinensis* (ADO95201), *S. lycopersicum* (AEK99074), *N. tabacum* (BAF96938) and *V. vinifera* (XP_002267640).

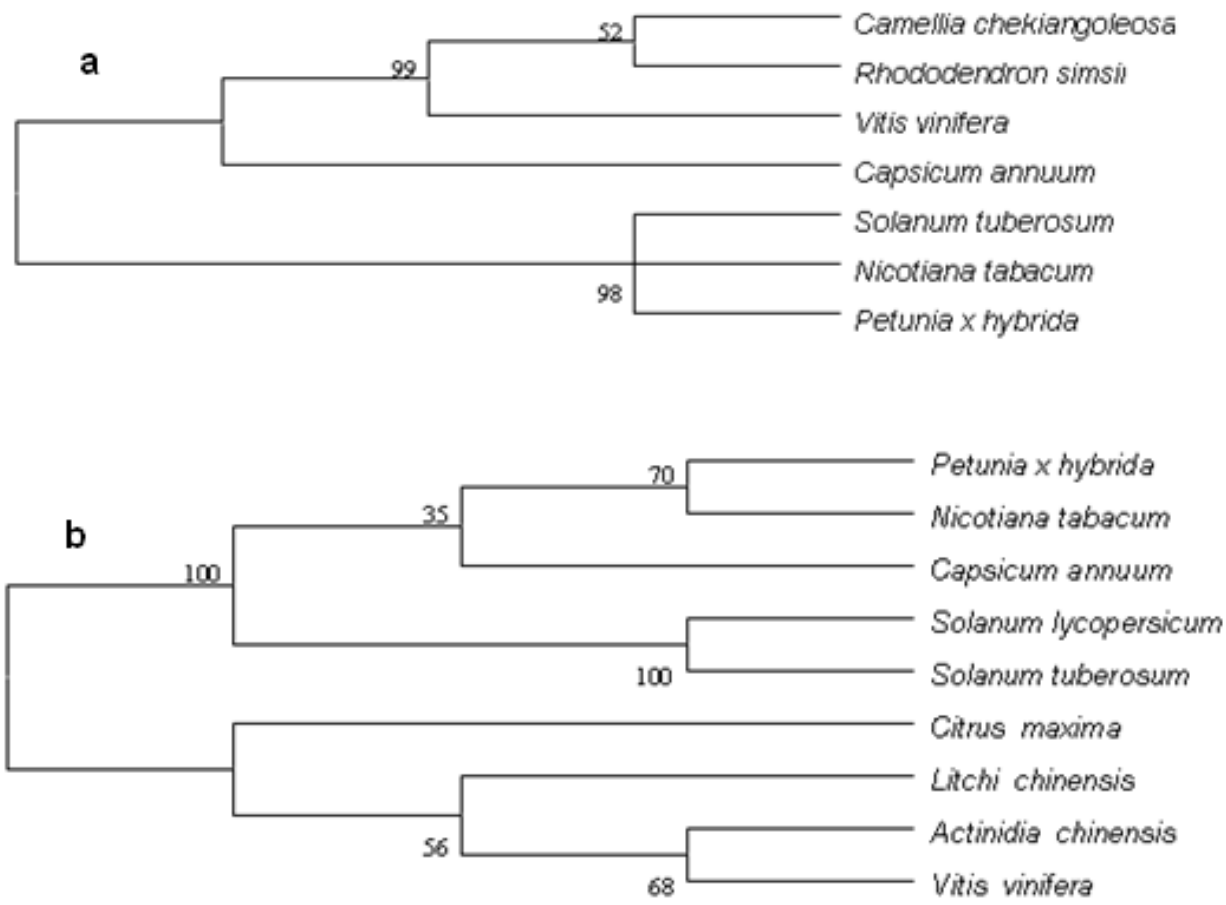

Figure 6. Phylogenetic tree for pepper *CHS* and *F3H* genes. a, *CHS*; b, *F3H.*

tuberosum, *C. maxima*, *L. chinensis*, *A. chinensis* and *V. vinifera* (Figure 6).

mRNA tissue-specific expression profile

Semi-quantitative RT-PCR was performed in seven pepper tissues to determine relative expression levels of pepper *CHS* and *F3H* mRNA. The continuously expressed gene (*Actin*), served as an endogenous reference for determination of targeted mRNA profiles. Results reveal that pepper *CHS* gene was over-expressed in pericarp, moderately in stem and flower, weakly in leaf and placenta, and hardly expressed in root and seed. The pepper *F3H* gene was over-expressed in pericarp; weakly in leaf, flower and seed, and hardly expressed in root, stem and placenta (Figure 7).

DISCUSSION

Comparative genomics determines the relationship of genome structure and function of different species (Hardison, 2003). Several researchers have shown that *CHS* or *F3H* proteins from different species are highly conserved (Suh et al., 2000; Davies, 1993; Charrier et al., 1995; Gong et al., 1997; Deboo et al., 1995). At the same time, these genes have been characterized at the genetic, chemical and enzymological levels (Ferrer et al., 1999; Jez and Noel, 2000; Niesbach-Klosgen et al., 1987; Koes et al., 1989; Pelletier and Winkel-Shirley, 1996; Shen et al., 2006; Meldgaard, 1992). However, cloning of *CHS* and *F3H* genes from pepper has not yet been reported.

