Cyanobacteria as “Filler” for loading Nitrocellulose Capillaries: An innovative method for High Pressure freezing of Pollen

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In recent years, haploid technology has contributed enormously for the plant breeding progress and has become one of the most successful biotechnological approaches. Barley (*Hordeum vulgare* L.) is considered as a model system for POEM but full interpretation of POEM is still ambiguous (Coronado *et al*., 2005). To elucidate and visualize the mechanism of structural changes of the POEM, we used Light Microscopy (LM) and Transmission Electron Microscopy (TEM). At this end, vacuolated immature pollen was isolated and incubated in starvation medium to undergo POEM, followed by culturing in suitable nutrient medium in which embryonic development takes place.
Chemical fixed and high pressure frozen probes of various POEM stages, ranging from 0 to 10 days, were resin embedded. Semi-thin sections were used under LM for histological analysis as well as 3-D reconstructions to carry out first basic structural examinations of the embryogenic pollen development into multicellular structures (Figure 1). The transformation of microspores into a callus started after four days with the first cell division. Ten days after isolation, the callus broke the exine layer at different locations, which always occurred in a dense cytoplasm area.
To minimize artifacts typically caused by chemical fixation (Studer *et al*., 1989), High Pressure Freezing followed by freeze substitution is one of the most challenging techniques in sample preparation for ultrastructural research was applied to the immature pollen. Due to the physical circumstances of the freezing process and the distinct biological properties it is difficult to carry out HPF using plant samples. Nevertheless we established a very useful HPF protocol for immature pollen. The inter-pollen-liquid spaces were minimized by employing small cyanobacterial cells as filler for loading nitrocellulose tubes. HPF and freeze substitution of the samples resulted in excellent ultrastructure preservation without freezing artifacts like ice crystal formation (Figure 2). The established protocol for HPF of isolated immature pollen will be further used for ultrastructural research, immunohistochemical studies and sophisticated cell biological methods such as 3D-electron tomography to gain a better understanding of POEM process.

1- Coronado M.J. et al., *Acta Physiologiae Plantarum* 27 (2005): p591-599.
2- Studer, D. et al., Scanning Microsc. Suppl. 3 (1989). p253–268; discussion 268–269

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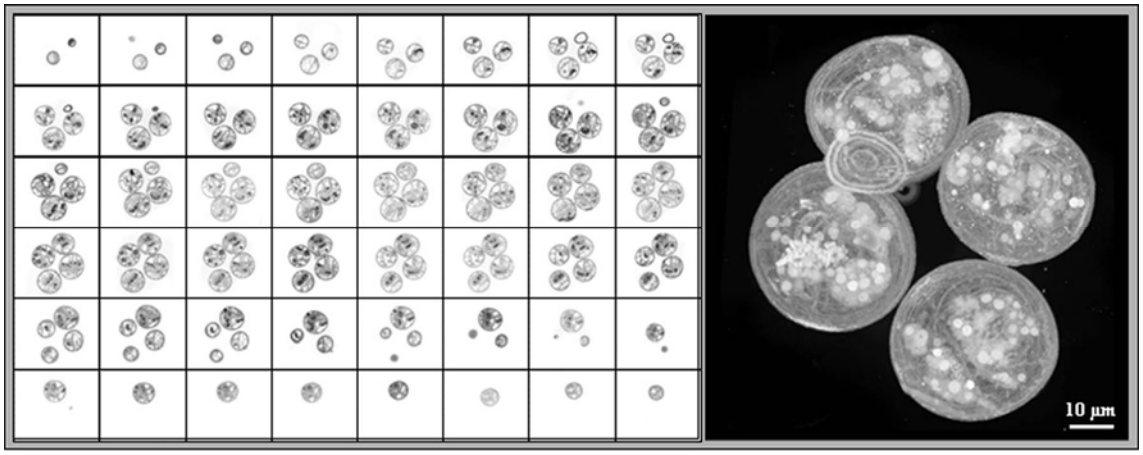

Figure 1. Series of 2d images constructed 3D image of cultured immature pollen immature pollen.

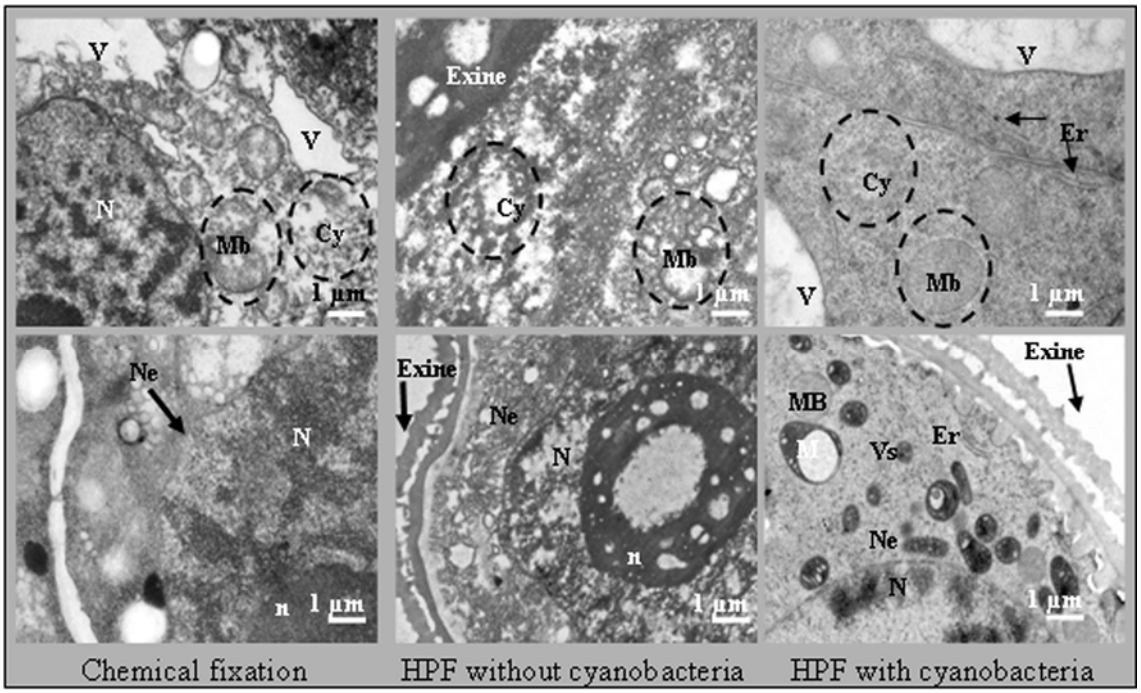

Figure 2. Comparison of chemical fixation und HPF without and with cyanobacteria of immature pollen. Cy = Cytoplasm, Er = endoplasmic reticulum, Mb = Microbody, N = Nucleus, Ne = Nuclear envelop, n = nucleolus, V. = Vacuole, Vs. = Vesicles.

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