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# Transcriptome profiling, and cloning and characterization of the main monoterpene synthases of *Coriandrum sativum* L.

Mariana Galata, Lukman S. Sarker, Soheil S. Mahmoud *

*Department of Biology, University of British Columbia Okanagan Campus, 3333 University Way, Kelowna, BC V1V 1V7, Canada*

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ABSTRACT

Terpenoids are a large and diverse class of specialized metabolites that are essential for the growth and development of plants, and have tremendous industrial applications. The mericarps of *Coriandrum sativum* L. (coriander) produce an essential oil (EO) rich in monoterpenes, volatile $C_{10}$ terpenoids. To investigate EO metabolism, the transcriptome of coriander mericarps, at three developmental stages (early, mid, late) was sequenced via Illumina technology and a transcript library was produced. To validate the usability of the transcriptome sequences, two terpene synthase candidate genes, *CsγTRPS* and *CsLINS*, encoding 558 and 562 amino acid proteins were expressed in bacteria, and the recombinant proteins purified by Ni–NTA affinity chromatography. The 65.16 (CsγTRPS) and 65.91 (CsLINS) kDa recombinant proteins catalyzed the conversion of geranyl diphosphate, the precursor to monoterpenes, to γ-terpinene and (S)-linalool, respectively, with apparent $V_{max}$ and $K_m$ values of 2.24 ± 0.16 (CsγTRPS); 19.63 ± 1.05 (CsLINS) pkat/mg and 66.25 ± 13 (CsγTRPS); 2.5 ± 0.6 (CsLINS) μM, respectively. Together, CsγTRPS and CsLINS account for the majority of EO constituents in coriander mericarps. Investigation of the coriander transcriptome, and knowledge gained from these experiments will facilitate future studies concerning essential and fatty acid oil production in coriander. They also enable efforts to improve the coriander oils through metabolic engineering or plant breeding.



## 1. Introduction

*Coriandrum sativum* (coriander) is a hardy annual plant belonging to the *Apiaceae* family, today cultivated in various temperate countries. The term cilantro refers to its leaf tissue, while the dry fruits are known as coriander mericarps (Reuter et al., 2008). Coriander mericarp oil is comprised of both fatty acids, as well as essential oil (EO); the leaves do not produce any EO. Lipid content of coriander mericarp oil is high, 28.4% of the total mericarp weight, which may be of great importance in the food industry (Ramadan and Morsel, 2002). Its EO has been shown to exhibit antimicrobial, anti-inflammatory and anti-hyperglycemic properties, to name a few (Gallagher et al., 2003; Kubo et al., 2004; Reuter et al., 2008).

Plant terpenoids, or isoprenoids, are made up of five carbon isoprene units, and are found in all plant tissues including leaves, flowers, buds, stems, roots, and seeds. Due to the cytotoxicity of many terpenoids (those which constitute EOs), plants have developed certain specialized structures for storage of these compounds. For example, flowers store terpenoids in glandular trichomes, while mericarps do so in secretory canals called vittae (Gross et al., 2006). In plants, terpenoids perform a variety of essential functions in growth and development (e.g., as growth regulators) and have crucial ecological roles (e.g., in defense and pollinator attraction) (Bakkali et al., 2008). These compounds are valuable in many industries, for example, medicine, agriculture (e.g., as pesticides), culinary (e.g., as flavorants) and hygiene (e.g., in scented products) (Chithra and Leelamma, 2000; Gallagher et al., 2003; Kubo et al., 2004; Lo Cantore et al., 2004).

There are seven major classes of terpenoids, categorized according to the number of isoprene units that make up their backbone structure (Yazaki, 2006). These include the mono-($C_{10}$), sesqui-($C_{15}$), di-($C_{20}$), sester-($C_{25}$), tri-($C_{30}$), tetra-($C_{40}$), and polyterpenes ($C_n$). These natural products are biosynthesized via two distinct pathways in separate cellular compartments. The 1-deoxyxylulose-5-phosphate (DXP) pathway is located in the plastid, and through this pathway, mono-, di-, and tetra-terpenes are produced (Hasunuma et al., 2008; Rodriguez-Concepcion, 2010). The mevalonate (MVA) pathway operates in the cytosol, where it is responsible for the production of sesqui-, tri-, and poly-terpenes (Ganjewala et al., 2009; Rodriguez-Concepcion, 2010). Each of these pathways yields universal terpenoid precursors, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate

* Corresponding author. Tel.: +1 (250) 807 8752.
*E-mail address:* soheil.mahmoud@ubc.ca (S.S. Mahmoud).

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(DMAPP). Further condensation of these two terpenoid precursors gives rise to prenyl diphosphates, geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranyl geranyl diphosphate (GGPP) (Supplementary Figure S1), the linear precursors to various isoprenoids. Specialized enzymes known as terpene synthases/cyclases convert these substrates to the great variety of terpenoids found in plants. Numerous terpenoids can be further modified via both enzymatic and non-enzymatic processes to increase plant terpene diversity (Mahmoud and Croteau, 2002; Rodriguez-Concepcion, 2010).

Coriander EO is a blend of mainly monoterpenes, as well as a small number of sesquiterpenes. (S)-Linalool (**1**) is the most abundant monoterpene found in coriander EO, making up approximately 72% of the oil (see Fig. 1). The mericarp EO composition changes as the mericarp matures; monoterpene alcohols (e.g., (S)-linalool (**1**)) increase, while monoterpene hydrocarbons, esters and ketones decrease (Msaada et al., 2009a).

