

The development of early somatic embryos of *Abies cephalonica*

Zuzana Vondráková, Jana Opatrná, Lucie Kumstýrová and Martin Vágner
Institute of Experimental Botany, Academy of Sciences of the Czech Republic
Rozvojová 135, Prague 6, 165 20, Czech Republic

E-mail: vondrakova@ueb.cas.cz

Introduction

Somatic embryogenesis /SE/ is studied as an efficient way of the propagation of conifers. It is problematic in *Abies cephalonica* as somatic embryos of this fir are not able to finish their development.

The process of SE of conifers consists in several developmental steps. The changes of cultivating media composition promote the transition from step to step of SE.

- 1) induction of embryonic suspensor mass /ESM/ -

we used immature zygotic embryos as the primary explants and induction medium ½ MS, gelrite 0,4%, sucrose 3%, BAP 2mM, kin 2mM

- 2) proliferation, in which ESM volume grows, proliferation medium ½ MS, kasein 1g/l, glutamin 0,5g/l, gelrite 0,4%, sucrose 3%, BAP 2mM, kin 2mM, 2,4-D 0,25mM

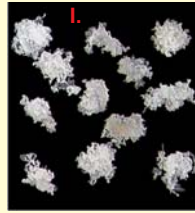
- 3) maturation, where somatic embryos develop into the cotyledonary stage, maturation medium ½ MS,

kasein 1g/l, glutamin 0,5g/l, gelrite 0,4%, maltose 4%, ABA 20mM.

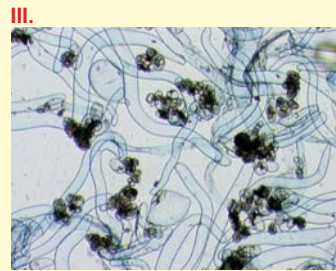
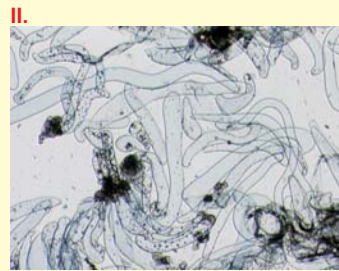
Somatic embryos in good quality can continue in the process of SE to the next steps - desiccation, germination and transition to ex vitro conditions.

This work is aimed to examine the changes of ESM after phytohormones /carbohydrates/

Makroskopolical pictures of ESM during proliferation



Proliferation

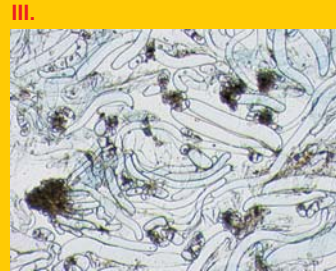
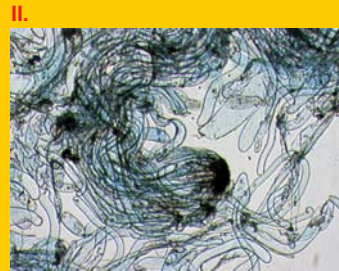


Proliferation

We can distinguish 3 types of ESM:

- I. hairy structured ESM arranged from the proembryos with very long suspensor cells connected with small meristematic centres
- II. granular structured of ESM with extremely small proembryos
- III. granular structured ESM composed of the mixture of suspensor and meristematic cells

Maturation



Maturation

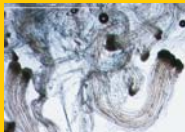
The changes of ESM structure after 3 weeks on maturation medium - auxin and cytokinins are replaced by ABA /20mM/, sucrose is replaced by maltose.

- I. small changes of somatic embryos structure, the suspensor cells are arranged into the long tails
- II. the greatest change of ESM arrangement, the whole somatic embryos are developed
- III. the differences between the ESM during proliferation and maturation are not evident, bigger meristematic centres are found during maturation

We will find the effects of phytohormones and carbohydrates on somatic embryos development during maturation. We will compare the development of embryos cultivated on basic and modifying maturation medium for 3 weeks..

The effect of phytohormones treatment

ABA treatment

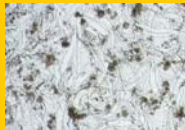


I. a tail-like structure formed from the suspensor cell mass connects to the large meristematic centres

II. no significant effect

III. very small embryos with well developed meristematic centres

Auxin treatment



I. small meristematic centres, high number of suspensor cells. A small fraction of suspensor cells remains not arranged in tail-structures.

II. Meristematic centres collapsed shrunk or destroyed, high number of non-organised suspensor cells has developed.

III. no significant effect

Cytokinins treatment



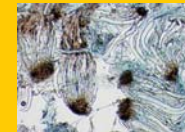
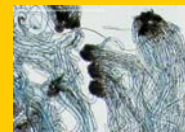
I. well developed meristematic centres connected to long tails composed of long highly organised suspensor cells

II. good quality somatic embryos; large meristematic centres, robust tails formed from highly organised suspensor cells

III. no significant effect

The effect of carbohydrates /maltose (4%) in maturation medium was replaced by lactose or sucrose of the same concentration/

The effect of lactose

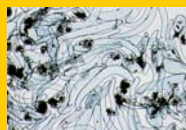
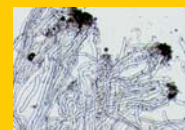


I. very weak stimulation of embryos development

II. stimulation of embryos development, somatic embryos with well organised suspensor tails

III. stimulation of embryos development, small embryos with well developed meristematic centres

The effect of sucrose



I. small meristematic centres, the suspensor tails formed of non-sufficiently organised suspensor cells

II. no significant effect

III. No effect, the complete embryos were not developed during maturation at all

Conclusions

We used 20 cell lines of ESM derived from *Abies cephalonica*. We found 3 types of cell lines. They differ in anatomical structure during proliferation and in the changes at the beginning of maturation.

Sugars can affect the maturation process very weakly. Lactose stimulates and sucrose inhibits the first steps of somatic embryos maturation. Sugars are not the main component in maturation medium controlling the next development of somatic embryos.

Phytohormones are the most important control substances in SE. ABA induces the maturation of somatic embryos, it controls the organisation of ESM and the development of meristematic centres. Auxin stimulates the growth of suspensor cells /amount of suspensor cell mass/. Cytokinins stimulate the arrangement of suspensor cells into the embryonic tail.

All these effects strictly depend on the cell line. The results were found in the beginning of the maturation phase only. The best developed somatic embryos were obtained:

- I. on basic maturation medium
- II. when cytokinins were added to the maturation medium
- III. on maturation medium richer in ABA