ROLE OF ACTIN IN PLANT EMBRYOGENESIS: IMPROVEMENT OF SPRUCE SOMATIC EMBRYO MATURATION BY TREATMENT WITH ANTI-ACTIN DRUG LATRUNCULIN B

Kateřina Schwarzerová[1], Zuzana Vondráková[2], Lukáš Fischer[1], Petra Bořiková[1], Erica Bellinvia[1], Kateřina Eliášová[2], Lenka Havelková[1], Jindřiška Fišerová[1], Martin Vágner[2], Zdeněk Opatrný[1]

[1] Charles University in Prague, Faculty of Science, Department of Experimental Plant Biology, Viničná 5, CZ 12844 Prague 2, Czech Republic
[2] Institute of Experimental Botany v.v.i., Academy of Sciences of the Czech Republic, Rozvojová 236, CZ 16502 Prague 6, Czech Republic
opat@natur.cuni.cz

Introduction

Embryogenesis in multicellular organisms comprises orchestrated processes that determine the spatio-temporal cell positioning in the developing embryo. In higher plants, which lack active cell movement, the structure and position of individual tissues are determined solely by the orientation of cell division, cell growth and developmentally regulated programmed cell death (PCD). Various cytoskeletal structures have been repeatedly demonstrated to play a key role in all these processes. In plants, actin cytoskeleton participates in the definition of cell polarity and orientation of cell division, cell elongation, cell wall development, transport processes, positioning of membrane receptors and in PCD. To fulfill such a variety of specific roles, the arrangement and dynamic of actin cytoskeleton must be precisely regulated including the composition of actin isoforms as well as the composition and activity of associated proteins.
To examine the role of actin cytoskeleton during somatic embryogenesis we applied the actin depolymerizing drugs latrunculin B (Lat B) and cytochalasin D (Cyt D) either to proliferating or maturating cultures of spruce and fir. The anatomical and macroscopical analysis of treated and untreated lines was evaluated together with expression analysis of four newly isolated actin genes.

Material and methods:

Embryogenic culture of *Picea abies*, genotype AFO 541 originated from AFOCEL, France, the results were verified using 5 genotypes of spruce (Kostelec nad Černými Lesy) and 5 genotypes of fir – *Abies alba, A. cephalonica (Jizerské hory, Orlik, Praha).*

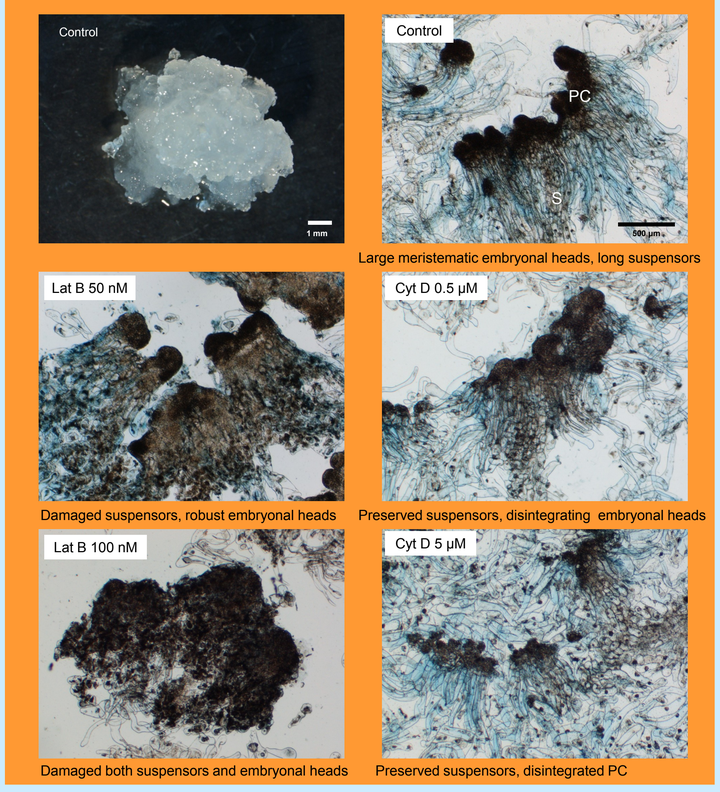
Cultivation: Embryogenic suspensor mass (ESM) was proliferated on medium GD (Gupta, Durzan 1986, In Vitro Cellular Developmental Biology 22(11):685-688) supplemented with sucrose, agar, 2,4-D, BAP and kinetin; maturation occurred on liquid medium GD supplemented with sucrose, PEG and ABA. Material was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

