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ORIGINAL ARTICLE

Cloning and characterization of squalene synthase and cycloartenol synthase from *Siraitia grosvenorii*

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KEY WORDS

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Cloning;
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Cycloartenol synthase;
Subcellular localization

Abstract Mogrosides and steroid saponins are tetracyclic triterpenoids found in *Siraitia grosvenorii*. Squalene synthase (SQS) and cycloartenol synthase (CAS) are key enzymes in triterpenoid and steroid biosynthesis. In this study, full-length cDNAs of *SgSQS* and *SgCAS* were cloned by a rapid amplification of cDNA-ends with polymerase chain reaction (RACE-PCR) approach. The *SgSQS* cDNA has a 1254 bp open reading frame (ORF) encoding 417 amino acids, and the *SgCAS* cDNA contains a 2298 bp ORF encoding 765 amino acids. Bioinformatic analysis showed that the deduced SgSQS protein has two transmembrane regions in the C-terminal. Both *SgSQS* and *SgCAS* have significantly higher levels in fruits than in other tissues, suggesting that steroids and mogrosides are competitors for the same precursors in fruits. Combined *in silico* prediction and subcellular localization, experiments in tobacco indicated that SgSQS was probably in the cytoplasm or on the cytoskeleton, and SgCAS was likely located in the nucleus or cytosol. These results will provide a foundation for further study of *SgSQS* and *SgCAS* gene functions in *S. grosvenorii*, and may facilitate improvements in mogroside content in fruit by regulating gene expression.

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1. Introduction

Siraitia grosvenorii, a traditional medicinal plant native to Guangxi Zhuang Autonomous Region, belongs to the Cucurbitaceae family. Its fruits have been used as medicine for the treatment of cough and lung congestion, and they are extremely sweet substances with low energy content and no toxicity[1]. The active components for sweetness are known as mogrosides, a kind of triterpenoid, and are extracted from the fruit. They exhibit anti-tumor, anti-diabetic, anti-oxidation, and hypoglycemic activities[2–5]. Our previous research proposed a mogroside biosynthetic pathway[6], with triterpenoids synthesized *via* mevalonate (MVA) and plastidial 2-*C* methyl-D-erthritol-4-phosphate (MEP) in the upstream pathway and involves farnesyl diphosphate (FPP) conversion into squalene and then to 2,3-oxidosqualene followed by sequential cyclization, oxidation and other modifications. Squalene synthase (SQS) catalyzes the conversion of two FPPs to squalene, the first committed entry step of sterol, triterpene and brassinosteroid (BRs) biosynthesis[7]. This enzymatic reaction occurs in the membrane of endoplasmic reticulum (ER), and sterols and BRs can play important roles in membrane fluidity and permeability, and also serve as signaling molecules in plant growth and development[8]. Cycloartenol synthase (*CAS*) and cucurbitadienol synthase (*CS*) are members of the oxidosqualene cyclase (*OSC*) gene family, and catalyze the cyclization of 2,3-oxidosqualene to cycloartenol or cucurbitadienol[9]. This step catalyzed by OSCs is the key branch-point leading to sterol or triterpenoid synthesis. Further modification by cytochrome P450-dependent monooxygenases (CYP450s) and glycosyltransferases (GTs) ultimately yields steroids and triterpenoids.

As there is no sequence or structural information on SQS and CAS in *S. grosvenorii*, we focused on these two genes (*SgSQS* and *SgCAS*). The full-length sequences of both genes were obtained and expression patterns were investigated. The subcellular locations were determined by experiment and expression in *Escherichia coli* was investigated. The results could provide foundation for further exploration of gene function in yeast or in *S. grosvneorii*, and help reveal the regulation of mogroside and sterol biosynthesis in *S. grosvenorii*.

