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ESSENTIAL OIL SECRETING STRUCTURES OF *ABELMOSCHUS MOSCHATUS* SEEDS

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Abelmoschus moschatus Medic. (Malvaceae) seeds are the source of musk-scented essential oil. Essential oil secreting structures are found in the testa. Oil secretion begins when the seeds start developing brown coloration on the seed coat. Osmiophilic droplets were first located in the cytoplasm as well as inside the dilated ER of basal cells. Later on, these droplets pass through the matrix of cell wall, plasmodesmata and accumulate in the collection cells, as the seeds become fully brown, hard and dry.

Key words : *Abelmoschus moschatus*, ambrette seed, essential oil, secretion.

Abelmoschus moschatus. (Malvaceae) are known for their musk scented seeds, which are used as substitute for Musk. Musk seed fragrance, though resemble animal musk, is essentially floral in character and the faecal note, sometimes found in animal musk is absent. The essential oil finds its apwlication in the preparation of perfumes. The characteristic musk like odour is mainly due to the presence of a ketone, ambrettelide, which is present in the crude oil to the extent of 0.3%. Essential oil is found in the seed coat. The crushed seeds by steam distillation yields 0.2-0.6% essential oil. A process for the extraction and purification of oil, with better yield of 1.5% has also been reported (Anonymous, 1985). Development of Ambrette seeds has been studied by Singh (1968). The presence of oil containing cells have been reported by Hedge *et al.*, (1984). In the present study, the essential oil secreting structures have been studied in detail by light, scanning and transmission electron microscopes to understand their process of secretion.

MATERIALS AND METHODS

Mature seeds were soaked for 3-4 hours in water. About 15 µm thick sections were cut by Leitz 1400 microtome and mounted in 50% glycerol for light microscopic observations. For localization of essential oil in seeds, fresh sections were stained with sudan IV as per Johnson (1940). Photographs were taken by Nikon optiphot-pol microscope with UFX II photomicrography attachment.

For EM studies, seeds of different stages were fixed in 3.5% gluteraldehyde (in phosphate buffer pH 7.2) for 2 hours and after washing three times in the same buffer were postfixed in 1% Osmium tetroxide till the specimen becomes black. Materials after washing with distilled water were dehydrated in graded series of ethanol. For SEM, materials were critically dried in Polaron critical point drier. The materials were then mounted on stubs with silver paint and coated with a thin layer of gold in Edwards coating system E 306A. For TEM, materials after dehydration, were cleared in Propylene oxide and infiltrated with araldite. Araldite blocks were cut with ultramicrotome LKB Nova and sections were stained with 2% uranyl acetate and 0.2% lead citrate. Sections were observed in Philips 420 scanning/transmission electron microscope.

OBSERVATIONS

Abelmoschus moschatus seeds are mainly tegmic, the main mechanical layer i.e. palisade layer is formed by the outer epidermis of the tegmen. The testa is outside this layer and it is undulated. Ridges of the testa in surface view, have rugose pattern with many raised rounded structures and pits (Figs 1.A, C). While, the troughs have reticulate pattern (Fig. 1A). In cross section, ridges are 3-4 cell thick and furrows are 2-3 cell thick (Fig.1B). Cells of the ridge regions are yellow in colour, while those present in the furrows are colourless in unstained sections. When these sections were treated with sudan IV solution, the cells of the ridge region, as well as inner wall of inner epidermis of the testa took the stain, indicating that oil is localised in these regions of the testa (Fig. 1C).

A typical oil containing structure is like a pitcher in cross section (Fig.1E). It has outermost single lid cell (LC), followed by single neck cell (NC), group of thick

