Bulgarian Journal of Agricultural Science, 13 (2007), 623-626
National Centre for Agrarian Sciences

Thidiazuron-induced Regeneration in Root Segments of White Poplar (*P. alba* L.)

I. TSVETKOV[1], J-F. HAUSMAN[2] and L. JOUVE[2]
[1]*Forest Research Institute, BG - 1756 Sofia, Bulgaria*
[2]*CRP Gabriel Lippmann, CREBS, 41, Department of Environment and Agrobiotechnology, Rue du Brill, L- 422 BELVAUX, Luxembour*

Abstract

TSVETKOV I., J-F. HAUSMAN and L. JOUVE, 2007. Thidiazuron-induced regeneration in root segments of white poplar (*P. alba* L.). *Bulg. J. Agric. Sci.*, 13: 623-626

Root segments isolated from stably proliferating *in vitro* clone of white poplar (*P. alba* L.) were cultivated on modified MS medium supplemented with different thidiazuron concentrations. The thidiazuron concentration significantly affected the regenerative capacity of the explants. The lower doses firmly stimulated the regenerative ability of the root segments, while the stronger ones demonstrated inhibitive organogenic effect. The highest number of regenerated shoots was formed on medium supplemented with 0.56 μM thidiazuron, but longest shoots were produced at 0.02 μM thidiazuron.

Key words: root explants, *in vitro* regeneration, thidiazuron, *Populus* spp.
Abbreviations: IAA – indole-3-acetic acid, BAP – 6-benzylaminopurine, MS – Murashige & Skoog nitrient medium, TDZ (thidiazuron) – 1-Phenyl-3-(1,2,3-thiadiasol-5-yl)urea

Introduction

Poplars (*Populus spp.*) are important elements of the riparian ecosystems and target of scientific interest due to number of valuable qualities (fast growth, easiness of sexual and asexual propagation, potential for inter-specific crossability, etc.). Thanks to the advantages offered, between 1994 and 2004 *Populus* has been the second most used tree genus in biotechnology studies in general (after *Pinus*) and the most used in genetic modification worldwide (Marchadier and Sigaud, 2005). White poplar (*P. alba* L.) is a widespread species found throughout the Mediterranean basin, Central Europe and the Middle East Selected cultivars of white poplar (*P. alba* L.) have been introduced and widely used in commercial scale in a number of countries from Europe, North Africa, Near and Middle East during the second half of the twentieth century (Confalonieri et al., 2000).

E-mail: tsvet_i@yahoo.com

Among the possible initial explants, roots have proven to be a suitable starting point for elaboration of organogenic systems for *in vitro* regeneration in different species, forest ones inclusive (George, 1993). Besides of being useful for micropropagation and somatic embryogenesis, the root culture could be successfully applied for germplasm preservation (Chaturvedi et al., 2004b). In addition, this approach offers attractive opportunities for studies on *Agrobacterium rhizogenes*-mediated gene transfer (Tsfira et al., 1996).

Results from a pilot study on thidiazuron abilities for provoking shoot regeneration in *in vitro* grown root segments of white poplar (*P. alba* L.) are presented in the paper.

Materials and Methods

Root segments 10-15 mm in length (root tips excised) obtained from stabilized *in vitro* culture of white poplar (*P. alba* L.) were used as initial explants. MS (Murashige and Skoog, 1962) nutrient medium supplemented with 0.59 mM adenine, 3 % (w/v) sucrose and solidified with 0.8 % (w/v) agar was used in all experimental treatments.

The effect of five thidiazuron concentrations (0.02, 0.11, 0.56, 2.8 and 14.1 μM) on the initial morphogenic reactivity of root explants was tested. Growth regulator-free medium was used as a control. Segments were placed in baby food jars (180 ml) with 40 ml nutrient medium. Each treatment consisted of six jars, with five explants being placed in every vessel.

The pH of media was adjusted to 5.7 before being autoclaved for 20 min at 120 °C. The cultures were pretreated in dark for two weeks and then maintained at conventional growth conditions in a culture room (40 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by cool white fluorescent lamps (Sylvania, GroLuxä, Germany), 16h photoperiod and temperature 23 ± 1 °C).

After 7-week period of time the frequency of initial regeneration reactivity of explants, average number of regenerated shoots per explant and average length of the longest shoot, were recorded. Analysis of variance (ANOVA) was used to estimate the putative factorial effects. Non-parametric test of independence (χ^2) was used for analysing the frequency data. Significance of differences between treatments means was assessed by Duncan's multiple range test (Sokal & Rohlf, 1996).

