The role of auxins in somatic embryogenesis of *Abies alba*

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Introduction

Somatic embryogenesis /SE/ is studied as an efficient way of the propagation of conifers. It consists in five developmental steps: induction, proliferation, maturation, desiccation and germination. Growth regulators play the central role in this process. Cytokinins and auxins control induction and proliferation. ABA is the control regulator of maturation. The germination stage is phytohormones free step of SE. In fir the SE process is more complicated and rarely produces well-developed emblings. Embryonic suspensor mass /ESM/ induction in fir is initiated by cytokinins with the absence of exogenous auxins. The effect of exogenous auxins during proliferation depends on the plant material used.

The aim of our work is to establish the role of auxins during the proliferation and the maturation of *Abies alba*. We compare the material proliferated on medium supplemented with auxin (2,4-D) and the material cultivated on auxins free medium.

Material and methods:

Embryonic suspensor mass 0R 4 used in this experiment was derived from immature zygotic embryos of *Abies alba*. Cultivation: /fresh medium every 2 weeks/

Proliferation
25⁰C, darkness
½ MS medium, 3% sucrose
0,4% Gelrite
2µM BAP, 2µM kinetin
With or without 0,25µM 2,4-D

Maturation
25⁰C, darkness
½ MS medium, 4% maltose, 3,75% PEG
0,4% Gelrite
20µM ABA

Auxin analysis: using HPLC with fluorimetric detection (Eder et al 1988)

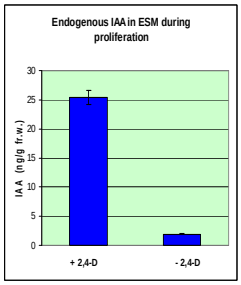
The non-fixated **anatomical preparations** were stained by trypan blue, image analysis system Lucia from Laboratory Imaging was used for visual characterization.

Proliferation

+ 2,4-D

The macroscopic structure of ESM treated by 2,4-D

- white hairy structures
- rapid growth
- large amount of ESM

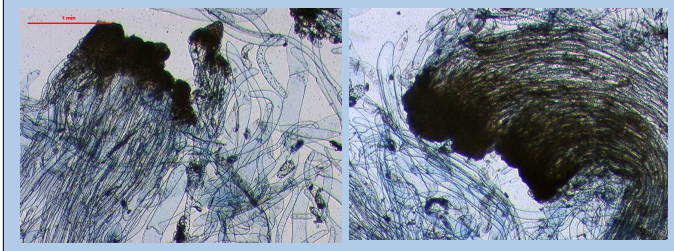


The macroscopic structure of ESM proliferated without 2,4-D

- compact structures
- short hairy structures
- slow growth of ESM

- 2,4-D

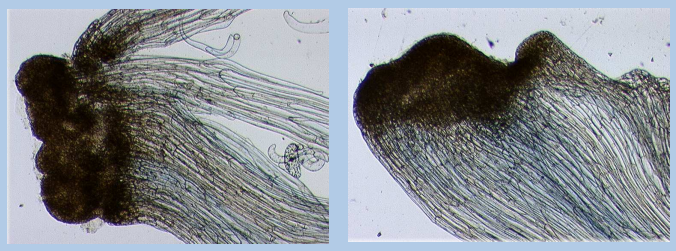
Proliferation - the microscopic structure of ESM /+ 2,4-D/



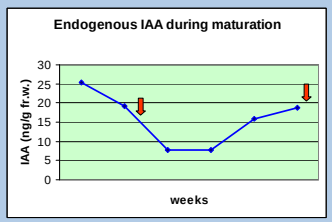
- large somatic embryos
- polyembryony
- robust suspensor tails
- long suspensor cells

Maturation

Somatic embryos after 1 week of maturation

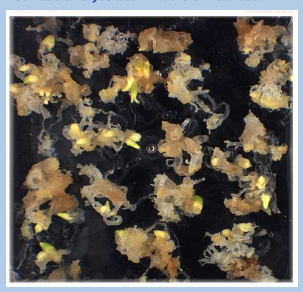


- large somatic embryos
- compact suspensor tails
- all suspensor cells organised in suspensor tails

