Polyketide synthases in Cannabis sativa L

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Summary

Cannabis sativa L. plants produce a diverse array of secondary metabolites, which have been grouped in cannabinoids, flavonoids, stilbenoids, terpenoids, alkaloids and lignans; the cannabinoids are the best known group of natural products from this plant. The pharmacological aspects of this secondary metabolite group have been extensively studied and the cannabinoid biosynthetic pathway has been partially elucidated. Although, it is known that the geranyl diphosphate (GPP) and the olivetolic acid are initial precursors in this route the biosynthesis of the olivetolic acid has not been found yet. It has been suggested that the olivetolic acid biosynthesis could be initiated by a polyketide synthase (PKS). This thesis was focused on the characterization of PKSs in cannabis plants.

More than 480 compounds have been identified from *C. sativa* but only 247 are considered as secondary metabolites. These latter are grouped into cannabinoids, flavonoids, stilbenoids, terpenoids, alkaloids and lignans. However, what do we know about their biosynthesis and role in the plant? **Chapter 1** summarizes the natural compounds in cannabis from a biosynthetic view. It seems that enzymes belonging to the polyketide synthase group could be involved in the biosynthesis of the initial precursors from the cannabinoid, flavonoid and stilbenoid biosynthetic pathways.

The Polyketide Synthases (PKSs) are condensing enzymes which form a myriad of polyketide compounds. In plants several PKSs have been identified and studied. Aspects such as specificity, reaction mechanisms, structure, as well as evolution are reviewed in **Chapter 2**.

In **Chapter 3** polyketide synthase (PKS) enzymatic activities were analyzed in crude protein extracts from cannabis plant tissues. Differences in activities of chalcone synthase (CHS), stilbene synthase (STS) and olivetol-forming PKS were observed during the development and growth of glandular trichomes on the female flowers. Although, cannabinoid biosynthesis and accumulation take place in glandular trichomes no activity for an olivetolic acid-forming PKS was

detected in this tissue. Content analyses of cannabinoids and flavonoids from different tissues revealed differences in their distribution, suggesting a diverse regulatory control on the biosynthetic fluxes of their biosynthetic pathways in the plant.

Chapter 4 reports *in silicio* expression analysis of a *PKS* gene isolated from glandular trichomes. The deduced amino acid sequence showed 51-72% identity to other CHS/STS type sequences of the PKS family. Further phylogenetic analysis revealed that this PKS (PKSG2) grouped with other non-chalcone and stilbene-producing PKSs. Homology modeling analyses of this cannabis PKS predicts a 3D overall fold similar to alfalfa CHS2 with small steric differences on the residues that shape the active site of the cannabis PKSG2.

Cannabis sativa cell culture induction has been reported for several purposes. However, cannabinoids have not been detected in cell cultures so far. Although, elicitation has been employed in the cell cultures for inducing and/or improving secondary metabolites there are no reports concerning elicitation effect on secondary metabolite production in *C. sativa* cell cultures. In **Chapter 5** the effect of elicitation on secondary metabolism of the plant cell cultures is reported. Metabolic profiles analyzed by ^{1}H-NMR spectroscopy and principal component analyses (PCA) showed variations in some of the metabolite pools. However, no cannabinoids were found in both control and elicited cannabis cell cultures. *THCA synthase* gene expression was monitored during a time course. Results suggest that other components in the signaling pathway can be controlling the cannabinoid pathway.