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Research article

Molecular characterization of *Glycine max* squalene synthase genes in seed phytosterol biosynthesis

Hanh T.M. Nguyen [1], Anjanasree K. Neelakadan [2], Truyen N. Quach [1], Babu Valliyodan, Rajesh Kumar [3], Zhanyuan Zhang, Henry T. Nguyen*

Division of Plant Sciences and National Center for Soybean Biotechnology, University of Missouri, Columbia, MO 65211, USA

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ABSTRACT

The reaction catalyzed by squalene synthase (EC.2.5.1.21) that converts two molecules of farnesyl pyrophosphate to squalene represents a crucial branch point of the isoprenoid pathway in diverting carbon flux towards the biosynthesis of sterols. In the present study two soybean squalene synthase genes, *GmSQS1* and *GmSQS2*, were identified in the soybean genome and functionally characterized for their roles in sterol biosynthesis. Both genes encode a deduced protein of 413 amino acids. Complementation assays showed that the two genes were able to convert yeast sterol auxotrophy *erg9* mutant to sterol prototrophy. Expression of *GmSQS1* and *GmSQS2* was ubiquitous in roots, stem, leaves, flower and young seeds of soybean, however *GmSQS1* transcript was preferential in roots while *GmSQS2* transcript was more in leaves. Their expression was lower in response to dehydration treatments suggesting they might be negative regulators of water stress adaptation. Transgenic *Arabidopsis* plants overexpressing *GmSQS1* driven by either constitutive or seed-specific promoters showed increases in the major end product sterols: campesterol, sitosterol and stigmasterol, which resulted in up to 50% increase in total sterol content in the seeds. The increase in the end product sterols by *GmSQS1* overexpression was at the level achievable by previously reported overexpression of individual or combination of other key enzymes in the sterol pathway. Together the data demonstrate that soybean *SQS* genes play an important role in diverting carbon flux to the biosynthesis of the end product sterols in the seeds.

1. Introduction

Sterols are found primarily in cell membranes of all eukaryotic organisms. In plants, the dominant sterols are 24-alkyl sterols which comprise sitosterol, stigmasterol and campesterol while the other non-methylated C-24 sterols, such as cholesterol, are present in relatively low amounts [1]. Sterols play multiple roles in developmental stages in higher plants [2]. Most of the sterols exist as free sterols and serve as components of the cell membrane. A small amount of sterols, specifically campesterol, are precursors to brassinosteroids, the critical hormones for plant growth and development [2]. Sterols have been reported to have roles in the adaptation of plants to environmental stress. The abundance of free sterols, sitosterol in particular, are associated with the adaptability of potato to low temperature [3] and down-regulation of *Arabidopsis* SQS increased susceptibility to pathogen attack [4].

Sterols are majorly derived from the mevalonate pathway of isoprenoid biosynthesis (Fig. 1). Catalyzing the first committed reaction in sterol biosynthesis [5], squalene synthase (SQS, EC.2.5.1.21) acts in the condensation of two farnesyl pyrophosphate (FPP) molecules to produce squalene which occur in two steps. The first step is the head-to-head condensation of two FPP molecules to form presqualene diphosphate, which is then rearranged and reduced by NADPH to form squalene in the second step [5]. Squalene is further metabolized to synthesize end product sterols including sitosterol, stigmasterol and campesterol. Because FPP is also the substrate to synthesize other non-sterol isoprenoids (Fig. 1), including ubiquinones, sesquiterpenoids and geranylgeranyl diphosphate in plant cells [6,7], regulation of SQS has been considered important for redirecting carbon flux to end product sterol biosynthesis, which was reflected by a number of evidences.

* Corresponding author. Tel.: +1 573 882 549; fax: +1 573 882 1469.
E-mail address: nguyenhenry@missouri.edu (H.T. Nguyen).
[1] Present address: The Center for Plant Science Innovation, University of Nebraska, Lincoln, NE 68588, USA.
[2] Present address: Department of Genetics, Development, and Cell biology, Iowa State University, Ames, IA 50011, USA.
[3] Present address: National Research Center on DNA Fingerprinting, NBPGR, New Delhi 110012, India.

