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# 水蕨(*Ceratopteris thalictroides*)查尔酮合成酶基因的克隆和表达

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**摘 要**: 查尔酮合酶(chalcone synthase, CHS)是植物类黄酮化合物合成的关键酶, 有关蕨类植物 *CHS* 基因的序列及功能信息尚不完善。本研究采用快速扩增 cDNA 末端(RACE)技术克隆获得了模式蕨类植物——水蕨(*Ceratopteris thalictroides*) *CtCHS* 基因(GenBank 登录号: JX027616.1), 其 cDNA 序列全长为 1616 bp, 具有 3 个外显子和 2 个内含子, 开放阅读框(ORF)为 1215 bp, 编码 404 个氨基酸。进化树分析表明, *CtCHS* 与问荆(*Equisetum arvense*)、松叶蕨(*Psilotum nudum*)和 3 种薄囊蕨的查尔酮合成酶基因聚为一枝, 说明这些蕨类植物亲缘关系较近且为单系起源。通过构建原核表达体系成功获得 CtCHS 蛋白的多克隆抗体并用于免疫印迹分析, 结果表明 *CtCHS* 基因的表达明显受紫外光(UV)诱导。*CtCHS* 基因的克隆与表达分析为进一步研究水蕨类黄酮化合物的合成及其调控机制提供了依据。

**关键词**: 查尔酮合成酶(CHS); RACE; 水蕨

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# Isolation and Expression Profiling of Gene Encoding Chalcone Synthase in *Ceratopteris thalictroides*

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**Abstract**: Chalcone synthase (CHS) is a key enzyme in the synthesis of plant flavonoids. However, information on *CHS* genes in ferns is still unclear. In this study, rapid amplification of cDNA ends (RACE) was used to isolate the full-length sequence of the *CHS* gene from model fern *Ceratopteris thalictroides* (*CtCHS*, GenBank accession number: JX027616.1). Sequence analysis showed that the full length of the *CtCHS* gene was 1616 bp, with three exons and two introns. Its ORF region was 1215 bp, encoding 404 amino acids. Phylogenetic analysis indicated that *CtCHS* was clustered with the other ferns, including *Equisetum arvense*, *Psilotum nudum* and three leptosporangiate ferns, which reflected the monophyletic feature of the ferns according to Smith's system. Western blot analysis showed that the expression of this gene was significantly affected by ultraviolet (UV) treatment. In this study, the full-length sequence of *CtCHS* was cloned and the function of the CtCHS protein was studied, thus providing molecular information for further studies on the effect of *CtCHS* on flavonoids production.

**Key words**: Chalcone synthase (CHS); Rapid amplification of cDNA end (RACE); *Ceratopteris thalictroides*

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Flavonoids are widely distributed in terrestrial plants, including most of the bryophytes, all pteridophytes (fern allies and ferns) and spermatophytes[1]. So far, more than 10 000 flavonoids have been identified in the plant kingdom[2-5]. The biosynthetic pathway of flavonoids is well known in spermatophytes, but unclear in spore-bearing plants. Chalcone synthase (CHS) is a key enzyme that catalyzes the first step in the flavonoid biosynthetic pathway[6]. It is a member of the plant polyketide synthase (PKS) superfamily[7]. So far, several hundred *CHS* genes have been cloned from plants, and the structure and reaction mechanism of higher plant *CHS* genes have been studied[8-12]. The *CHS* gene has become an attractive model for studying the regulation of gene expression and evolution of gene families[13-16].

In pteridophytes, the *CHS* genes of *Equisetum arvense*, *Psilotum nudum* and three leptosporangiate ferns have been cloned; however, the functions of these genes have not been studied. In the present investigation, the fern *Ceratopteris thalictroides* was selected to clone the chalcone synthase gene and analyze its function. *Ceratopteris* ferns are regarded as model plants for studying the genetics, biochemistry, and cell biology of basic biological processes[17]. The high-efficiency stable transformation of the genus *Ceratopteris* has also been established[18,19], which provides the possibility for studying gene functions of ferns. The cloning and expression of the *C. thalictroides* chalcone synthesis gene can provide useful information for studying flavonoid biosynthesis and clarifying the flavonoid biosynthetic pathway in ferns.

