Comparative studies on leaf structure and ultrastructure of Peperomia species *in vivo* and *in vitro*

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The genus *Peperomia* has several hundred species, mostly epiphytic, in the tropical and subtropical areas, making it one of the largest genera of basal angiosperms [1].

Leaves from genus *Peperomia* have three distinct layers of parenchyma tissues [2]. An adaxial epidermis is differentiated as a multiple epidermis and is described as a water storage tissue because of its lack of intercellular air space, large cell size, and low chlorophyll content. The abaxial spongy mesophyll is distinctive for each species in thickness, cell size, and volume of air space. All leaf stomata are in the lower epidermis in almost every species. A dark green palisade mesophyll is described as a median mesophyll for its position between the multiple epidermis and spongy mesophyll [3].

In this report we compared the structure and ultrastructure of leaves from two species: *P. metallica* (L. Ding and Rodigas), *P. pedunculosa* (C. DC.) *in vivo* and *in vitro*. Several observations of the ultrastructure of theses species of *Peperomia* have implications in regard to water relations, carbon metabolism, and organic acid fluctuation. Further, the differences in physiology among these two species of *Peperomia* may be related not only to specific functions in each of the leaf tissues but also to anatomical and ultrastructural differences in the distinct parenchyma types.

We aimed to use a set of different *Peperomia* species to analyse plastid morphology, the relationship between cell and chloroplast size and the regulation of plastid size and number in different tissues.

The most recent, fully expanded leaves were selected for examination. Pieces of $1mm^2$ were fixed in 2,5% glutaraldehyde on cacodylate buffer, pH 7,2 for 4 hrs at room temperature. Post-fixation was in 1% OsO_4 on the same buffer for overnight at 4°C. The tissue were dehydrated in series of ethanol and propylene oxide and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Sections were examined with LEO transmission electron microscope at 80 kV.

The cellular ultrastructure of each tissue type is similar among these two species, including as *in vivo* and *in vitro* investigations, indicating a specialised function for each tissue. However, the differences in leaf anatomy were in presence of a huge mount, gigantic lipid bodies in median mesophyll cells, which occupies almost all cytoplasm space in the "specialised oil cells" within this tissue and were found only in *P. metallica* species (Fig.1, a-c). Cells of only *P. metallica* in the median mesophyll were distinctive for their accumulation of gigantic lipid bodies (spherosomes), bounding by single-membrane. Nuclei, mitochondria and elements of endomembrane system mostly smooth ER were present in the cells of both species *in vivo* (Fig.2 a-c). The cells of median mesophyll tissue *in vitro* contain numerous membrane–bound vesicles or channels, probably part of the predominant vacuolar system, especially in *P. pedunculosa*, where they have been observed numerous. Nuclei, mitochondria, microbodies and ER were observed as well.

The ultrastructural examination of so called "specialised oil" or "fat-storing" cells in median mesophyll tissue confirms that reserve lipid production and storage in higher plants

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involves mainly plastids, endoplasmic reticulum (ER), and lipid bodies, which is in accord with the biochemical evidence [4,5].

Proceeding from the investigations carried out on the ultrastructural basis an idea was proposed that gigantic oil bodies in *P. metallica* play a reserve role during the dehydration, where the long-term drought occur seasonally and the epiphytic growth form of most species would expose them to periodic drought like many other epiphytic plants [6].

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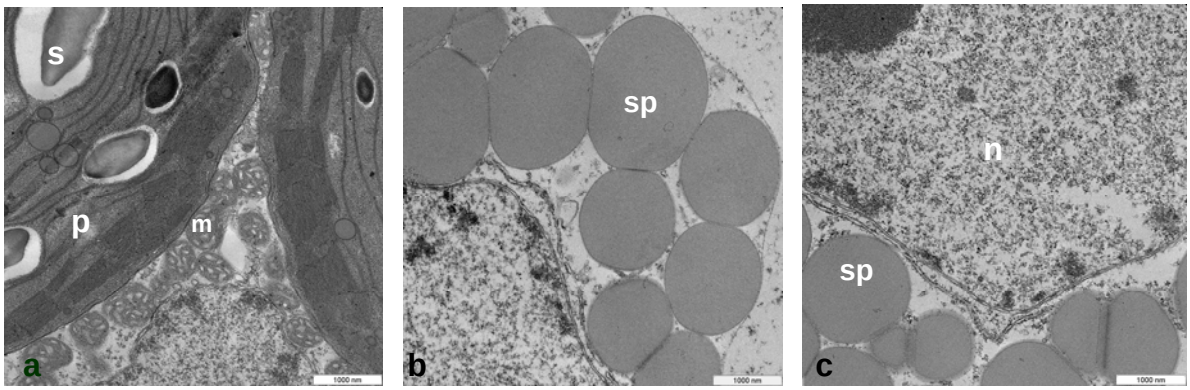

Figure 1 (a-c). Transmission electron micrographs of *Peperomia metallica L.D&R in vivo.* Electron micrograph of a palisade parenchyma cell with two big amilochloroplasts, nucleus and a numerous round, oval and elongated condense mitochondria in cytoplasm. Note gigantic numerous lipid globules (spherosomes) in cytoplasm.
Abb.: n- nucleus; m- mitochondria; p- plastid; s- starch grain; sp- spherosome

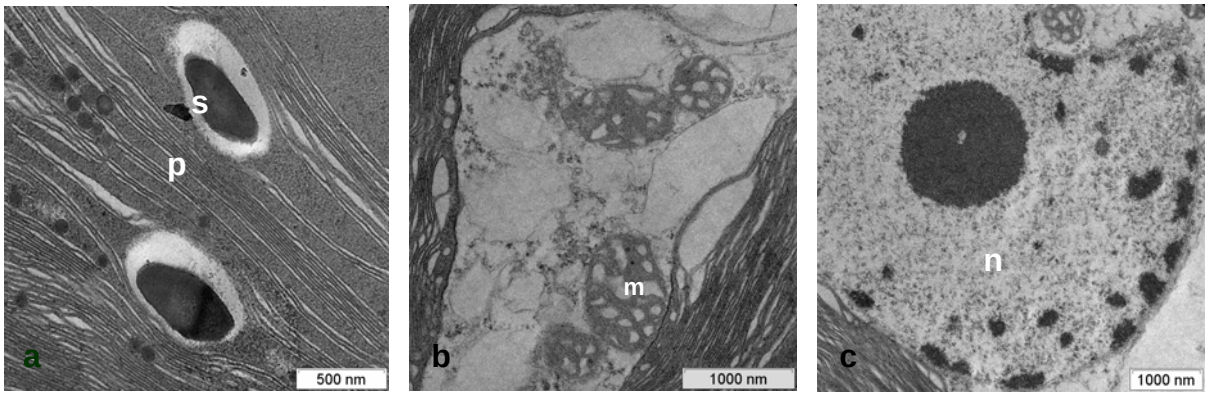

Figure 2 (a-c). Transmission electron micrographs of *Peperomia pedunculosa C.DC. in vivo.* Electron micrograph of a palisade parenchyma cell with big amilochloroplasts, nucleus and a few round, oval condense mitochondria in cytoplasm.
Note absence of lipid globules in cytoplasm and less number of mitochondria.
Abb.: n- nucleus; m- mitochondria; p- plastid; s- starch grain

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