Polyketide synthases in Cannabis sativa L

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Concluding remarks and perspectives

In the phytochemistry from *Cannabis sativa* L., six secondary metabolite groups (cannabinois, flavonoids, stilbenoids, terpenoids, alkaloids and lignans) have been identified. Pharmacological aspects of the best known group of the secondary metabolism of this plant, cannabinoids, have been extensively studied. Other studies have been focused on the elucidation of the cannabinoid biosynthetic pathway. Although, it has not been completely elucidated, and the same applies for other secondary group biosynthetic pathways in the plant, it has been suggested that a polyketide synthase (PKS) catalyzes the synthesis of the first precursor of the cannabinoid pathway, the olivetolic acid. However, the identification of flavonoids and stilbenoids in the plant involve the presence of more than one PKS.

In this study, the interest was focused on PKSs, their functions in the cannabinoid and flavonoid biosynthesis and the identification of *PKS* genes. Activity of an olivetol-forming PKS and activities of PKSs type CHS and STS were identified from plant tissues. These activities showed to be different in plant tissues. Olivetol-forming PKS activity seemed to be related to the growth and development of the glandular trichomes (hairs) on the female flowers and cannabinoid biosynthesis, a higher cannabinoid accumulation in the bracts than other cannabis plant tissues was shown. Although, type-CHS activity preceded the accumulation of flavonoids in the female flowers and it seemed to be also related to the growth and development of the glandular trichomes on female flowers CHS activity was lower than olivetol-forming PKS activity. The biosynthetic fluxes from cannabinoid and flavonoid pathways seemed to be differentially regulated; differences in the accumulation of these two compounds during the growth and development of the glandular trichomes on the female flowers were observed. Significant activity of type-CHS PKS in roots could not be correlated with flavonoid biosynthesis. Metabolic profilings during development and growth of the cannabis roots to identify the main secondary metabolite groups should be performed to correlate the PKS activities identified in roots. It seemed that stilbenoid accumulation depends on the STS activity, the basal activity of type-STS PKS detected during the growth and development

of the glandular trichomes on female flowers was related to the absence of stilbenoids.

One PKS cDNA (*PKSG2*) was characterized and identified in leaves and glandular trichomes, according to expression analyses by RT-PCR. The expression of the known cannabis CHS-type PKS (*PKS1*) was not tissue-specific, as it was identified in flowers (female and male) and glandular hairs; and from previous studies in leaves and roots by Northern blot. PKSG2 seems to be a non-chalcone and non-stilbene forming enzyme and PKS1 a chalcone forming enzyme, according to the phylogenetic analysis. Furthermore, the substrate specificity of PKS2 is different from CHS and VPS, according to the homology modeling analysis. Although, PKSG2 is 97% similar to cannabis PKS (PKS-1) recently identified, which biosynthesizes hexanoyl triacetic acid lactones, according to the homology analyses the biochemical characterization of the protein encoded by *PKSG2* needs to be carried out. As cannabinoids with different side-chain moiety lengths have been identified in cannabis plants and the detection of THCA, a pentyl-cannabinoid, and THVA, a propyl-cannabinoid, in a same plant tissue, as it was shown on the cannabinoid profile from female flowers highlights the necessity to analyze the biochemical characteristics of PKSG2.

No cannabinoids were produced by cannabis cell suspension, calli or embryo cultures; neither did elicited cannabis cell cultures, as it was shown by LC-MS and ^{1}H-NMR spectroscopy. During a time course the *THCA synthase* gene expression was not detected in the cell cultures corroborating no cannabinoid biosynthesis. In cannabis plants, cannabinoid pathway seemed to be linked to tissue-specificity and/or developmental controls, as it was shown only in cannabis plant tissues containing glandular trichomes such as leaves and flowers the expression of *THCA synthase* gene was observed and it was linked to the development and growth of glandular trichomes on flowers. As cannabinoids are cytotoxic compounds they should be biosynthesized and stored into the glandular trichomes, studies about the development and metabolism of glandular tissues should be considered to increase product yield. Knowledge about the regulatory control of secondary metabolite biosynthetic pathways and gland differentiation may be required to generate successfully these compounds in cell or tissue cultures. Cannabis glandular tissue should be considered as a model system for research.