## 1 Materials and methods

### 1.1 Isolation of total RNA and genomic DNA

The spores of *C. thalictroides* were collected from plants in the botanical garden of the Shanghai Normal University. The spores were surface sterilized with 5% sodium hypochlorite solution for 3 min. After rinsing three times with distilled water, the spores were sown on MS medium in culture dishes. These dishes were placed in an artificial climate chamber under conditions of 24℃ and a light: dark schedule of 18 h : 6 h. After 2 to 4 weeks, the prothalli of *C. thalictroides* were collected. Total RNA was extracted from the prothalli (0.5 g) using the TRIzoL Reagent (Invitrogen, USA) method. The cDNA was synthesized from 2 μg of mRNA using Superscript Ⅲ reverse transcriptase (Invitrogen, USA) and the T17Ap primer according to the manufacturer's instructions. Following RNase H treatment, the resulting single-strand cDNA mixture was used as a template for polymerase chain reaction (PCR). Subsequently, genomic DNA was extracted from the prothalli using the CTAB method. The concentration and quality of the RNA and DNA were all measured by agarose gel electrophoresis and spectrophotometer analysis before use.

### 1.2 Generation of the full-length DNA sequence of *CtCHS*

For PCR amplification of the core region of the cDNA encoding CHS-like enzymes, one set of the degenerate oligonucleotide primers was synthesized according to the highly conserved amino acid sequences of chalcone synthases (GenBank). The first PCR was performed using LA *Taq* DNA polymerase (Takara, Japan) with the set of degenerate primers (*CtCHS*-P1: 5'-GARAARTTCAAGCGCATGTG-3' and *CtCHS*-P2: 5'-GTAGTCRGCSCCRGGCATGT-3'). The PCR program was: 5 min of pre-denaturation at 94℃, 35 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 61.7℃, 30 s of extension at 72℃, and 10 min of final extension at 72℃.

The core sequence thus obtained was used

to design specific primers for the rapid amplification of cDNA ends (RACE) to determine full-length nucleotide sequences including 5'- and 3'-untranslated regions of cDNA. Both 5'- and 3'-RACE were performed using a cDNA amplification kit (Invitrogen, USA). For 3'-RACE, total RNA was reverse-transcribed using the adapter primer (5'-TTGATGGCCTTGGTAGCAGCCTCCTTG-3'). The PCR program was: 5 min of predenaturation at 94℃, 30 cycles of 30 s of denaturation at 94℃, 30 s of annealing at 63℃, 30 s of extension at 72℃, and 10 min of final extension at 72℃, with the universal amplification primer as the antisense primer. Likewise, 5'-RACE procedures including terminal deoxynucleotidyl transferase tailing were performed according to the manufacturer's protocols using primer (5'-TTGATGGCCTTGGTAGCAGCCTCCTTG-3'). The PCR program was the same as above apart from the annealing temperature, which was 66.7℃. The PCR amplifications were carried out three times and the PCR products were ligated into pEASY-T5 zero cloning vector (TransGen Biotech, Beijing) and cloned in Trans1-T1 phage resistant competent cells followed by sequencing from both sides.

### 1.3 Construction of the expression vector pET32a-*CtCHS* in Escherichia coli

A pair of specific primers, that is, *CtCHS*-P3 (5'-CGGGATCC ATG CCGGCCCATAG-3', start codon boxed and *Bam*H Ⅰ restriction enzyme site underlined) and *CtCHS*-P4 (5'-CCAAGCTT TTA GCTTGCGGTAAGGGG-3', stop codon boxed and *Hind* Ⅲ restriction enzyme site underlined), were designed and synthesized to amplify the coding region of *CtCHS* cDNA by PCR with the incorporation of the restriction enzyme site and protective base, which would simplify later vector construction. Using the cDNA as a template, the PCR program was the same as above apart from the annealing temperature, which was 57.5℃. The PCR product was purified, digested with *Bam*H Ⅰ and *Hind* Ⅲ, and then ligated into expression vector pET32a, which was pre-digested with the same enzymes. The resulting recombinant plasmid pET32a-*CtCHS* was then sequenced from both strands to confirm that the open reading frame was correct. Subsequently, pET32a-*CtCHS* was transferred into host strain BL21 for protein expression.

