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**RELATIONSHIP BETWEEN EXOGENOUS ABA SUPPLIED TO SOMATIC EMBRYOS OF HYBRID LARCH AND INTERNAL ABA LEVELS MEASURED BY ELISA**

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**SUMMARY**

Somatic embryogenesis of hybrid larch (*Larix x leptoeuropaea*) offers a good tool to produce large amount of plants from selected genotypes. As with many gymnosperm species, exogenous ABA is required to control the synchronicity and the level of maturation of somatic embryos. In order to optimize the maturation process of this culture we investigated the optimal exogenous ABA amount that was needed to apply to somatic embryos during the maturation process. The aim of this study is to determine the relationship between exogenous ABA and internal ABA level present in somatic embryos during the culture.

Samples were collected 4 weeks after culture on a medium (1) containing 0 (control), 20, 40 and 60µM ABA. ABA levels were measured using a methanolic extraction followed by a SepPak™ purification, an HPLC fractionation, an ELISA quantitation (2,3) and a GCMS identification (4).

Results show that internal ABA levels are directly correlated to the concentration of ABA exogenously supplied. In our culture conditions, undetectable levels of endogenous ABA (detection limit : 300 femtomol/sample corresponding to 25pmol/g FW) were found in control four-week cultured hybrid larch somatic embryos. Quantitatively, about 1% of the available exogenous ABA (at 40µM) was present in the embryos after 4 weeks of culture. As a direct consequence of the experiment we defined the optimal concentration of ABA in the culture medium to be about 40µM. Over this level, internal ABA measurements showed that somatic embryos could be ABA-saturated.

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- (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.
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