Endogenous phytohormone profiles during Norway spruce somatic embryogenesis

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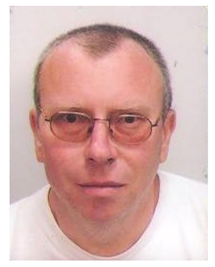
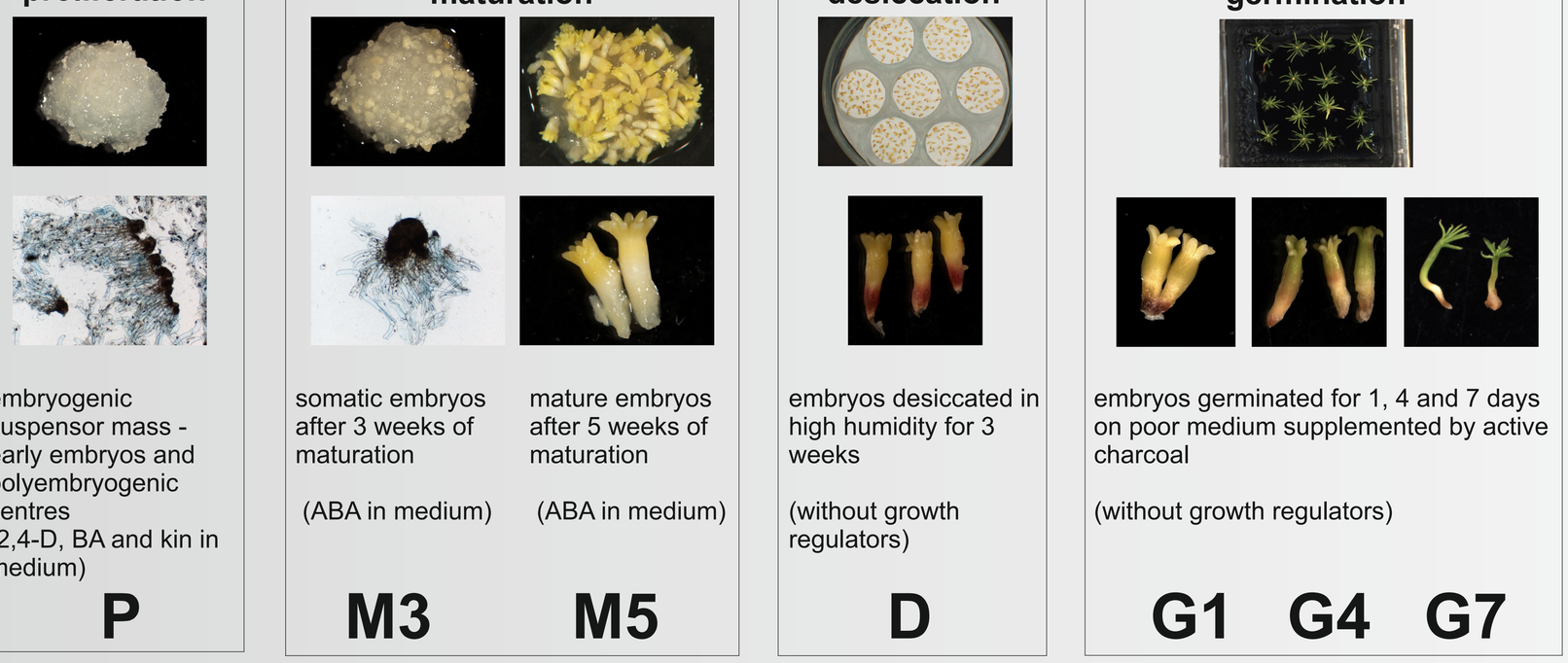


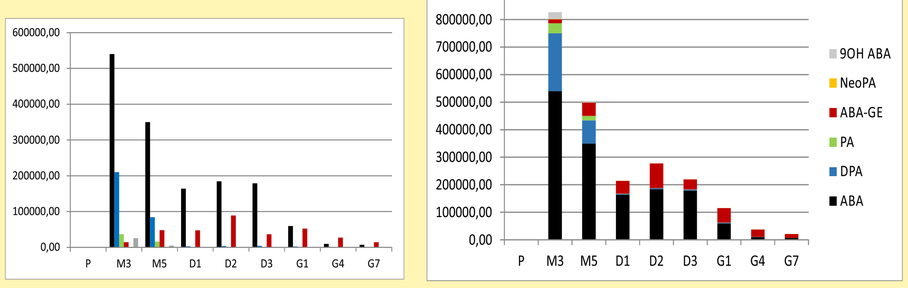
Somatic embryogenesis in *Picea abies* (AFO 451 embryogenic culture)



