The development of Norway spruce somatic embryos visualized with different microscopic tools

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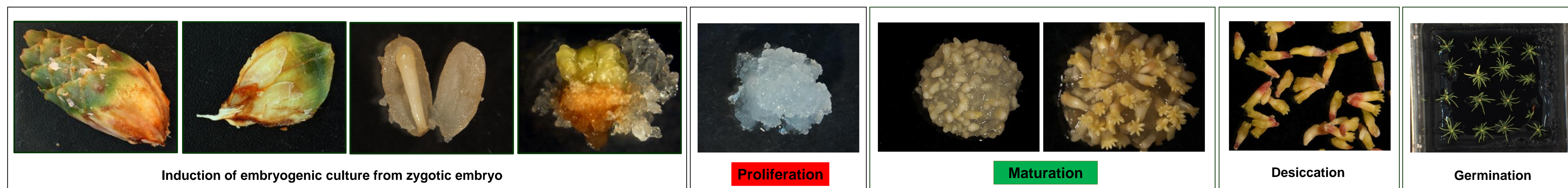
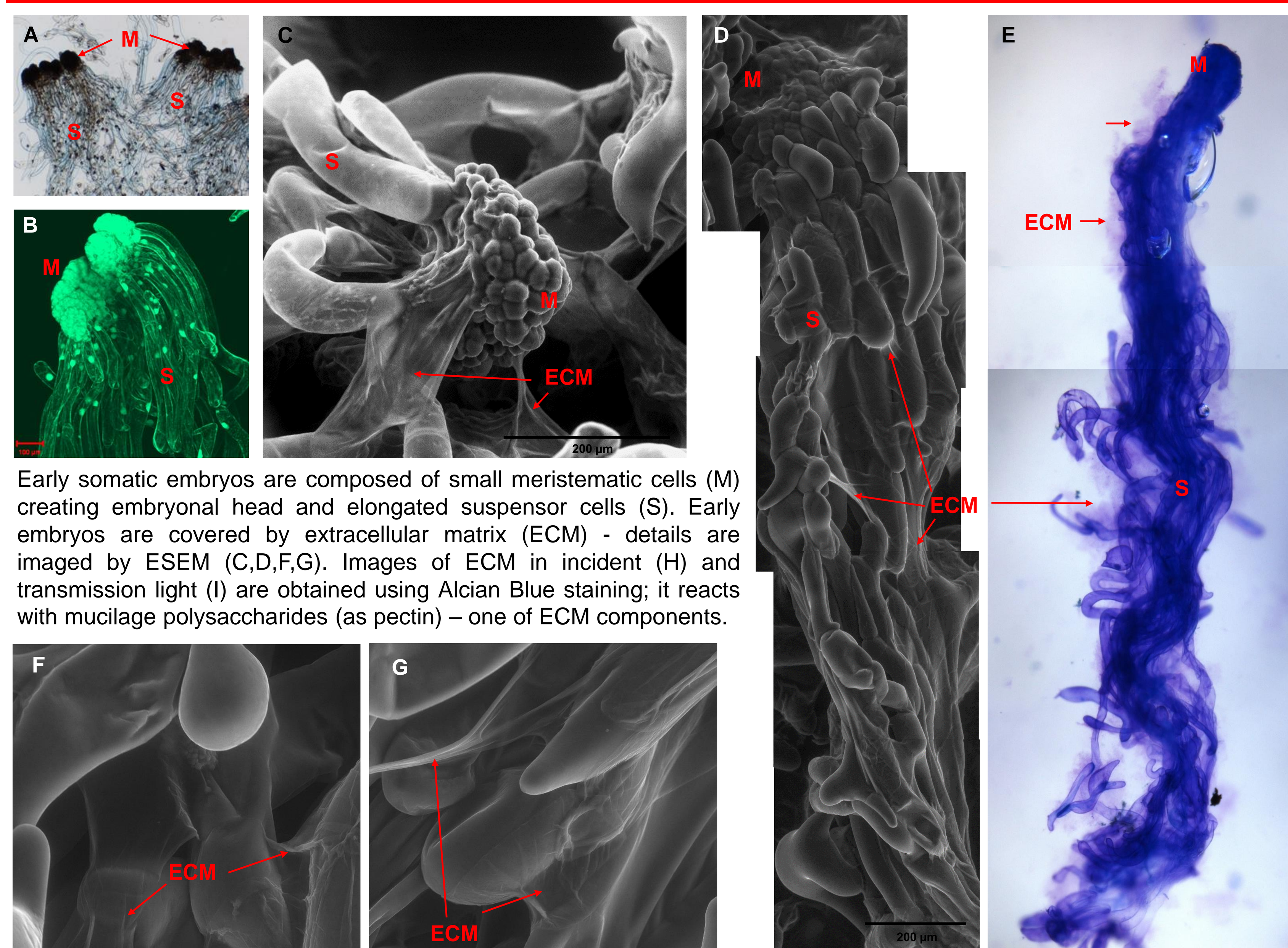
Kateřina Eliášová[1], Vilém Neděla[2], Jiří Runštuk[2], Zuzana Vondráková[1]
eliasova@ueb.cas.cz
[1]Institute of Experimental Botany CAS, Rozvojová 263, Praha 6 - Lysolaje, 165 02, Czech Republic
[2]Institute of Scientific Instruments CAS, Královopolská 147, Brno, 612 64, Czech Republic



