

MEJA ELICITATION OF SECONDARY METABOLITES IN INDUSTRIAL HEMP: BIOCHEMICAL AND RNA-SEQ SURVEYS

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Hemp, *Cannabis sativa* (L.), is a commercial crop, well known for its industrial, food, medical, and recreational applications, being a rich source of bioactive secondary metabolites (SMs). In plants, SMs accumulate at low concentration (less than 1% DW), but their level increases in response to adverse stimuli. Elicitation with jasmonate-related phytohormones (JA, MeJA) has been reported to positively stimulate the plant defence mechanisms, and, consequently, the accumulation of SMs. In hemp, an estimated 545 chemical compounds of different classes have been identified up to now, including phenols, cannabinoids and terpenoids, mainly accumulated in female inflorescences.

The aim of this work was to maximise the hemp content of phenols, cannabinoids, and terpenoids by using the MeJA elicitor (alone or conveyed through chitosan nanoparticles) on female plants of a dioecious variety (Tiborszallasi) during flower development. A pilot field trial was carried out with the following treatments: control, 1mM MeJA, 10mM MeJA, chitosan nanoparticles (CHTnp), and CHTnp loaded with 1mM MeJA. Inflorescence samples were harvested at two different developmental stages (DS): beginning of flowering and mature flower. Within each DS, inflorescences were collected at two harvest times (HT): 6h and 6 days after treatments. Overall, DS, HT, and treatments significantly affected both total phenolic and cannabinoid contents. Focussing on cannabinoids, the highest levels of cannabidiolic acid (5,01% CBDA) were found in mature inflorescences harvested 6 days after treatment with 10mM MeJA, doubling the amount of the control. Consistently, RNA-Seq analysis revealed that, among all the

treatments, 10mM MeJA elicitation produced the greatest transcriptome responses in terms of differentially expressed genes (independent of DS). Moreover, the analysis highlighted a strong up-regulation of an *olivetol synthase* (*OLS*), a key biosynthetic gene upstream of the cannabinoid route, in mature inflorescences harvested 6 days after treatment with 10mM MeJA. Finally, the gene regulatory networks underlying MeJA elicitation of SMs production is being dissected by transcript-metabolite correlation, clustering of gene expression and gene co-expression network (GCN) analyses.