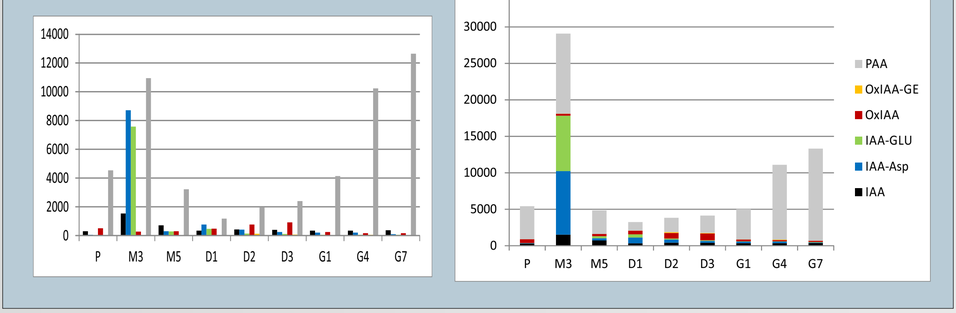
Somatic embryogenesis (SE) in conifers is governed by a complex network of hormonal metabolic and signaling pathways. Changes in the patterns and concentrations of endogenous phytohormones including auxins, cytokinins (CKs), abscisic acid (ABA), jasmonates and salicylic acid (SA) were analyzed in the course of SE in Norway spruce (*Picea abies*). Taking advantage of advanced HPLC-ESI-MS/MS, as yet the most comprehensive overview of the plant hormonome in somatic embryos was provided here that revealed substantial variations in the levels of particular phytohormone classes during proliferation, maturation, desiccation and germination. The peak in concentration of endogenous ABA and its inactive catabolite, dihydrophaseic acid, at the start of maturation reflected the presence of exogenous ABA in the medium and showed its efficient perception and deactivation by the embryos as a prerequisite for their further development. The concentration maxima at maturation were also shown for most of auxins, both indole and non-indole, suggesting their role in embryo polarization. For the first time, endogenous jasmonates are reported in conifer somatic embryos reaching their highest levels at germination. The involvement of some other phytohomone derivatives such as the non-indole auxin phenylacetic acid, cis-zeatin- and dihydrozeatin-type CKs and SA in the process of SE was demonstrated here for the first time as well. Aforementioned data together with substantial quantitative and qualitative changes in concentrations of individual CK forms during SE indicated potential correlations between endogenous phytohormone profiles and particular developmental stages of somatic embryos.

Concentration of phytohormones (pmol/g DW)

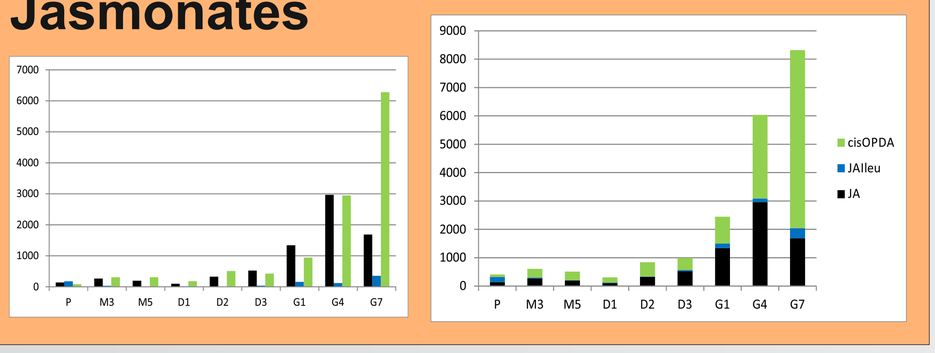
Abscisic acid



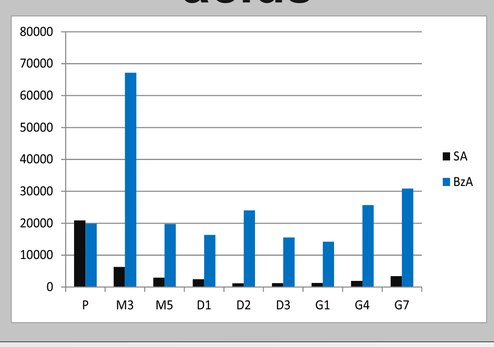
Auxins



Jasmonates



Salicylic and Benzoic acids



Summary:

P - endogenous levels of most phytohormones are low, although an intense hormonal regulation of multiplication/destruction processes of embryogenic structures is presumed.

