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DEVELOPMENT OF PHYTOMELANIN LAYER IN FRUIT WALL OF *TAGETES PATULA* L. (ASTERACEAE)

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Development of phytomelanin layer in the fruit wall of *Tagetes patula* L. (Asteraceae) was studied using both light and electron microscopy. At the organised female gametophyte stage, the ovary wall is distinguishable into two zones, outer comprises epidermis and hypodermis while inner includes developing fibre cells. And parenchymatous cells. After fertilization, a schizogenous space develops in between the outer and inner zones. Phytomelanin begins to deposit in the space created. During phytomelanin accumulation, abundant smooth endoplasmic reticulum is present in the hypodermis. At maturity, entire schizogenous space is filled with phytomelanin which cements the two zones separated earlier. It is hypothesized that the precursors of the phytomelanin are synthesized in the endoplasmic reticulum and these precursors migrate into the schizogenous space where the phytomelanin is polymerised.

Key Words: *Tagetes patula*, Asteraceae, achenes, phytomelanin layer.

Phytomelanin is a mechanically hard, black coloured resistant layer in the achene wall of taxa belonging to the tribes Eupatorieae and Heliantheae of family Asteraceae (Pandey *et al.*, 1989; Pandey and A. Singh, 1994). Interestingly, phytomelanin layer is absent in other tribes of the Asteraceae. This layer is formed, after fertilization, in the schizogenous space which develops between hypodermis and developing fibre layer. In immature achenes, the phytomelanin layer is pliable but it becomes dense and rigid during fruit maturation (Kiewnick, 1964). The characteristic morphology of the phytomelanin layer results from the contour and surface structure of the fibre and hypodermal layers. Controversy centres around the question that which tissue in the developing pericarp is responsible for the formation of phytomelanin layer. Phytomelanin layer has been described variously as carboniforous, resinous, tanniniferous and amorphous (Hanausek 1902, 1907, 1911, 1913; De Vries 1948; Pandey and Singh 1982; Pandey and Kumari, 1987; Pandey *et al.*, 1986, 1989, Pandey and A. Singh 1994). The phytomelanin layer is noncellular, very resistant to degeneration and frequently has diagnostic sculpturing on its surface that in some cases appears to be useful taxonomically (Stuessy and Liu, 1983). Phytomelanin is of special interest because it has been found to deter insect predation in *Helianthus annuus* (Carlson and Witt, 1974; Rogers and Kreitner 1983). De Vries (1948) studied phytomelanin development in some Asteraceae including *Tagetes patula* but his observations were based on light microscopic studies only. The present study on the phytomelanin development is based on both light and electron microscopic studies in order to findout the role of fruit wall cell layers in deposition of phytomelanin.

MATERIALS AND METHODS

To ascertain the stages when phytomelanin begins to deposit in the pericarp and the mode of its deposition, flowers and achenes of *T. patula* at various stages of development were fixed in formalin-acetic-alcohol. Customary methods of dehydration in tertiary-butyl-alcohol series and embedding in paraffin wax were employed. Sections cut between 8-20 µm thickness were stained in safranin-fastgreen combination.

For electron microscopy, individual flowers and fruits were fixed in 3% glutaraldehyde (v/v) in 0.1M sodium phosphate buffer at pH 7. Fixation was carriedout at room temperature under vacuum for 2 hr. Following several buffer rinses, the material was post-fixed overnight in 1% (w/v) osmium tetraoxide in buffer at 4°C, After a brief rinse, the material was dehydrated in acetone and embedded in epoxy resin. Thin sections cut with a diamond knife were post-stained with lead citrate, with or without uranyl acetate. Specimens were viewed with a Philips CM-10 transmission electron microscope operated at 80 kV.

For the study of spines, pore and striations, mature achenes were heated in 15% NaOH for 4 hr at 50°C.

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Achenes were then washed thoroughly in water and viewed under the compound microscope. This NaOH treatment was found to be helpful in clearing the epidermis, and to some extent the hypodermis to have a clearer view of the phytomelanin pattern which are sometimes masked by the overlying cells of the pericarp.

For SEM studeis mature achenes (both treated and untreated with NaOH) were fixed on aluminum studs with silver paint. Material was then coated with a thin layer of gold (100 Å) in sputter-coating unit. Achenes were then viewed in a Jeol-JSM 36C Scanning electron microscope at National Botanical Research Institute, Lucknow.

