Membrane Structures in the Integumentary Cell Walls of the Ovule of *Nerium oleander*

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Summary. Ultrastructural changes in the integumentary cell walls of *Nerium oleander* L. were observed, starting with the beginning of nucellus degeneration. The cell walls in direct contact with the nucellus, followed in a regular progression by those of the next 2–3 cell layers, were seen to increase rapidly in thickness and, in contact with the plasmalemma, to develop a peculiar layer characterized by the presence of numerous membrane-like structures. Morphological and cytochemical findings indicate a membraneous nature of these wall structures; the structures exhibit a marked affinity to potassium permanganate, ruthenium red and phosphotungstic acid, and possess a three-layered configuration. Moreover, the structures were found to be disorganized by phospholipase C. Some of the wall structures appear to be pitted, sac-shaped formations; others to be single sheets. Both types exhibit a direct continuity with the plasmalemma after digestion of the wall material by cellulase. The origin and development of these structures are discussed.

Introduction

The ultrastructure of the innermost integumentary cell walls of the oleander ovule has been described in a previous note (Gori, 1971). The salient characteristic of these cell walls is the presence of numerous, membrane-like structures in the part nearest the plasmalemma. The purpose of the present work has been to investigate more thoroughly the nature and origin of these wall structures. Preliminary data were presented at the Botanical Meeting “De l’ovule à la graine” held at Siena in October, 1972 (Gori, 1973).

Materials and Methods

Routine Fixation, Embedding and Staining Procedures. The material was collected from white, red and pink-flower plants of *Nerium oleander* L. The ovules were fixed for 2 h in 3% glutaraldehyde in 0.075 M Sorensen’s phosphate buffer, pH 6.9, and postfixed for 3 h in 1% osmium tetroxide in the same buffer. They were then dehydrated through a graded ethanol series, and embedded in Epon-Araldite (Mollenhauer, 1964). Sections were cut with glass knives on an LKB ultramicro-
membrane structures in the ovule of Nerium. Stained with uranyl acetate (Watson, 1958), and observed with a Zeiss EM9A electron microscope at 60 kV.

Potassium-permanganate Fixation. Ovules were fixed for 20 or 40 min in unbuffered potassium permanganate (Mollenhauer, 1959). Thin sections were viewed unstained.

Ruthenium-red Staining Procedure. Ovules were fixed in 3% glutaraldehyde and 1% osmium tetroxide in 0.075 M sodium-cacodylate buffer, pH 7.2, containing 0.1% ruthenium red (Luft, 1966). Thin sections were viewed unstained. In control experiments, no ruthenium red was added to the fixatives.

Cellulase Digestion. Ovules were prefixed for 15–20 min in 3% glutaraldehyde, washed for 40 min in phosphate buffer, and incubated in 0.05% or 0.1% cellulase in 0.05M acetate buffer, pH 4.5, for 12–16 h at 35°. They were washed in acetate buffer and then in 0.05 M sodium-cacodylate buffer, pH 7.2, for 10 min each, and finally postfixed with osmium tetroxide-ruthenium red solution as described above. Sections were observed unstained.

Alternatively, postfixation was carried out in an osmium tetroxide solution not containing ruthenium red. In this case, thin sections were mounted on gold grids and stained at 35° with 5% phosphotungstic acid (Benedetti and Bertolini, 1963) after bleaching for 30 min in 1% periodic acid at 35°.

Phospholipase-C Digestion. Ovules prefixed in glutaraldehyde and then incubated in cellulase as described above were washed for 10 min in 0.05 M phosphate buffer, pH 7.2, and incubated in 0.05 or 0.1% phospholipase C. Incubation was continued for 1, 2 or 4 h at 36°, and followed by washing in 0.05 M sodium-cacodylate buffer, pH 7.2, for 10 min prior to postfixation with osmium tetroxide-ruthenium red solution. Thin sections were examined unstained.

Controls were carried out at the temperatures and time periods mentioned but in incubation media lacking cellulose or phospholipase C.

Key to Abbreviations in Figures

CW cell wall; Cyt cytoplasm; L lipids; N nucleus; Ne nucellus; pl plasma-lemma; pw1 paraplasmatic wall layer. GAld glutaraldehyde; KMnO4 potassium permanganate; OsO4 osmium tetroxide; PTA phosphotungstic acid; RR ruthenium red. The scale marks in the figures = 0.25 μm, except in Fig. 12 (1 μm) and Figs. 1, 7, 8, 13 (5 μm).

Results

General Observations

In the oleander, nucellus degeneration occurs early during the ovule development. At that time, ultrastructural changes can be observed in the

Fig. 1. Longitudinal section of oleander ovule. To the left, nucellar cells in degeneration. In the integument the walls of the cells in direct contact with the nucellus show the first ultrastructural changes, whilst the walls of cells further away are as yet unchanged with a clear middle lamella (arrows). GAld—OsO4 fixation, section stained with uranyl acetate. × 6000

Fig. 2. Modified walls of adjacent cells: a well-defined layer containing pitted structures and three-layered membrane fragments (arrows) can be seen in contact with each plasma membrane. GAld—OsO4—RR fixation, unstained. × 93,000