Previous studies on coriander have primarily focused on fatty acid biosynthesis and essential oil composition; however, coriander mericarp EO biosynthesis remains understudied, and the terpene synthases responsible for monoterpene production in this plant have not been described. The main objective of the work presented here was to clone and functionally characterize the main coriander terpene synthases, in particular (S)-linalool synthase, which is responsible for the production of (S)-linalool (**1**), the main component of coriander EO.

Given that the production of several monoterpenes is regulated, at least in part, at the transcriptional level (Lane et al., 2010), it was anticipated herein that transcript levels for (S)-linalool synthase would also increase as coriander mericarps mature. Illumina sequencing technology was thus used to obtain sequence information for the entire transcriptome of the *C. sativum* mericarpacross three developmental stages (early, mid and late). This information facilitated cloning of terpenesynthases from coriander, and enabled investigation of expression patterns of two key regulatory genes, DXP synthase (DXS) and HMG-CoA reductase (HMGR), in isoprenoid biosynthesis.

The sequence information obtained from this study will aid in novel gene discovery and for development of molecular markers for improvement of *C. sativum* through plant breeding. Additionally, knowledge regarding EO biosynthesis in coriander mericarps may aid future efforts to improve plant mericarp EO content via genetic engineering. Mericarps are excellent storage vessels, as they maintain oil integrity and have minimal storage requirements (Misharina, 2001). This makes plant mericarps great targets for research concerning production of industrially valuable terpenoids.

## 2. Results and discussion

### 2.1. Scanning electron microscopy (SEM)

High resolution images were obtained of coriander whole mericarp and cross-section (Fig. 2A and B). Four vittae, or secretory canals, are clearly visible in Fig. 2B and a close-up in Fig. 2D. These vittae are the storage sites of coriander mericarp EOs (Parthasarathy and Zachariah, 2008; Purseglove et al., 1981), unlike some angiosperms which store EOs in glandular trichomes (Turner and Croteau, 2004) and gymnosperms which store EOs in resin ducts (Wu and Hu, 1997). Typical stomata, complete with guard cells, are present on the surface of the coriander mericarp (Fig. 2C). The presence of stomata on mericarps is uncommon, although it has been previously reported (e.g., in *Bauhinia* and *Eschscholzia*) (Jernstedt and Clark, 1979; Rugenstein and Lersten, 1981).

To date, the exact function of stomata on plant mericarps has not been elucidated with certainty. Proposed functions include facilitation of gas exchange in photosynthesizing mericarps, during embryo development, as well as playing a role during imbibition (Jernstedt and Clark, 1979; Paiva et al., 2006; Werker, 1997). Green plant tissues, including green plant mericarps, have entered the greening process and are actively photosynthesizing (Tschiersch et al., 2011). According to the RNA-seq data, coriander mericarps strongly express all photosynthetic genes (Supplementary Table S1). This, coupled with the observation that developing coriander mericarps are green, indicates that these tissues are actively photosynthesizing during their development. Once coriander mericarps have matured and fallen from the parent plant they become desiccated and their seed coat browns and hardens. Photosynthesis is likely inactive at this point yet the stomata may still function

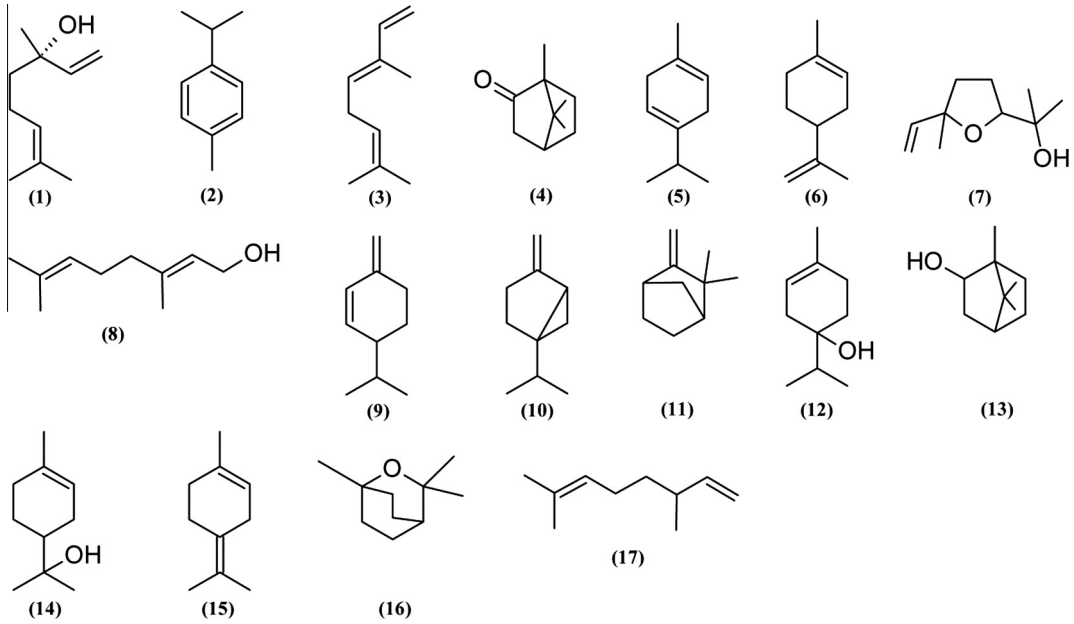


**Fig. 1.** Structures for volatile terpenes found in *C. sativum* mericarps use in this study.

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