Lacandonia granules: ultrastructure of a nuclear nanoribonucleoprotein particle

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Lacandonia granules are 32 nm in diameter nuclear particles first observed in the nuclei of several cell types of the plant *Lacandonia schismatica*, a species displaying flower sex organs spatially inverted. We have been characterizing these particles and here we present a summary of these studies using microscopy approaches including electron and atomic force microscopy. The presence of these nanometer in size ribonucleoprotein (nanoribonucleoprotein or nanoRNPs) structures in other species, suggest that they may be of more widespread distribution in nature.

**Keywords:** Atomic force microscopy; chromatin; Lacandonia granules; *Lacandonia schismatica*; nucleus; RNA; RNP

1. The plant *Lacandonia schismatica*

*Lacandonia schismatica* E. Martínez & C.H. Ramos is a rare monocotyledonean plant first found only in the Lacandon rainforest, in southeastern Chiapas [1], México but later localized in another locality also within the Lacandon rainforest [2]. Its most conspicuous feature is the inverted spatial distribution of the sex organs, with the androecium in central position and apocarpous gynoecium at the periphery [1, 3-4], a unique characteristic initially resulting in the proposal to classify this species within a new family of plants called Lacandoniaceae [1, 5]. The recent finding of another species in Brazil, namely *Lacandonia brasiliana* A. Melo & M. Alves [6], as well as other studies including an exhaustive searching for natural populations displaying the inverted position of sex organs [7], a comparative development study of *Lacandonia schismatica* and *Triuris brevistylis* [8], and a molecular analysis of the ABC function revealing the causes of the inverted position [9] placed both species within the already existing family Triuridaceae. In Fig. 1 a view of the Lacandon rainforest is shown and *L. schismatica* plants are displaying among the leaf litter.

2. Studies on the biology of *Lacandonia schismatica*

Due to the extraordinary inverted position of anthers and carpels found in *L. schismatica*, several studies on the biology of this species have been performed. Fig. 2 shows images of specimens of *L. schismatica* taken with a camera or with a dissection microscope. Flowers in preanthesis or postanthesis are observed. Tepals display papillae. In Fig. 3, papillae are observed by scanning electron microscopy. Papillae correspond to individual cells. The chromosome number has also been determined as n=9 [10] and populations display low genetic variation [11]. The endotrophic micorrhyza in *L. schismatica* is similar to the type vesicular-arbuscular [12]. In addition, detailed studies on reproductive biology and embryology have revealed that *L. schismatica* is characterized by a reproductive pattern known as preanthesis cleitogamy [13] and a unique type of megagametophyte development [14].

3. Microscopy and cell biology of *Lacandonia schismatica*

Cell biology studies of *L. schismatica* revealed that cytoplasm contains organelles also present in other plants as nucleus and nucleolus, mitochondria, Golgi apparatus, ribosomes and rough endoplasmic reticulum [15]. Chloroplast are not present, but instead plastids are observed. Photosynthesis is not carried out by *L. schismatica* [15]. Cell walls and plasmodesmata are common structures. Also, a large nucleus is generally present in each cell of the plant.

3.1 The cell nucleus of *Lacandonia schismatica*

The interphase nucleus in *Lacandonia schismatica* cells is reticulated, according to the spatial arrangement of compact chromatin as large strands [16]. Fig. 4 shows a longitudinal section of a carpel stained with toluidine blue and observed by bright field. Strands are observed within the cell nucleus which corresponds to DNA as shown by Feulgen staining, followed by a 100 nm width optical section achieved by confocal microscopy. Among strands, several spaces are observed.
Fig. 1 Lacandon rainforest in Chiapas, México (a), and plants of *Lacandonia schismatica* (b) present among leaf litter. Arrow indicates a flower of *L. schismatica*. 
Fig. 2  Dissection microscopy images of Lacandonia schismatica plants. (a) Flower (F), stem (S) and root (R) of an entire plant. (b) an open flower (large arrow) and a bud (small arrow). (c) a flower of L. schismatica showing ovaries or carpels (O), anthers (A), cauds (C), bracteae (B) and tepals (T). Tepals show papillae (arrow).
Fig. 3  Scanning electron microscopy of *L. schismatica* flowers. (a) Anthers (A), ovaries (O) and tepals (T) show papillous texture. Inset in (a) shows papillae corresponding to cells. (b) High magnification of carpels and anthers. Close arrows point an individual cell of a carpel.
3.1.1 Lacandonia granules

Samples of *Lacandonia schismatica* flowers prepared for transmission electron microscopy were examined searching for ribonucleoprotein (RNP) particles in the nucleoplasm. Several particles had been described previously, including the so-called perichromatin fibres, perichromatin granules and interchromatin granule clusters, all of them involved in gene expression pathways [17-21]. Observations on the nucleoplasm in several cells from the teguments and receptacle of *L. schismatica* revealed a novel particle intermediate in size and distribution to that of the mentioned perichromatin and interchromatin granules, so it was proposed as an additional ribonucleoprotein structure [22]. This novel structure was named Lacandonia granules. It is 32 nm in diameter and is composed by RNA and proteins. It is widely distributed in the nucleoplasm and it is present as large clusters of particles. In addition, closed related species as *Triuris brevistyris* also displayed these particles. Further ultrastructural, immunoelectron microscopy and high resolution *in situ* hybridization studies indicated that they contain SR proteins and poly(A)$^+$ RNA [23-24] and suggested that they are
Fig. 5  Transmission electron microscopy of a cell from the tegument of the carpel of *L. schismatica*. Image shows a portion of the cell nucleus (N) and cytoplasm (Cy). Inside the nucleus, compact chromatin is shown as strands (Ch) and spaces of nucleoplasm among them display large clumps of Lacandonia granules (arrow). In the cytoplasm, ribosomes are shown to compare the size to Lacandonia granules. CW, cell wall.

equivalent to perichromatin and Balbiani ring granules present in polytene nuclei in some cells of insects [24-25]. Interestingly, the finding that cells from the gymnosperm tree *Ginkgo biloba* also present Lacandonia granules strongly suggests that these particles may be more widely distributed among plant species [26]. Fig. 5 shows Lacandonia granules contrasted with the standard uranyl acetate-lead citrate technique for transmission electron microscopy. In addition, the EDTA regressive method preferential for ribonucleoproteins [27-28] results in a high contrast indicating the basic composition of these granules. In Fig. 6, a portion of a cell nucleus is shown where nucleoplasm among chromatin strands contains abundant granules. Cytoplasm is also shown to compare nuclear granule size to that of ribosomes. An image of high magnification suggest a complex structure that has already been studied by atomic force microscopy [29-31]. In summary, Lacandonia granules are ribonucleoprotein (RNP) particles 32 nm in diameter, -i.e. they are nanometric in size and therefore we include them as nanoRNP particles- that are present in very distant plant
species, playing a putative role in intranuclear RNA metabolism. Gene expression may be associated to a nanoRNP in plants.

Fig. 6 Lacandonia granules in the nuclei of *L. schismatica*. (a) Individual Lacandonia granules (arrow) are present in the nucleoplasm as large clumps. b) Lacandonia granules are contrasted by a technique for ribonucleoproteins (RNPs). c) Lacandonia granules (arrow) are present within a fibrous environment.

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References