### 1.4 Expression and characterization of recombinant *CtCHS* in *E. coli*

A single colony of the *E. coli* strain BL21 harboring plasmid pET32a-*CtCHS* was inoculated and cultured at 37℃ in Luria-Bertani (LB) liquid medium containing ampicillin (100 mg/L) with shaking (250 r/min). When the optical density (OD600) of the cultured cells reached ~0.6, protein expression was induced by the addition of isopropyl-β-D-thiogalactoside (IPTG) in the medium to a final concentration of 1 mmol/L. The cultivation of *E. coli* strain BL21 with pET32a-*CtCHS* was continued for 0.5, 1, 2, 4 and 6 h, respectively, and the protein expression levels were assessed by analyzing the total protein on SDS-PAGE followed by coomassie brilliant blue staining. The *E. coli* strain BL21 cells harboring pET32a-*CtCHS* were also analyzed without IPTG as the control. Taking advantage of 6*His-tag, the fusion proteins were purified by AKTA protein purifier (GE, USA). The purified protein was sent to the company to make protein antibodies.

Western blot analysis was subsequently carried out to verify the correct expression of the CtCHS protein. After protein electrophoresis, the gel was electro-blotted onto a membrane using the trans-blot electrophoretic transfer cell system (GE, USA). The membrane was then blocked in TBS containing 5% skim milk powder for over 1 h. The primary anti-rabbit CtCHS antibody was add-

ed to the membrane at a 1∶20000 dilution in TBS containing 5% skim milk powder. After washing, the membrane was treated with a goat anti-rabbit secondary antibody (NEB, China) at a 1∶2000 dilution. Finally, the membrane was soaked in Amersham ECL prime western blotting detection reagent (GE, USA) to detect the immobilized specific antigens conjugated to horseradish peroxidase (HRP) labeled antibodies.

### 1.5 UV treatment and western blot analysis of CtCHS

For UV treatment, the prothalli after 21 days growth were exposed to UV irradiation treatment in the dark in a closed chamber, and samples were collected at 0, 1, 2, 3, 4 and 5 h of treatment. Subsequently, total protein was extracted from all treated tissues using phosphate buffer (10 mmol/L Hepes, 5 mmol/L phosphate buffer pH 7.5, 10 mmol/L $MgCl_2$, 10 mmol/L NaCl, 25% glycerol). According to the Bradford (Bio-Rad, USA) method for protein quantitative, the CtCHS protein expression levels were assessed though western blotting, using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the control.

## 2 Results

### 2.1 Generation and characterization of the full-length DNA of *CtCHS*

A set of degenerate primers and cDNAs prepared from total mRNA as the template yielded a 300 bp (approx.) core sequence. Full-length cDNA sequences including the 5'- and 3'-untranslated regions were obtained by employing the RACE method with primers derived from the core sequence. This cDNA contained an open reading frame (ORF) of 1215 bp, encoding 404 amino acids. ExPASy Compute PI/MW server analysis showed that the molecular weight (MW) was 44.33 kD, and the isoelectronic point (pI) was 6.28. Sequence analysis showed 80% of the amino acid sequence of CtCHS (GenBank accession number JX027616.1) was consistent with the CHS of *E. arvense*, reflecting their close evolutionary relationship.

Is it worth noting that three exons and two introns were found in the *C. thalictroides* genomic *CtCHS* sequence by comparing the genomic and cDNA sequences. Exon 1 (197 bp), exon 2 (689 bp) and exon 3 (329 bp) were separated by intron 1 (312 bp) and intron 2 (89 bp). The putative splicing sites obeyed the gt/ag rule as in most species (Fig. 1).