Results and Discussion

The thidiazuron was highly efficient in inducing shoot formation on white poplar root segments, thus confirming its high biological activity and promotive abilities demonstrated in previous tissue culture research (Huettemann, Preece, 1993; Mok et al., 1987). No regeneration occurred on the control variant. Three weeks after the start of the experiment regeneration events have been observed on the variants supplemented with low thidiazuron doses (0.02–0.56 μM). It was found that the regeneration frequency of root segments was strongly affected by the thidiazuron concentration (Table 1). The lower doses appeared to be more effective in promoting high frequencies of bud/shoot regeneration, with maximal value being registered on media supplemented with either 0.11 or 0.56 μM thidiazuron (Figure 1). Negligible organogenic reactivity was noticed on the variants with the highest inductive doses of TDZ applied (2.8 and 14.1 μM). Both the number and length of the regenerated

Table 1

Effect of TDZ concentration on some performance parameters of shoots regenerated from root segments of white poplar (P. *alba* L.) grown *in vitro* . The data were recorded 7 weeks after strat of the experiment

TDZ concentration, μM	Explants with regenerated shoot(s), %	Number of formed shoots, per vessel	Length of the longest shoot, mm
0.02	53.3	5.0 ± 1.1^{c}	14.8 ± 3.3^{a}
0.11	96.7	20.5 ± 1.0^{b}	7.5 ± 2.4^{b}
0.56	96.7	31.8 ± 0.4^{a}	4.0 ± 0.9^{b}
2.8	3.3	2.4 ± 0.2^{c}	1.6 ± 0.2^{b}
14.1	-	-	-

Fig. 1. *In vitro* regeneration of shoots from *P. alba* L root segments on MS medium supplemented with 0.11 μM thidiazuron after 7 weeks in culture. (bar = 5mm)

shoots were significantly influenced by the TDZ supplement ($P<0.001$ and $P<0.01$, respectively). While small number of short shoots (1-2mm) were produced on medium supplemented with 2.8 μM TDZ, only callus formation was observed after applying the strongest TDZ dose. Development of significantly higher ($P=0.05$) average number of shoots (> 6 per explant) was provoked on medium supplemented with 0.56-μM thidiazuron. Chaturvedi et al. (2004a) reported similar differentiation rate per explant (7.8) for *Populus deltoids* root culture stimulated by combined action of BAP and IAA (0.25 mg l^{-1} each). The lowest concentration of TDZ (0.02 μM) induced formation of significantly longer shoots ($P=0.05$), with shoot length progressively decreasing together with rising of the TDZ concentration. Thus, our findings are in general concordance with those of Sankhla et al. (1996) regarding the TDZ effect on regenerative ability of *Albizzia julibrissin* root segments.

The experimental data indicate that *P. alba* root segments could be successfully used as initial explants for establishing a reliable system for direct *in vitro* regeneration. Further experiments are needed to optimise the protocol, which could be effectively integrated in proper schemes for gene transfer.

Acknowledgements

The study was partly financially supported by EC project ESTABLISH QLK5-CT-200-01377.

References

Chaturvedi, H. C., A. K. Sharma, B. Q. Agha, M. Jain and M. Sharma, 2004a. Production of cloned trees from *Populus deltoids* through *in vitro* regeneration of shoots from leaf, stem and root explants and their field cultivation. *Indian J. of Biotechnology*, **2**: 203-208.

Chaturvedi, H. C., M. Sharma, A. K. Sharma, M. Jain, B. Q. Agha and P. Gupta, 2004b. *In vitro* germplasm preservation through regenerative root culture for conservation of phytodiversity. *Indian J. of Biotechnology*. **3**: 305-315.

Confalonieri, M., B. Belenghi, A. Balesirazzi, S. Negri, G. Facciotto, G. Schenone and M. Delledonne, 2000. Transformation of elite white poplar (*P. alba* L.) cv. "Villafranca" and evaluation of herbicide resistance, *Plant Cell Reports*, **19**: 978-982.

George, E. F. 1993. Plant Regeneration by Tissue Culture, 2nd Ed., Exegetics Ltd., Part I, pp. 574.

Huettemann, C. A. and E. Preece, 1993. Thidiazuron: a potent cytokinin for plant tissue culture, Plant, *Cell Organ and Tissue Culture*. **33**: 1105-119.

Marchadier, H. and P. Sigaud, 2005. Poplars in biotechnology research. Unasylva, N 221, vol. 56, 2. In: http://www.fao.org/docrep/008/a0026e/a0026e00.htm

Mok, M. C., D. W. S. Mok, J. E. Turner and C. V. Mujer, 1987. Biological and Biochemical Effects of Cytokinin-active Phenylurea Derivatives in Tissue Culture Systems. *HortScience* **22(6):** 1194-1196.

Murashige, T. and K. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plantarum*. **15:** 473-497.

Sankhla, D., T. D. Davis, and N. Sankhla, 1996. *In vitro* regeneration of silktree (*Albitzzia julibrissin*) from excised roots. *Plant Cell, Tissue and Organ Culture*. **44:** 83-86.

Sokal, R. R. and F. J. Rohlf, 1996. Biometry: The Principles and Practice of Statistics in Biological Research. 3rd edn.: 865 p. W.H. Freeman & Co., New York.

Tsfira, T., H. Ben-Meir, A. Vanstein and A. Altman, 1996. Highly efficient transformation and regeneration of aspen plants through shoot-bud formation in root culture. *Plant Cell Rep.*, **14:** 94-97.

Received August, 2, 2007; accepted September, 23, 2007.