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Ultrastructure of the leaf cuticle in *Cycas circinalis* Linn.

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ABSTRACT

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The organization and development of the cuticular membrane (CM) in juvenile as well as mature pinnae of *Cycas circinalis* Linn. have been investigated under TEM. In the juvenile pinnae the CM is very thin with faint structural organization, whereas in the mature pinnae the structural organization of the CM can be distinctly divided into three zones, i.e., a polylamellate zone, an amorphous zone and a reticulate zone. Stages of liberation and transportation of lipophilic secretion have also been observed.

Key-words—*Cycas circinalis*, cuticular membrane, ultrastructure, cuticle development, lipophilic bodies.

साइकस सर्सिनेलिस लिन. में पर्ण उपचर्मों की परासंरचना

ऊषा बाजपेई

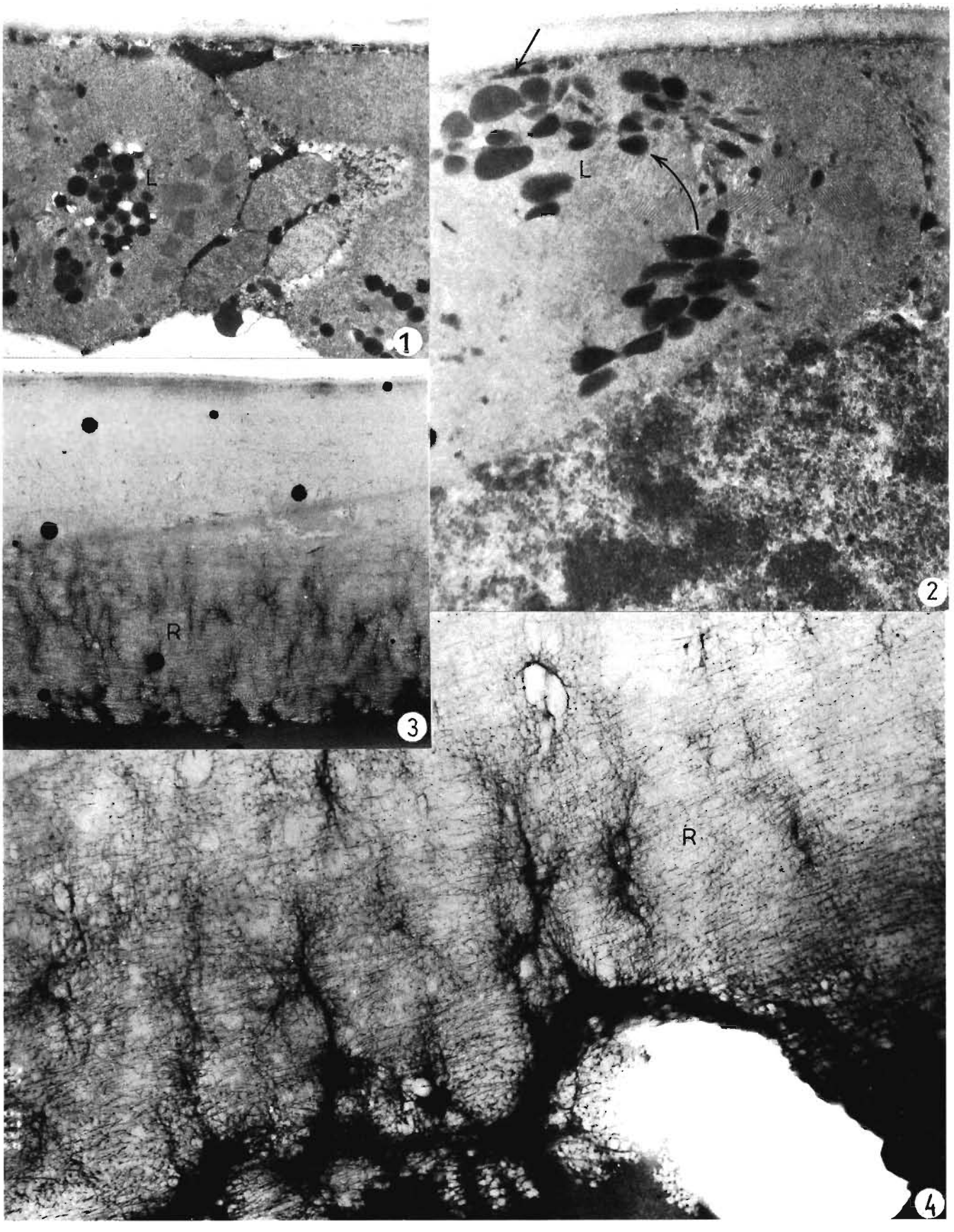
सारांश

साइकस सर्सिनेलिस लिन. के नव्य एवं परिपक्व पिच्छकों में उपचर्मीय झिल्ली के संगठन एवं विकास का विश्लेषण संचारित इलेक्ट्रॉन सूक्ष्मदर्शी द्वारा किया गया. नव्य पिच्छकों में उपचर्मीय झिल्ली अस्पष्ट संरचनात्मक संगठन युक्त तथा अत्यन्त पतली है, जबकि परिपक्व पिच्छकों में उपचर्मीय झिल्ली को सुस्पष्ट रूप से तीन मण्डलों- बहुपटलिकीय मण्डल, अक्रिस्टलीय मण्डल तथा जालिकारूपी मण्डल में विभक्त किया जा सकता है. इनमें वसास्नेही स्राव की विमोचन एवं परिवहन प्रावस्थाएँ भी प्रेक्षित की गयी हैं.

संकेत शब्द—*साइकस सर्सिनेलिस*, उपचर्मीय झिल्ली, परासंरचना, उपचर्मीय विकास, वसास्नेही तत्व.

INTRODUCTION

THE epidermis, the outermost layer of the plant, is covered by a cutin layer, which varies in thickness, both between different species and within individual species, depending on the conditions under which the plants grow (Martin & Juniper 1970; Holloway, 1982). Martin and Juniper (1970) discussed various definitions of this non-cellular layer, in general called the cuticle, at length. They used the terms 'cuticular membrane' to denote the material lying above the pectinaceous layer, 'cuticle proper' to describe the outer mainly cutinous layer, and 'cuticular layer' to describe the inner layer of cutin encrusted on cellulose.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Fossil gymnosperms and angiosperms, like their modern counterparts, have resistant cuticles. Primary chemical composition of the cuticles determines their preservation potential (Tegelaar *et al.