식물학회지 제34권 제1호
Korean J. Bot. 34(1) : 1~8

Ultrastructure of the Developing Epicarp in Fruit of *Nerium indicum* Mill. (Apocynaceae)-I

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ABSTRACT

A sequential sub-cellular study of the epicarp of *Nerium indicum* has been performed. Outer epidermis of the ovary wall is covered externally with a thin cuticle. Cytoplasm possesses most of the cell organelles in the ovary stage itself. Outermost zone of the pericarp is the epicarp, developing from the outer epidermis. In the developing fruit, cell organelles are found with its maximum intensity. In mature fruit, the epicarp becomes multilayered due to the additional development of few collenchymatous cells close to the outermost layer. Epicarpic cell possesses large central vacuole, around which a thin layer of cytoplasm is present. Number of cell organelles are considerably reduced in the mature fruit. In the ovary stage starch grains are electron transparent, while in the mature fruit it is electron transluscent.

INTRODUCTION

As one of the most important organs of the plant, the fruit is somewhat neglected for sub-cellular details even though a series of isolated light microscopic studies have been performed in many families. Pericarpic tissue gives nutrients and protection to the developing seeds and also helps the seeds in dispersal at maturity (Roth, 1977). Most fruit studies carried out by Scott *et al.*,(1963), Ben-Aire *et al.* (1979), Platt-Aloia *et al.* (1980), Platt-Aloia and Thomson (1981), Vinarova and Chalukova (1983) are along physiological and biochemical lines, utilizing experimental methods that take into account the whole tissue. These researches point to a need for more study at the inter-cellular and sub-cellular levels. Thomas (1989) has studied the developmental anatomy of the follicle of *Nerium* under light microscope. The present articleincorporates the ultrastructural study of ovary wall and epicarp of developing and mature fruit of *Nerium indicum*.

MATERIALS AND METHODS

Ovary and developing and mature fruits of *Nerium indicum* are fixed in paraformaldehyde-gluteraldehyde in cacodylate buffer at pH 7.2 (Karnovsky, 1965). After repeated washing in cacodylate buffer post-fixation is done in 2% O_5O_4 in the same buffer for overnight. They are then dehydrated in a graded series of acetone and embedded in Spurr's resin (Spurr, 1969) and kept in the oven for 12 hrs at 70℃. 1 μm thick sections are cut on Dupont Sorvall JB-4 microtome using glass knives. Sections are stained with 1% Toluidine Blue prepared in 1% Borax (O'Bren and McCully, 1981). Semi-thin sections are cut in Reichert OM U3 ultramicrotome with glass and diamond knives. Ultra-thin sections are stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined and photographed at 80 KV with a Philips STEM 400 and JEOL JSM 100 electron microscopes.

RESULTS

Ovary wall. Outermost zone of the ovary wall is the outer epidermis formed of a layer of radially elongated, compactly arranged cells (Figs. 1-3). Both radial and tangential walls show almost same thickness with distinct plasmalemma closely associated to the primary wall. Plasmalemma is smooth or sinuous with membranes lying in between the cell wall and membrane. Invaginations of the plasmalemma containing small projecting strands of cytoplasm occur in the outer epidermis of the ovary