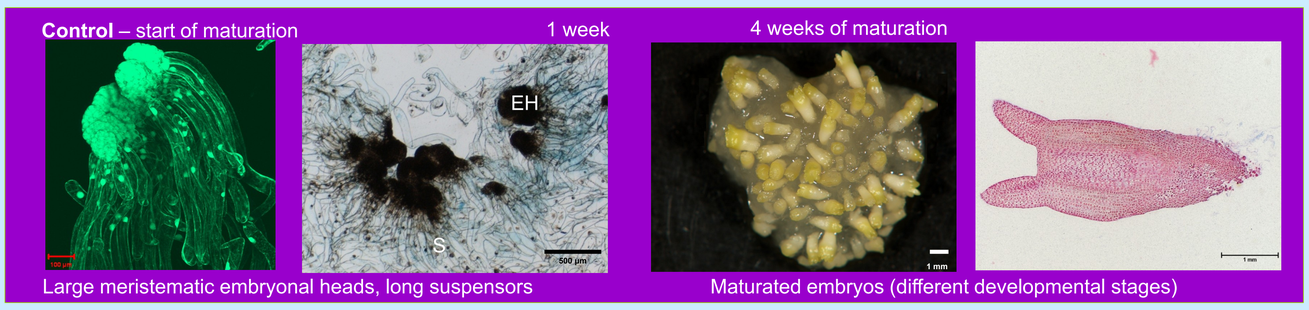
The process of spruce somatic embryogenesis consists of 5 steps (induction of ESM, proliferation, maturation, desiccation and germination). It is strictly controlled by the exogenous treatments by plant growth regulators – see above. Desiccation and germination are phytohormones free steps of somatic 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.

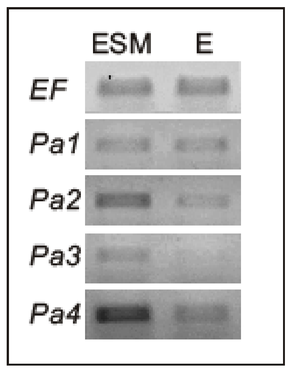
Drug application: Drugs were dissolved in DMSO in concentrations 50, 100 and 200 nM (Lat B) and 0,5 ; 5 µM (Cyt D) and added into maturation media. The cultures were growing on media supplemented with drugs all the time of cultivation. Untreated cultures were used as controls. The highest concentration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

Anatomy and histology: Clusters of ESM were stained with 0.04 % trypan blue . Cell viability – staining by 0.02 % (w/v) fluorescein diacetate (FDA). Actin was visualised using rhodamine – phalloidin according to Blancaflor 2000 (J.Plant Growth Regul. 19:406-414). Matured somatic embryos were embedded in paraffin, sectioned longitudinally and stained by alciane blue and nuclear fast red.
Microscopy: transmission light microscope Zeiss Jenaval equipped 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.
Isolation and expression analysis of actin isoforms: Actin genes were isolated by RT-PCR (using specific and degenerated primers) from RNA isolated from the maturating embryogenic culture. The expression analysis was done by semiquantitative RT-PCR with EF-1a as an internal standard.