### 2.2 Construction and expression of the recombinant vector pET32a-*CtCHS*

A specific PCR-amplified fragment for protein expression was the coding sequence of *CtCHS* (1215 bp). After digestion with *Bam*HⅠ and *Hind* Ⅲ, which did not cut within the coding region, the amplified fragment was ligated into the *Bam*HⅠ and *Hind* Ⅲ pre-digested pET32a vector to generate the recombinant plasmid pET32a-*CtCHS* (Fig. 2). The complete nucleotide sequence of the PCR-amplified fragment was confirmed correct by sequencing from both strands before it was transferred into *E. coli* strain BL21.

Upon induction by IPTG, CtCHS was expressed as a major protein product in total cellular proteins of the recombinant vector. Simultaneously SDS-PAGE patterns of total cellular proteins, visualized by coomassie brilliant blue staining, showed the time course for expression of the target protein (Fig. 3a). The maximal level of the protein expression was obtained 6 h after IPTG induction. The molecular weight of the expressed recombinant protein was estimated to be about 63 kD fused with His-tag, therefore the size of the expressed CtCHS protein was in good agreement with that deduced from the amino acid

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   1 ATGCCGGCCC ATAGCGCAGC CACGTTTCCT CCTTGCGCTC GTAAGATGGA GCGGGCCGAC
  61 GGCCCAGCTA CCGTGCTCGC CATTGGTACT GCCAATCCGC CCAACGTGTT TCAGCAGAGC
 121 GAATATCCGG AATTCTACTT CAACATCACT AACAGCAACC ATATGTCAGA CCTGAAGGAG
 181 AAATTTCAGC GCATGTgtaa gtccaccctt tcctgcatac ggaagttgca agcctagtac
 241 acattcactg cgcgagaagc tcgtatgttt catgctgcta ggtgtaagtg catttcttct
 301 cgtctccaca ctcagcaatt gaattgtcta ctgcttttat gaactgttcg atgggggatc
 361 catgagtcca agctcgcgac gtatcaacaa gttgaggacg atatacgatg tcaaaagttc
 421 tgagaagcat ttcctcatgc gtaggatgtc tcacgagcct tgaatgtagc ggctaacagg
 481 atttttgtcc tttgatctgc ttttgtagGT GACAAGTCCG GAATCAAGAA GAGATACATG
 541 TACCTGAATG AGGAGATTCT CAAGGCCAAC CCAAACATGT GTGCTTACTG GGAGAAGTCC
 601 CTCGACGTGA GGCAGGACAT GGTGGTCGTG GAGGTTCCCA AGTTGGGCAA GGAGGCTGCT
 661 ACCAAGGCCA TCAAAGAATG GGGACAGCCC AAGTCCAAGA TCACGCATCT CATATTTTGC
 721 ACCACCAGTG GCGTCGACAT GCCCGGCGCT GATTGGGCCC TAACCAAGCT CCTTGGGCTC
 781 CGCCCTAGCG TGAAGCGGCT CATGATGTAT CAACAAGGCT GCTTCGCCGG AGGGACGGTG
 841 ATGAGGATCG CGAAAGACCT TGCAGAGAAC AACAAGGGTG CACGTGTTCT TGTTGTTTGT
 901 AGCGAGCTGA CGGCAGTGAC GTTTCGTGGA CCCAGTGAGA CGCATTTGGA TAGCCTTGTC
 961 GGACAGGCGC TTTTCGGCGA TGGCGCTTCA GCAGTGATCG TCGGCTCAGA TCCAATTCCA
1021 GAAGTGGAGA GGCCATGGTT CGAGATCCAT TATTGTAGCG TCCACATTCT CCCAGACAGT
1081 GACGGCGCCA TCGATGGCCA TCTGCGCGAA GTAGGGCTCA CCTTCCATCT CATGAAGGAT
1141 GTGCCTGGGA TCATCTCGAA GAACATCGGC ACTGTGCTGA AGGACGCATT CGAGAAGgta
1201 catagtggag ctccatagtg ttgtgagtca aagtctcttg atttgagatg cgacagaaga
1261 tcaaatgcgg tctctgattt ctgcagGTTT TCGGGAACGA AGATGGTGGC TTGCCGGGGT
1321 TCAACGACGT ATTCTGGATC GCACATCCGG GAGGGCCTGC AATTCTCGAC CAAGTCGAGC
1381 AGAAGCTCCA ACTGAAGCCT GAGAAGATGG CAGCGAGCCG TCAAGTTCTC TCTGACTACG
1441 GAAACATGTC GAGCGCATGT GTTCTCTTCA TCATGGACCA CATGCGGAAG AAGTCTGTCG
1501 AGCAGAACCT CAGTACTTCT GGAGAAGGCC ATGAGTGGGG GCTCCTACTG GGTTTCGGTC
1561 CAGGGCTAAC GTGCGAGACT GTTTTGCTGA GAAGTGTCCC CCTTACCGCA AGCTAA
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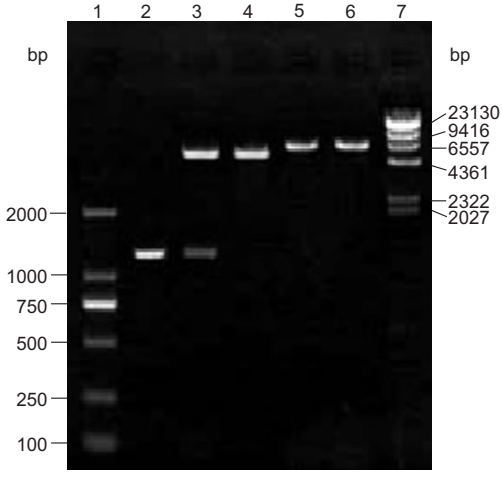
Three exons (uppercase) were separated by two introns (lowercase). Putative intron splicing sites gt/ag were boxed.

