ENVIRONMENTAL SCANNING ELECTRON MICROSCOPE AS A TOOL FOR IMAGING OF NATIVE STATE SOMATIC EMBRYOGENESIS

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Introduction

Somatic embryogenesis is a developmental process characterized by formation of structures that resemble zygotic embryos, but starting from somatic cells. Embryogenic cells of somatic embryos are covered by special extracellular cell wall layer called extracellular matrix (ECM) at their early developmental stages.

The aim of this work was the detailed ESEM studies of native ECM of early conifer embryogenic tissues (Abies numidica and Pinus sylvestris) without artefacts in specific conditions of observation given by our methodology (gas type, gas humidity and temperature of sample) using the BSE YAG detector and a specially designed ionization detector.

Material and Methods

The embryogenic tissue of Abies numidica De Lann. and Pinus sylvestris L. was initiated from immature zygotic embryos of cones from open-pollinated trees. Immature seeds were surface-sterilized for 10 min in 10% (v/v) H_2O_2, and rinsed with sterile distilled water. Megagametophytes of Abies numidica De Lann. containing embryos were cultured on SH medium and the medium for Pinus sylvestris L. was the DCR medium. The cultures were grown in darkness at 25 ± 1°C and transferred to a fresh medium at 2–3 week intervals.

All experiments were carried out under constant operating conditions of the AQUASEM II microscope (beam accelerating voltage 20 kV, probe current 80 pA) and in the gas environment with relative humidity equal to 40% and 550 Pa of air. The microscope is equipped with the single crystal YAG:Ce^{3+} (yttrium aluminium garnet activated with trivalent cerium), used for detection of backscattered electrons (BSEs) and the ionization detector for secondary electrons (SEs) signal.

Native samples of conifer were placed on a cooled specimen holder (Peltier stage) and their temperature was reduced and kept at -18/-22 °C. Due to the relatively low temperature conductivity of the conifer sample the real temperature of the sample surface was reduced to approximately -5 °C.

Results

Our methodology enables to image well preserved structures of plant samples without any artifact resulting from chemical fixation. This is espetially remarcable in the case of the extracellular matrix which remains without any damage. The Fig. 1 shows the early somatic embryos of Abies numidica De Lann., Fig. 2 and Fig. 3 show the embryonic tissues of Pinus sylvestris L.

The comparison of micrographs obtained by the BSE-YAG and the ionization detectors enables to study morphology as well as the material composition. The lower parts of all figures show acceptable image resolution of embryogenic and suspensor cells and the native surface of extracellular matrix achieved by the ionization detector. On the contrary, with the BSE-YAG detector (see the upper part of figures), a high distinction of chemical and material composition can be achieved.