2. Materials and methods

2.1. Plant materials

The Nongyuan B6 variety of *S. grosvenorii* tissue culture seedlings are maintained in our laboratory. Fresh root, stem, leaf and fruits of *S. grosvenorii* from 5 to 50 days were harvested in Guangxi Botanical Garden of Medicinal Plant, Guangxi Zhuang Autonomous Region. All samples were cut into small pieces, frozen immediately with liquid nitrogen and stored at −80 °C for further use.

2.2. RNA extraction, cDNA synthesis and cloning of full-length SgSQS and SgCAS gene

Total RNA was extracted from the fruits of *S. grosvenorii* using Trizol (Invitrogen, USA) as described by Tang et al.[6]. First-strand cDNA was reverse-transcribed using 1 μg of total RNA and SMARTer™ RACE DNA Amplification Kit (Clonetech Laboratories Inc., Mountain View, CA, USA) according to the manufacturer's protocol. All the primers for rapid amplification of cDNA ends by PCR are shown in Table 1.

The first-strand cDNA for full-length cloning was synthesized using DNase I–treated RNA, Oligo dT primers and PrimeScript II 1st Strand cDNA Synthesis Kit (Takara, Dalian, China). The specific primers for amplification of these two genes were designed by Primer Premier 5 (Table 1). PCRs were conducted in a total volume of 50 μL, containing 1 μL of cDNA, 10 μmol/L of forward and reverse primers, and 25 μL Taq Plus MasterMix (Tiangen, China). PCRs were carried out using the cyclic parameters as: initial denaturation at 94 °C for 5 min followed by 35 cycles of 20 s at 94 °C, 20 s at 56 °C and 1 min at 72 °C, and final extension of 10 min at 72 °C. The PCR products were purified and cloned into the pMD19-T (Takara, Dalian, China) vector for sequencing.

2.3. Bioinformatic analysis

Open reading frames (ORFs) were determined using NCBI online tools (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The physical and chemical parameters, such as molecular mass (MW), theoretical p*I* and stability of the deduced amino acids were predicted by ProtParam software online (http://web.expasy.org/protparam/), while conserved domains of both SgSQS and SgCAS were identified by ScanProsite (http://www.ebi.ac.uk/Tools/pfa/iprscan/). The signal peptide, subcellular localization and transmembrane regions were identified using SignalP4.1 Server (http://www.cbs.dtu.dk/services/SignalP/), PSORT (http://wolfpsort.org/) and TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Table 1 List of primers used in this study.

Gene	Primer name	Sequence (5′–3′)
SgSQS 5′-RACE primer	*SgSQS*-5′GSP1	TCGCCATAATCTGAGGAATTGCAC
SgSQS 3′-RACE primer	*SgSQS*-3′GSP1	CCGTGCAATGCCTCAACGATTTGGTC
SgSQS ORF cloning	*SgSQS*-ORF1	ATGGGCAGCTTGGGGGCGAT
	SgSQS-ORF2	TCATACAGGTTGGTTAGCCGGT
SgSQS qRT-PCR primer	*SqSQS*-qPCR1	CTGAGACACCCAGATGACT
	SqSQS-qPCR2	GAGGGCTCGCAGAACAAGA
SgCAS 5′-RACE primer	5′RACE-*CAS*1	AGAACCGAACATTGTGCTTGGGCC
SgCAS 3′-RACE primer	3′RACE-*CAS*2	GAGGCAGTAACTGGCACTCTAAGAAGGG
SgCAS qRT-PCR primer	*SgCAS*-ORF1	ATGTGGCATCTCAAGATTGG
	SgCAS-ORF2	TTAAGGGGCTCGCAGTACC
SgCAS qRT-PCR primer	*SgCAS*-qPCR1	CAAATACAACATGCTCACC
	SgCAS-qPCR2	TAGCCCTTCTTAGAGTGCC
Reference gene primer	*SgUBQ*-qF	ATAAAAGACCCAGCACCACATTC
	SgUBQ-qR	CCCTTGCCGACTACAACATCC

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