Fig. 1　Genomic DNA sequence of the *Ceratopteris thalictroides* chalcone synthase gene



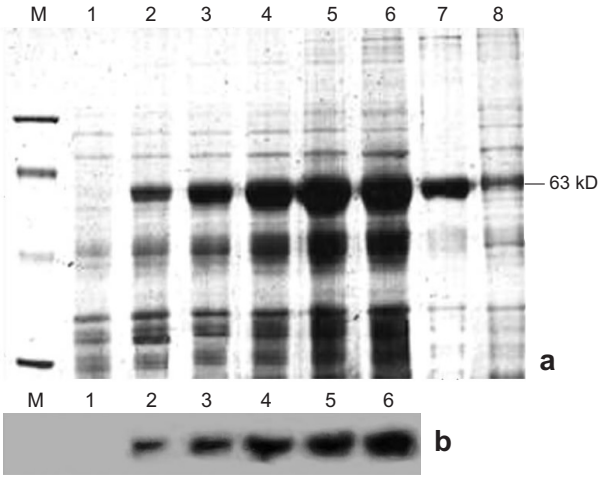

Lane 1: DL2000 DNA Marker; Lane 2: PCR product of *CHS*; Lane 3: pET32a-*CHS* digested by *Bam*H Ⅰ/*Hind* Ⅲ; Lane 4: pET32a digested by *Bam*H Ⅰ/*Hind* Ⅲ; Lane 5: pET32a-*CHS* digested by *Bam*H Ⅰ; Lane 6: pET32a-*CHS* digested by *Hind* Ⅲ; Lane 7: λ*Hind* Ⅲ DNA Marker.

Fig. 2　Gel electrophoresis appraisal figure of vector construction

sequence of CtCHS. However, a similar molecular weight band was found at the position of the recombinant protein for the *E. coli* strain BL21 without induction. Western blotting of samples confirmed its specific immune activity to the anti-rabbit CHS antibodies, while the control with a similar band did not react (Fig. 3b), confirming the correct expression of the chalcone synthase in *E. coli*. Taking advantage of the 6*His-tag, we obtained highly purified CtCHS protein and antibody, and laid the foundation for *CtCHS* function research.