*, 1991). It is an indubitable fact that features of the fossil cuticle, as studied under the light and the electron microscopes, contain information valuable for taxonomic studies (Kerp, 1990), for taphonomic studies (Collinson *et al.*, 1998), for correlation of dispersed organs (Clement-Westerhof, 1984) and for ecological interpretations (Kerp & Barthel, 1993). The ultrastructure of the cuticular membrane (CM) has been investigated in a large number of taxa of extant angiosperms, particularly those of agricultural interest (Kirkwood *et al.*, 1982; Price, 1982; Krüger *et al.*, 1996).

The ultrastructure of the CM of extant gymnosperms also needs detailed investigation to allow comparative study of the CM of fossil gymnospermous plants for taxonomic studies, to determine the degree of cuticle preservation (taphonomy), and for interpretation of the environment in which the plant lived (Archangelsky *et al.*, 1995; Maheshwari & Bajpai, 1996). According to Taylor (1999) "the use of cuticle ultrastructure in palaeobotany may also provide an indirect method of determining how cuticles have evolved". Ultrastructure of the cycadalean cuticle has been studied in *Encephalartos lehmanni* Lehmann (Villar de Seone, 1997) and *Stangeria paradoxa* Moore (Artabe & Archangelsky, 1992). To generate more data on the ultrastructure of cycadalean cuticles, investigations were carried out on the ultrastructure of the cuticular membrane (CM) in mature and juvenile pinnae of *Cycas circinalis* Linn. (Cycadaceae).

MATERIAL AND METHODS

The material was collected from an old plant growing in the campus of Birbal Sahni Institute of Palaeobotany, Lucknow.

For preparation of ultrathin sections, very small pieces of whole pinnae were fixed over night at room temperature in 4 % glutaraldehyde in 0·1M cacodylate buffer at 7·2 pH and were post-fixed in 2 % buffered Osmium tetraoxide for 4 hours. The material was dehydrated in graded ethanol series and acetone. After dehydration the pieces were passed through various combinations of acetone and Spurr's low viscosity medium, each step being of 10-12 hours; embedding was done in Spurr's low viscosity medium (Spurr, 1969). Serial sections were cut with glass knives on an ultramicrotome, and stained with uranyl acetate and lead citrate.

