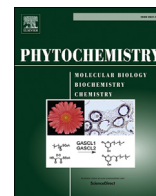




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## Transcriptome profiling of the Australian arid-land plant *Eremophila serrulata* (A.DC.) Druce (Scrophulariaceae) for the identification of monoterpene synthases

Octavia Natascha Kracht<sup>a</sup>, Ann-Christin Ammann<sup>a</sup>, Julia Stockmann<sup>a</sup>, Daniel Wibberg<sup>b</sup>, Jörn Kalinowski<sup>b</sup>, Markus Piotrowski<sup>c</sup>, Russell Kerr<sup>d</sup>, Thomas Brück<sup>e</sup>, Robert Kourist<sup>a,\*</sup>

<sup>a</sup> Junior Research Group for Microbial Biotechnology, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany

<sup>b</sup> Centre for Biotechnology, University of Bielefeld, 33615 Bielefeld, Germany

<sup>c</sup> Chair of Plant Physiology, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany

<sup>d</sup> Marine Natural Products Lab, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada

<sup>e</sup> Chair of Industrial Biocatalysis, Technical University of Munich, Lichtenbergstraße 4, 85748 Garching, Germany

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### ABSTRACT

Plant terpenoids are a large and highly diverse class of metabolites with an important role in the immune defense. They find wide industrial application as active pharmaceutical ingredients, aroma and fragrance compounds. Several *Eremophila* sp. derived terpenoids have been documented. To elucidate the terpenoid metabolism, the transcriptome of juvenile and mature *Eremophila serrulata* (A.DC.) Druce (Scrophulariaceae) leaves was sequenced and a transcript library was generated. We report on a first transcriptomic dataset of an *Eremophila* plant. IlluminaMiSeq sequencing ( $2 \times 300$  bp) revealed 7,093,266 paired reads, which could be assembled to 34,505 isogroups.

To enable detection of terpene biosynthetic genes, leaves were separately treated with methyl jasmonate, a well-documented inducer of plant secondary metabolites. In total, 21 putative terpene synthase genes were detected in the transcriptome data. Two terpene synthase isoenzymatic genes, termed ES01 and ES02, were successfully expressed in *E. coli*. The resulting proteins catalyzed the conversion of geranyl pyrophosphate, the universal substrate of monoterpene synthases to myrcene and Z-(b)-ocimene, respectively.

The transcriptomic data and the discovery of the first terpene synthases from *Eremophila serrulata* are the initial step for the understanding of the terpene metabolism in this medicinally important plant genus.

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### 1. Introduction

Due to the geographical isolation and the diversity of geological and climate conditions, the Australian continent has developed its unique indigenous flora (Sadgrove and Jones, 2014). Therefore, the proportion and composition of the essential oils produced by Australian plants differ highly from the flora found on any other continent (Sadgrove and Jones, 2014).

The genus of Australian plant *Eremophila* (*eremos* – dry, *philos* – loving) belongs to the order of Lamiales and the Scrophulariaceae family and encompasses more than 200 different species

commonly found throughout the Australian arid-lands and temperate grasslands (Chinnock, 2007; Sadgrove and Jones, 2014). The species *Eremophila serrulata* (A.DC.) Druce (*E. serrulata*) is widely distributed in central-western New South Wales through South Australia to the west coast of Western Australia (Chinnock, 2007). Some species of *Eremophila* are established in traditional Aboriginal medicine to alleviate symptoms such as skin lesions and sore throat (Ghisalberti, 1994; Ndi et al., 2007). Various studies demonstrated antibacterial effects of different *Eremophila*-derived terpenoid structures against a wide range of bacteria. The plant extracts of some species have also been shown to exhibit antiviral, anti-inflammatory, antimalarial and cytotoxic effects (Barnes et al., 2013). *E. serrulata* was reported to contain five serrulatane diterpenoids with antibacterial activities against Gram-positive bacteria

\* Corresponding author.

E-mail address: [kourist@tugraz.at](mailto:kourist@tugraz.at) (R. Kourist).

(Ndi et al., 2007). The final mechanistic route for the formation of these diterpenoids cannot be proposed as the corresponding enzyme systems producing these compounds in the *Eremophila* genus are still unknown. In plants, terpenes are formed either by the mevalonate pathway operating in the cytosol or by the methylerythritol phosphate (MEP) pathway operating in the plastids (Oldfield and Lin, 2012) whereat mono- and diterpenes are usually produced by the MEP pathway.

Monoterpenes are the C<sub>10</sub> members of the terpenoid family of natural products and play an important role in plant's defense mechanism against different herbivores (Dudareva, 2003). In addition, such terpenes are currently used for industrial applications such as flavoring agents, perfumes and insecticides (Mahmoud and Croteau, 2002). The isomers myrcene and Z-(b)-ocimene are present in the essential oils of many plant genera (Dudareva, 2003; Zini et al., 2002) and take part in the immune defense (Arimura et al., 2004; Navia-Gine et al., 2009). They are also commonly reported for the essential oils of the *Eremophila* genus (Smith et al., 2010; Youssef et al., 2014) and Z-(b)-ocimene is one of the major components of the oil of *Eremophila maculata* (Youssef et al., 2014).