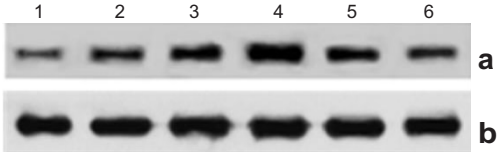
### 2.3　Western blotting analysis of *CtCHS* under UV treatment

Following prothalli treatment under UV, the *CtCHS* was obviously induced (Fig. 4). Under UV




**a**: Accumulation of recombinant CtCHS protein in *E. coli* strain BL21. SDS-PAGE patterns of total cellular protein under induced conditions to perform a time course, visualized by coomassie brilliant blue R250 staining. Lane 1: Sample collected before induction by IPTG; Lanes 2–6: Samples collected after induction by IPTG for 0.5, 1, 2, 4 and 6 h, respectively; Lane 7: Soluble protein after 6 h; Lane 8: Insoluble protein after 6 h; Lane M: Protein molecular weight marker. Dashed line (63 kD) indicates protein of recombinant CtCHS. **b**: Western blotting of recombinant protein. Western blotting patterns of total protein under induced conditions to perform a time course, visualized by staining with ECL prime western blotting detection reagent.

Fig. 3 Expression of recombinant *CtCHS* in *E. coli* strain BL21




**a**: Lane 1: Samples collected before UV irradiation; Lanes 2–6: Samples collected after UV irradiation for 1, 2, 3, 4 and 5 h, respectively. **b**: *C. thalictroides* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used in western blotting as the control.

Fig. 4 Expression profile of *CtCHS* under UV treatment

treatment, *CtCHS* accumulation reached the highest level at 3 h and then gradually declined, but still showed higher levels than that without treatment (0 h). The decrease in *CtCHS* may be caused by injury from UV treatment to the young gametophytes of *Ceratopteris*.

## 3 Discussion

In general, *CHS* genes are highly conserved and usually contain one intron and two exons, with the only exception being *Antirrhinum majus*, in which the *CHS* gene contains two introns[20]. The position of the intron in *CHS* genes is usually conserved. Exon 1 usually encodes 37–64 amino acid residues and exon 2 encodes almost all the active sites. The length of the intron varies in different plant species, ranging from less than 100 bp to more than 1 kb (Fig. 5)[16].

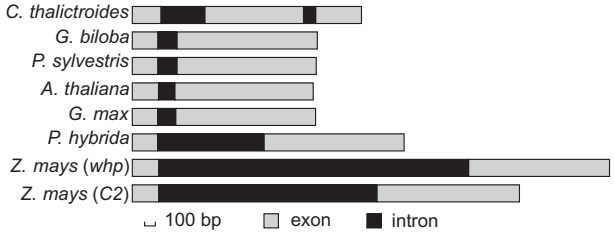



Fig. 5 Comparison of chalcone synthase gene organisation in *Ceratopteris thalictroides*, *Gingko biloba*, *Pinus sylvestris*, *Arabidopsis thaliana*, *Glycine max*, *Petunia hybrida*, *Zea mays* (*whp*) and *Zea mays* (*C2*)

The strictly conserved *CHS* active site residues, Cys170, His315, and Asn348[8], as well as the highly conserved *CHS* signature sequence, $G^{384}$FGPG[9], were found in *CtCHS* (Fig. 6). The two Phe residues ($Phe^{221}$ and $Phe^{271}$), important in determining the substrate specificity of *CHS*[10], were also found in the present species. Both *P. nudum* and *Physcomitrella patens* were contained in these active sites, and exhibited enzyme activity[21, 22].