Introduction

Somatic embryogenesis represents useful system for the study of the process of embryogenesis as well as the convenient system for clonal propagation of conifers. It consists of 5 steps: induction of embryogenic culture from somatic cells of zygotic embryos; proliferation, i.e. the development and multiplication of early somatic embryos; their maturation, desiccation and germination followed by embling´s development. Our study was focused on morphology of somatic embryos, viability of embryonal cells and on the changes in starch distribution in embryos during their development. We aimed our investigation at the development of surface cell layers on early embryos during proliferation and on embryos during maturation. We used light and confocal laser-scanning microscopy (CLSM) as well as environmental scanning electron microscopy (ESEM).

Proliferation

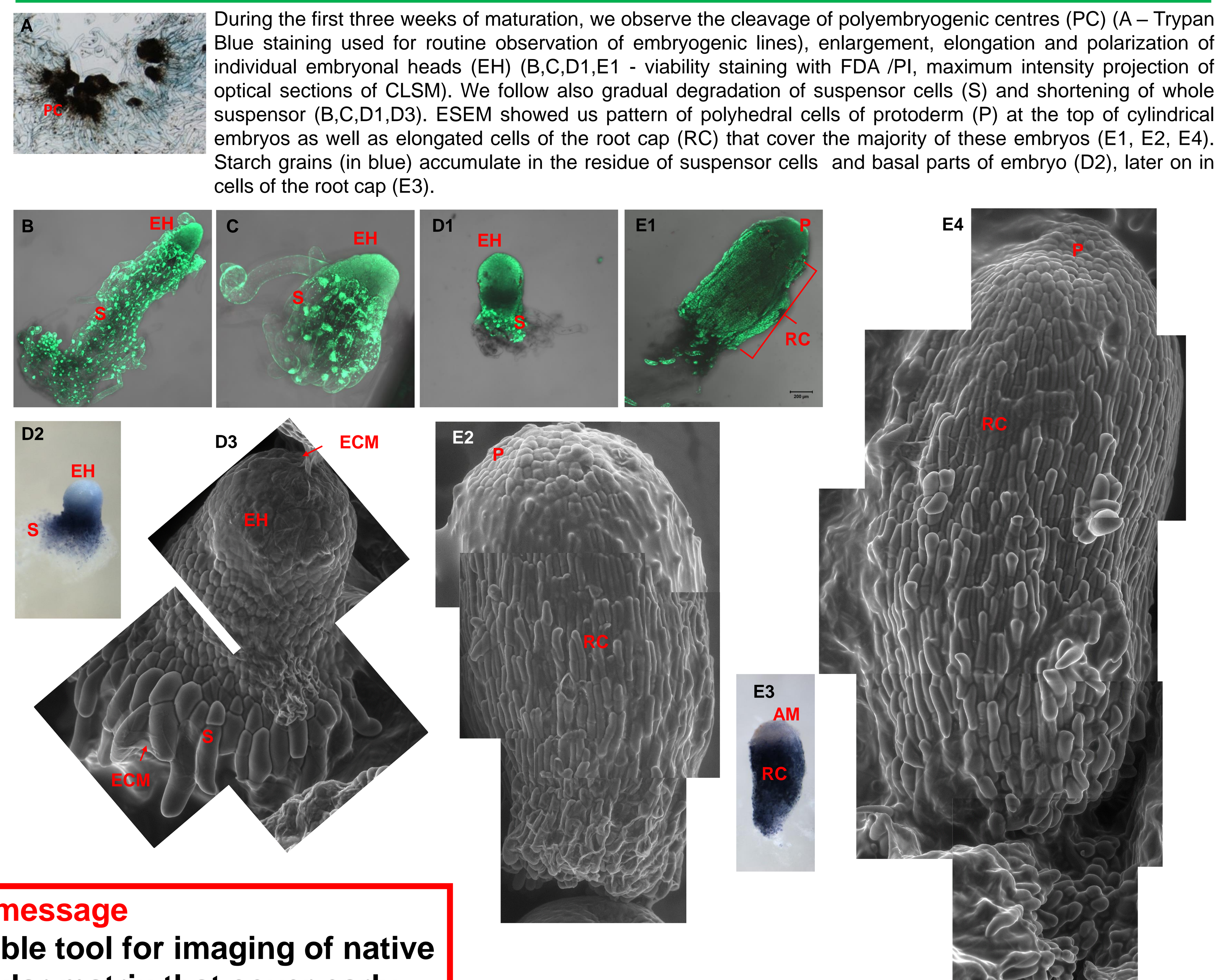


Early somatic embryos are composed of small meristematic cells (M) creating embryonal head and elongated suspensor cells (S). Early embryos are covered by extracellular matrix (ECM) - details are imaged by ESEM (C,D,F,G). Images of ECM in incident (H) and transmission light (I) are obtained using Alcian Blue staining; it reacts with mucilage polysaccharides (as pectin) – one of ECM components.