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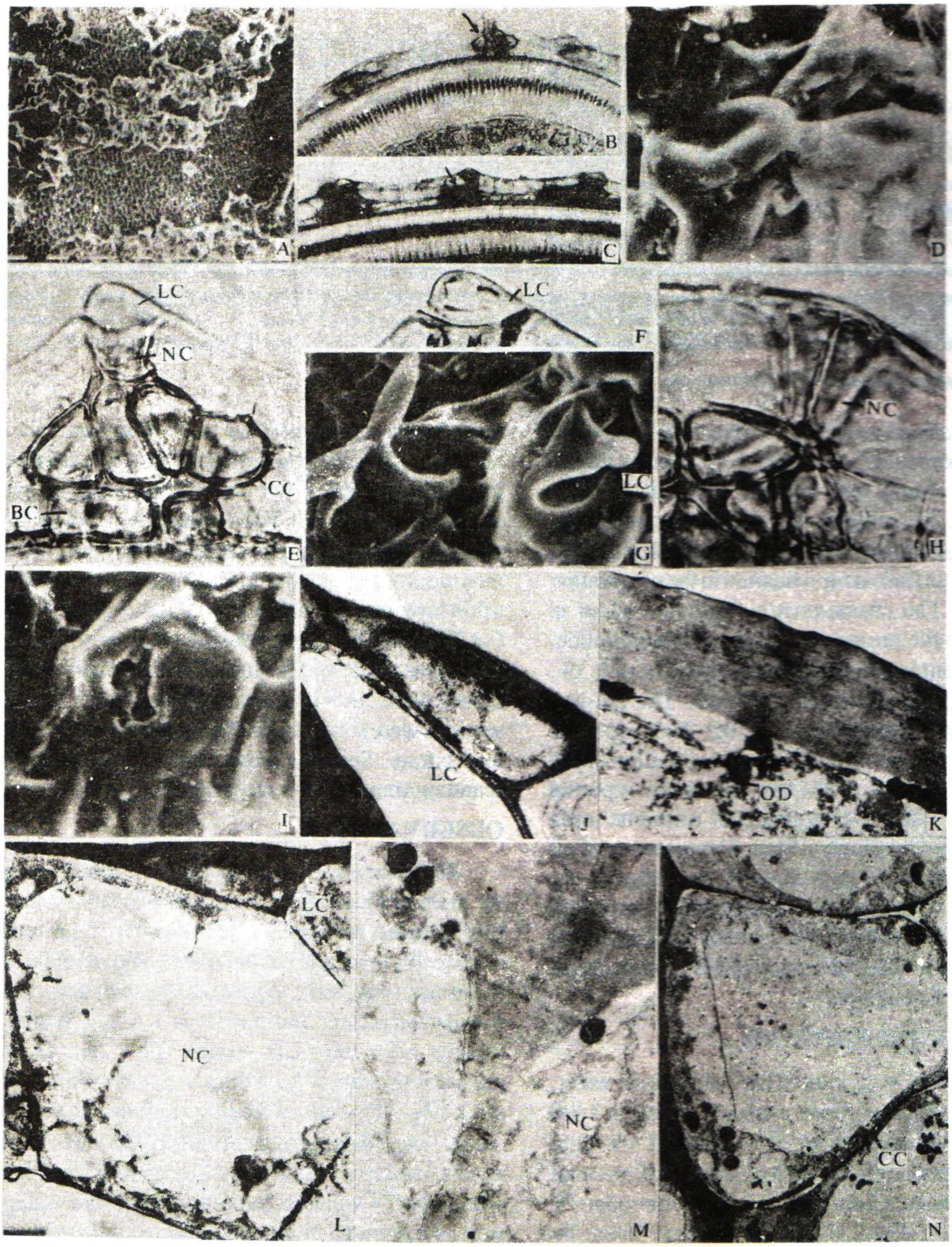


Figures 1A-N: Essential oil bearing structure of *Abelmoschus moschatus* testa, A. Seed surface with ridges and furrows (X 80); B. Unstained cross section of testa through ridge, arrow indicates the essential oil bearing structure (X 112); C. Stained cross section of testa, arrow indicates the location of essential oil (X 112); D. Closed oil bearing structures in surface view (X 750); E. Essential oil bearing structure of testa in cross section with lid cell (LC), neck cell (NC), collection cells (CC) basal cells (BC) (X 560); F. LC separating from NC (X 560); G. Separated LC in surface view (X 750); H. NC without LC (X 560); I. Pit in the neck cell in surface view (X 750); J. Lid cell (X 6350); K. Outer wall of mature lid cell, osmiophilic droplets (OD) in cytoplasm and in between plasmalemma and cell wall, arrow indicates laminations and osmiophilic Inclusions in outer part of the wall (X 18500); L. LC and NC (X 4900); M. Radial wall between NC and adjacent cell (X 10500); N. young collection cell (X 3800).

walled collection cells (CC) and the innermost 1-3 basal cells (BC). As the name indicates, LC functions like a lid. After the seed becomes fully mature, one side of this cell gets separated from the lower cell on a slight disturbance forming an opening and the underlying cells thus, come in direct contact with the outer environment (Figs. 1 F,G). Sometimes, the opening is in the form of a pore, in such case LC was not observed (Figs. 1H, I) and the NC directly opens through the pore. LC in the seeds, which are still green show dense granular cytoplasm with few vacuoles; cell organelles are few and less prominent and without any osmiophilic droplets (Fig. 1J). At the onset of browning of the seeds, cytoplasm gets precipitated and gradually degenerates. However, some rough endoplasmic reticulum (ER) can be seen (Fig. 1K). LC have thick outer and thin inner walls. The outer margin of the outer cell wall is sharp and inner margin is slightly granulated. The outer matrix of this wall shows laminations with dark osmiophilic inclusions (Fig. 1K). Radial walls of LC are mostly unequal in length and are more thickened towards outside (Fig. 1J). Thin radial and inner wall of LC may help the cell to open like a lid after maturation.