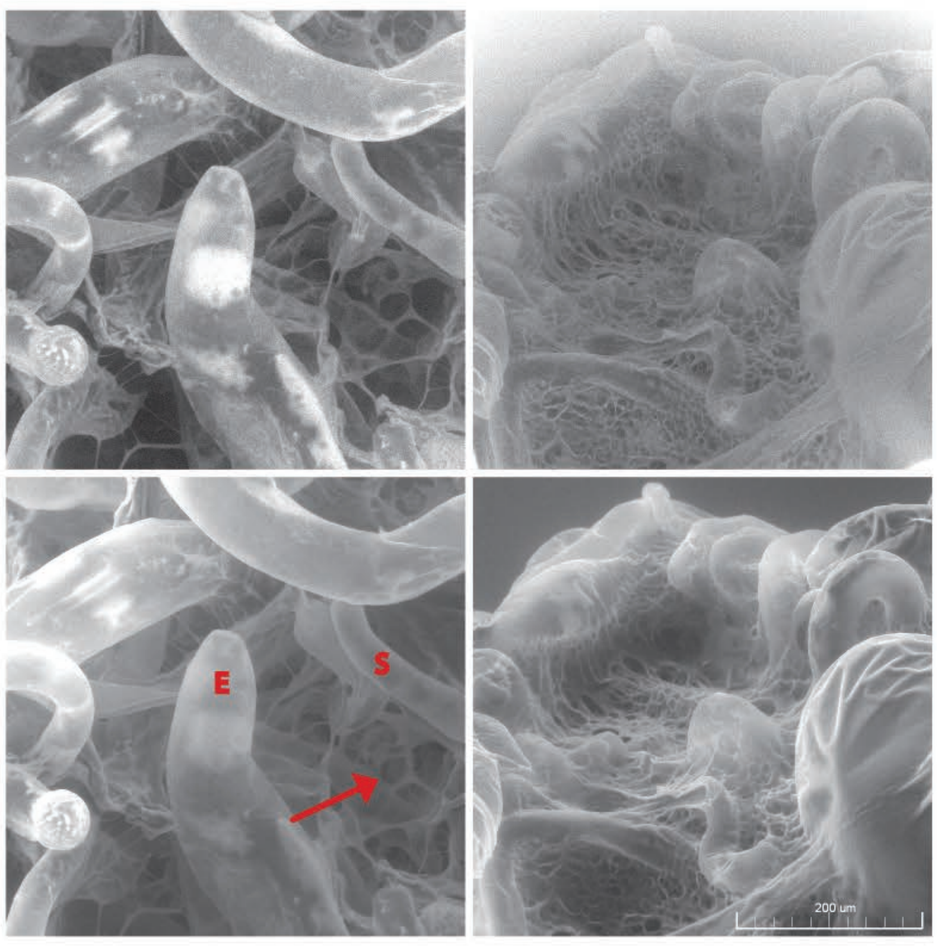

Fig. 1: Comparison of ESEM observation with two detectors in embryogenic tissue of Abies numidica De Lann. (upper part – BSE YAG detector; lower part – ionization detector); the presence of extracellular matrix -indicated by arrows; E-embryonic cell, S-suspensor cell.

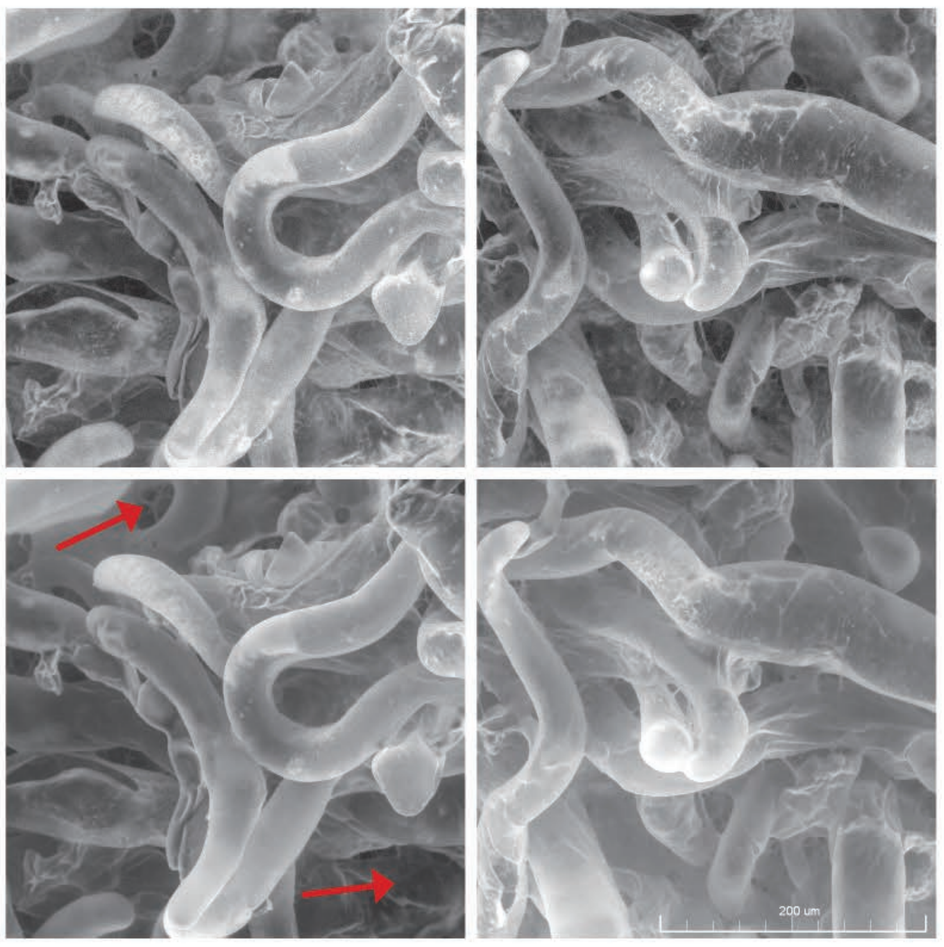

Fig. 2: Comparison of ESEM observation with two detectors in embryogenic tissue of Pinus sylvestris (upper part of figure – BSE YAG detector); lower part – ionization detector; extracellular matrix-indicated by arrow.