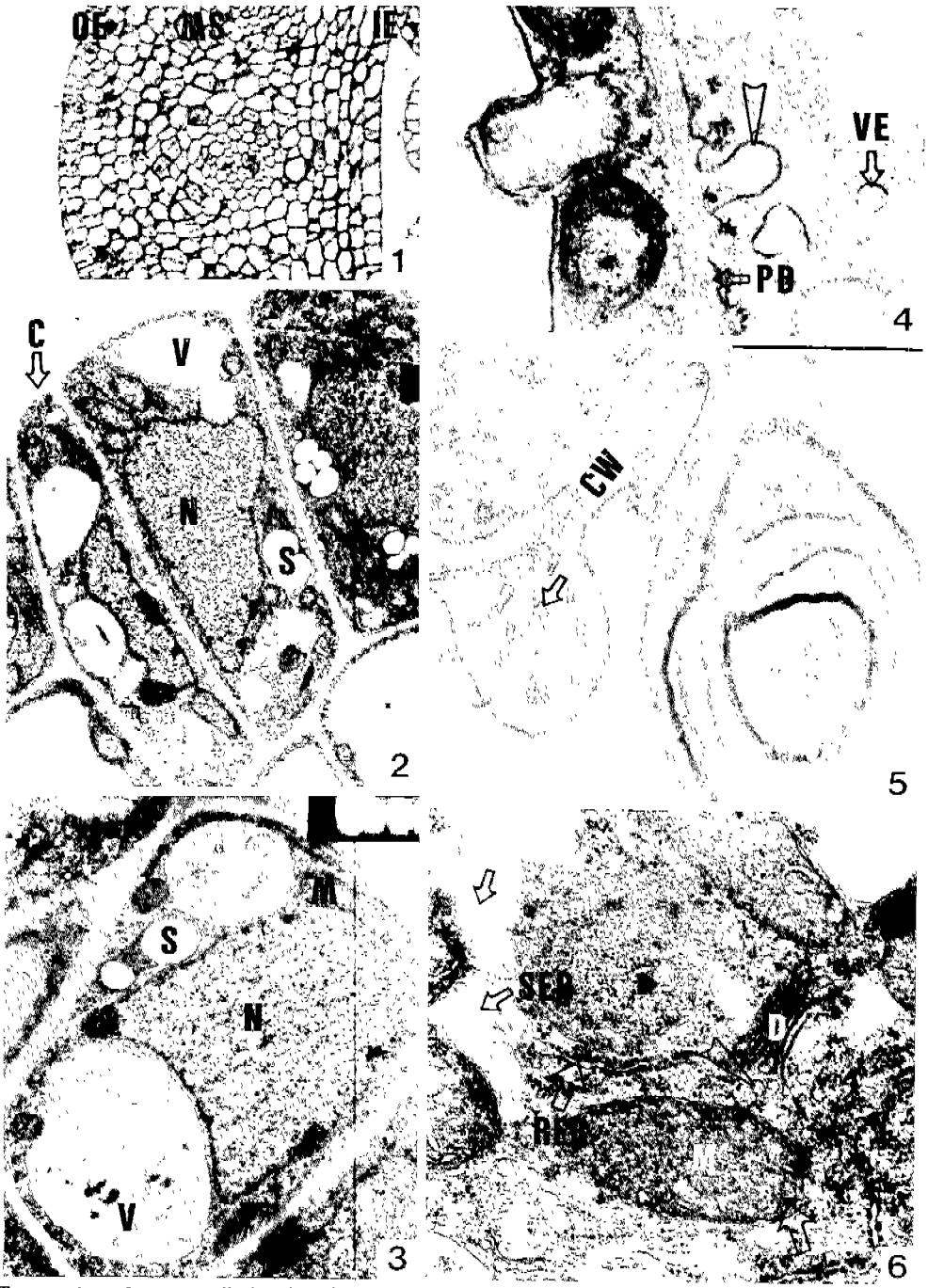

Fig. 1. Transection of ovary wall showing tissue zones such as outer epidermis, mesodermis and inner epidermis. × 165.

Figs. 2, 3. Outer epidermis is radially elongated covered with a thin cuticle. Cytoplasm possess most of the cell organelles. × 4957; × 5136.

Figs. 4, 5. Invagination of plasmalemma enclosing granules (arrow) or concentric membraneous layers. × 38,333; × 40,000.

Fig. 6. Blebbing of vesicles from Golgi cisternae (arrow head) and from SER (small arrow). Mitochondria possess deeply stained globules (large arrow). × 34,571.

wall (Fig. 4). Vesicular structure from plasmalemma possesses concentric membraneous layers inside (Fig. 5). Plasmodesmata traverse both radial and inner tangential walls, i.e. between adjacent epidermal cells and the epidermal and mesodermal cells. Number of plasmodesmata vary from cell to cell. The newly formed wall of the dividing cell possesses numerous plasmodesmata.

