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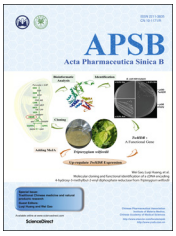

ORIGINAL ARTICLE

# Molecular cloning and functional identification of a cDNA encoding 4-hydroxy-3-methylbut-2-enyl diphosphate reductase from *Tripterygium wilfordii*

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**KEY WORDS**

*Tripterygium wilfordii*;
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4-Hydroxy-3-methylbut-2-enyl diphosphate reductase;
Complementation;
Gene expression

**Abstract** The 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) is the last step key enzyme of the methylerythritol phosphate (MEP) pathway, synthesizing isopentenyl diphosphate and its allyl isomer dimethylallyl diphosphate, which is important for regulation of isoprenoid biosynthesis. Here the full-length cDNA of *HDR*, designated *TwHDR* (GenBank Accession No. KJ933412.1), was isolated from *Tripterygium wilfordii* for the first time. TwHDR has an open reading frame (ORF) of 1386 bp encoding 461 amino acids. TwHDR exhibits high homology with HDRs of other plants, with an N-terminal conserved domain and three conserved cysteine residues. *TwHDR* cDNA was cloned into an expression vector and transformed into an *Escherichia coli hdr* mutant. Since loss-of-function *E.coli hdr* mutant is lethal, the result showed that transformation of *TwHDR* cDNA rescued the *E.coli hdr* mutant. This complementation assay suggests that the *TwHDR* cDNA encodes a functional HDR enzyme. The expression of *TwHDR* was induced by methyl-jasmonate (MJ) in *T. wilfordii* suspension cells. The expression of *TwHDR* reached the highest level after 1 h of MJ treatment. These results indicate that we have identified a functional TwHDR enzyme, which may play a pivotal role in the biosynthesis of diterpenoid triptolide in *T. wilfordii*.

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## 1. Introduction

*Tripterygium wilfordii* Hook. F., also known as Lei Gong Teng or thunder god vine, is native to eastern and southern China[1]. This vine-like plant belongs to the Celastraceae family, and has a long history of use in traditional Chinese medicine when treating autoimmune diseases and inflammatory dermatoses, such as psoriasis[2], erythema nodosum[3], rheumatoid arthritis[4], and systemic lupus erythematosus[5]. The research for the medicinal value of *T. wilfordii* has found out that the plant possesses anti-HIV, anti-inflammatory, antitumor, and anti-Parkinsonian effects[6–9], which arouses great interest in the field of medicine. The major active compound responsible for its medicinal functions is believed to be triptolide. Currently, only limited information on the biosynthesis of triptolide is available.

Triptolide is a diterpenoid triepoxide derived from isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP)[10]. There are two independent pathways leading to the biosynthesis of both IPP and DMAPP localized in different cellular compartments which are the cytosolic mevalonic acid (MVA) pathway and the plastidic 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway[11,12]. While the MVA pathway is responsible for synthesizing sesquiterpenes and triterpenes, the MEP pathway is in charge of the biosynthesis of monoterpenes, diterpenes, and tetraterpenes[13]. As the last enzyme in the MEP pathway for isoprenoid biosynthesis, 4-hydroxy-3- methylbut-2-enyl diphosphate reductase (HDR) catalyzes (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP) into a mixture of 5:1 IPP and DMAPP (Fig. 1). Silencing of *HDR* gene in *Nicotiana benthamiana* can make the isoprenoid-derived chlorophyll and carotenoid pigments decrease to less than 4% of the control plants[14]. And overexpression of *HDR* gene contributes to increasing the production of isoprenoid-derived carotenoid and over-producing taxadiene up to 13-fold of the control group in transgenic *Arabidopsis*, proving its vital role in metabolic regulation of plastidial isoprenoid biosynthesis[15].

