THE ROLE OF ACTIN IN NORWAY SPRUCE SOMATIC EMBRYOGENESIS

K. Eliášová [1], Z. Vondráková [1], K. Schwarzerová [2], L. Fischer [2], E. Bellinvia [2], L. Havelková [2], J. Fišerová [2], J. Špačková [1], Z. Opatrný [2], M. Vágner [1]

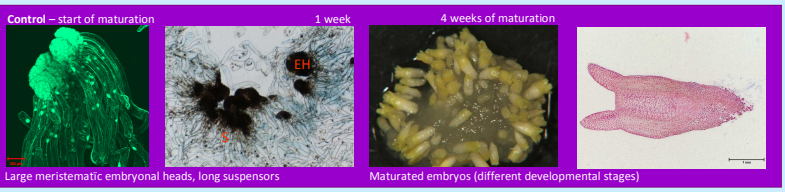
[1] Institute of Experimental Botany, AS CR, Rozvojová 263, CZ 16502 Prague 6, Czech Republic

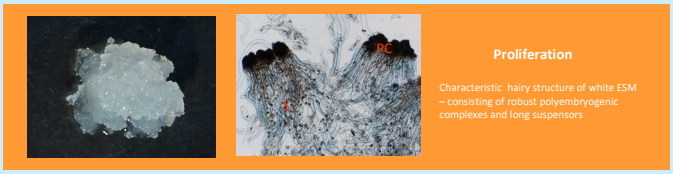
[2] Departement of Plant Physiology, Faculty of Science, Charles University Prague

INTRODUCTION: The roles of microtubules and actin cytoskeleton in processes as cell wall shape control, cell division or intracellular transport which are essential for correct morphogenesis are established. Embryogenesis comprises series of processes that determine the spatiotemporal position of embryogenic cells and their descendents in the developing embryo and in the organism. The structure and the position of tissues and organs are determined solely by oriented cell division. The origin, development and maturation of a plant embryo are controlled by the origin and the development of specific cell populations and their programmed death.

The process of spruce somatic embryogenesis consists of 5 steps (induction of embryogenic suspensor mass (ESM), proliferation, maturation, desiccation and germination). It is strictly controlled by the exogenous treatments by plant growth regulators. ESM is treated by cytokinins and auxin during induction and proliferation. The embryo maturation occurs on medium supplemented by ABA. Desiccation and germination are phytohormones free steps of somatic embryogenesis (SE).

During the proliferation the ESM of AFO 541 line is composed of early somatic embryos, free suspensor cells and large polyembryogenic complexes (PC). The meristematic embryonal heads (EH) are robust and are linked with large suspensors (S) constituted of highly vacuolated suspensor cells. At the beginning of the maturation single somatic embryos are separated from the polyembryogenic complexes. All early somatic embryos start their development. During three weeks the meristematic embryonal heads grow and elongate; the suspensors are shorter and prone to breaking. The best-developed embryos located on the surface of the ESM clusters consist of long meristematic head linked with several suspensor cells only. The cotyledons are formed and the somatic embryos are ready to be desiccated and germinated after 5 weeks of maturation.

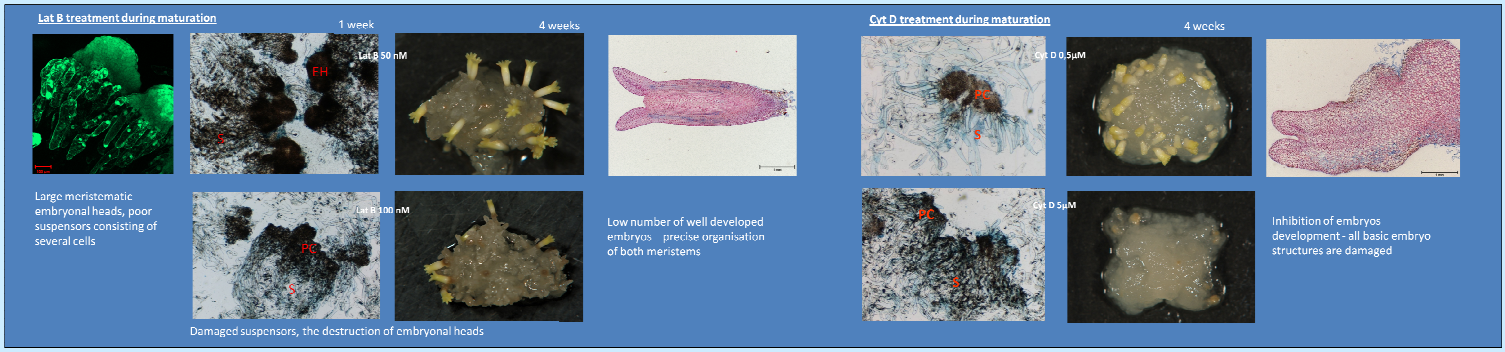


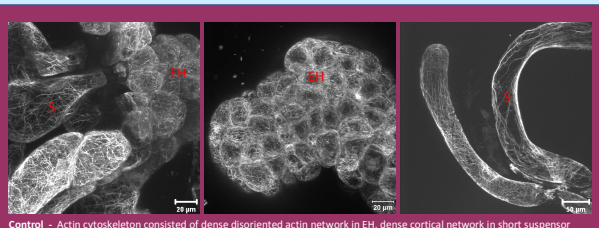


AIMS: To investigate the effect of actin destabilization on two different cell types - meristematic cells of embryonal heads and vacuolised suspensor cells - during maturation of AFO 541 ESM using different concentrations of anti-actin drugs with different mode of action – latrunculin B (Lat B), blocking monomer actin polymerisation and cytochalasin D (Cyt D), inducing F-actin depolymerization.