Metachromatic staining of mucilage polysaccharides in ECM with Toluidine Blue (E)

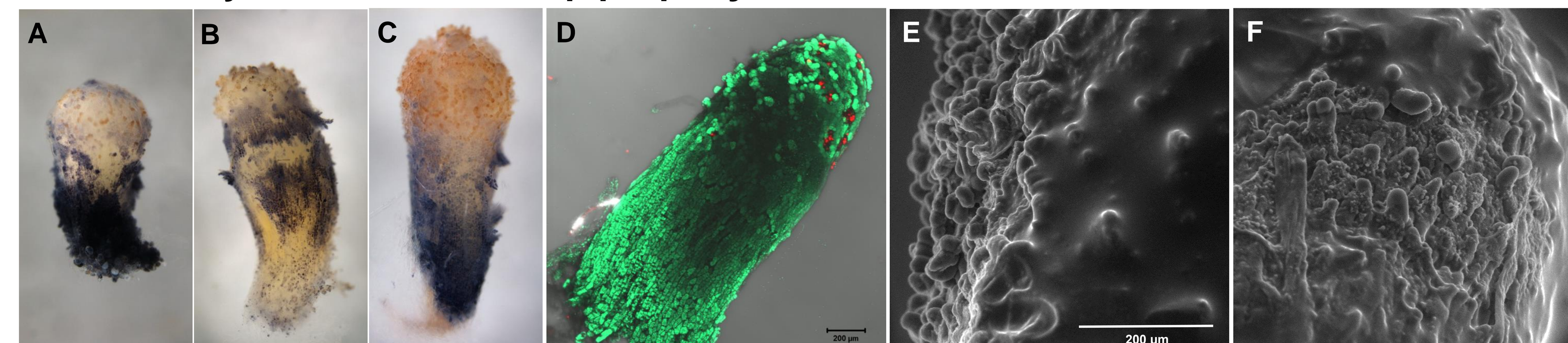
Start of maturation (1 – 3 weeks of maturation)

During the first three weeks of maturation, we observe the cleavage of polyembryogenic centres (PC) (A – Trypan Blue staining used for routine observation of embryogenic lines), enlargement, elongation and polarization of individual embryonal heads (EH) (B,C,D1,E1 - viability staining with FDA /PI, maximum intensity projection of optical sections of CLSM). We follow also gradual degradation of suspensor cells (S) and shortening of whole suspensor (B,C,D1,D3). ESEM showed us pattern of polyhedral cells of protoderm (P) at the top of cylindrical embryos as well as elongated cells of the root cap (RC) that cover the majority of these embryos (E1, E2, E4). Starch grains (in blue) accumulate in the residue of suspensor cells and basal parts of embryo (D2), later on in cells of the root cap (E3).



Take-home message
ESEM is a unique and irreplaceable tool for imaging of native embryonal cells and extracellular matrix that cover early somatic embryos and interconnect their suspensor cells.

