

## 5. CONCLUSIONS

- The development of somatic embryos on maturation medium can be divided at anatomical level into four developmental stages, differing in the inner structure: stage of early somatic embryo, cylindrical stage, precotyledonary stage and cotyledonary stage.
- The prolonged cultivation of cotyledonary embryos on maturation medium leads to disintegration of the root cap, connected with starch and phenolic compounds deposition.
- The time course of discrete developmental stages is affected by PEG 4000; use of 3,75 % PEG speeds up the process of maturation by two weeks. Increased level of PEG (7,5%) leads to the formation of ruptures in embryo hypocotyls.
- The content of non-structural saccharides increases during the cultivation of embryogenic culture on maturation medium due to increasing level of sucrose in developing embryos. The content of hexoses in embryos is low.
- The addition of 3,75 % PEG 4000 into maturation medium increases the sucrose to hexoses ratio in somatic embryos, the total amount of saccharides slightly decreases. 7,5 % PEG leads to hexoses accumulation in embryos.
- Insertion of pre-maturation phase (without growth regulators) between proliferation and maturation increases total amount of developed embryos in a majority lines tested. Cultivation of embryogenic cultures of rafts with polypropylene membrane has a similar effect and it also speeds up the process of embryo development.
- We set up the model protocol for handling with Norway spruce embryogenic cultures, covering all phases of somatic embryogenesis from induction to *ex vitro* conversion. The protocol for cryopreservation is included.
- Expression of PaVP1 transcription factor gene is detectable in embryogenic lines irrespective to their ability to develop mature embryos. *PaVP1* expression is not detectable in non-embryogenic culture.

- In embryogenic lines, the *PaVP1* expression is induced by the presence of ABA in maturation medium. The omitting of ABA from maturation medium leads to rapid disappearance of *PaVP1* expression and to the disintegration of embryos.
- The increase in *PaVP1* expression is only temporary in the line with low embryogenic capacity during maturation. The subsequent decrease of *PaVP1* expression is connected with the disintegration of meristematic centres.
- The expression of *PaVP1* in highly embryogenic lines keeps on a high level during the maturation process, reaching the maximum in early cotyledonary stage of embryo development.
- *PaVP1* probe specifically hybridises with two transcripts differing in length. Their ratio varies during the development of embryogenic cultures. That indicates the possible role of alternative splicing in regulation of PaVP1 protein synthesis.