In this study we identified the two first isoenzymatic terpene synthases (TPS) generating myrcene and Z-(b)-ocimene from the Australian desert plant *E. serrulata*.

## 2. Results and discussion

### 2.1. Transcriptome sequencing, annotations and phylogenetic analysis

Transcriptome sequencing of a normalized cDNA library made from a mix of *Eremophila* juvenile and matured leaves was performed on the IlluminaMiSeq system yielding 7,093,266 paired reads. After processing and quality control, 7,084,170 reads remained. Assembly of these reads was carried out by applying the gsAssembler (Roche Applied Science) version 2.8 using the cDNA mode and default settings. In total, 34,505 generated isogroups

(103,404 isotigs) were imported into the Sequence Analysis and Management System (SAMS) 2.0 (Bekel et al., 2009). Automatic annotation was performed and functional annotations were created. The annotation pipeline assigned more than 3000 gene names and 6000 EC numbers to the isogroups. The GC content of the assembled isogroups was 41.16%.

In total, 53.4% of the proteins in the transcriptome did not show significant amino acid sequence similarity to known enzymes in the KEGG database (Figs. 1 and S1). However, 12.2% of the proteins were annotated to play a role in protein biosynthesis and modification, 8.3% appear to have a general function and 7.7% are involved in general metabolism. Furthermore, 4.4% of the proteins showed similarity to proteins responsible for signal transduction and 3.5% appear to be involved in the localization and transport system. Altogether, 1.8% of the proteins are assumed to be involved in reproduction, growth and death of the cells. Furthermore, 1.2% of the genes play a role in the secondary metabolism of the plant and 0.3% of the total proteins are hypothesized to participate in the immune defense. Lastly, 6.2% of the proteins are assumed to carry out remaining functions.

First, genomic DNA was used to validate the taxonomic position of *E. serrulata*. Reconstructing the internal transcribed spacer 1 (ITS) region using degenerated primers and phylogenetic analysis of the resulting gene suggested a close phylogenetic relationship to *Myoporum laetum*, *Myoporum sandwicense* as well as two other *Eremophila* species (see Fig. 2).

Furthermore, phylogenetic studies were performed on the citrate synthase protein sequence derived from the transcriptome data. Due to a lack of citrate synthase sequences of the Scrophulariaceae family, analysis of this house-keeping gene indicated a close relationship of *E. serrulata* with *Erythranthe guttatus* which also belongs to the order of Lamiales (see Fig. 3).

### 2.2. Terpene synthase candidate genes

To find novel terpene synthases, transcripts were analyzed with antiSMASH 3.0 (Weber et al., 2015) as well as with BLASTx (NCBI

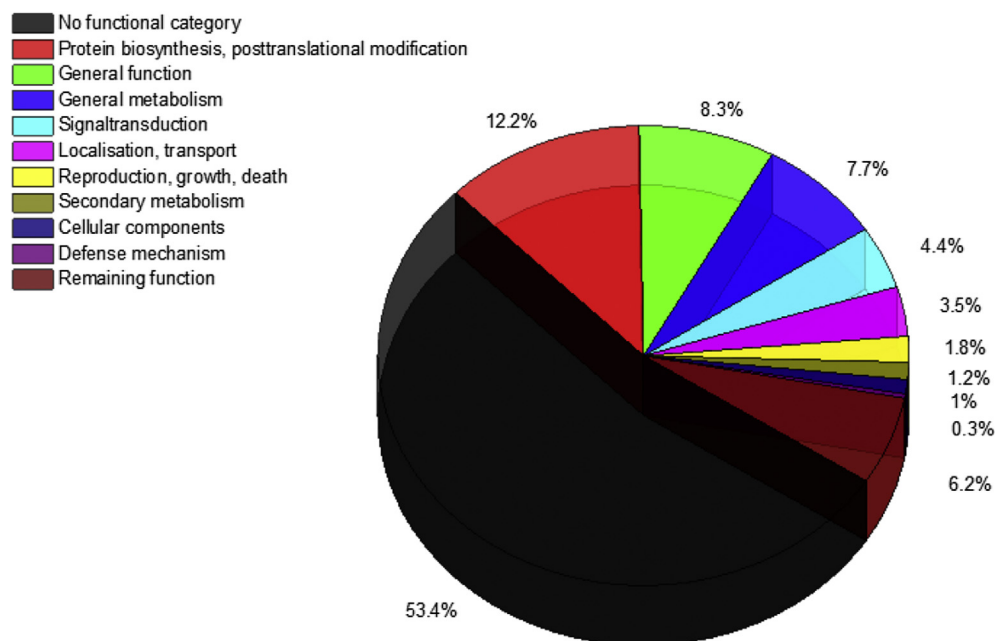


Fig. 1. Overview of the transcriptomic data of *E. serrulata*. In total, 53.4% could not be classified to a functional category by annotation and 1.2% of the genes are supposed to play a role in the specialized metabolism.

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