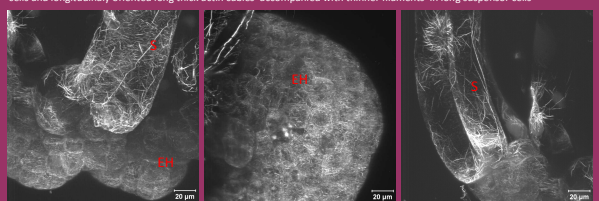
CONCLUSIONS:

- **The big differences in the effect of latB and cyt D on SE differentiation are demonstrated**
- **Lat B preferentially affected cortical actin of suspensor cells. The effect of 50-100 nM latB on actin filaments of meristematic cells was mild, higher concentrations – up to 200 nM - caused fragmentation and depolymerisation of actin in both types of the ESM cells.**
- **Cyt D affects cortical actin in meristematic cells markedly more than in suspensor cells. The effect of higher concentrations (5 µM) is full depolymerisation of actin filaments in meristematic cells.**
- **The positive effect of lat B treatment on embryo development is due to the inhibition of deficiently developed embryos. The destruction of suspensors is lethal for their next development. The yield of maturated embryos is lower than in control but their quality is higher.**
- **The negative effect of cyt D treatment on embryo development is due to the immediate effect on the meristematic heads. The yield of embryos is low and the embryos are often malformed.**



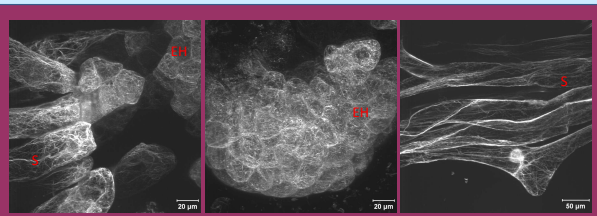


Control - Actin cytoskeleton consisted of dense disoriented actin network in EH, dense cortical network in short suspensor cells and longitudinaly oriented long thick actin cables accompanied with thinner filaments in long suspensor cells

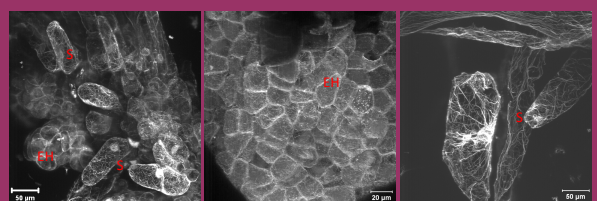


Latrunculin B 50 nM (1 hour of treatment) - minor changes of actin organisation in EH contrary to severe fragmentation of long actin cables in S

Latrunculin B 200 nM (1 hour of treatment) – actin filaments in EH fragmented into shorter bundles or fully depolymerised, actin cables in S almost completely destroyed into short non-oriented rods



Cytochalasin D 0,5 µM (1 hour of treatment) – disorganised dense actin network with some evidences of actin filament depolymerization in EH, well conserved longitudinal actin cables accompanied with thinner cortical actin filaments in S



Cytochalasin D 5 µM (1 hour of treatment) - actin filaments in EH were fragmented into shorter thin filaments or fully depolymerised, actin cables as well as cortical actin network in S sustained with only minor changes

MATERIAL & METHODS:

Embryogenic culture of *Picea abies* L. (Karst.) , genotype AFO 541 originated from AFOCEL, France

Cultivation: ESM was proliferated on medium GD (Gupta, Durzan 1986, In Vitro Cellular Developmental Biology 22(11):685-688) supplemented with sucrose, agar, 2,4-D, BAP and kinetin; maturation occurred on liquid medium GD supplemented with sucrose, PEG and ABA. Material was cultivated under the continuous darkness at 25°C. It was transferred onto the fresh medium in the interval of 1 week.

Drug application: Drugs were dissolved in DMSO in concentrations 50, 100 and 200 nM (Lat B) and 0,5 ; 5 µM (Cyt D) and added into maturation media. The cultures were growing on media supplemented with drugs all the time of cultivation. Untreated cultures were used as controls. The highest concentration of the Lat B (200 nM) was almost lethal.

Anatomy and histology:

a)Clusters of ESM were stained with 0.04 % trypan blue . Cell viability – staining by 0.02 % (w/v) fluorescein diacetate (FDA) .

b)Actin was visualised using rhodamine – phalloidin according to Blancaflor 2000 (J.Plant Growth Regul. 19:406-414).

c)Matured somatic embryos were embedded in paraffine , sectioned longitudinaly and stained by alciane blue and nuclear fast red

Microscopy: transmission light microscope Zeiss Jenaval equiped with DS-5M digital camera Nikon, confocal laser scanning microscope Zeiss LSM 5 Duo (excitation at 561 nm, emission filter set LP 575 for rhodamine-phalloidin; excitation at 488 nM, emission filter set LP 505 for FDA)

The project under the frame of COST 871 was supported by the Ministry of Education, Youth and Sports (OC 158).