In the ovary stage itself cytoplasm of the outer epidermis possesses most of the cell organelles such as golgi bodies, ER, plastids, mitochondria and ribosomes with great intensity (Fig. 6). Golgi bodies are usually seem close to the cell wall (Fig. 7) with 4-7 cisternae and the cisternae are separated from one another by intercisternal space. From the rim of the cisternae, golgi bodies appear to produce vesicles in large number (Figs. 6, 7). Golgi vesicles originate in two ways: (i) direct proliferation of golgi cisternal rim (Fig. 7 arrowhead), and (ii) from sac like structures that develop from golgi cisternae (Fig. 7 large arrow). Granular material is present in the vesicles produced from golgi bodies. Golgi vesicles appear to be moving in a direction towards the newly formed cell wall of the dividing cell (Fig. 7 small arrow).

Mitochondria with variable shape are frequently found in the cytoplasm (Fig. 6); mostly appearing globular, dumb-bell shaped or rarely elongated. Mitochondrial envelop is clearly visible and numerous ribosomes are scattered in the mitochondrial matrix. Other than the ribosomes certain dark globular bodies are also noticed in the matrix (Fig. 6 large arrow). Mitochondria occur in groups nearer to the cell wall or the plastids (Fig. 8). Plastids are observed in all the cells with great diversity in number and shapes such as round, oblong or dumb-bell shaped. In sections plastid contains 3-8 electron transparent starch grains of different sizes (Fig. 8). Plastid possesses plastoglobuli, which vary in number from 2-5.

Nucleus is spherical or oval in outline occupying about one-third of the entire cell area, found at the centre or lateral side of the cell (Figs. 2, 3). Nucleus is surrounded by a nuclear membrane, which encloses the homogenous nuclear contents with randomly dispersed chromatin in the nucleoplasm. Nucleolus stains deeply and is found in the lateral side of the nucleus. Deeply stained chromatin in aggregation are present in association with the nuclear envelop (Fig. 3). Outer epidermal cell possesses one or more vacuoles situated near the cell wall. Some of the vacuoles situated near the cell wall. Some of the vacuoles possess vesicles (Fig. 3). Endoplasmic reticulum is abundant in the cell and occurs in the form of typical ribosome studded cisternae-the rough ER. Smooth ER is also observed (Fig. 6). Continuity of the ER with nuclear membrane is observed, but the connection with plasmamembrane is not found. It is noticed that vesicles cut off from the ER, are smaller than those cut from the plasmalemma. Free ribosomes are rich in ground cytoplasm and aggregate to form polyribosomes.

Developing fruit. After fertilization ovary develops into fruit, which is typically a follicle. Many enzymes released after fertilization lead to an increment in the cell activity. Cytoplasm possesses a large vacuole, but cell organelles are present in abundance indicating the activity of the cell.

Epicarp, the outermost zone of the fruit wall is developing from the outer epidermis of the ovary wall. In addition, few mesocarpic cells become collenchymatous and contribute to epicarp formation (Fig. 9). Thus, the epicarp becomes multilayered. Epicarpic cells are radially elongated with thick radial and tangential walls (Fig. 10). Microfibrils are distinctly evident and are parallel to each other. Plasma membrane stains more deeply than the microfibrils. A thick cuticle covering the outer tangential wall of the epicarp is wavy in outline with a deeply stained boundary (Figs. 10, 11 at arrow). Cuticle is traversed by microfibrillar extensions from the epicarpic cell walls (Fig. 11, arrow head). Sometimes cuticle develops an interspace in between two adjacent cells with granular material inside (Fig. 11). This space has connection with the walls of the adjacent cells.

Epicarpic cells are considerably large in comparison with the outer epidermis of the ovary wall. Cytoplasm possesses a large vacuole (Fig. 10). In addition to the large vacuole few small vacuoles are also present. In many cells formation of a large vacuole by fusion of small vacuoles is noticed. Vacuole possesses small vesicles. Nuclei are larger and are found adjacent to the cell wall. Nucleoplasm stains uniformly. Nucleolus and nucleolar organizer are very prominent. Nuclear membrane is associated with chromatin materials. Golgi bodies are found adjacent to the wall. Cisternae produce numerous vesicles. These vesicles often remain close to the cell wall. ER appears in both rough and smooth form. ER is also blebbing vesicles. Ribosomes are present as polyribosomes. Number of plastids is more in the epicarpic cells. There is a considerable enlargement in the size of the plastid. Both the membranes of the plastid are highly distinct when compared to the plastids in the outer epidermal cells of the ovary wall. In many of the plastids, number of starch grains is lesser than those in the outer epidermis. Number of plastoglobuli varies from 4-6 (Fig.

