

NEW AGRONOMIC PRACTICES TO IMPROVE HEMP SECONDARY METABOLITES ACCUMULATION

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Hemp, *Cannabis sativa* (L.), is a commercial crop, well known for industrial, food, medical, and recreational applications. It contains a large number of bioactive secondary metabolites (SMs), including cannabinoids, terpenoids and phenolic compounds, which mainly accumulate in female inflorescences. Hemp is conventionally classified in drug and fiber types based on content ratios between delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). However, there is also a growing interest in compounds other than cannabinoids, which are associated with powerful health benefits and may act synergistically, contributing to the “entourage effects” of cannabis-based medicines. In plants, SMs usually occur at low concentrations and their synthesis is induced by adverse stimuli. A strategy to raise SMs content in plant cell cultures is the use of elicitors, molecules able to trigger plant defence response. Specifically, the jasmonate-related phytohormones (jasmonic acid, JA, and methyl jasmonate, MeJA), have been reported to positively stimulate secondary biosynthetic pathways workflows, leading to increased production of various SMs. The effect of these molecules could be even increased by the use of nanoparticles as carriers.

The aim of this work was the optimization of SMs hemp productivity (total phenols, cannabinoids, terpenoids) by supplying MeJA as such (10mM) or carried by chitosan nanoparticles (CHTnp+1 mM MeJA) to female plants of the dioecious variety 'Tiborszallasi'. Small-scale field experiments included treatments with MeJA, CHTnp+MeJA, CHTnp (mock), and control supplied to

inflorescences from pre-flowering onwards. Inflorescences were collected at three development stages. A biochemical characterization of the samples in terms of total phenol content, antioxidant capacity, CBD and THC content has been carried out. The cannabinoid content was significantly affected by the elicitation treatments and by the sampling time. Namely, 10mM MeJA treatment gave rise to 80% CBDA increase compared to the control, and CHTnp+1 mM MeJA led to 88% CBDA increase compared to CHTnp (mock). Moreover, a significant interaction between treatment and sampling time was observed. A parallel RNA-Seq analysis revealed that both MeJA and CHTnp+MeJA treatments were effective in producing common and treatment-specific transcriptomic responses. However, GO enrichment analyses did not include terpenoid-related terms as expected; hence, a deeper survey was addressed to differential expression of genes in cannabinoid-related routes.

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