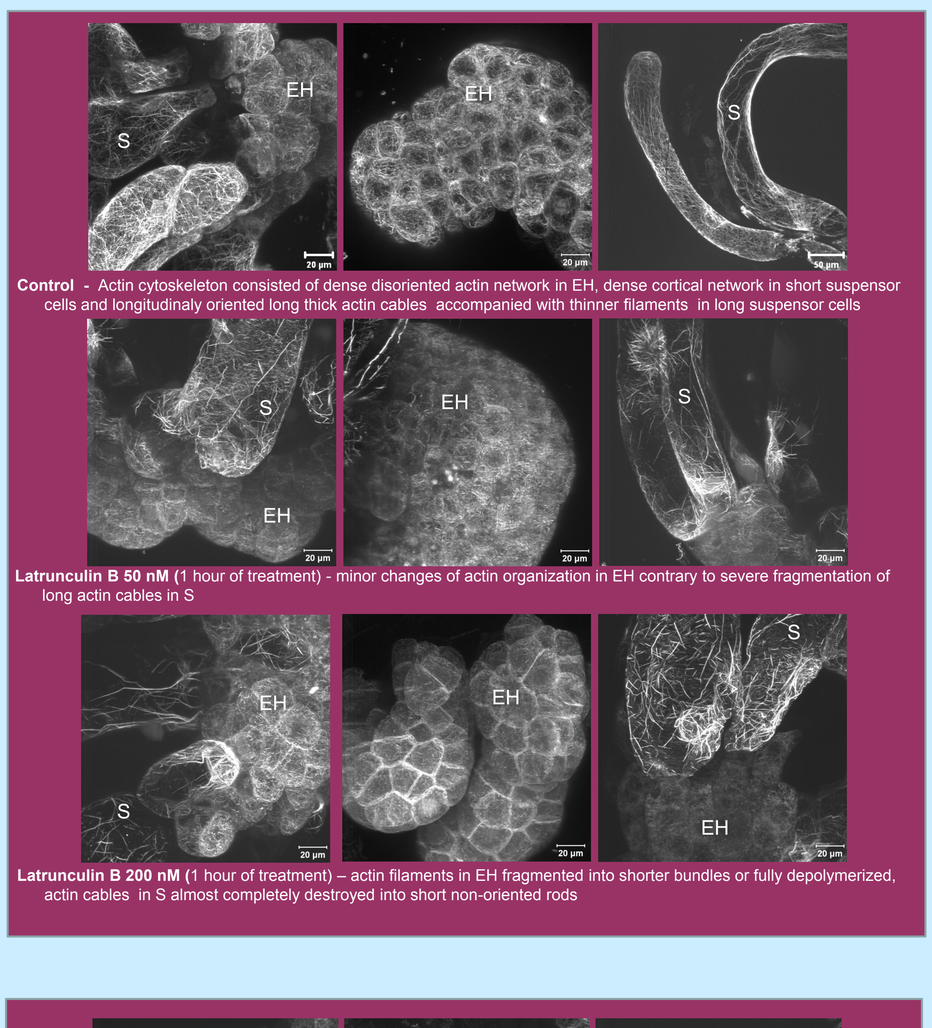
Proliferation







Expression of the actin isoforms in control ESM and isolated embryos determined by semiquantitative RT-PCR. Transcript levels in non-fractionated ESM culture (ESM) and in isolated embryos (E) after 2 weeks of maturation on the control media.
(EF, gene for elongation factor ef1α (internal standard); *Pa1-4, Picea abies* actin isoforms 1-4)

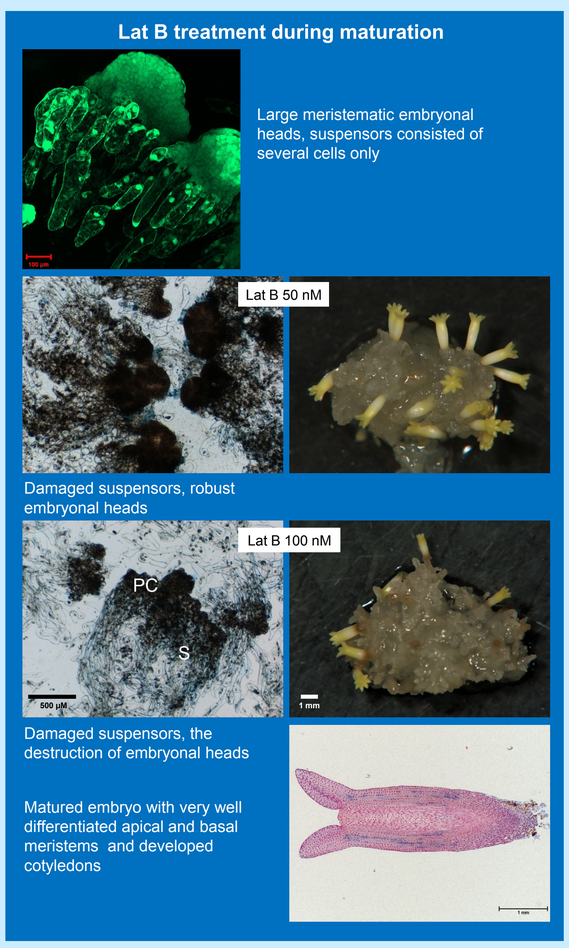


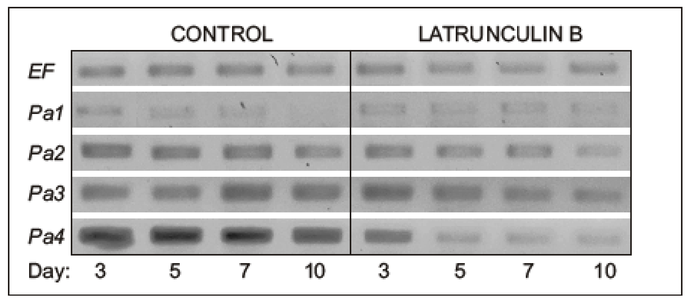
Control - Actin cytoskeleton consisted of dense disoriented actin network in EH, dense cortical network in short suspensor cells and longitudinaly oriented long thick actin cables accompanied with thinner filaments in long suspensor cells