OBSERVATIONS

The gynoecium is bicarpellary and the syncarpous., unilocular ovary contains a single anatropous, unitegmic and tenuinucellate ovule (Fig. 1A). The ovary wall at the organised female gametophyte stages is 22-25 cell layers thick. The ovary wall at this stage is distinguishable into outer and inner zones (Fig. 1B). The outer zone includes the epidermis which is one cell-layer thick and the hypodermis which contains two cell layers. The cells of the hypodermis are prominent as compared to epidermal cells owing to the presence of dense cytoplasmic contents. The inner zone includes profibre and paranchymatous cell layers (Fig. 1B).

After fertilization, the outer zone begins to separate from the inner zone resulting in the formation of a schizogenous space between the profibre cells and inner hypodermis. The small papillae begin to develop from the outer tangential walls of the fibres. The papillae are broader towards base and taper towards end which connects the fibre zone with hypodermal zone. Except for the attachment points of the papillae, the fibres and the hypodermal cells are completely separated throughout the length of the ovary. At this stage, both outer and inner hypodermal cells have large central vacuoles.

After the formation of schizogenous space and the papillae, phytomelanin begins to deposit on the cell wall due to secretory activity of the hypondermal cells. The hypodermal cells contain abundant smooth and apparently tubular endoplasmic reticulum (Fig. 2D). The phytomelanin is detected first on the cell walls of the fibres including the papillae and on the inner hypodermal walls where the papillae are attached. Phytomelanin covers the fibre cell walls prior to the coating of hypobdermal cells (Fig. 2C) Continuous deposition of phytomelanin results in complete filling of schizogenous space.

