Transmission Electron Microscopy (TEM) protocol for the observation of the ultrastructure 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

Resin Embedding

Step 1.

Tissue was embedded in medium hardness Spurr's resin (Polysciences Inc., PA, USA) according with manufacturer as follows:

Each recipe maked about 21 g.

V.C.D. 5.0 g + + D.E.R. 3.0 g + N.S.A. 13.0 g + D.M.A.E. 0.4 g

Stir the mixture as preparing. The accelerator (DMAE) must be added at last and keeped at 4 °C.

At 4 °C

Dehydrated tissues in ethanol 100% were transferred to:

3:1 (ethanol: Spurr's resin), 1:1 (ethanol: Spurr's resin), 1:3 (ethanol: Spurr's resin), 100 % Spurr's resin for 2h each.

Secretory spines were orientated for longitudinal sections in flat embedding moulds and polymerised at 60 °C for 24 h.

Sectioning and staining

Step 2.

Semi thin longitudinal and transverse sections (1-2 µm) of the spine were obtained with an ultra-microtome (Ultracut, Reichert-Jung) using glass knifes. The sections were stained with 1% toluidine blue as following:

Reagents:

1% Toluidine Blue and 2% Borate in Distilled Water

Toluidine Blue O ---------- 1 g

Sodium Borate (Borax) ---------- 2 g

Distilled Water ---------- 100 m

Dissolve the sodium borate in the water, then add the toluidine blue powder and stir until dissolved.

Collect and dry sections down on a glass slide by placing the slide on a slide warmer at 60 °C. After the sections are completely dried, cover them with a few drops of staining solution (with the heat source still on) for 1-2 minutes depending on the intensity the darkness of staining you would like to achieve. Rinse off excess stain gently with distilled water. Air dryi the slide. Coverslip with oil immersion medium.

Ultrathin (80 nm) sections were obtained with an ultra-microtome (Ultracut, Reichert-Jung) with 6 mm glass knife and collected on 200 mesh copper grids coated with formvar/carbon (Agar Scientific). Double staining was performed by immersion of sections in 2% uranyl acetate in ethanol 70% for 40 min followed by three 30 min rinsed in double distilled water. Second staining with Lead Citrate solution prepared according with Reynolds (1963) cited by Hayat (1970), for 2 min and followed by three 2 min washes and three 10 min rinsed in double distilled water according with Zavaleta-Mancera et al., (1999).

Reynolds (1963) lead citrate prepared as follows.

Lead nitrate [Pb $(NO_3)_2$] ..... 1.33 g

Sodium citrate [Na $(C_6H_5O_7)$ $2H_2O$]..... 1.76 g

CO_2 free distilled water .....30 mL

Boil distilled water for 10 min to obtain CO_2 free distilled water.

Pour into a 50 mL volumetric flask 1.33 g of lead nitrate and 1.76 g of sodium citrate and 30 mL of CO_2 free distilled water. Shake the mixture at intervals for about 30 min until a milky suspension is obtained. Add 8 ml of 1 n NaOH (prepared with CO_2 free distilled water). Bring to volume 50 mL with CO_2 free distilled water and mix by inversion until lead citrate dissolves completely and the solution clears up completely. Use it fresh.

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Observations and photomicroscopy

Step 3.

Observations were performed with a TEM microscope (JEOL JEM 12000 EII) at 80 Kv, and images were digitally recorded.