Cloning and functional analysis of HMGR gene in *Ligularia fischeri*

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**Abstract:** 3-hydroxy-3-methylglutaryl CoA reductase cDNA genes have been successfully isolated from *Ligularia fischeri* and named as HMGR with 2031bp (Gen Bank accession number: DQ916106), which encodes a predicted polypeptide of 579 amino acids. The pBSHMGR gene vector was successfully constructed. The HMGR gene is over expressed through agrobacterium transformation. Southern and Northern analyses of transgenic plants showed that the HMGR gene was integrated into the Ligularia fischeri genome and expressed at the transcriptional level. HPLC analysis showed that the Shionone content in transgenic roots and stems of Ligularia fischeri were increased 16.67% and 12.25% as compared with the control.

### 1. Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) is the first rate-limiting enzyme regulating the synthesis of terpenoids upstream of the mevalonate (MVA) pathway. In higher plants, members of the HMGR genes families play an important role in plant growth and development and in response to various environmental stresses<sup>[1,2,7]</sup>. HMGR gene has been cloned from the potato<sup>[3,4]</sup>, cotton<sup>[5]</sup>, tomato<sup>[6]</sup> and other plants. The ethanolic extract of *Ligularia fischeri* has anti-inflammatory activity. Hepatic protective effects of *Ligularia fischeri* and *Aronia melanocarpa* (AM) against alcohol were investigated in vitro and in vivo test<sup>[8]</sup>. This project is the first attempt to study the synthesis of asterone from the perspective of functional genes and specific regulatory genes molecular mechanism and important regulatory points of the formation pathways of cloning terpenoids. HMG-COA reductase(HMGR) family determine their enzyme activities, study their gene characteristics, functions, expression sites. The tissue and induction conditions were correlated with the transcriptional level and the content of astanone. It provides a theoretical basis for elucidating the synthetic pathway of the terpenoids. It provides a new idea and theoretical basis for the production of shionone by genetic engineering.

### 2. Materials and methods

The full-length cDNA sequence of HMGR gene was cloned. The specific primers were designed at both ends of the cDNA sequence open reading frame (ORF). The underlined part of the primer represents the restriction enzyme(BamH I and Kpn I )cutting site respectively. Recombinants of pBSHMGR were identified by double enzyme digestion Bam HI and KpnI. It is used for the construction of plant expression vector. Agrobacterium tumefaciens was used the genetic transformation.

Medium for agrobacterium-mediated transformation of HMGR gene.

Preculture medium: MS+6-BA 0.5mg/L+ 2,4-D 0.5 mg/L.

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Co-culture medium: MS+6-BA 0.5mg/L +2,4-D 0.5 mg/L.
Screening medium: MS+6-BA 1.5mg/L +Cef 500mg/L.
Subculture medium for callus: MS+6-BA 0.5mg/L +Htg 5mg/L+Cef 500mg/L.
Callus screening medium: MS+6-BA1.5mg/L +Htg 20mg/L+Cef 500mg/L.
Meristematic medium: MS+6-BA 2.5mg/L+NAA 0.05mg/L+Htg 5mg/L+Cef 500mg/L.
Root medium: 1/2MS+ NAA 0.5mg/L+Htg 5mg/L. The above media are all in 121℃ sterilization 20min.

3. results
The 1759 base coding region of HMGR cDNA was amplified by RECE gene method. See Figure 1 for the results. The molecular evolutionary trees of HMGR genes were compared to HMGR with cloned 3'-The hydroxyl-3'-ethyl glutaryl coenzyme A. See Figure 2 for the results.

![Figure 1](image1.png)  
**Figure 1** PCR amplification of the whole sequence of HMGR. M, DL2000 marker; 1, The PCR product of the whole sequence of HMGR.

![Figure 2](image2.png)  
**Figure 2** Molecular phylogenetic tree of HMGR from plants

Plant expression vector pBSHMGR was constructed. See Figure 3 for the results.

![Figure 3](image3.png)  
**Figure 3** Expression vector pBSHMGR

In vitro culture of *Ligularia fischeri.* and establishment of plant regeneration system. See Figure 4 for the results.
Figure 4 In vitro regeneration and plant establishment of *Ligularia fischeri*.  
A: Explants were placed on MS medium supplement with 2,4-D 2.5μM after 2 weeks of culture.  
B: Four-week-old culture showing well-developed shoots on MS+BA(0.5μM).  
C: Proliferation of shoots on MS+BA(5.0μM)+NAA(0.5μM).  
D: The acclimatised plantlet in soil.  
E: The plantlets of *Ligularia fischeri*.  

The Southern blotting and Northern blotting showed that the HMGR gene had been transformed into plant. See Figure 5,6 for the results.

Figure 5 Southern blot analysis digested by BamH I and Kpn I (lane1-4) probe for hybridization of HMGR. Genomic DNA from *Ligularia fischeri* was respectively and after blotting.

Figure 6 Northern blot analysis of HMGR. 1: leaf.  2: stem.  3: root.

The results indicated that the transferred HMGR gene could be normally transcribed and translated into resveratrol synthase capable of normal function, and catalyzed the production of resveratrol by substrates in plants. The high resolution of HPLC enables more accurate analysis of these compounds, as shown in Figure 6: These compounds show different retention times on the HPLC. The results showed that the content of asta none in the root of *Ligularia fischeri* is 1.80 mg/g and asterone in the stem content is 1.60 mg/g. There are too many impurities in the blade. 10m the peak area was too large and the content of asterone could not be measured. See Figure 7 for the results.
Figure 7  HPLC analysis of trans-resveratrol Shionone of *Ligularia fischeri*. A: reference substance B: root samples C: stem samples D: leaf samples.

4. Conclusion

Southern hybridization analysis showed that HMGR found in the genome of the *Ligularia fischeri*. Northern hybridization results showed that the transcription level of HMGR in roots and stems was relatively high. The expression level cannot be calculated in the blade. The content of asterone in different parts of plants was determined. HPLC the determination results showed that the content of asterone in roots and stems was obvious higher than the blade. HMGR gene may be associated with the biosynthesis of asterone. Different plants HMGR gene phylogenetic relationships derived from amino acid sequences do not truly reflect the diffuse nature of plants natural evolutionary relationships over a long period of time. The results still have a bearing on how plants are related to each other definite reference significance.

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