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Short communication

Cannabinoid accumulation in *in vitro* multiplied shoots and acclimatized plants of *Cannabis sativa* L.

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Introduction

Cannabis sativa L. is a medicinal plant that produces diverse bioactive secondary metabolites, particularly cannabinoids synthesized in glandular trichomes abundant on female inflorescences. The principal pharmacologically active compounds include Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and their acidic precursors Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA), (Stefkov et al., 2022). The therapeutic potential of *Cannabis sativa* L. has been associated with a wide range of biological activities, including pain relief, reduction of chemotherapy-associated side effects, antispasmodic effects, and supportive treatment of epilepsy, eating disorders, and inflammatory diseases (Wrobel et al., 2020). Cannabinoid profile is highly dependent on genotype, developmental stage, and environmental factors such as light and temperature (Stoilkovska-Gjorgievska et al., 2022).

Conventional propagation methods often result in variability in phytochemical profiles, while plant tissue culture offers a controlled alternative for producing uniform plant material. *In vitro* systems provide a controlled and sterile environment that enables precise manipulation of plant growth regulators, which can significantly affect both biomass production and secondary metabolite accumulation. However, cannabinoid biosynthesis capacity varies among *in vitro* systems, including shoot cultures, and regenerated plantlets (Wrobel et al., 2020).

The aim of this study was to compare the cannabinoid content in *in vitro* multiplied shoots and acclimatized plants grown under *in vivo* conditions, with a focus on their cannabinoid production potential.

Material and methods

Seeds of *Cannabis sativa* L. (cv. Bubba Kush × OG Kush) were surface-sterilized and germinated *in vitro* as previously described (Trajkovska et al., 2025). Cultures were maintained on MS medium supplemented with B_5 vitamins under controlled conditions (26 ± 1 °C, 16 h photoperiod).

Apical segments were cultured on MS/B_5 medium supplemented with thidiazuron (0.05-1.0 mg·L^{-1} TDZ) for shoot multiplication. Shoots were rooted on hormone-free medium and subsequently acclimatized by gradual reduction of humidity under controlled

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environmental conditions before transfer *ex vitro* into pots filled with a mixture of vermiculite and perlite (1:1).

Cannabinoids were extracted from powdered plant material (0.1 g) using ethanol with ultrasonication, followed by centrifugation and filtration. Extracts were evaporated to dryness, re-dissolved in methanol, and analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD) according to the German Pharmacopoeia method.

Results and Discussion

The production of major cannabinoids (CBDA, CBD, Δ⁹-THC and Δ⁹-THCA) was confirmed in both *in vitro* multiplied shoots and acclimatized plants of *Cannabis sativa* L., demonstrating distinct patterns of accumulation between the two systems. *In vitro* shoot cultures showed enhanced cannabinoid production, particularly under low TDZ concentrations, with the highest Δ⁹-THC content (49.23 $mg \cdot g^{-1}$) observed at 0.05 $mg \cdot L^{-1}$ TDZ. This indicates that low cytokinin levels may stimulate secondary metabolism, while higher TDZ concentrations reduced cannabinoid levels, consistent with previous findings (Wróbel et al., 2020). In contrast, acclimatized plants exhibited a more developmentally regulated cannabinoid distribution, differing between vegetative and reproductive organs. After six months, Δ⁹-THC levels in leaves reached 49.38 $mg \cdot g^{-1}$, exceeding those of the mother plant (30.63 $mg \cdot g^{-1}$), whereas inflorescences of regenerated plantlets contained lower Δ⁹-THC (82.81 $mg \cdot g^{-1}$) and Δ⁹-THCA levels (225.78 $mg \cdot g^{-1}$) compared to the mother plant (107.20 and 251.16 $mg \cdot g^{-1}$, respectively). At the same time, CBD and CBDA remained consistently lower in both leaves and inflorescences of acclimatized plantlets.

Overall, these results highlight that the *in vitro* shoots are characterized by hormonally driven enhancement of cannabinoid production, while acclimatized plants exhibit organ-specific and developmentally regulated cannabinoid profiles. These differences can be attributed to underlying physiological and structural factors. *In vitro* conditions may promote metabolite accumulation through controlled stress and hormonal regulation, but lack the complexity of whole-plant development. In contrast, acclimatized plants develop fully functional glandular trichomes, strongly influenced by plant maturity and environmental conditions (Stoilkovska-Gjorgievska et al., 2022). Additionally, cannabinoid profiles may further change post-harvest, as storage conditions, particularly temperature, can promote decarboxylation, leading to decreased Δ⁹-THCA and increased Δ⁹-THC (Davkova et al., 2023).

Conclusion

In vitro multiplied shoots of *Cannabis sativa* L. exhibit a clear capacity for cannabinoid production under controlled and aseptic conditions, although their profiles may differ from those of acclimatized plants. The observed differences likely reflect variations in hormonal regulation, tissue differentiation, and glandular trichome development. These findings support the complementary application of both *in vitro* shoot cultures and acclimatized plants as biotechnological platforms for cannabinoid-rich biomass production.

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