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Endosperm of rattan batu(Calamus subinermis, H. Wendland ex Beccari): Ultrastructural studies

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Rattan batu (Calamus subinermis) is a dioecious plant belonging to the palm family (Arecacea) and widely distributed in the Southeast Asia regions. In spite of an increasing demand for rattan as raw materials for furniture, baskets, mats, and sporting goods in the region, the biology of the rattan seed is poorly understood. Because of its economic importance to the regions, the programs for genetic improvements and multiplication of rattan batu have been initiated for Association of Southeast Asian Nations(ASEAN). Only few studies have been made on the ultrastructure of mature endosperm of the palm family (DeMason, et al. 1983; DeMason, 1986), but none with rattan endosperm. This is probably because of technical difficulties involved in electron microscopy of the hard, thick-walled endosperm of the palm family. Rattan, like the other members of the palm family, stores polysaccharides in the form of thickened primary cell walls in the endosperm (Easu, 1977).

In this paper we describe the ultrastructure of the rattan batu endosperm before and after extraction of storage carbohydrates with KOH. Combination of KOH extraction with scanning electron microscopy (SEM) has provided a better understanding the ultrastructure of rattan endosperm.

The plant material used was rattan batu (Calamus subinermis) seeds obtained from Sandakan, Malaysia. Freshly broken pieces of rattan seeds were fixed, posted-fixed, dehydrated, and embedded in the Araldite resin (Pease, 1964). Thick and thin sections were cut for light and electron microscopic observations, respectively. For SEM fixed specimen was dehydrated and sputter-coated with gold.

Washed rattan seeds were grounded to small pieces with a mortar and pestle and incubated with 7% KOH at room temperature to extract storage carbohydrates (Meier, 1958). After extraction, pieces of endosperm were repeatedly washed with distilled water to remove KOH before examination with light, TEM, or SEM.

The rattan seed coat is brownish and convoluted into the endosperm which is creamy in color. The hilum is found on top of the seeds and the operculum on the side has an elongated projection. On the average each rattan seed weighs about 0.3 g. Strong force is required to break the seed.

Just below the seed coat lies a single layer of cells with a large lumen. Underneath this layer starts the endosperm proper. The size of the endosperm cells tends to increase toward the center and their shape is multi-faced (usually 5-6-sided) and angular rather than round. The endosperm cell wall of rattan seeds consists of three layers as in the date and ivory nuts (Meier, 1958); a thin middle lamella, a thick intermediate lamella, and a thin inner lamella bordering the lumen.

The primary cell walls were thickened with the reserved carbohydrates except the primary pit-fields and the volume of the cytoplasm is accordingly reduced and shrunken into the center of the cell. Because of a dense staining of the cytoplasm, the cellular organelles are in general difficult to discern. No branching of plasmodesmata has been observed in the endosperm. The diameter of plasmodesmata varies depending on the angle and location of sections; a section near the neck or opening of the plasmodesmata was about 50 nm in diameter compared with 90 nm for sections through the middle.

SEM pictures of freshly broken rattan endosperm also show the shrunken cytoplasm, the thickened primary cell walls, and pit-fields. The surface of the endosperm cells after extraction reveals dome-shaped pit-fields, protruding above the plane of the surface with a slight depression in the center. When they are viewed from inside, pit-fields represent a depression in the cell wall, extending to the cell surface where they form a " dome" . Each of these domes comes into contact with a dome from an adjacent cell and completes the cytoplasmic connections between neighboring cells.

A pit-field is perforated with the plasmodesmata pores. KOH extracted the "contents" (the plasma membrane, desmotubule, and the cytoplasmic sleeves) of plasmodesmata, leaving behind the plasmodesmata pores (Esau, 1977). The pores are not uniformly distributed in and around the pit-field. On the average a pit-field has about 150 plasmodesmata of 70 to 130 nm in diamter.