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**EXTENDED ABSTRACTS  
FIRST MEETING OF THE COST 822  
WORKING GROUP**

**MECHANISMS AND MARKERS OF  
REGENERATION  
AND  
GENETIC STABILITY**

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## Somatic embryogenesis : control of somatic embryo maturation in hybrid larch

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### Aim

Somatic embryogenesis of hybrid larch (*Larix x leptoeuropaea*) has been successfully developed at INRA of Orléans (France) since 1990. The maturation is an important step as the germination and the plantlet recoveries are depending on the quality of the somatic embryos. For the conifers, as for the angiosperms, a great improvement was obtained by the addition of abscisic acid (ABA) to the culture medium. This gave rise to well-developed somatic embryos with typical morphology (considering the zygotic embryo), synchronous development and no precocious germination (1). Changes in content in ABA during maturation of hybrid larch somatic embryos were followed using an enzyme immunoassay (ELISA).

### Materials and Methods

Somatic embryogenic lines of hybrid larch (*Larix kaempferi* x *L. decidua*) were obtained in 1990 from immature zygotic embryos and since subcultured every two weeks on basal MSG medium as previously described (2). The maturation consisted in the obtention of cotyledonary somatic embryos well-developed. The maturation medium consisting in MSG medium supplemented with 0.2 M sucrose, 1  $\mu$ M IBA and 60  $\mu$ M ABA (2). After 3 weeks of culture; cotyledonary somatic embryos were transferred to germination medium which is a growth regulator-free MSG medium. After 5 weeks of germination, germinated somatic embryos which developed needles were considered as plantlets. For the hormonal analyses, the somatic embryos which underwent embryogeny were removed after 0, 1, 2, 3, 4 and 5 weeks of maturation. Analyses using an enzyme immunoassay (ELISA) were realised as previously described (1).

### Results

Development of cotyledonary somatic embryos was greatly improved by the presence of ABA in the culture medium (1). However in hybrid larch, the extension of the period of maturation in presence of ABA from 3 weeks to 4 or 5 weeks resulted in a significant decrease in both germination and plantlet frequencies (figure 1). The highest germination rates (90%) were obtained for 3 weeks-old somatic embryos. In contrast prolongation of maturation period to 5 weeks resulted in reduced germination (38%); this was also observed for the plantlet recovery: the development of the aerial parts was reduced from 70 to 10% for respectively 3 and 5 weeks-old somatic embryos. In presence of ABA in the culture medium, the ABA level of the somatic

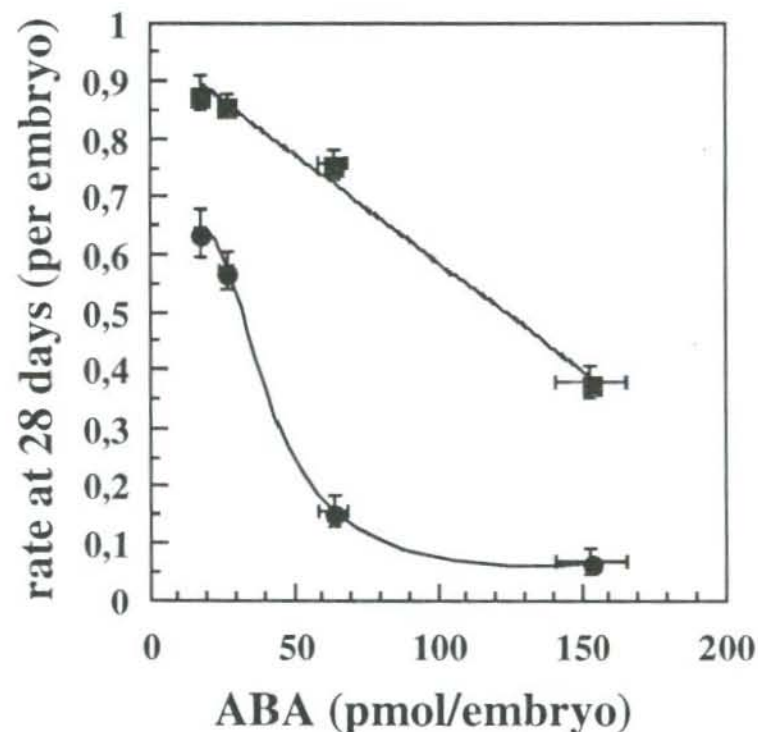
embryos increased during their development to reach a maximum value at week 5 (153 pmol/embryo, figure 1). As a consequence, we assume that it was the level of ABA *in planta* that was responsible for the inhibition.

## Conclusion

ABA acted in both a stimulatory and an inhibitory manner. If ABA promoted the obtention of high quality somatic embryos in large numbers, it also had a deleterious effect on the subsequent development, i.e. germination and plantlet recoveries. The results stressed the importance of the maturation procedure and duration.

## Literature

- (1) LELU MA and LABEL P (1994). *Physiol. Plant.*, 92, 53-60.
- (2) LELU MA, BASTIEN C, KLIMASZEWSKA K, WARD C and CHAREST P (1994). *Plant Cell Tissue Organ Culture*, 36, 107-115.



**Figure 1:** Relationship between endogenous ABA levels and growth rates of somatic embryos. ■ : germination rate at 28 days of culture ; ● plantlet rate at 28 days of culture. Bars represent standard errors on the mean (n=5 for ABA, n=60 for rates).