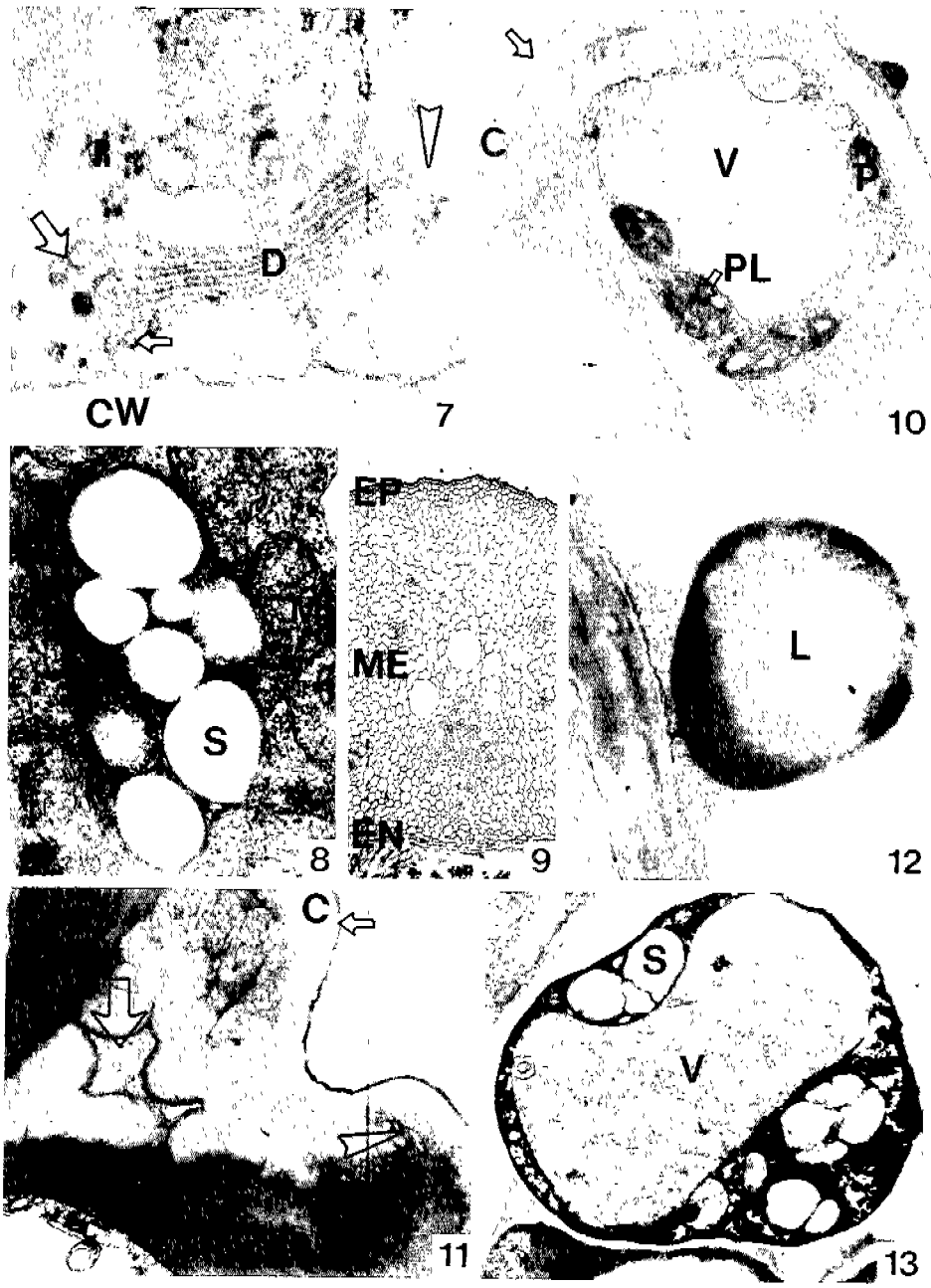

Fig. 7. Blebbing of vesicles from Golgi bodies by direct proliferation of cisternae (arrow head) or from sac like structures (large arrow). Note the vesicles are moving towards the cell wall (small arrow). × 61,111.
Fig. 8. Plastid with electron transparent starch grains. × 37,714.
Fig. 9. Transection of young fruit (paraffin processed) under light microscope. × 72.
Fig. 10. Epicarp of young fruit showing thick cuticle with dark limiting zone (arrow). Note the large central vacuole and cytoplasm with cell organelles around it. × 72.
Fig. 11. Outer tangential wall of the epicarp show uneven thickness (arrow head). Large arrow indicating the incomplete cutin deposition and small arrow indicating the limiting zone of the cuticle. × 6111.
Fig. 12. Lipid globule in the epicarp. × 16,428.
Fig. 13. Penolic substances (dark) and starch. × 6643.

