Radula marginata; a prospective liverwort an alternate source of cannabinoid-like compounds

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INTRODUCTION

The liverwort Radula marginata belongs to the bryophyte division of land plants. Bryophytes are an early-diverged group of land plants. The division comprising of around 20,000 species and are divided into three phyla: mosses, hornworts and liverworts. Among them liverworts are the most abundant consisting of 6000-8000 species. Radulales is an order of subclass Jumengnannidae in this phylum. Radulaceae is the only family in this order, comprises Radula genus and 283 species. Radula marginata is of significant interest since perrottetinene and perrottetenic acid 1-2 had been reported. These compounds are structural analogs to tetrahydrocannabinol (Δ9-THC), a psychopharmacological compound in Cannabis sativa L. Recently, agonistic activity of these compounds with cannabinoid receptor 1 (CB1) 3-5 further confirmed the potential of this species in cannabinoid-based pharmaceutical industry. Therefore genetic-level understanding of secondary metabolic pathways that lead to the synthesis of cannabinoid-like natural compounds would be desirable.

RESULTS

i) de-novo assembly and annotation

Paired-end Illumina HiSeq-2000 platform generated 30 million raw reads for each pair. Raw reads filtered and quality trimmed. Trinity was used for de-novo Paired-end Illumina Hiseq-2000 platform generated 30 million raw reads for each pair.

Table 1: Sequencing Summary

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<tr>
<th>Description</th>
<th>Value</th>
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<td>No of assembled transcripts</td>
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<td>Minimum length (bp)</td>
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</table>

Assembled transcripts were BLAST against publically available databases, followed by scanning with InterProScan (IPR) for protein signatures, assigning Gene ontology (GO) term and finally map against KEGG database for active pathways in Radula marginata (Figure 1).

ii) Identification of precursor genes for the bioynthesis of cannabinoid-like compound

Figure 2: Candidate precursor genes for cannabinoid biosynthesis in Radula marginata: A) Identification of candidate genes for cannabinoid biosynthesis in R. marginata. GFF-transcripts and all the unique genes were identified. Genes with Rm_tct_0 prefix were identified in the study. B) Transcriptome expression of identified genes determined from TPM. Relative expression of candidate-gene by real time quantitative PCR (RT-qPCR).

iii) Characterization of stilbene synthase

Figure 3: A) Crystal structure of stilbene synthase model compared with Drosophila synthase-like polypeptide (Left, 3awv_i) and Stilbene carboxylate synthase from Matrinia polyophaga (right, 2pux_1A). B) Quality estimate of local similarity, Z score of the predicted STS model in Radulamarginata, C) Alignment of the identified STS in Radula marginata with the respective template model, blue boxes are the best streets, yellow boxes in the alignment are the variable sites for both models used as well as in dynamic acid from Cannabis sativa.

iv) Metabolomic detection of CBGA-analog

The CBGA analog was identified with the HFLC-ESI-MS-MS approach. The positive ESI mass spectrum of the CBGA bibenzyl analog showed an [M+H]+ ion at m/z 395, a predominant [M+H2O]+ion at m/z 377, and fragment ions at m/z 271 [M+H-C9H16] and m/z 267 [M+H2O-C9H4]. The ion m/z 253 is probably the result of degradation processes eliminating two protons, resulting in [M+H-H2O-C9H4]. Together these indicate the presence of the CBGA analogues’ bibenzyl structure in the methanolic extracts of R. marginata (Figure 4).

Conclusion and future prospects

This is the first transcriptomic study for the Radulaceae family. We captured, assembled and annotate the R. marginata transcriptome. Identification of the precursor genes for the cannabinoid-like compound, validation of these genes through quantitative real time expression analysis, characterization of the central precursor and finally detection of compound from metabolomics approach lead us to speculate that the cannabinoid-like pathway is likely to be conserved in lower and high land plants with the exception of first intermediate. However, these findings require further experimental work to confirm this proposed novelty. Overall, this study would serve as a reference transcriptomic resource for Radulaceae family to further explore especially Radula marginata. Moreover, this study also proposes R. marginata as an alternate to Cannabis sativa for cannabinoid-like compounds.

Acknowledgment

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References


For further details: