THE EFFECT OF DIFFERENT DESICCATION TREATMENTS ON POLYAMINE METABOLISM OF SPRUCE SOMATIC EMBRYOS

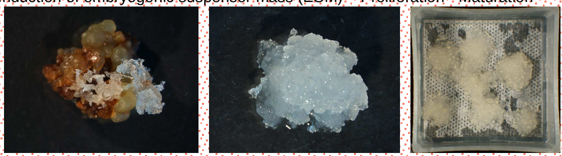
<u>Zuzana Vondráková</u>, Alena Trávníčková, Jiří Malbeck and Milena Cvikrová

Institute of Experimental Botany AS CR, Rozvojová 263, 165 02 Prague 6, Czech Republic

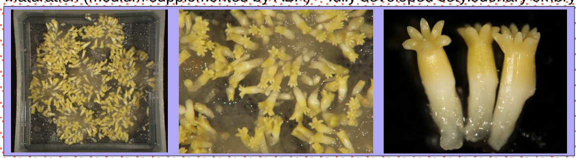
E-mail:vondrakova@ueb.cas.cz

Somatic embryogenesis of spruce

Induction of embryogenic suspensor mass (ESM)   Proliferation - Maturation



Maturation (medium supplemented by ABA) – fully developed cotyledonary embryos



Desiccation (on paper)

Germination

Put	Spd	Spm
122.58	764.06	335.85

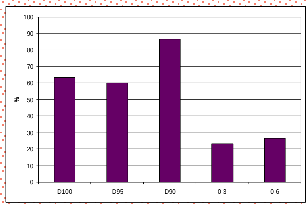
Endogenous polyamines in matured somatic embryos at the end of maturation = at the start of desiccation

Contents of PAs are expressed in **µg/g of dry weight.**

Introduction

Plants have evolved complex strategies to deal with abiotic stresses. They control the synthesis of regulatory molecules – especially abscisic acid, synthesis of osmolytes and production of key protective compounds including polyamines (PAs) - the diamine putrescine (Put), the triamine spermidine (Spd) and the tetraamine spermine (Spm). We aimed our study at the effect of osmotic stress in the somatic embryogenesis of *Picea abies*. Desiccation is one step of somatic embryogenesis which is necessary for the successful development of emblings but it may represent a sort of osmotic stress in somatic embryos. We evaluated the development of embryos treated by the different desiccation conditions (90%, 95% and 100% humidity). We compared the changes in the metabolism of PAs and ABA during desiccation with the changes in morphology of desiccated embryos and emblings.

The effect of desiccation on root formation

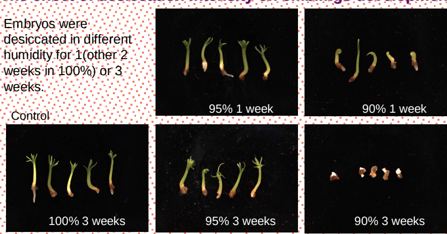


When embryos were desiccated (3 weeks in different humidity – D100%,D95%, D90%), the root formation occurred in approx. 60 – 85% of emblings after 3 weeks of germination. In contrast only 20% of embryos starting germination just after maturation were able to form roots after 3 weeks of germination (03). The % of rooted emblings was not enhanced after 6 weeks of germination (06).

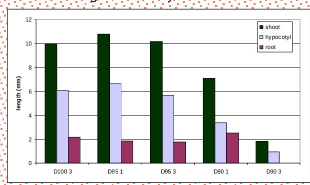
Emblings – 3 weeks of germination

The effect of desiccation intensity on emblings development

Embryos were desiccated in different humidity for 1(other 2 weeks in 100%) or 3 weeks.



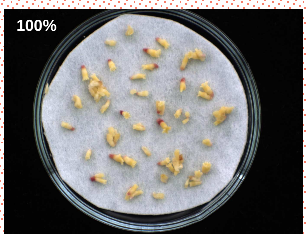
Growth of emblings from embryos desiccated in different conditions



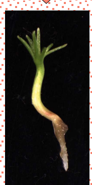
Shoot growth was inhibited in emblings developed from embryos after strong desiccation (90% humidity – 1 week). Root growth and formation was less affected. The development of emblings from embryos desiccated for 3 weeks in 90% humidity was fully inhibited.