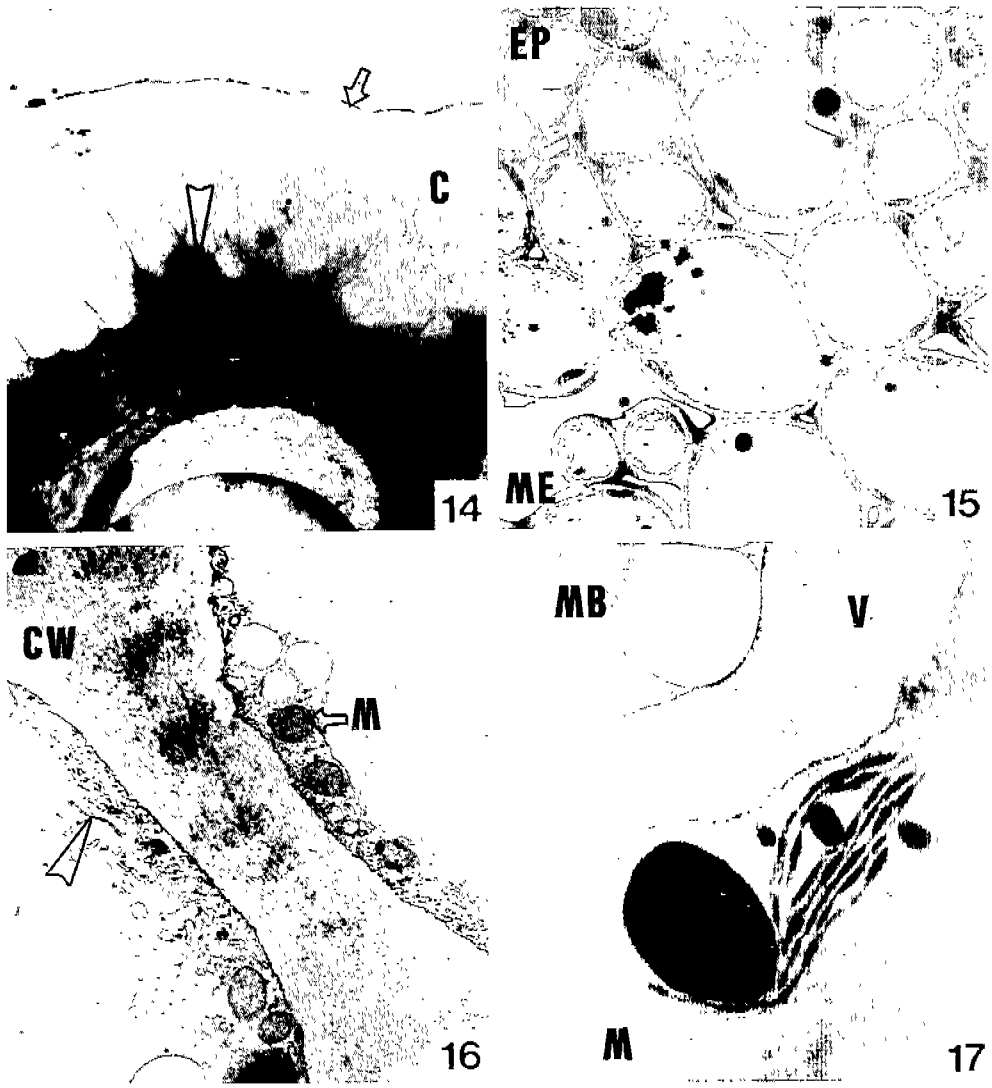

Fig. 14. Thick cuticle over the epicarp showing microfibrillar extensions (arrow head). Small arrow showing the limiting zone of the cuticle. × 11,143.

Fig. 15. Epicarpic and mesocarpic cells of mature fruit. × 1779.

Fig. 16. Both number and size of cell organelles are highly reduced in mature fruit. Arrow head showing ER. × 8452.

Fig. 17. Plastid with electron opaque starch grains and plastoglobuli. × 6643.

Symbols : CU, Cuticle; CW, Cell wall; EP, Epicarp; G, Golgi; L, Lipid; M, Mitochondria; ME, Mesocarp; MB, Multivesicular body; N, Nucleus; PD, Plasmodesmata; PL, Plastoglobuli; R, Ribosomes; RER, Rough ER; S, Starch; SER, Smooth ER; V, Vacuole; VE, Vesicle.

10). Because of the presence of abundant plastids in abundance, young fruits are green in colour. Epicarp possesses one or two lipid globules adjacent to the cell wall (Fig. 12) with a ring of cytoplasm around. Number of mitochondria is increased and they are usually globular in outline. Many of the epicarpic cells possess tanniniferous contents (Fig. 13). These cells have a large vacuole. Plastids with starch grains are present in these cells (Fig. 13).

Mature epicarp. Cell walls of epicarp become very thick. Outer tangential walls show uneven thickness. Cuticle on the outer tangential walls become very thick tra-

versed by the microfibrilar extensions from the epicarpic cell (Fig. 14). Limiting zone of the cuticle stains deeply (Fig. 14).

There is no intercellular space present in between epicarpic layers (Fig. 15). Cells have highly vacuolated cytoplasm in which few cell organelles can be seen nearer to the cell wall. Number and shapes of mitochondria, plastid, nuclei and ER are highly reduced (Fig. 16). Plastid possesses one or two large electron translucent starch grains (Fig. 17) and 3-4 large plastoglobuli. Golgi bodies are not observed in the mature fruit. Multivesicular bodies are present in the vacuole (Fig. 17). Most of the cells possess lipid globules.

DISCUSSION