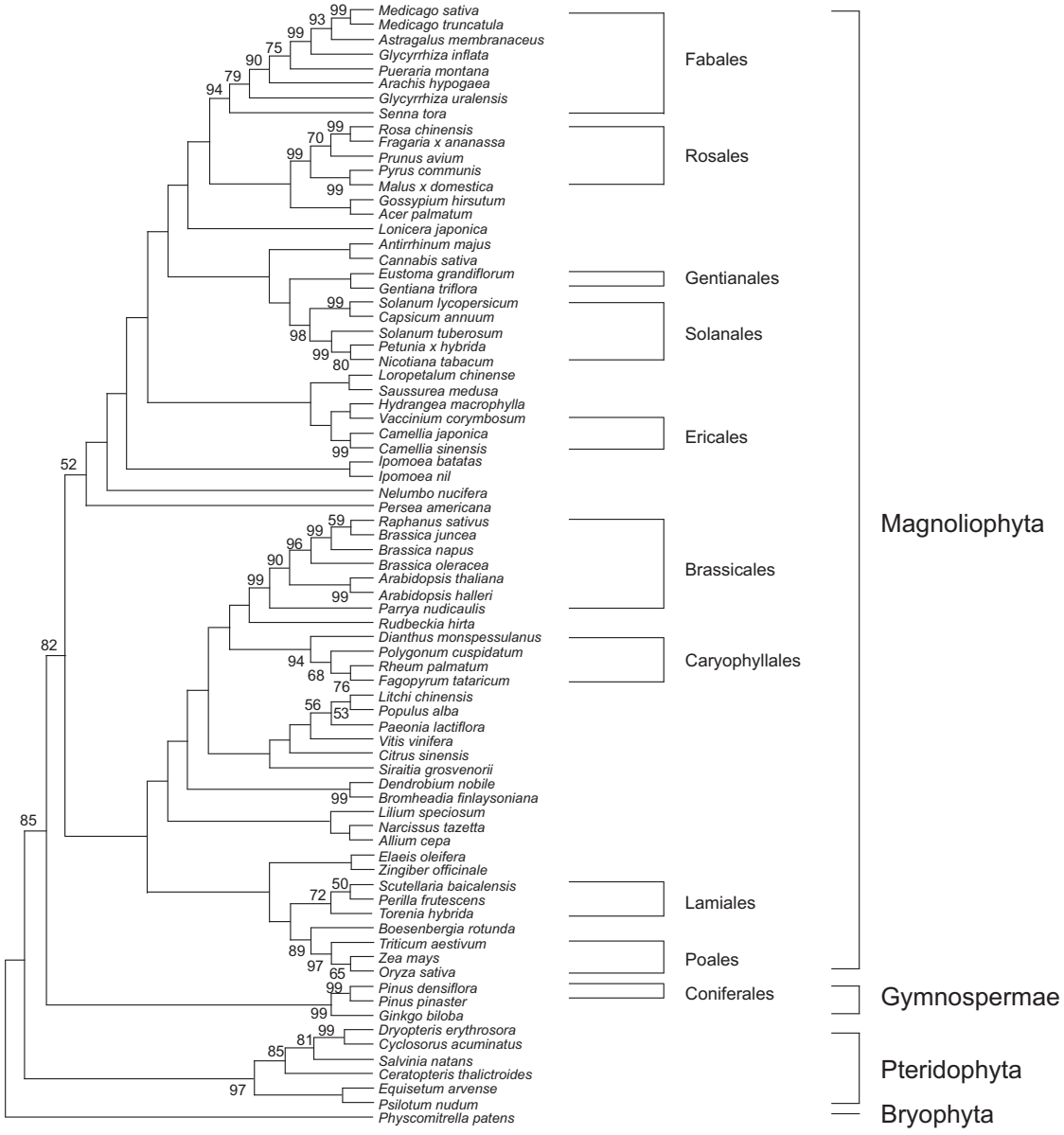
In almost all cases, the evolution of the multigene family of *CHS* has been analyzed with exons. We produced a neighbor-joining (NJ) phylogenetic tree using ClustalX and MEGA5 software packages. Phylogenetic analysis (Fig. 7) indicated that *C. thalictroides* and *P. nudum*, *E. arvense*, *Dryopteris erythrosora*, *Cyclosorus acuminatus*, and *Salvinia natans* formed a separate cluster based on exon nucleotide sequences analysis, which is in agreement with their molecular evolutionary relationship according to Smith's system [23].