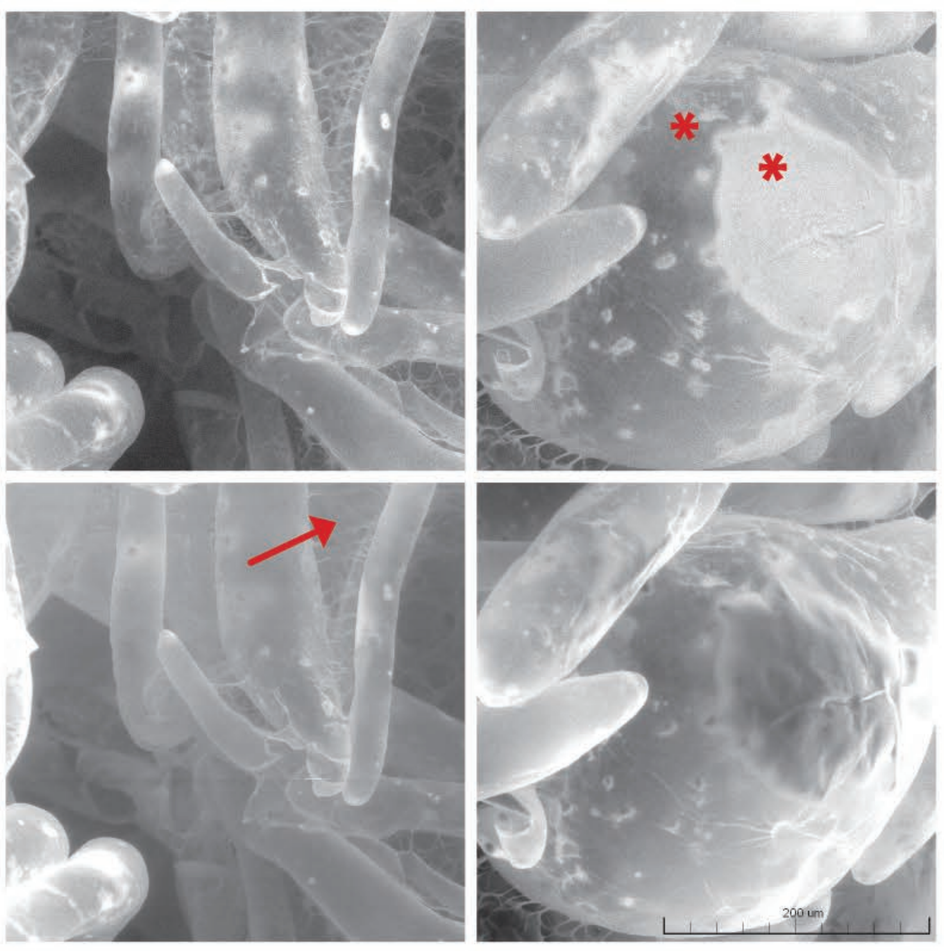

Fig. 3: Comparison of ESEM observation with two detectors in embryogenic tissue of Pinus sylvestris (upper part of figure – BSE YAG detector); lower part – ionization detector; extracellular matrix-indicated by arrow, material contrast by asterisks.

Conclusions

We have found that the environmental scanning electron microscope operated by the use of our methodology is useful and very suitable for the observation of native state plant tissue. Additionally, plant tissue free of chemical fixation procedures allows the observation of the extracellular matrix in its native state. This method is fast and simple, more over relatively inexpensive. We suppose it will be a generally applicable tool in the field of plant research.

References

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[4] This work was supported by the Grant Agency of the Czech Republic: grant No. GAP 102/10/1410, and by the EU project No. CZ.1.07/2.3.00/20.0103.

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