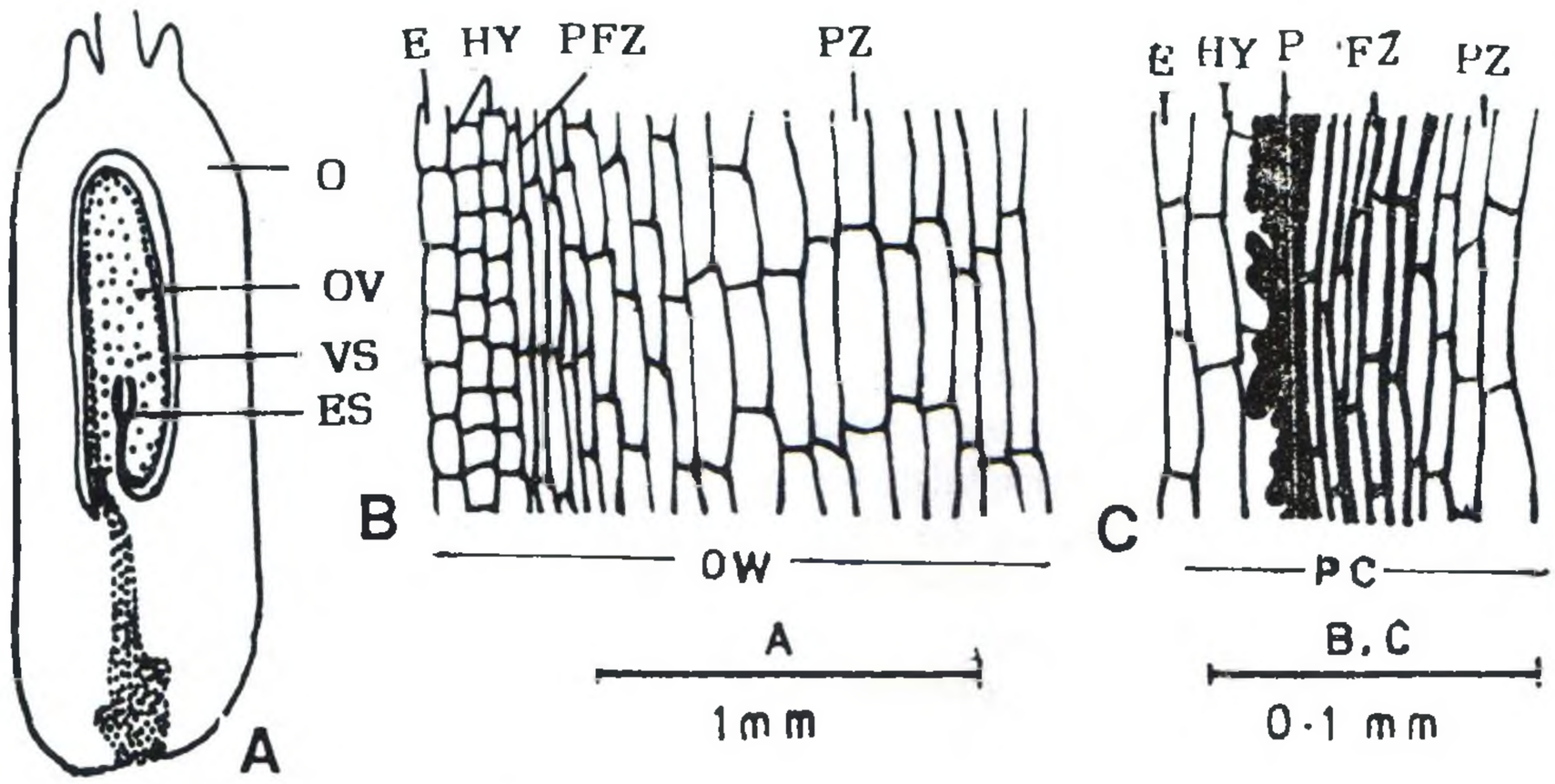

Figure 1 A-C *Tagetes patula*. A-L.S. ovary and ovule at organised female gametophyte stage. B-L.S. part of ovary wall at organised female gametophyte stage. C-L.S. part of mature pericarp. Note phytomelanin layer between hypodermis and fibre layers (E-epidermis; ES-embryo sac; FZ- fibre zone; HY- hypodermis; P-phytomelanin; PC- pericarp; PFZ profibre zone; PZ-parenchymatous zone; 0-ovary; OV-ovule; OW-ovary wall; VS- vascular supply)

