The Zinc Iodide-Osmium Tetroxide (ZIO) Method Reveals Variation In The Distribution And Population Of Organelles In Colleter Epithelial Cells

Silvia Rodrigues Machado [1,2*], Fernanda Tresmondi [3], Elza Guimarães [2], Yve Canaveze [4]

1. Electron Microscopy Center (CME), Institute of Biosciences of Botucatu (IBB), São Paulo State University (UNESP), Botucatu City, SP, Brazil
2. Department of Biostatistics, Plant Biology, Parasitology and Zoology, Institute of Biosciences of Botucatu (IBB), São Paulo State University (UNESP), Botucatu City, SP, Brazil
3. Postgraduate Program in Biological Sciences (Botany), Paulo State University (UNESP), Brazil
4. Department of Botany, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro City, RJ, Brazil
*silvia.machado@unesp.br

Studies on plants gland ultrastructure as well as on the relationship between the subcellular organization and mechanisms of secretion are numerous [1]. However, most of those investigations use conventional techniques, which do not always allow a clear definition of variations in the population and topography of organelles in the secretory stage. The zinc iodide-osmium tetroxide method (ZIO) is suitable for general impregnation of the endomembrane system of many plant, algal, and fungal tissues [2]. However, the use of this method to study specialized plant cells is not common [3]. In this work, we report on the applicability of the ZIO method to study variations in the ultrastructure of the epithelial cells in the same stage of the secretory cycle. Based on a previous study [4], we chose *Palicourea rigida* as a study model, a species of Rubiaceae exhibiting standard-type colleters producing a secretion predominantly lipophilic. Samples were fixed for 12 h with 2.5% glutaraldehyde-4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, and incubated for 22 h at 10 ºC in a solution containing Zn, I, TRIS-aminomethane, and OsO_4 [5]. Then, samples were dehydrated using a graded acetone series and embedded in Araldite resin. Polymerization was performed at 60 ºC for 48 h. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Tecnai Spirit Transmission Electron Microscope (FEI Company) at 80kv. The ZIO method revealed epithelial cells rich in profiles of smooth endoplasmic reticulum (SER) side by side to epithelial cells rich in dictyosomes (Fig. 1). SER were in a parallel arrangement and exhibited a peripheral distribution, mainly in the distal pole of the epithelial cells (Fig. 2). ZIO-positive SER profiles with dilated extremities and associated vesicles occurred to near the plasmalemma (Fig. 3). Dictyosomes were poorly developed (Fig. 4). Plasmodesmata were not stained by the ZIO method (Fig. 5). In those cells with predominance of dictyosomes (Fig. 1, 6), the SER profiles were absent or did not stained with ZIO. Dictyosomes were located predominantly in the central region of the cell (Fig. 6). Dictyosome polarization, which comprises a forming face (*cis*) and a secretion face (*trans*), was clearly visible (Fig.7). Vesicles of different sizes filled with dense deposits could be seen near the *trans* cisternae (Fig.7). Dictyosomes showed

different ZIO impregnation intensities. In those well-structured dictyosomes with numerous cisternae and associated vesicles, deposits of the reaction products were seen only in the *trans*-Golgi network (Fig. 7) from which proteins are packaged into vesicles destined to lysosomes, secretory vesicles, or the cell surface. Small vacuoles containing cell debris were associated with ZIO-positive dictyosome cisternae/vesicles (Fig. 8-9). These findings suggest that dictyosomes vesicles/vacuoles are engaged in endocytosis activity resulting in the sequestration and degradation of cytoplasmic material. The findings of this study show the multiplicity of functions of individual cells within the same gland and open new perspectives in studies of secretion dynamics in plants.

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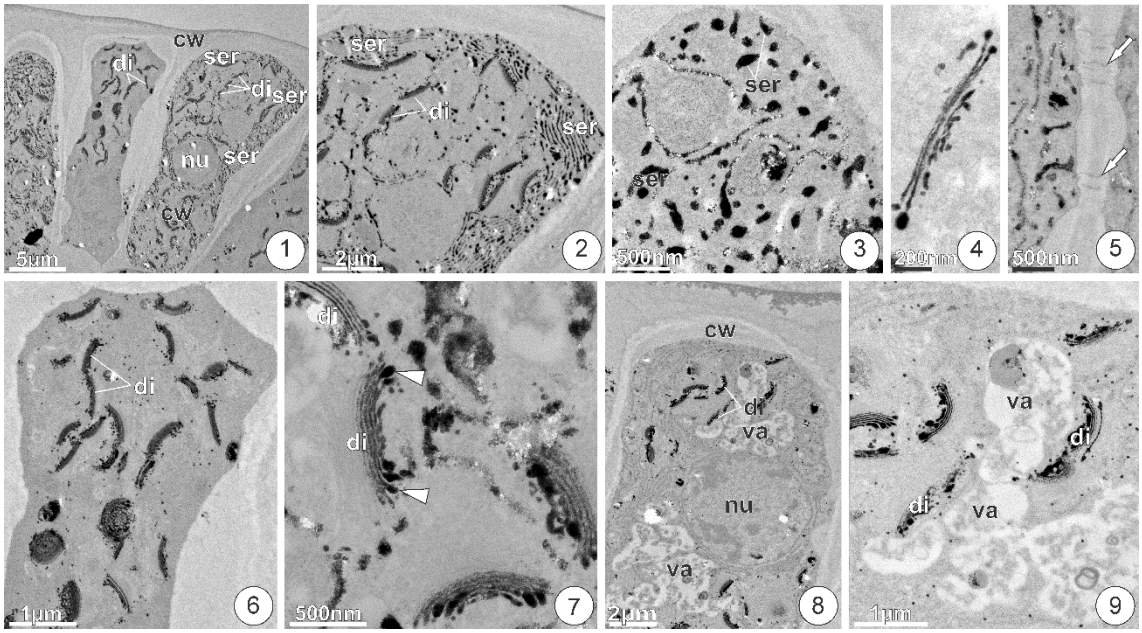

Figures 1-9. ZIO method in the epithelial cells of the colleters in *Palicourea rigida*. **1.** Epithelial cells side by side with variation in the organelle population. **2-5.** Details of the figure 1. **2.** SER in a parallel arrangement with a peripheral distribution. **3.** ZIO-positive SER profiles with dilated extremities, associated vesicles, near to the plasmalemma. **4.** Dictyosomes. **5.** Plasmodesmata. **6.** Detail of the figure 1 showing cell with predominance of dictyosomes. **7-9.** Details of the figure 6. **7.** Dictyosomes with different ZIO impregnation intensities. Note dictyosome polarization and vesicles with dense deposits near the *trans* cisternae. **8.** Small vacuoles containing cell debris **9**. Autophagic vacuole associated to ZIO-positive dictyosome cisternae/vesicles. cw: cell wall; di: dictyosome; nu: nucleus; ser: smooth endoplasmic reticulum; va: vacuole; arrow: plasmodesmata; headarrow: vesicles.