Ultraviolet (UV) light is a potential hazard for organisms because it can damage DNA and im-

| | | | | |
|---|---|---|---|---|
| *C. thalictroides* | -MMYQQGCFAGG- | -FWIAHPGGPA- | -SDYGNMSSAC- | -LLGFGPGLT- |
| *M. sativa* | -MMYQQGCFAGG- | -FWIAHPGGPA- | -SEYGNMSSAC- | -LFGFGPGLT- |
| *P. sylvestris* | -MMYQQGCFAGG- | -FWIAHPGGPA- | -SDYGNMSSAC- | -LFGFGPGLT- |
| *A. thaliana* | -MMYQQGCFAGG- | -FWIAHPGGPA- | -SEYGNMSSAC- | -LFGFGPGLT- |
| *E. arvense* | -MMYQQGCFAGG- | -FWIAHPGGPA- | -SEYGNMSSAC- | -LLGFGPGLT- |
| *P. nudum* | -MLYQQGCFAGG- | -FWIAHPGGPA- | -ADYGNMSSAC- | -LFGFGPGLT- |
| *M. paleacea* | -MLYQQGCFGGA- | -FWCVHPGGRA- | -YNYGNMSGAC- | -VVGFGPGLT- |
| *P. patens* | -MMYQTGCFGGA- | -FWAVHPGGPA- | -SEFGNMSSAS- | -FIGFGPGLT- |
| | 170 | 315 | 348 | 387 |

Sequences around the active site residues, Cys, His, and Asn, and the signature GFGPG loop are shown. Residue numbers are those of *C. thalictroides* chalcone synthase.

Fig. 6 Alignment of *CHS* sequence parts from *Ceratopteris thalictroides* (JX027616.1), *Medicago sativa* (P30074), *Pinus sylvestris* (CAA43166), *Arabidopsis thaliana* (CAI30418), *Equisetum arvense* (Q9MBB1), *Psilotum nudum* (BAA87922), *Marchantia paleacea* (BAD42328), and *Physcomitrella patens* (ABB84527)




Numbers below branches indicate bootstrap values.

Fig. 7　Neighbor-joining phylogenetic tree based on exon nucleotide sequences of plant chalcone synthase

pair several physiological processes[24]. Like all other organisms, plants have counter-measures to protect themselves from UV damage. One of the most common responses of plant seedlings to UV light is the transcriptional activation of flavonoid biosynthetic genes[25,26]. Flavonoids are strongly UV-absorbing and accumulate mainly in epidermal cells after UV treatment, suggesting that they function as a protective shield[25]. The expression of *CHS* genes is regulated by light through a photoreceptor-mediated mechanism[27]. Studies in a wide range of species, such as *Ligustrum vulgare*, *Vitis vinifera*, petunia, and *Arabidopsis* have provided new evidence that UV light induces the synthesis of flavonol compounds[28-33]. The western blot analysis of *CtCHS* under UV treatment showed that the expression of the *CtCHS* gene increased. This phenomenon might be due to an acute need for extra material in terms of UV protection. The flavonoid skeleton present hydroxy group is the main structural feature responsible for chelating metal ions such as iron, copper, and zinc, and hence inhibits the formation of free radicals and protects plants from UV irradiation[33]. This may explain why the *CtCHS* gene expression in young prothalli increased transiently under UV-induction.

In conclusion, we cloned the *CHS* gene from the *C. thalictroides* fern, and analyzed the nucleic acid sequence. Phylogenetic analysis indicated that *C. thalictroides CtCHS* had a close relationship to fern allies *Equisetum arvense* and *Psilotum nudum*, but not to the spermatophytes. The *CtCHS* gene was introduced into an expressed plasmid pET-32a vector and the fusion protein was highly expressed in *E. coli* strain BL21 with IPTG induction. The expressed CtCHS protein had a molecular weight of about 45 kD, a size matching that predicted by bioinformatic analysis. Western blot analyses revealed that UV irradiation treatment increased *CtCHS* expression in *C. thalictroides*. The present investigation provides base data for understanding the flavonoid biosynthetic pathway of ferns.

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