Proceedings of the IUFRO Working Party 2.09.02 conference
"Integrating vegetative propagation, biotechnologies and genetic improvement for tree production and sustainable forest management"
June 25-28, 2012. Brno, Czech Republic

Native state of extracellular matrix of early conifer embryogenic tissue imaged by environmental scanning electron microscope

V. Neděla[1], J. Hřib[1], B. Vooková[2] , J. Runštuk[1] , S. Svidenská[3]

[1]Institute of Scientific Instruments of ASCR, Brno 61264, Czech Republic, vilem@isibrno.cz;[2]Institute of Plant Genetics and Biotechnology SAS, Nitra 95007, Slovak Republic; [3]Department of Electrotechnology, Brno University of Technology, Brno 61600, Czech Republic

Keywords: ESEM, detectors, methodology, in situ study, plant biology

An environmental scanning electron microscopy is described that by using our newly introduced methodology allows observation of conifer somatic embryos in their native state. This research method prevents morphological changes during sample dehydration and avoids appearance of the artifacts that are commonly created by traditional preparation techniques needed for the study of biological samples in classical scanning electron microscopy.

Environmental scanning electron microscopy (ESEM) opens up a wide range of new applications in the field of electron microscopy. Some of these biological applications rely on us being able to image samples in, or close to, their native state [1, 2]. In ESEM the specimens can be observed in a wide range of pressure, from 0,001 Pa (comparable with the one used in SEM) to over a thousand Pa in the specimen chamber [3]. In high pressure conditions very wet non-conductive samples can be observed free of artifacts caused by charging and without a conductive coating covering their surface. If the gas pressure is sufficiently increased or the sample's temperature reduced, its natural and fully hydrated surface structure is preserved [4, 5, 6].This study is focused on introduction of methodology enabling the creation of suitable conditions for the study of ECM *in situ*. Early somatic embryogenic tissues of selected conifers (*Abies alba, Abies numidica* and *Pinus sylvestris*) were observed free of sputter coating with an electrically conductive layer, without use of any chemical fixation or preparation technique which means the material was in a really native state. Our method was the following: our samples were placed on a cooled Peltier stage with a temperature range from -18 °C to -22 °C with the specimen chamber of the ESEM under high pressure (550 Pa). To observe the natural surface of early conifer somatic embryogenic tissues a specially designed ionization detector of secondary electrons and an yttrium aluminum garnet activated with a trivalent cerium (YAG: Ce^{3+}) detector of backscattered electrons were used. Fig. 1. (a-f) shows native early embryogenic tissue of *Abies alba*, *Abies numidica* and *Pinus sylvestris* covered by a network of fibrillar material forming ECM layer.

We found that an environmental scanning electron microscope that uses the above introduced methodology is useful and very convenient for observing the native state of plant tissue, even though we suppose that this method is unsuitable for observing animal tissue without freezing damages. Additionally, plant tissue free of chemical fixation procedures allows observing the extracellular matrix in its native state. This method is fast and simple and, moreover, relatively inexpensive. We expect that it will be a generally applicable tool in the field of plant research.

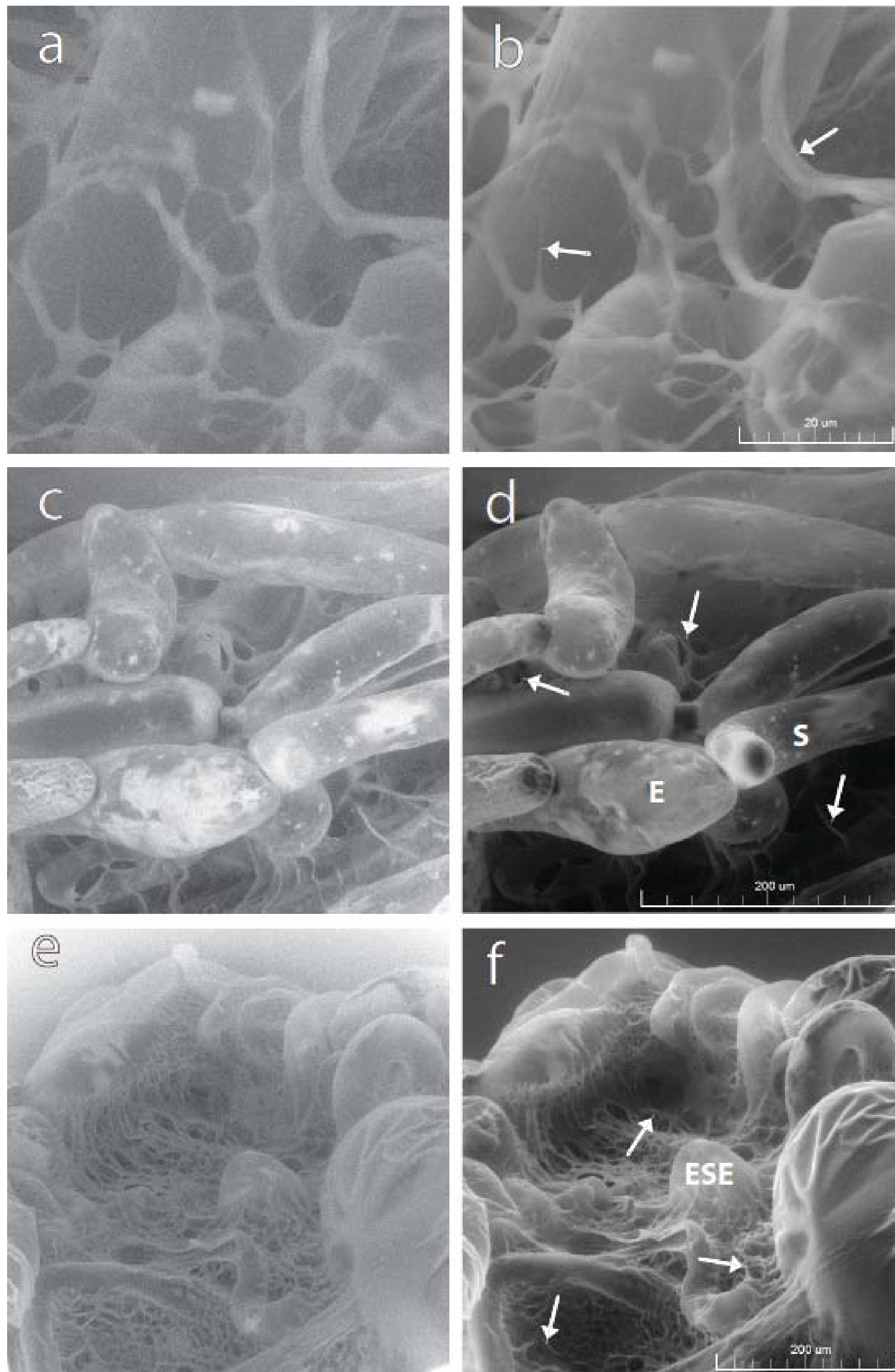

Fig. 1. *Comparison of ESEM observation with two detectors of embryogenic tissue of conifers. Abies alba (a, b); Abies numidica (c, d) and Pinus sylvestris(e, f). On the left side of the figure is the ionization detector and on the right side is the BSE YAG detector (the presence of ECM is indicated by arrows; E-embryonic part; ESE-early somatic embryo; S-suspensor cell).*

Acknowledgements: This work was supported by the Grant Agency of the Czech Republic: grant No. GAP 102/10/1410, Slovak Grant Agency for Science, Project No. 2/0076/09 and by the EU project No. CZ.1.07/2.3.00/20.0103.

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