Cannabinoids Analysis of Laser-Microdissected Trichomes of Cannabis sativa L. by LC-MS and Cryogenic NMR

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Abstract

Trichomes of Cannabis sativa have been reported as the main sites of cannabinoids production. A comprehensive study of cannabinoids was performed on capitulate-stalked and capitulate-sessile trichomes, and on capitulate-stalked glands and stems harvested by laser microdissection (LMD) during flowering time (week 4-8). LC-MS and cryogenic-NMR analysis were used for qualitative and quantitative assessment of cannabinoids in the collected cells. δ-9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabinolic acid (CBDA) were identified as the major cannabinoids in all tested samples, while THCA, cannabidiolic acid (CBDA) and cannabinolic acid (CBDA) were present in lower concentrations. Cannabinol (CBN) and cannabinolic acid (CBN) were detected as minor compounds only in intact capitulate-stalked trichomes in week 8. Based on the cannabinoids levels, discrimination of capitulate-stalked and capitulate-sessile trichomes at flowering time was possible. The study demonstrated the possibility of other spots for cannabinoids production besides the gland of the capitulate-stalked trichomes. In particular, the presence of cannabinoids in the stem of capitulate-stalked trichomes is reported for the first time. The combined use of LMD, LC-MS and cryogenic NMR constitutes a valuable method for the comprehensive assessment of plant metabolites at the cellular level.

Introduction

Cannabis sativa has long been used as a medicine in Asia, mainly in India, before the Christian era [1]. The main responsible components for biological activities of Cannabis are cannabinoids, a unique group of sesquiterpene-carboxylic compounds possessing psychotropic and medicinal modes in their molecular structures. Recently, new insights into the cellular localization of the cannabinoids production were revealed, focusing on trichomes [2,3]. Small protuberances of epidermis on the surfaces of leaves and other organs of plants. In C. sativa, trichomes are the main sites of cannabinoids production, and classified into three types, namely capitulate-stalked, capitulate-sessile and foliar trichomes. Capitulate-stalked trichome contains highest cannabinoids levels and consist of two parts: the gland (head) and the stem. The head contains cannabinoids precursors to be the site of cannabinoid production and surrounded by the dikaryotic cell wall, while the stem is formed by trichome cells and, when mature, consists of capitate (stems, pedicels) and stalked trichomes. Laser microdissection (LMD) is a powerful tool for isolating specific tissues, cell types, and organelles from whole-mount biological samples in a manner conducive to the extraction of RNA, DNA, proteins, or secondary metabolites [4]. LMD uses a laser beam to micro-dissect and cut out the cells of interest from surrounding cells and tissues. The wavelength of visible laser is 1077-340 nm. It is slightly higher than the absorption peak of proteins and nucleic acids but does not harm tissue integrity or the neighbouring cells [5].

Experiments

Floral leaves and bracts of C. sativa

Figure 1. Experimental workflow

Results and discussion

Cannabinoids profiles in all the investigated samples were qualitatively similar, THCA, CBDA, CBN, THC, CBD, and CBG were detected in all samples during the flowering stages. While CBG and CBN were only identified in the intact capitulate-stalked trichomes, and their heads at week 8.

THCA, CBDA, and CBN were present in high concentrations in all tested samples relative to other cannabinoids. Meanwhile, THC, CBD, and CBG were detected at lower concentrations.

CBDA, the presence of which could not be ascertained by LC-MS as its reference compound was unavailable, was effectively confirmed by cryogenic NMR.

The discovery of cannabinoids in the stem of capitulate-stalked is new and may indicate the existence of additional sites of cannabinoid production besides the trichome heads.

Conclusions

In this research we demonstrated a promising method of cannabinoid analysis in trichomes of medicinal Cannabis sativa combining LMD, LC-MS, and cryogenic NMR, LMD allowed isolating specific trichomes and their individual parts without contaminations from neighboring cells or tissues. Subsequent application of LC-MS provided for effective detection and measurement of low amounts of secondary metabolites in dissected trichome samples. Finally, cryogenic NMR was used to confirm structures of the identified trichome-specific cannabinoids. Moreover, the successful detection of CBCA by the latter analytical method showed that cannabinoid NMR can be efficiently applied in metabolite identification, even in absence of appropriate reference compounds.

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References