Because of the high toxicity, obtaining the effective components from *T. wilfordii* by traditional chemical methods is difficult spending much time and labor. And now the current studies regarding key enzymes of triptolide biosynthesis in *T. wilfordii* are few, and the production of triptolide still cannot be synthesized through biosynthesis methods. Based on the above issues, we present the cloning of full-length *HDR* cDNA of *T. wilfordii* (*TwHDR*) for the first time, proving it having the function of IspH and may acting a role as a potential key enzyme for the biosynthesis of triptolide.

## 2. Materials and methods

### 2.1. Plant material

*T. wilfordii* cell suspensions were cultured in Murashige and Skoog (MS) medium containing 30 g/L sucrose and 8 g/L agar with 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 0.1 mg/L kinetin (KT), and 0.5 mg/L indole-3-butytric acid (IBA). All suspension cell cultures were maintained at 25 ± 1 °C with shaking by orbital shaker (DZ-100, Suzhou experimental equipment Co., Ltd., Suzhou, China) at 120 rpm in the dark.

### 2.2. RNA isolation

The 10-day-old *T. wilfordii* suspension cells were treated with MJ for 0, 1, 4, 12, 24, 48 and 72 h at a final concentration of 50 μmol/L.

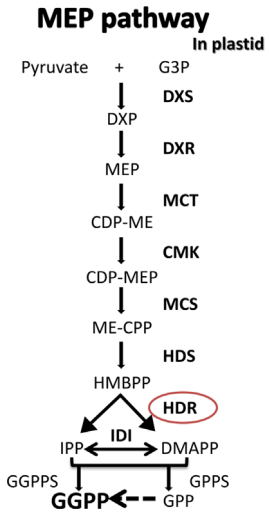


**Figure 1** Schematic MEP pathway for GGPP. Multiple steps are indicated with striped arrows. G3P, glyceraldehyde 3-P; DXP, deoxyxylulose 5-P; MEP, methylerythritol 4-P; CDP-ME, 4-diphosphocytidyl-methylerythritol; CDP-MEP, CDP-ME 2-P; ME-CPP, methylerythritol 2,4-cyclodiphosphate; HMBPP, hydroxymethylbutenyl 4-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate. Enzymes are indicated in bold: DXS, DXP synthase; DXR, DXP reductoisomerase; MCT, MEP cytidylyltransferase; CMK, CDP-ME kinase; MCS, ME-cPP synthase; HDS, HMBPP synthase; HDR, HMBPP reductase; IDI, IPP isomerase; GPPS, GPP synthase; GGPPS, GGPP synthase.

Subsequently, the suspension cells were harvested for RNA isolation. The total RNA was isolated using the cetyltrimethylammonium bromide (CTAB) method[16].

### 2.3. Cloning of TwHDR full-length cDNA

Total RNA was reverse transcribed into first-stand cDNA with PrimeScript 1st Strand cDNA Synthesis Kit (Takara Biotechnology (Dalian) Co., Ltd., Dalian, China) due to the manufacturer's instruction. The full-length primers were designed based on the transcriptome sequencing data of *T. wilfordii* obtained previously. The prime pairs were as follow: *TwHDR*-F 5′-CTGTTCCACGCATTTTTCAACACAG-3′ and *TwHDR*-R 5′-GAGCCTAGAG GTAAAAACTGCGGTC-3′. The product was purified and cloned into the pMD19-T vector (Takara Biotechnology (Dalian) Co., Ltd., Dalian, China). The vector was transformed into *E. coli* DH5*α* cells and cultured in Luria–Bertani (LB) medium at 37 °C in dark. The positive colonies were sequenced and assembled to verify the correct *TwHDR* insertion.

### 2.4. Sequence alignment of HDR/IspH proteins

The nucleotide sequence was analyzed using Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website. The ORF and amino acid sequence of TwHDR was deduced using the ORF finder. HDR/IspH amino acid sequences from *T. wilfordii*, *Aquilaria sinensi* (AHE93332.1), *Arabidopsis thaliana* (AAN87171.1), *Salvia miltiorrhiza* (AFQ95412.1), *Nicotiana tabacum* (AAD55762.2), *Camptotheca acuminate* (ABI64152.1), *Hevea brasiliensis* (BAF98297.1), *Synechocystis* (WP_010873388.1), *Rhodobacter*

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