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

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Mariola Dreger, Milena Szalata, Małgorzata Górska-Paukszta, Grażyna Mańkowska, Karolina Wielgus

Institute of Natural Fibres & Medicinal Plants, Department of Biotechnology, Wojska Polskiego 71b, 60-630 Poznań, Poland

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 (Hu *et al.* 2017; Shukla *et al.* 2014). This study explored the effect of auxin transport inhibitors such as 1-*N*-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) application on shoot induction in hemp.

Industrial hemp field culture

Material and Methods

Epicotyls (hybrid isolated from 7-day-old seedlings were used as explants. Explants were cultured on shoot regeneration media composed of Murashige and Skoog basal medium (Murashige and Skoog 1962) 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}. The data were statistically analysed using one-way analysis of variance (ANOVA) and the statistical significance was determined applying Tukey's test (p-value of 0.05).

Results

Table 1. Effect of NPA and TIBA on shoot induction from *Cannabis sativa* epicotyl explants after 3 weeks of cultivation

Medium	Inhibitor	Survival rate (%)	Mean no. of shoots per explant	Explant forming callus (%)
MS+TDZ (0.5 mg L^{-1})	0.0 (control)	100	3.4 ± 1.10^{ab}	87
	NPA (5 mg L^{-1})	100	3.6 ± 0.86^{bc}	50
	NPA (10 mg L^{-1})	100	**4.3 ± 1.07^{c}**	26
	NPA (20 mg L^{-1})	97	3.1 ± 1.25^{ab}	54.8
	TIBA (0.05 mM)	100	2.9 ± 1.08^{a}	40.6
	TIBA (0.1 mM)	97	2.0 ± 1.07^{e}	20
	TIBA (0.5 mM)	42	0^{d}	0
MS+mT (1.0 mg L^{-1})	0.0 (control)	100	2.8 ± 0.93^{ab}	35.5
	NPA (5 mg L^{-1})	100	3.4 ± 1.02^{bc}	26.7
	NPA (10 mg L^{-1})	100	**3.7 ± 0.49^{c}**	10
	NPA (20 mg L^{-1})	97	2.8 ± 1.45^{ab}	3.1
	TIBA (0.05 mM)	100	3.1 ± 1.13^{abc}	23.3
	TIBA (0.1 mM)	90	2.5 ± 1.17^{a}	26.7
	TIBA (0.5 mM)	23	0^{d}	0

Values are mean ± standard error of 15 replicates per treatment in three repeated experiments. Mean values with column with the same letter are not significantly different at p = 0.05

Effect of NPA (10 mg L^{-1}) and TDZ on shoot induction from epicotyl explants

Unfavorable effect of TIBA (0.1 mM) on shoot induction and growth

Conclusions

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. 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.

References

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