After anthesis, a series of enzymes are released which favour the development of pericarp from the ovary wall (Eames and McDaniels, 1947). Outer epidermis of the ovary wall transforms into the epicarp of the fruit wall. In the developing fruit of *Nerium*, the epicarp is single layered, but it becomes multilayered towards the maturity by the addition of few collenchymatous layers.

Outer epidermal cells are radially elongated, dividing anticlinally only. In the ovary stage microfibrils in the cell walls are not distinct, but in the young and mature fruit microfibrils are very distinct and are found arranged parallel to each other. The cell wall of unripened tomato fruit has compact microfibrillar structure (Bhavannarayana *et al.*, 1982). Microfibrillar extension to the cuticle is analogue to the microchannels as described by Lyshede (1978). These microchannels are supposed to function as pathways for cutin and wax precursors (Lyshede, 1978). Outer tangential wall of the ovary is covered with a thin smooth cuticle which becomes thick towards the maturity of the fruit. Kuriachen (1989) noticed corrugated cuticle in the follicle of *Asclepias*. The cuticle of fruits is invariably much more waxy and heavy than those of the corresponding leaves (see Martin and Juniper, 1970). The cuticular gaps found in the fruit of *Nerium* have connection with the tangential wall of the epicarp. Gunning and Steer (1975) suggested that the cuticular gaps which are in close association with the cell walls may function as channels for cutin and wax transportation. According to Joel and Juniper (1982) there are two ways in which cuticular gaps might be formed: (i) defective deposition of cutin, (ii) separation or tearing of cutin from the cell wall. Cuticle over the young and mature fruit of *Nerium* are traversed by microfibrillar extensions from the outer tangential walls of the epicarp as found in the fruit of *Asclepias*. Such fibrillar material is observed in the leaf cuticle of *Eucalyptus* and in the petiole cuticle of *Apium* (see Martin and Juniper, 1970).