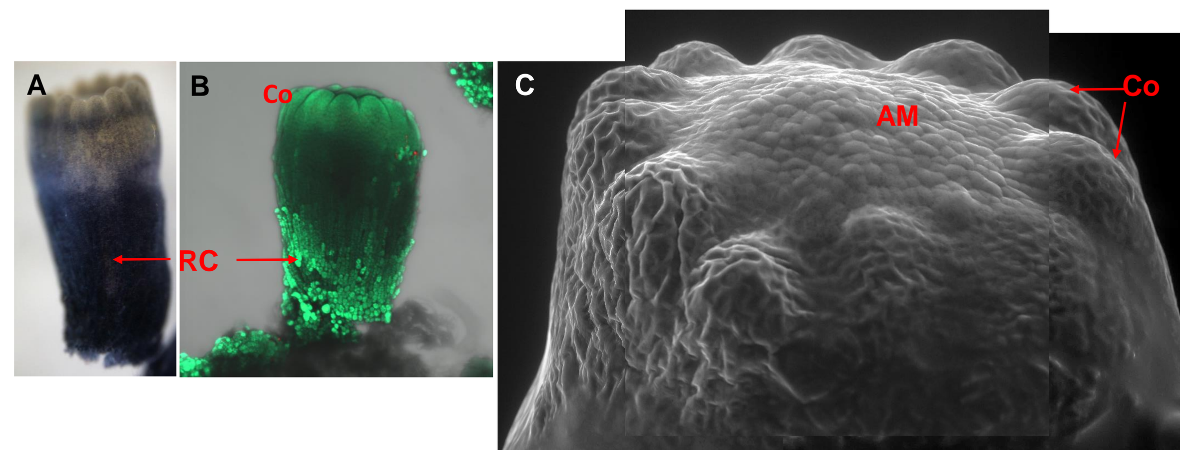
When embryo does not develop properly…

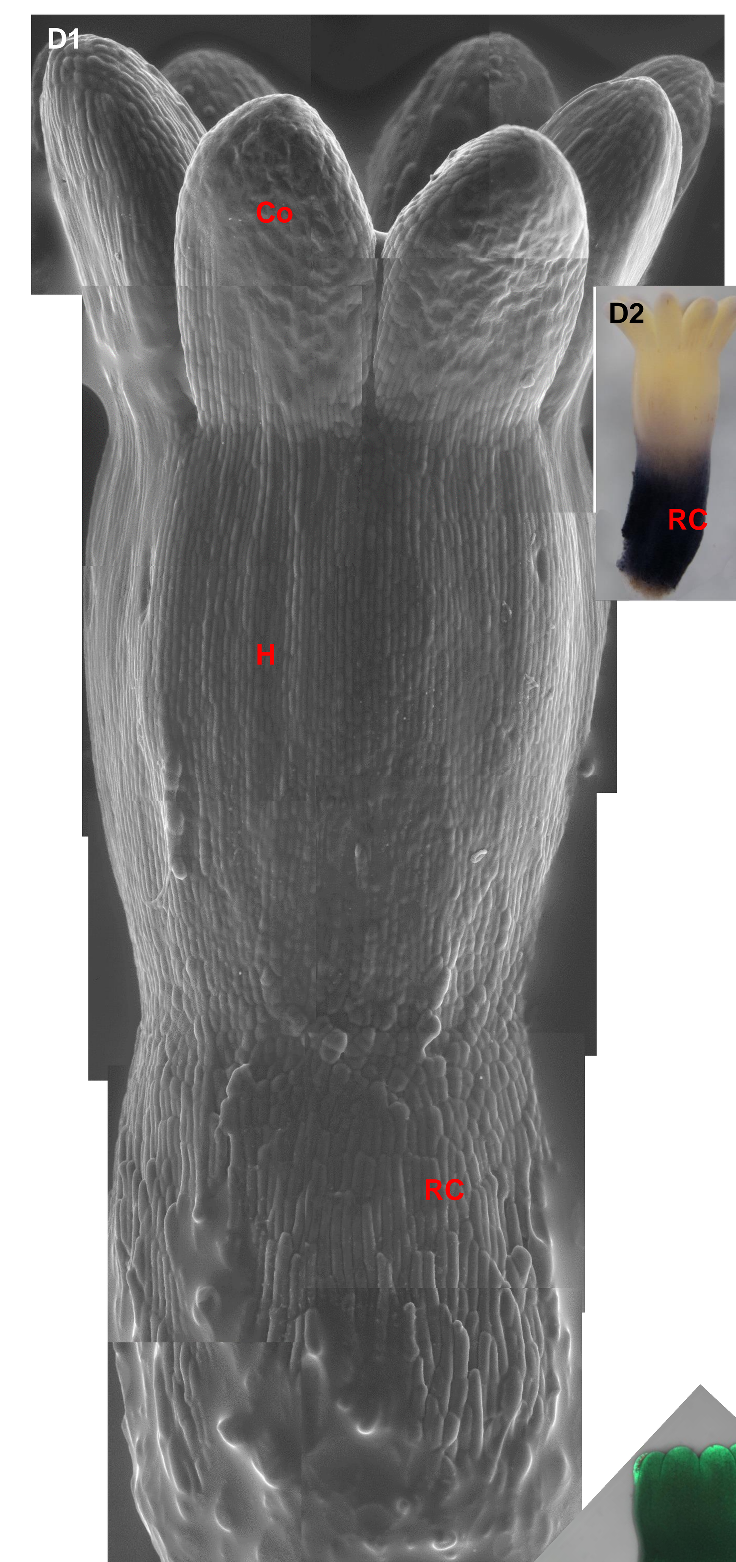


Light microscopy (A,B,C - histochemical staining of starch), confocal microscopy (D - viability assessment) as well as ESEM (E,F) show the defects in the establishment of protoderm on embryo surface and accumulation of starch grains not only in the root cap cells but also in the upper part of embryo. On the images from light microscope accumulation of phenolic compounds on the top of embryos is obvious (brown colour).

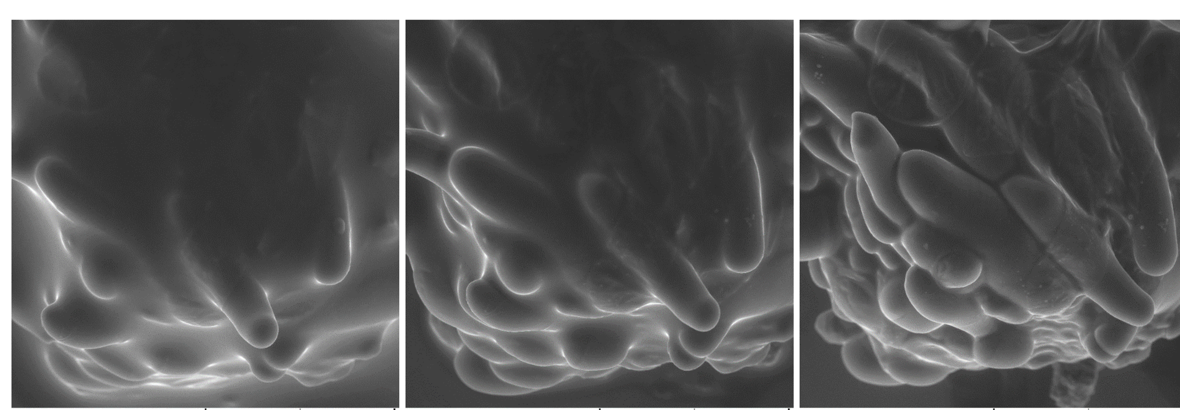
End of maturation (4 – 6 weeks of maturation)

Mature embryos are characterized by the ring of cotyledons (Co) around the apical meristem (AM), by the elongated hypocotyl (H) and radicle. Starch grains are primarily accumulated in the whole embryo (A – staining by Lugol solution). In fully developed embryos with long cotyledons (D1) starch grains are present abundantly in the root cap (RC) (D2) . Accumulation of starch in RC is obvious also after vital staining (B and D3 as a strong fluorescent signal in the RC). Surface of cotyledonary embryos is covered by polyhedral or elongated protodermal cells (C,D1) .





D3



Gradual evaporation of thin water layer from suspensor cells surface as a result of controlled reduction of water vapour pressure at a constant sample temperature in the ESEM AQUASEM II.