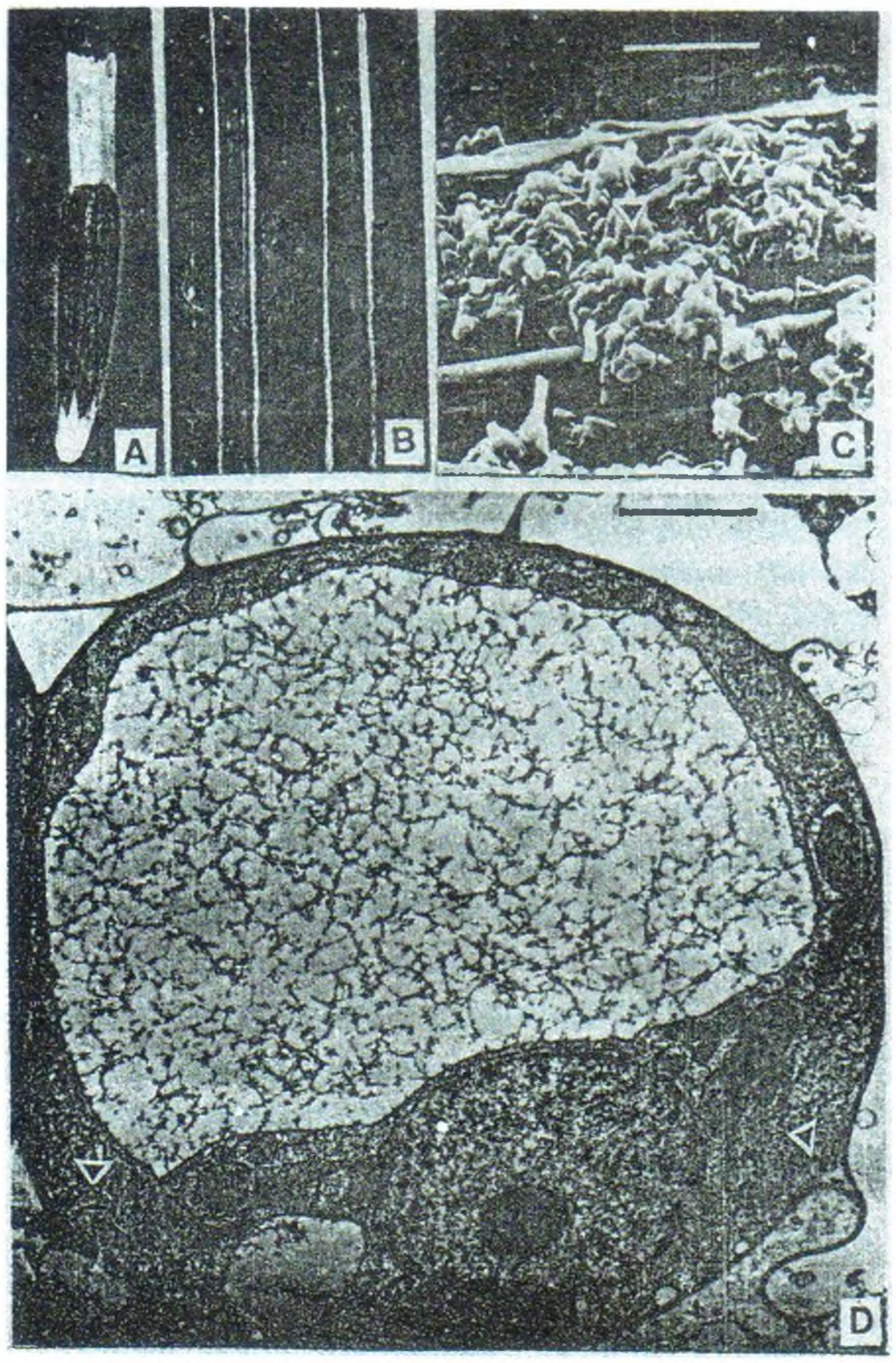

Figure 2A-D. *Tagetes patula*. A. Mature achene (x 5). B. Fruit wall in surface view. Note striations (x 300). C. Scanning electron micrograph of developing fruit in which epidermis and hypodermis have been dissolved by NaOH treatment. The phytomelanin (arrow) is NaOH resistant and it remains as layer over the outer fibre walls (Bar= 20 µm). D. Hypodermal cell. Note smooth and tubular endoplasmic reticulum (arrow) present in abundance (Bar= 1 µm).

As the fruit develops further, the phytomelanin increases in quantity and by the time fruit matures, the entire space is filled with this substance. The mature pericarp is distinguishable into an epidermis, two layers of hypodermis, a solid phytomelanin layer, fibre zone and a few layers of parenchymatous cells (Fig, 1C). The mature achenes become black due to presence of phytomelanin (Fig. 2A). The mature achenes show striations in surface view (Fig. 28). These striations are gaps which develop due to presence of parenchymatous ray-like uniseriate cells.

In mature achenes phytomelanin forms a protective covering around the embryo. When mature achenes were placed for germination in petriplates, it was observed that radicle comes out of the achene from the side where achenes are attached to the receptacle. The pores present in the phytomelanin layer allow entry of water inside the seed resulting in the swelling of the embryo. The embryo germinates without much disturbing the phytomelanin covering.

DISCUSSION

The formation of a schizogenous space in the ovary wall is a prerequsite for the deposition of phytomelanin. Development of schizogenous space in the ovary has been reported in members belonging to tribes Eupatorieae and Heliantheae (Pandey *et al.*, 1989; Pandey and A. Singh, 1994). De Vries (1948) reported that in *Tagetes* phytomelanin is formed consecutively in three different ways, viz., (a) as a thin brown "pellicle" covering the outer side of the fibre bundles, (b) as "boundary strips" and "dendrites" outside the pellcle, (c) as "drops" at the inner surface of the hypodermis. In the present study phytomelanin accumulations are first observed along the papillae and on the outside of the fibre walls that face the cavity. Simultaneously, the cytoplasm of the outer hypodermal cells become densely packed with smooth and tubular endoplasmic reticulum, a feature also observed in *Ageratum conyzoides* (Pandey *et al.*, 1989) and often been found in secretory cells (see Luttge and Schnepf 1976; Robards and Stark, 1988). During the onset of phytomelanin secretion, the protoplasts of the fibres and many of inner parenchymatous cells do not change or develop extensive endoplasmic reticulum. Pandey *et al.* (1989) hypothesized that in *Ageratum conyzoides* phytomelanin precursors are synthesized in abundant endoplasmic reticulum of the outer hypodermis and these precurosrs migrate to the walls facing the cavity where they are polymerised into phytomelanin by enzymes in the walls or in the subjacent protoplasts. It is quite likely that the route of movement of the precursors is by secretion from the hypodermal protoplasts into the cell walls facing the space, rather than by movement through palsmodesmata between the hypodermal and fibre cells. This is also true for phytomelanin deposition in *T. patula* (present study).

Hanausek (1912) opined that phytomelanin is formed by transformation of the middle lamella and that substance for its formation is supplied by sclerenchymatous cell wall. This observations is not

true. The phytomelanin always develops from the hypodermal cells of the pericarp. The present study also gets support from the obserations of other authors that the hypodermis is source of the secretion of phytomelanin (Pandey and Singh,1982; Stuessy and Liu, 1983; Pandey and Kumari 1987; Pandey and A.Singh 1994; Pandey *et al.*, 1989).

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