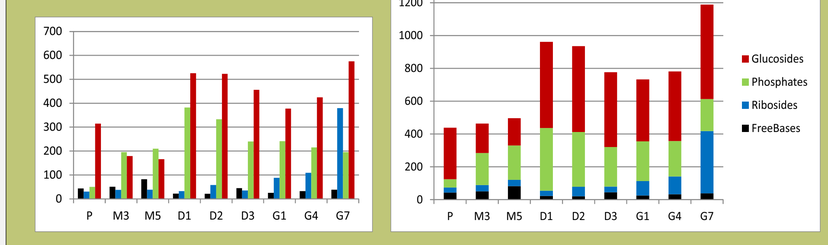
M - significant increase in endogenous concentrations of auxins, ABA and BzA was recorded. The 3rd week of maturation in which embryo polarization and formation of cotyledonary embryos occur, represents a key moment of somatic embryos development.

D - no dramatic changes in the phytohormone contents; a formation of „reserve pool" of storage CK and ABA derivatives represented the only apparent characteristic of this step balancing probably a reduction or a loss of respective bioactive CK and ABA forms.

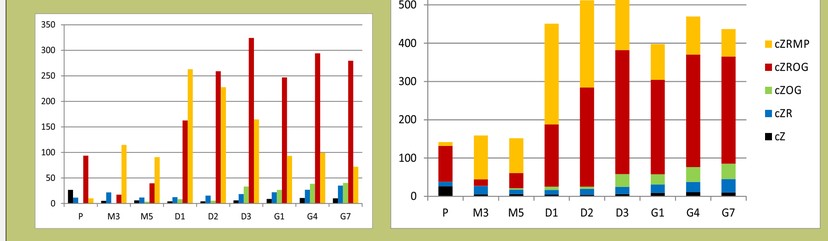
G - germination and the start of emblings development include a sequence of complex timely and spatially balanced events associated with increased levels of the non-indole auxin PAA, total CKs and jasmonates and with a drop of ABA and its derivatives. This complexity is evidently a result of a crosstalk between phytohormones leading to establishment and maintenance of hormonal homeostasis during the germination process.