Plasmalemma is smooth or irregular with particles found in between the membrane and cell wall. Invaginations of the plasmalemma containing small projecting strands of cytoplasm occur in some plants (Grun, 1963). The invaginations of plasmalemma are termed variously as lamasomes, plasmalemmasomes, paramural bodies or multivesicular bodies (Cox and Juniper, 1973; Merchant and Robards, 1968). As found in the ovary wall of *Nerium*, Rao and Catesson (1987) observed irregular membrane structures such as vesicles, tubules or lamellae in the plasmalemma invaginations of the cambial cells of *Aesculus* and used the term "myelin-like bodies". According to Rao and Catesson (1987) the plasmalemmal invaginations carrying electron dense substances may be endocytotic vesicles. They also suggested that the formation of plasmalemmal invagination with vesicles is due to the excess production of vesicles from dictyosomes and are fused. Plasmalemmal invaginations carrying vesicles are later cut into the peripheral cytoplasm and carried to the central vacuole.

Cell organelles like mitochondria, golgi bodies, ribosomes, vacuoles, nucleus, plastid etc. are well-developed in the ovary stage itself. Frequency of cell organelles in the developing fruit is maximum and in the mature fruit its number and size are considerably reduced. In the ovary of *Asclepias* most of the cytoplasmic inclusions are in the developing stages, but in the young fruits all the cell organelles are well-developed. Golgi bodies are well-developed in the ovary of *Nerium* and are found adjacent to the cell wall. Golgi cisternae are producing numerous vesicles in the ovary and young fruit. Golgi vesicles are used in the formation of cell plate (Evert and Deshpande, 1970) or they may be used in the later stages of cell wall development in plants (Mollenhauer *et al.*, 1961). Pickett-Heaps (1968) concluded that Golgi vesicles transport hemicellulose, pectin and lignin from cytoplasm to the sites of cell wall thickenings. Occurrence of more Golgi vesicles nearer to the cell wall of ovary and young fruit, and the absence of Golgi bodies in the mature fruit of *Nerium* support the above statements.

Plastids occur abundantly both in the ovary wall and young fruit, while in mature fruit its number is highly reduced. Starch grains are electron transparent in the ovary stage, but become electron opaque in the mature fruit. In the follicles of *Asclepias* (Kuriachen, 1989) the

starch grains are electron opaque. Plastid possesses plastoglobuli. Number and size of the plastoglobuli are increasing towards fruit maturity. The significant point in the presence of plastoglobuli is that they represent a reservoir of excess lipid contents (Greenwood *et al.*, 1963). ER of *Nerium* are of smooth and rough type blebbing many vesicles. A possible function of SER seems to be the transport of hydroxyproline rich protein from cytoplasm to cell wall (Roberts and Northcote, 1972). Outer epidermis in *Nerium* possesses one or more vacuoles and the number and size is increasing towards the maturity of the fruit. The function of vacuoles may include providing motive force for cell growth by turgor pressure they exert when full of water (Barnett, 1973).

Tannins are large group of a heterogenous group of polyhydroxy phenolic substances. Tannins are found both in the cytoplasm (Harris, 1971) and in vacuoles (Endress and Thompson, 1976) of plant cells. In *Nerium* it is found in the vacuoles. The phenol compounds have an antitoxic role (Buvat, 1969) and some role in plant metabolism (Mueller and Beckman, 1976).

ACKNOWLEDGEMENT

Authors acknowledge the authorities of University Grants Commission, New Delhi, for financial assiatance.

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(Received September 6, 1990)