In this study, pepper *CHS* and *F3H* genes were isolated and characterized. The isolated *CHS* cDNA is 1209 bp long and encodes 402 amino acids. Our comparison of its amino acid sequence showed high homologies (90%) to *N. tabacum*, *R. simsii*, *Petunia* × *hybrida*, *S. tuberosum*, *V. vinifera* and *C. chekiangoleosa*. The *F3H* cDNA is 1098 bp long, encodes 365 amino acids and has high homology (more than 81%) with the proteins of eight species: *N. tabacum*, *Petunia x hybrida*, *S. tuberosum*, *S. lycopersicum*, *L. chinensis*, *A. chinensis*, *C. maxima* and *V. vinifera*. Our results are in accordance with the results with *A. thaliana* (Pelletier and Winkel-Shirley, 1996) and *Pueraria Lobata* (Suh et al., 2000). This indicates that genes on the pathway at the primary steps controlling the secondary metabolites of anthocyanin biosynthetic

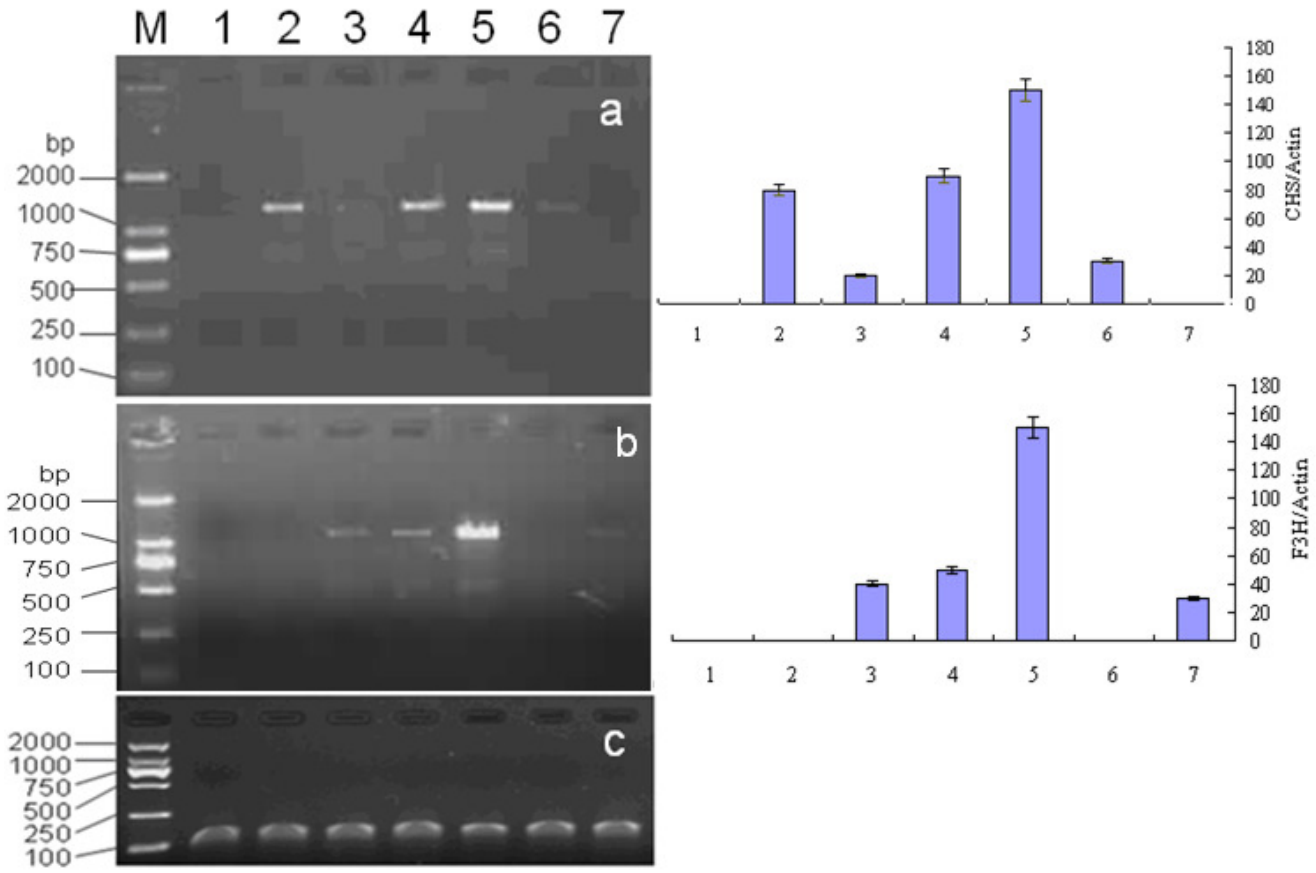

Figure 7. Tissue expression profile of pepper *CHS* and *F3H* genes. 1, root; 2, stem; 3, leaf; 4, flower; 5, pericarp; 6, placenta; 7, seed. The *Actin* expression level is used for the internal control. M, DL2000 DNA marker; a, *CHS*; b, *F3H*; c, *Actin*.

pathway are very conservative during the plant evolution. Gene expression shows that *CHS* and *F3H* genes are expressed with tissue specificity (mostly in photoautotrophic cells) (Todd and Vodkin, 1996). Our results suggest that *CHS* and *F3H* were obviously differentially expressed in various tissues (over-expressed in pericarp, moderately or weakly in leaf and flower). The plant of Yunnan Purple Pepper No.1 is violet black, especially the fruit. The *CHS* and *F3H* proteins that are accumulated in pericarp, leaf and flower are in accordance with anthocyanin accumulation in these tissues, but the reason why *F3H* was not checked in stem is unclear. *CHS* expressed in seed and *F3H* expressed in placenta may have other function(s).

In summary, we firstly isolated pepper *CHS* and *F3H* genes and performed some necessary analysis. Our results will be extremely important in elucidating the molecular mechanism of anthocyanidins biosynthesis.

ACKNOWLEDGEMENTS

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