Fixation and dehydratation protocol for Scanning Electron Microscopy (SEM), for the observation of morphology of secretory spiness

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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.

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Protocol

Stock solutions

Step 1.

Prepared with deionised water.

A) 0.2 M of NaH_2PO_4 (Sodium phosphate monobasic anhydrous, CAS Number: 7558-80-7) BioUltra, ≥99.0% (T) (Sigma-Aldrich S3139).

B) 0.2 M of Na_2HPO_4 (Sodium phosphate dibasic anhydrous, CAS Number: 7558-79-4) Ultra ≥99.5%, (Sigma-Aldrich S7907).

To make 200 mL of phosphate buffer solution (0.1 M, pH 7.2), mix 28 mL of stock solution A + 72 mL of stock solution B and 100 mL of deionised water. Adjust pH with solutions of 1N HCl or 5 M NaOH if necessary.

Buffered glutaraldehyde solution

Step 2.

To prepare 100 mL

Mix 10 mL of glutaraldehyde solution (25% for electron Microscopy, Merck, Germany) with

90 ml of phosphate buffer Sorensen's solution (0.1 M, pH 7.2).

1% Osmium tetroxide in deionised water

Dissolve 0.25 g of OsO_4 in 25 mL of deionised water (use fume hood). Protect the solution from light and store at 4 °C.

Scanning Electron Microscopy (SEM)

Step 3.

Critical/point dried and SEM observations.

Samples were critically-point dried using a Samdri-780 (Tousim Research Corporation, Rockville, USA), mounted on cooper studs with a carbon conductive tape, double coated (Ted Pella I.N.C U.S.A) and then sputter-coated with gold-palladium with a Fine coat ion sputter (JFC-1100, Jeol Ltd., Tokyo, Japan). Observations were performed using a JEOL JSM 6390 scanning electron microscope operated at 25 kV.