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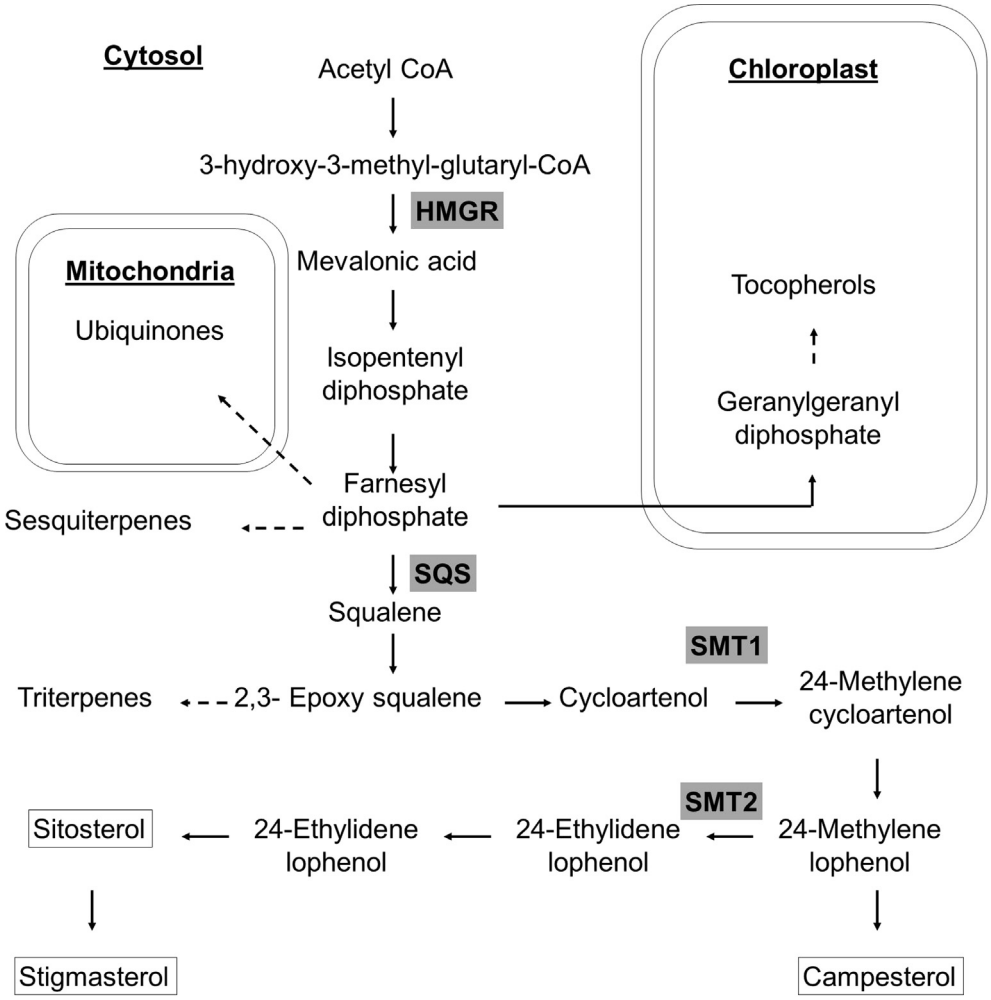

Fig. 1. Major sterol biosynthesis pathway in plants. Shaded boxes represents rate limiting enzymes that have been reported to enhance total sterol biosynthesis in plants. Major sterol products are boxed. Dotted arrow marks represent multiple enzymatic steps.

Cholesterol level of human fibroblasts was found to be modulated by SQS activity [8,9]. Changes in the activity of SQS in the yeast *Saccharomyces cerevisiae* sterol-auxotrophic mutants of upstream genes were associated with the treatment of exogenous sterol [10]. In plants, there is an association of SQS activity and the partitioning of FPP between sterol and sesquiterpenoid biosyntheses, which compete for this prenyl diphosphate [11–13]. Lowering sterol levels resulted from treatments with SQS inhibitor were found to trigger increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) an upstream enzyme in the pathway [14]. Overexpression of squalene synthase was reported to increase the major phytosterols up to 200% in *Panax ginseng*, *Eleutherococcus senticosus* and *Euphorbia tirucalli* [15–17] while down-regulation of SQS reduced the level of stigmasterol in tobacco [4].

Soybean is the world's leading oilseed crop and provides the largest source of processed vegetable oil. Sterol content in soybean crude oil is about 300 mg/100 g [1] and present mainly as free sterols [1]. Consumption of plant sterols is considered beneficial to human health due to their proposed inhibitory effects on lung, stomach, ovarian and breast cancers, and their promotion of anti-oxidant enzyme activities thereby reducing oxidative stress [18]. Phytosterols also inhibit and lower cholesterol absorption in human and consequently reduce cardiovascular risk [19]. With the given benefits, improving sterol content in the soybean oil could increase the soybean nutritional values. In this study, we aimed to investigate the function of two soybean SQS genes, *GmSQS1* and *GmSQS2*, in the regulation of end product sterol biosynthesis. *GmSQS1* (GenBank accession number AB007503) was previously cloned from soybean (Hata, 1997, unpublished); however, its function in seed sterol biosynthesis has not yet been investigated. *GmSQS1* and *GmSQS2* encoded two highly identical proteins and they were able to convert yeast *erg9* mutant, which is defective in SQS, to ergosterol autotrophic. Transgenic *Arabidopsis* plants over-expressing *GmSQS1*, driven by either constitutive or seed-specific promoter, were able to enhance the production of phytosterols in the seeds. Use of seed-specific expression of soybean SQS can potentially avoid negative effect on drought adaptation that may be associated with the elevated overexpression of SQS in the vegetative tissues.

2. Results

2.1. Identification and sequence information of soybean squalene synthases

Using known GmSQS1 protein (NCBI accession BAA22559) sequence as a query, Phytozome TBLASTN searches against soybean genome showed two distinct high scored hits of 1.8e−39 and 9.5e−32 in chromosome 12 and 11, respectively. Other BLAST hits landed on non-genic regions or resulted in substantially low scores.