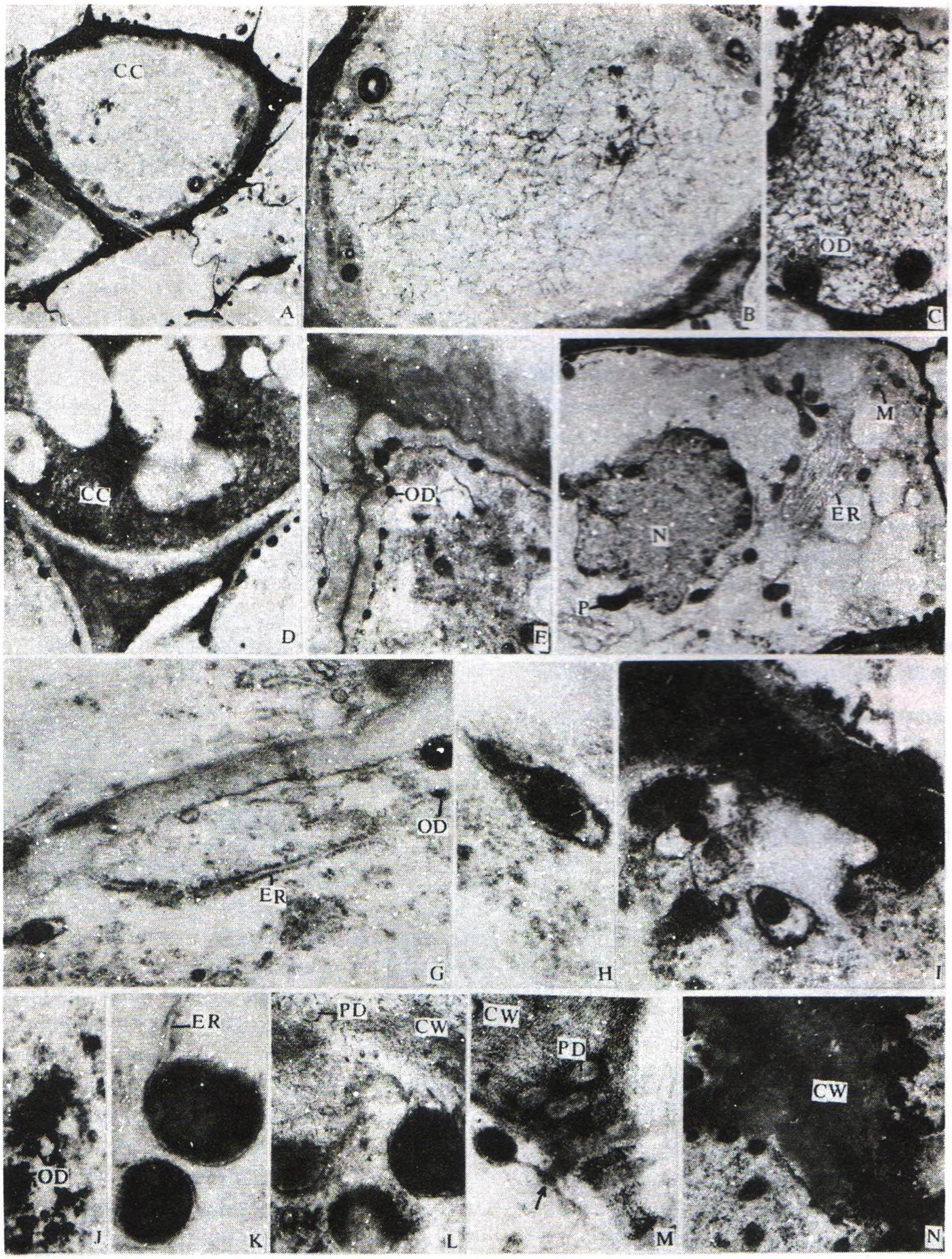
NC are empty, slightly elongated and funnel shaped (Fig. 1 H,L). The cytoplasm forms a thin layer along the walls and the central part of the cell is mostly occupied by a big vacuole. Smaller vacuoles are also present in the cytoplasm. Before browning of the seeds, ER are rough and long but are shorter in brown seeds. Chloroplasts and mitochandria are very few in number. Initially chloroplasts contain big starch but gradually it degenerates. In brown seeds, the cytoplasm looks like precipitate. Osmiophilic droplets can be seen in between the cell wall and plasma membrane (PM) and also in the cytoplasm. The droplets in the cytoplasm are usually contained with in a unit membrane. Radial walls of these cells usually have numerous plasmodesmata (Fig. 1M).