Spruce somatic embryos at the 11th day of desiccation

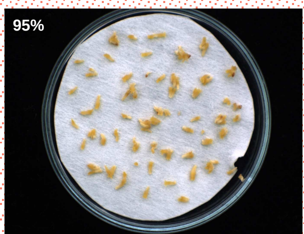
The extent of desiccation was determined as the decrease of water content in embryos together with the changes of embryos morphology after 11 days of desiccation. The degree of stress reaction of embryos correlated with the changes in endogenous polyamine levels. Stressed embryos finished the 3 weeks desiccation at 100% humidity (for the next 10 days) and continued in further development. However, many malformed emblings were obtained from the embryos desiccated at 90% humidity.



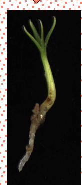
Dry weight of desiccated embryos : 23%



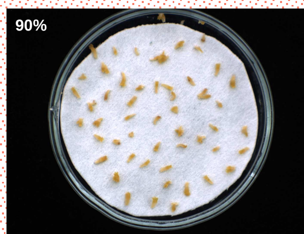
Put	Spd	Spm
13.62	696.91	500.90



Dry weight of desiccated embryos: 54%

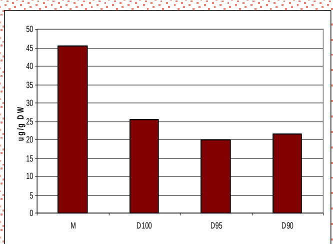


Put	Spd	Spm
3.42	216.12	212.44



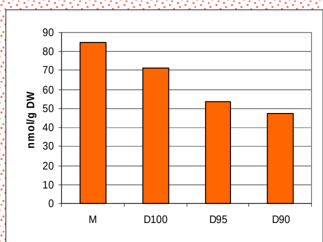
Dry weight of desiccated embryos: 83%

Put	Spd	Spm
50.74	452.65	165.32



Endogenous level of ABA in somatic embryos at the 11th day of desiccation in comparison to that at the end of maturation (M)

The ABA content is lower in all embryos from desiccation as in maturated embryos. The endogenous level was extremely high during maturation (on medium supplemented with ABA) and it decreased after media changing. The stress reaction pronounced in the changes in ABA content was not observed.



The malondialdehyde (MDA) content in somatic embryos at the 11th day of desiccation in comparison to that at the end of maturation (M)

Measured rates of lipid peroxidation (which are determined by monitoring changes in the levels of MDA) can be related to the antioxidant activity balance within a given cell or tissue. Decline in MDA content in embryos in the course of desiccation may coincide with decreased metabolic activities under decreased humidity conditions.

Methods

<u>An embryogenic culture of *Picea abies*</u>, genotype AFO 541, was obtained from AFOCEL, France. Cultivation in detail is described in : Gemperlová, L., Fischerová, L., Cvikrová, M., Malá, J., Vondráková, Z., Martincová, O., Vágner, M.: Polyamine profiles and biosynthesis in somatic embryo development and comparison of germinating somatic and zygotic embryos of Norway spruce, Tree Physiology 29(10): 1287-1298, 2009. <u>The control of humidity</u> in desiccation was performed according to: Roberts, D.R., Sutton, B.C.S., Flinn, B.S.: Synchronous and high frequency germination of interior spruce somatic embryos following partial drying at high relative humidity, Can. J. Bot. 68: 1086-1090, 1990.
<u>Extraction and HPLC analysis of benzoylated polyamines</u> was performed according to : Slocum, R.D., Flores, H.E., Galston, A.W., Weinstein, L.H.: Improved method for HPLC analysis of polyamines, agmatine and aromatic monoamines in plant tissue, Plant Physiology 89: 512-517, 1989. <u>ABA extraction</u> is described in: Kosová, K., Prášil, I.T., Vítámvás, P., Dobrev, P., Motyka, V., Floková, K., Novák, O., Turetková, V., Rolčík, J., Pešek, B., Trávníčková, A., Gaudinová, A., Galiba, G., Janda, T., Vlasáková, E., Prášilová, P., Vaňková, R.: Complex phytohormone responses during the cold acclimation of two wheat cultivars differing in cold tolerance, winter Samanta and spring Sandra, J Plant Physiol. 169:567-576, 2012.
<u>The malondialdehyde (MDA) content</u> of the samples was determined using the NWLSS-Malondialdehyde Assay kit (cat. no. NWK-MDA01, Northwest Life Science Specialties, LLC, Vancouver, Canada) as described in detail by: M. Cvikrová, M., Gemperlová, L., Martincová, O., Prášil, I.T., Gubis,J., Vaňková,R.: Effect of heat stress on polyamine metabolism in proline-over-producing tobacco plants, Plant Sci. 182: 49–58, 2012.

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