

# I. Summary

The literature review concerning *Cannabis sativa* L. was prepared with focus on the biologically active compounds. The scope of cell cultures biotechnology and application of modern instrumental analytical methods in the field of metabolomics was discussed.

Based on the NMR and MS results, no cannabinoids were detected in the control or elicited cell suspension cultures of *Cannabis sativa* L. Also other secondary metabolites were not detected by NMR. Other more sensitive analytical methods and their combinations should be used for absolute evaluation of other secondary metabolites, if they are present. It can be concluded, that biosynthesis of the cannabinoids was not induced by pectin or jasmonic acid as elicitors in the suspension, callus or embryogenic tissue cultures of *Cannabis sativa* L.

Main metabolites of certain growth phases were evaluated based on the quantification of produced metabolites and prepared growth phase curves. The impacts of elicitations were interpreted by PCA statistical method, with subsequent metabolite identification or structure elucidation. Maxima of possible  $^1\text{H}$  NMR signals were identified and 2D NMR experiments were applied for signals of unknown compounds.

The results from NMR measurements combined with PCA analysis brought comprehensive picture of the cultures' primary metabolism, which is not however directed through biosynthetic pathways to the complex structures of secondary metabolites. Unambiguously was induced metabolism of shikimic acid pathway leading to the aromatic building stones, which are indeed not part of the cannabinoids metabolism. On the other hand detection of terpene signals in chloroform fraction is proof of deoxyxylulose and mevalonate metabolic pathways leading to  $\text{C}_5$  isoprene units. Compartmentization is also one of the major requirements for secondary metabolites production, which can be handicap of successful experiment in case of the nondifferentiated cell cultures. For cannabis the presence of glandular trichomes with specific enzymatic apparatus is necessary condition. Based on this knowledge we can expect that even with sufficient supply of the cannabinoids biosynthetic building stones, the necessary enzymatic apparatus representing by olivetolic synthase is not activated in early stages and therefore olivetolic acid as major precursor is not synthesized. In the field of research also remains characterization of other polyketide synthase isoforms, which could be involved in the biosynthesis of cannabinoids or rearrange metabolic pathway to the other secondary metabolites using acetate as building block like flavonoids or stilbenoids <sup>257</sup>).

By our analysis was comprehensively studied metabolism of the suspension cultures and achieved NMR results will be used in next experiments with the biological material. Integral part will be comparison of the data with intact plant extracts, which represent distinctively much more complex system from the  $^1\text{H}$  NMR analysis point of view. Our data will be used in subsequent experiments of chemical or molecular biology way, especially concerning early stages of cannabinoids metabolism, namely in the study of polyketide synthase izoforms<sup>258</sup>).