Material and methods

Embryogenic culture of Norway spruce (*Picea abies* L. Karst.) was studied using:

- Light microscopy (stereomicroscope Nikon SMZ 1500, and Jenaval transmission light microscope Zeiss) – study of morphology (fresh tissue stained with 0.04% Trypan Blue); visualization of mucilage polysaccharides (histochemical staining with 0.05% Toluidine Blue in acetate buffer (pH 4.5) and 0.1% Alcian Blue in 3% acetic acid); study of starch grain localization (staining with Lugol solution).
- Confocal microscopy (CLSM LSM5 Duo, Zeiss) – assessment of embryonal cell viability using double staining with fluorescein diacetate (FDA) and propidium iodide (PI). These two dyes enable to distinguish living cells (green fluorescence of FDA; ex. 488 nm/ em. BP 505-550 nm) from dead ones (red fluorescence of PI; ex. 561 nm/ em. LP 650 nm)
- Environmental scanning electron microscopy (ESEM) – morphological study of native samples in wet state with higher resolution and large depth of field using non-commercial microscope AQUASEM II (see the figure on the left) equipped with ionization detector of secondary electrons and Peltier cooling stage. Beam energy 20 keV, beam current 60 pA, dwell time 61μs, Peltier stage temperature -1°C, water vapour pressure from 620 Pa to 770 Pa.

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