Effect of auxin transport inhibitors on shoot organogenesis of hemp (*Cannabis sativa* L.) epicotyl explants

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Abstract: Industrial hemp (*Cannabis sativa* L.) is economically valuable crop used in a production of nutraceutical supplements, functional food, pharmaceuticals, cosmetics etc. However, the large scale propagation of this plant has been so far limited by the challenges regarding low regeneration rate and variety-/genotype-dependent response of the explants. Previously, it was shown that elevated endogenous auxin levels are inhibitory for the shoot organogenesis and the use of auxin transport inhibitor may improve shoot regeneration in some recalcitrant species. This study explored the effect of auxin transport inhibitor such as 1-*N*-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) application on shoot induction in hemp. Epicotyls isolated from 7-day-old seedlings were used as explants. Explants were cultured on shoot regeneration media composed of Murashige and Skoog basal medium enriched with *meta*-topolin (mT) and tidiazuron TDZ (control), as well as media supplemented with combination of TDZ or mT with auxin transport inhibitors NPA (0.0-20 mg L^{-1}) and TIBA (0.05-0.5 mM). Shoot regeneration proceeded at 25°C± 2°C with a 16 h photoperiod under a photosynthetic flux of 80 μmol m^2 s^{-1}. After three weeks of culture the following data were recorded: percentage of survival explants, percentage of explants producing axillary shoots, and their mean number per explant and percentage of callusing and malformed explants. The use of medium supplemented with NPA at concentration 10 mg L^{-1} for both hormonal treatments resulted in the higher number of shoots per explant as compared with control (4.3 vs. 3.4 for TDZ and 3.7 vs. 2.8 for mT). The regeneration rate for TIBA treatment was lower than in the control medium. Moreover, inhibition of growth and necrosis of explants was observed. The results of this study demonstrated the promotional effect of NPA on shoot organogenesis in hemp in vitro cultures. Further studies on various plant material (different genotypes/cultivars) and the effects of auxin transport inhibitors are recommended in order to establish the optimal protocol.

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