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"L'embryogenèse somatique : approches cellulaire et moléculaire"

SOMATIC EMBRYO GERMINATION AND PLANTLET RECOVERY OF HYBRID LARCH IN RELATION TO ENDOGENOUS ABA LEVELS MEASURED BY ELISA

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SUMMARY

Somatic embryogenesis offers a solution to produce large quantities of propagules especially in hybrid larch (*Larix* x *leptoeuropaea*) species which has great potential for reforestation programmes. Somatic embryogenesis has been successfully achieved of hybrid larch at INRA of Orléans (France).

The maturation, which leads to the development of cotyledonnary somatic embryos, was improved when abscisic acid (ABA) was added to the culture medium (1). However, germination and plantlet development were strongly affected by the duration of the maturation process (i.e. the time the somatic embryos were cultured on the maturation medium). The highest germination rate (90%) and plantlet recovery (70%) were achieved when the somatic embryos were matured for a 3 week period. Extension of the maturation period on medium with ABA beyond 3 weeks resulted in a significant decrease of the germination and plantlet frequencies.

Changes in ABA content have been studied during somatic embryogenesis of hybrid larch. The technique used was a methanolic extraction followed by a SepPak™ purification, an HPLC fractionation, an ELISA quantitation (2,3) and a GCMS identification (4). The ABA level of somatic embryos dramatically increased during the first two weeks of maturation followed by a drop at the 3rd week. Then, the ABA content showed a second steep rise to reach its highest value after the 5th week of maturation. In consequence, the decrease of the germination and plantlet frequencies were consistent with the increase in ABA content.

In conifer species, this is the first evidence of a relation between ABA and the control of somatic embryo germination.

(1) Lelu M.-A., Bastien C., Ward C., Klimaszewska K. and Charest P. 1993. An improved method for somatic plantlet production in hybrid larch (*Larix* x *leptoeuropaea*) part 1. Somatic embryo maturation. Plant Cell Tissue Organ culture (in press).

(2) Leroux B., Maldiney R., Miginiac E., Sossountzov L. and Sotta B. 1985. Comparative quantitation of abscisic acid in plant extracts by gas-liquid chromatography and an enzyme-linked immunosorbent assay using the avidin-biotin system. Planta **166** : 524-529.

(3) Maldiney R., Leroux B., Sabbagh I., Sotta B., Sossountzov L. and Miginiac E. 1986. A biotin-avidin-based enzyme linked immunoassay to quantify three phytohormones : auxin, abscisic acid and zeatin riboside. J. Immun. Meth. **90** : 151-158.

(4) Hirai N. 1986. Abscisic acid. In Chemistry of plant hormones. Edited by N. Takahashi. CRC Press, Boca Raton. pp. 201-248.