OBSERVATIONS

The study was carried out on juvenile and mature pinnae. The cuticle was observed at two stages of leaf development. In the juvenile (3-4 days old) pinnae the cuticle is generally thin with a very faint structural composition (Pl. 1·1). The epidermal cells are comparatively less vacuolated, the cytoplasm is full of different cell organelles and numerous lipophilic 'bodies'. The lipophilic 'bodies' are rounded and elongated in shape, and vary in size from 2 to 120 microns. Small and large accumulations of lipophilic secretions are evident in the cytoplasm of the epidermal cells. It has been suggested by earlier studies that precursors for the development of the cuticle, such as, the lipophilic 'bodies', are synthesised in the epidermal cells (Kolattukudy, 1980). To reach the surface, these lipophilic bodies move through the plasmalemma by molecular diffusion. At the time of liberation in the cytoplasm the lipophilic 'bodies' are in monomeric form, and on transportation to the outer surface of the epidermal cell they esterify to form a polymer (Mahlberg & Kim, 1991). In the 8-10 day old pinnae, the lipophilic 'bodies' move outward (Pl. 1·2) and fuse with the sub-cuticular surface whereupon their contents get deposited on the cuticle in large quantities. These precursors solidify rapidly and lead to the thickening of the CM (Pl. 1·1, 2).

Sections of the CM of mature pinnae reveal three distinct zones, i.e., the outer polylamellate zone (at the leaf-air interface), a middle amorphous zone, and an inner reticulate zone. The well-developed polylamellate zone is made up of compactly arranged, parallel running, 8-9 electron dense lamellae alternating with 7-8 electron lucent lamellae (Pl. 1·3). The lamellae are mostly uniformly wide, but some lamellae run continuously while others run only for a short distance. The polylamellate zone is underlain by an amorphous matrix that seems to form the major part of the CM. Adjacent to this zone is another zone that comprises matrix of the same density with fine reticulations (Pl. 1·4). The orientation of the reticulum is mostly parallel to the polylamellate zone of the CM. Irregular masses of lipophilic secretion are seen to permeate at the sub-

PLATE 1

1-4. *Cycas circinalis* Linn. Transmission electron micrographs of ultrathin sections of the pinnae.

1. Section of a 3-4 days old pinna showing very thin cuticular membrane in contact with the wall of an epidermal cell, cytoplasm with various cell organelles and lipid bodies (L). x 14,200.

2. Section of an 8-10 days old pinna, arrow showing transfer and accumulation at sub-cuticular layer of lipid bodies (L) to the outer region of the epidermal cell. x 33,800.

3. Cuticular membrane of a mature pinna showing polylamellate zone (arrow) followed by an amorphous zone and a reticulate-fibrillate zone (R). x 8,800.

4. Reticulate zone in figure 3 enlarged to show extensive cutinisation and its addition to the sub-cuticular level. x 33,800.

cuticular surface contributing to the thickening of the CM. These secretions impart an irregular dendroid appearance to the sub-cuticular layer. The thick and irregular inner surface of the CM thus seems to be the result of coalescence of the lipophilic substance with the existing cuticle (Pl. 1-3, 4).

REMARKS

Except for minor differences in the thickness of the layers, the basic ultrastructure of the CM of both juvenile and mature leaves is apparently similar. The cuticular membrane of *Cycas circinalis* resembles, in basic pattern, type-1 of Holloway (1982) in which the CM is polylamellate and the outer region is sharply delineated against the inner, mainly reticulate, region. During the initial phase of CM development, the cytoplasm is non-vacuolated and full of different organelles and lipophilic substance. The lipophilic precursors migrate from cytoplasm to plasma membrane to leaf-air interface, where these monomer compounds volatize and form polymers by coming in contact with the atmosphere (Baker, 1982). The directional flow of lipophilic 'bodies' has been observed in a number of sections. The formation and apparent dynamic movement of these electron dense bodies is best seen in the juvenile pinnae.

While in general organisation, the cuticle of *Cycas circinalis* compares with that of *Encephalartos lehmanni* and *Stangeria paradoxa*, the outer polylamellate and the inner reticulate zones do not seem to be so well developed in the South American taxa. If this difference is not due to the difference in environments in which the investigated plants were growing, it could be of taxonomic significance. Could the reticulate zone of the CM possibly be an expression of xeromorphic nature of the plant?

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