Group of CC is present throughout the length of each concentric ridges of the seed (Fig.1A). Before browning of the seeds, these cells have thin wall, large central vacuole and cytoplasm forms a thin layer along the cell wall (Fig. 1N). Chloroplasts usually contain large starch. Few mitochondria and golgi-bodies were also observed. As the s•ed becomes brown, central vacuole gets filled with fibrillar material, (Figs. 2A,B), wall becomes thick and complete cell becomes filled with dark osmiophilic content (Figs. 2C-E).

The inner epidermis of testa iw a continuous layer both in ridge and trough regions (Figs. 1 B,C,E), but the cells which are present below the CC are yellow and contain oil are the basal cells (BC). These cells have dense cytoplasm with numerous vacuoles and more cell organelles as compared to the epidermal cells of trough regions, ER, from the young stage, are well-developed (Fig.2F). As the seeds start becoming brown, many osmiophilic droplets appear in the cytoplasm (Fig.2J), inside the ER (Fig.2G), dialated ends of ER form vesicles (Figs. 2G-I) and along the cell wall of BC (Figs. 2L-N). Droplets which are present along the cell wall are more in number and bigger in size than the normal droplets present in the cytoplasm (Fig. 2J-N). These droplets from BC passes through the cell wall matrix and plasmodesmata and get accumulated in CC (Figs. 2E,L M). In this process wall between BC and CC becomes completely occluded with oil and appears densely osmiophilic (shown by arrow in Figs. 2 E,N). As the seeds become fully mature and hard, the secretion of essential oil in BC gradually diminishes and latter becomes functionless. Finally the oil gets completely accumulated in CC (Fig. 2D).

DISCUSSION

Essential oils in plants are commonly secreted by specialised glandular structures such as oil cells, glandular trichomes, secretory cavities and ducts. Plastids are most common site of essential oil synthesis. However, it has also been reported to be synthesized in cytoplasm, rough and smooth ER, golgibodies, mitochondria and even nuclear membrane (Fahn, 1979). Singh (1968) reported that during the development of *A. moschatus* seeds, the cells of outer integument are almost free of contents. However, Hedge *et al.*, (1984) observed oil droplets and resinous substance in the gland cells of mature undried and dried seeds, respectively. The present study reveals that essential oil in *A. moschatus* seeds is secreted by the cells of inner epidermis of testa, mainly by those cells (BC) which are present below the ridges. Essential oil secretion occurs when the seeds start turning brown in colour. Before browning, the cells of testa do not show much differentiation in structure between the secretory and non-secretory cells. Since, the cytoplasm of BC contains osmiophilic droplets both with and without unit membrane, the site of essential oil synthesis appears to be both cytoplasm and rough ER. Very small osmiophilic droplets have been observed in normal, rough ER,



Figures 2A-N: Essential oil bearing structures of *Abelmoschus moschatus* testa, A. Collection cell (CC) just before browning of the seed and underlying basal cells (BC) (X 1750); B. CC magnified showing fibrillar structures in the vacuole (X 3800); C. oil droplets (OD) getting accumulated in CC (X 8200); D. CC full with oil at maturity (X 13500); E. Oil droplets (OD) attached to the radial and upper cell wall of BC (X 8200); F. Mature basal cell with endoplasmic reticulum (ER), plastids (P), mitochondria (M) and nucleus (N) (X 4900); G. Endoplasmic reticulum (ER), the site of oil synthesis, arrow indicates the oil droplets (X 30000); H. ER containing oil at dilated end (X 82000); I. Oil inside vesicles formed by ER in BC (X 49000); J. Oil droplets without membrane in the cytoplasm (X 30, 000); K. Fully grown membrane bound oil droplets still connected with ER (X 60,000); L. Oil passing through the cell wall (X 49000); M. Arrow indicates the oil passing through the plasmodesmata (PD) (X 82000); N. Wall (CW) between BC occluded with oil (X 10500).

which later dialates as the droplets increases in size. Later, both types of droplets come-out of the PM and get attached with the nearest cell wall. In this process membrane bound droplets, loose their membranes. Osmiophilic droplets appear to have some affinity with cell wall, since, mostly these were seen travelling along or through the walls. Some stray osmiophilic droplets have also been observed in LC and NC but no active site for synthesis has been observed in them. Opening of LC after maturation, with slight disterbance suggests that these openings help in the dessication of the seed as well as resininfication of the essential oil.

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