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

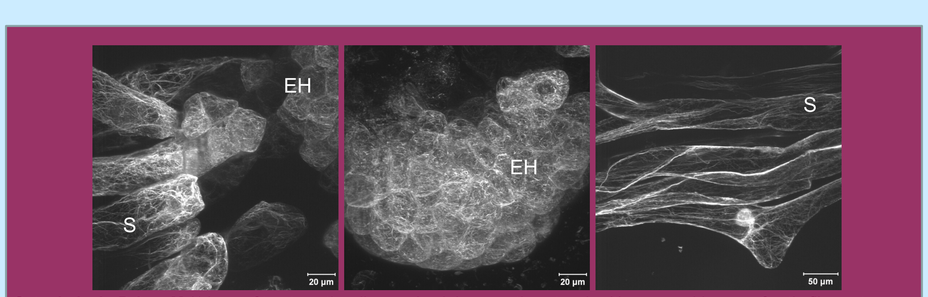
Latrunculin B 200 nM (1 hour of treatment) – actin filaments in EH fragmented into shorter bundles or fully depolymerized, actin cables in S almost completely destroyed into short non-oriented rods

Lat B treatment during maturation

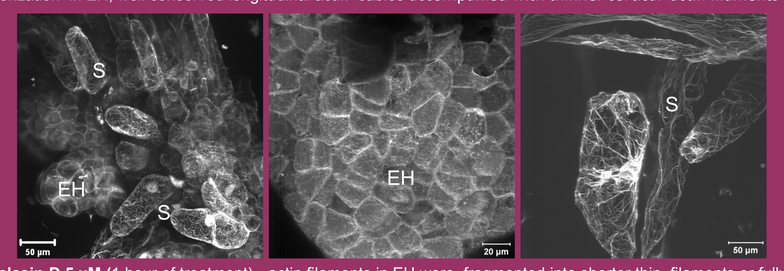




Expression of the actin isoforms during maturation in the presence of Lat B as determined by semiquantitative RT-PCR. Transcript levels in non-fractionated ESM cultures were monitored during 10-days maturation with or without addition of 100 nM latrunculin B (Lat B).
(EF, gene for elongation factor ef1α (internal standard); *Pa1-4, Picea abies* actin isoforms 1-4)

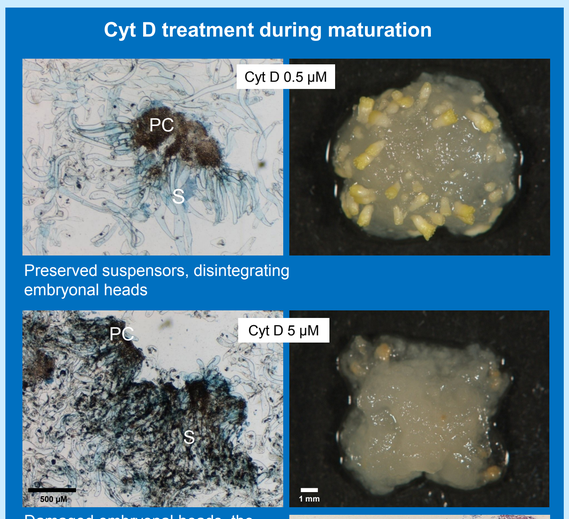


Cytochalasin D 0.5 µM (1 hour of treatment) – disorganized dense actin network with some evidences of actin filament depolymerization in EH, well conserved longitudinal actin cables accompanied with thinner cortical actin filaments in S



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

Cyt D treatment during maturation



Conclusions

Meristematic and suspensor cells were differentially affected by the treatment with the actin depolymerizing drugs.

Expression analysis of four newly isolated spruce actin isoforms revealed that 1 actin isoform was expressed in both cell types; 3 actin isoforms were expressed predominantly in suspensor cells.

Lat B preferentially affected cortical actin of suspensor cells. The effect of 50-100 nM Lat B on actin filaments of meristematic cells was mild, higher concentrations – up to 200 nM - caused fragmentation and depolymerization of actin in both types of the ESM cells.

The positive effect of Lat B treatment on embryo development was probably due to the inhibition of poorly developed embryos. The destruction of suspensors was lethal for their next development. The yield of maturated embryos was lower than in control but their quality was higher.

Cyt D affected cortical actin in meristematic cells markedly more than in suspensor cells. Higher concentrations (5µM) caused depolymerization of actin filaments in meristematic cells.

The negative effect of Cyt D treatment on embryo development was probably due to the immediate effect on the meristematic heads. The yield of embryos was low and the embryos were often malformed.

Two cytoskeletal drugs Lat B and Cyt D (causing depolymerization of actin cytoskeleton) strongly differed in their effects on SE differentiation, as was verified using other 4 embryogenic lines of spruce and 5 of fir.

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