Cytokinins

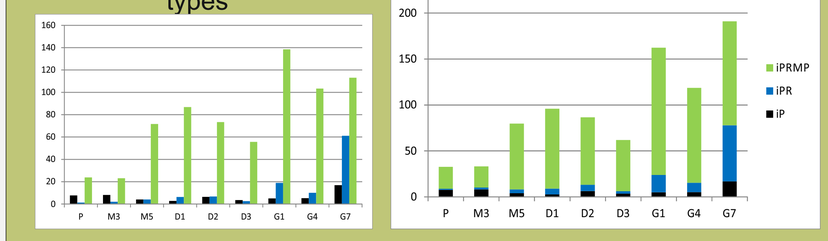
total amount



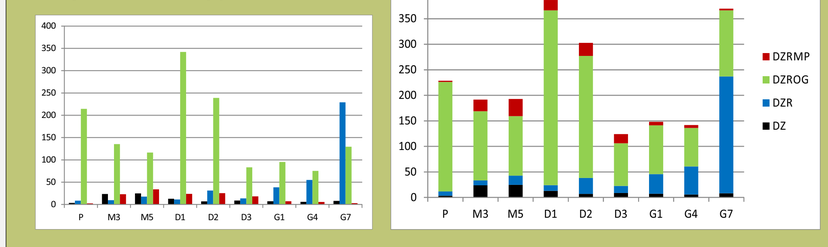
cis-zeatin types



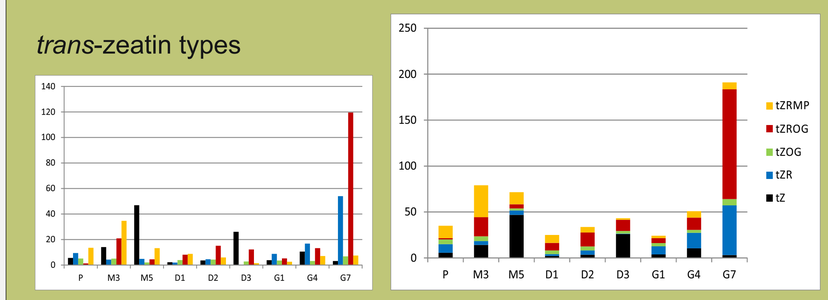
N^6-(Δ^2-isopentenyl)adenine types



dihydrozeatin types



trans-zeatin types



Methods - phytohormonal analysis

The concentrations of phytohormones were measured at nine time points over the course of the development of somatic embryos. The analysis was carried out as described in [1] and [2]. Briefly, an aliquot of ca. 100 mg fresh weight of frozen plant material was homogenized in liquid nitrogen, extracted with methanol/water/formic acid (15/4/1, v/v/v) reagent supplemented with a mixture of stable isotope labeled internal standards (10 pmol). Clarified supernatants were subjected to solid phase extraction using Oasis-MCX cartridges (Waters, U.S.A.). The eluates were evaporated to dryness and dissolved in 30 µl 10% methanol in water. Quantification was done on an Ultimate 3000 high-performance liquid chromatograph (HPLC; Dionex, U.S.A.) coupled to hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP, Applied Biosystems, U.S.A.) set in selected reaction monitoring mode. Quantification of phytohormones was performed using isotope dilution method with multilevel calibration curves. Data processing was carried out with Analyst 1.5 software (Applied Biosystems). Each of six independent biological samples for each time point was run in two technical replicates. Phytohormone concentration is related to 1 g of dry weight of plant material, due to the different water content in embryos in different developmental stages.

References:
[1] Djilianov DL, Dobrev PI, Moyankova DP, Vankova R, Georgieva DT, Gajdosova S, Motyka V (2013) Dynamics of Endogenous Phytohormones during Desiccation and Recovery of the Resurrection Plant Species Haberlea rhodopensis. J Plant Growth Regul. 32:564-574.
[2] Dobrev PI, Vankova R (2012) Quantification of abscisic acid, cytokinin, and auxin content in salt-stressed plant tissues. Methods in molecular biology (Clifton, NJ). 913:251-61.

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Abbreviations:

Auxins
IAA - indole-3-acetic acid
IAA-Asp - IAA-aspartate
IAA-GLU - IAA-glutamate
OxIAA - oxo-IAA
OxIAA-GE - oxo-IAA-glucosylester
PAA - phenylacetic acid
2,4-D - 2,4 dichlorophenoxyacetic acid

ABA
ABA - abscisic acid
ABA-GE - ABA-glucosylester
PA - phaseic acid
DPA - dihydrophaseic acid
NeoPA - neophaseic acid
9OH-ABA - 9-hydroxy-ABA

Cytokinins
cZ – *cis*-zeatin
cZOG – *cis*-zeatin O-glucoside
cZR – *cis*-zeatin 9-riboside
cZRMP – *cis*-zeatin 9-riboside-5'-monophosphate
cZROG – *cis*-zeatin 9-riboside O-glucoside
DHZ – dihydrozeatin
DHZR – dihydrozeatin 9-riboside
DHZRMP – dihydrozeatin 9-riboside-5'-monophosphate
DHZROG – dihydrozeatin 9-riboside O-glucoside
iP – *N6*-(Δ2-isopentenyl)adenine
iPR – *N6*-(Δ2- isopentenyl)adenosine
iPRMP – *N6*-(Δ2-isopentenyl)adenosine-5'-monophosphate
tZ – *trans*-zeatin
tZOG – *trans*-zeatin O-glucoside
tZR – *trans*-zeatin 9-riboside
tZRMP – *trans*-zeatin 9-riboside-5'-monophosphate
tZROG – *trans*-zeatin 9-riboside O-glucoside
BA - *N6*-benzyladenine

Jasmonates
JA - jasmonic acid
JA-Ile - JA-isoleucine
cisOPDA - cis-(+)-12-oxo-phytodienoic acid

Others
SA - salicylic acid
BzA - benzoic acid