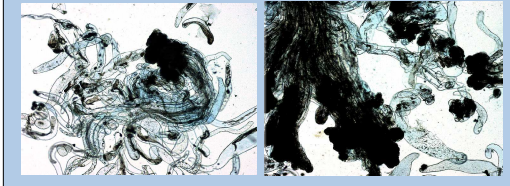


- decrease of endogenous auxin in the beginning of maturation
- increase of endogenous auxin at the end of maturation

Somatic embryos after 7 weeks of maturation



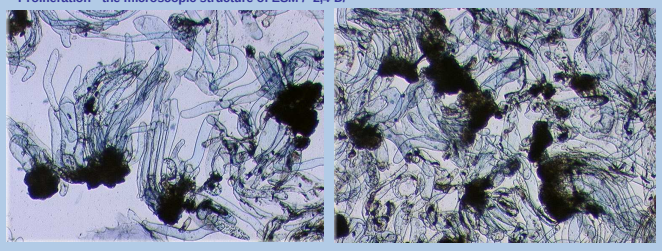
Somatic embryos after 1 week of maturation on the medium with PCIB



2O mg/l PCIB
auxin antagonist
/2-(p-chlorophenoxy) 2 methyl propionic acid/

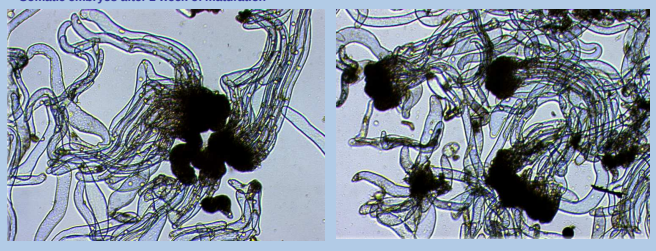
- destruction of suspensor tails
- destruction of meristems

Proliferation - the microscopic structure of ESM /- 2,4-D/

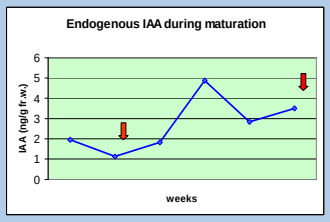


- small somatic embryos
- short suspensor tails
- free suspensor cells

Somatic embryos after 1 week of maturation

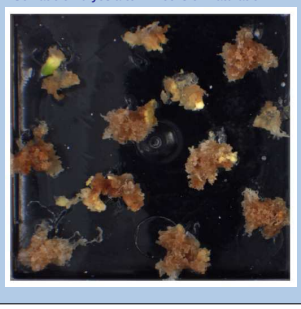


- small somatic embryos
- pure suspensor tails
- low organisation of suspensor cells



- low concentration of auxin
- decrease of endogenous auxin in the beginning of maturation
- increase of endogenous auxin at the end of maturation

Somatic embryos after 7 weeks of maturation



Conclusions

Proliferation
- ESM of *Abies alba* proliferates successfully on medium supplemented by auxin (2,4-D) and on auxin free medium
- Endogenous level of auxin (IAA) is 10 times lower on auxin free medium.
- Large early somatic embryos were obtained on medium with auxin(2,4-D).
- Polyembryony is stimulated by auxin treatment.

Maturation
- Endogenous level of auxin decreases at the beginning of maturation /in both materials/.
- The suspensor tails are constituted at the beginning of maturation. The development of somatic embryos is finished after 7 weeks of maturation.
- Endogenous level of auxin increases gradually during maturation /in both materials/.
- The yield of somatic embryos is higher from material treated by auxin during the proliferation phase.
- Somatic embryos from ESM treated by auxin affected by PCIB during maturation are not comparable to that from ESM proliferating on auxin free medium.
- The number and quality of maturated somatic embryos is regulated by the auxin treatment during the proliferation phase of SE .

Acknowledgement: This work was supported by the COST project 843.50.