Light microscopy protocol for the study of morphology of secretory spines

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Abstract

The presence and structure of EFNs in *Opuntia robusta* had not been investigated. We used light, scanning-electron, and transmission-electron microscopy to examine morphology, anatomy, and ultrastructure of the secretory spines in areoles in female and hermaphrodite individuals of *O. robusta*. Young cladodes develop areoles with modified and secretory spines as EFNs only active during the early growth phase of female and hermaphrodite individuals. EFNs are non-vascularized structures, with no stomata, that consist of three distinct tissues: a basal meristematic tissue; a middle elongation region; and an apical secretory cone (asc) formed by large globular epidermal cells, of sac shape, containing nectar and medullar elongated cells. TEM observations indicated the presence of Golgi apparatus, vesicles and plastids in the medullar and sup-epidermal cells of the asc, transporting nectar to the epidermal secretory cells. The nectar is transported through plasmodesmata, and then stored on the asc cells, to be secreted by breaking through the globular cells and pores.

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Protocol

Tissue fixation

Step 1.

Areole tissue was removed from the cladode and sectioned transversely to expose the secretory spine on longitudinal plane. Tissue was fixed on FAA solution for 72 h.

FAA fixative solution:

10 mL of formalin, formaldehyde 37 % in $_{H2O}$, contains 10-15% methanol as stabilizer to prevent polymerization (Sigma-Aldrich F16-35).

5 mL glacial acetic acid, ACS reagent, ≥99.7% (Sigma-Aldrich 695092)

50% ethanol 96% (Sigma-Aldrich E1860100)

25 mL double distilled water.

After 72 h tissue was washed three times (15 min) in deionised water.

Paraffin embedding and sectioning

Step 2.

Fixed tissue was dehydrated in ethanol solutions (50%, 70%, 80%, 96%, 100%, 100%) for 2 h. in each concentration. Then the tissue was infiltrated in 3:1, 1:1, 1:3 (ethanol : xilene) and xilene (two changes) for 4 h each, then to Paraplast plus (McCormick Scientific, Supplier: Leica Biosystems) two changes for 12h. Tissue was embedded in Paraplast, and considering the EFNs as a unit. We obtained 8 µm-thick longitudinal sections in the central area of the EFNs spine in each sample with a rotatory microtome (American Optical Company, USA).

Mounting and Staining

Step 3.

Paraffin sections were mounted on glass slides with chromium gelatine adhesive [1% gelatine, 0.1 % phenol, 0.05 % chrome alum (Cr K $(SO_4)_2$ ·12 (H_2O) in water]. After 12 h on a hot (60 °C) plate, paraffin sections were dewaxed in xylene (100%), three changes for 3 min each and hydrated in a serial of ethanol (100%, 96%, 70% and 50%) three minutes each. Staining was performed with a 0.5% safranin (Safranin O (C.I. 50240) for microscopy, Merck) in a saline (3% NaCl in water) solution for 2 h. Then sections were washed twice in deionised water for 3 min each and dehydrated in an ethanol series (50%, 70%, 90%, 100%) 2 min each. The sections were stained in 0.12 % fast green FCF (C.I. 42053 for Microscopy, Merck) in 95% ethanol for 1 min and the excess of colorant was removed with isopropanol (100%).

Sections were mounted in Eukit mounting medium and dried on a hot plate at 60 °C.

Photomicroscopy

Step 4.

We observed the sections with an Axiostar Plus light microscope (Carl Zeiss, Germany) and images were captured with an Axiocam MRc5 (Carl Zeiss, Germany).