J Indian Bot Soc 68 (1989)

Intracarpellary Secretory Cells and Their Role in *Ottelia alismoides*

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(Accepted January, 1988)

The structure and cytochemistry of secretory cells are studied with respect to their location, chemical composition and active period of secretion. The secretory cells are present in clusters of 10-90 cells inside the ovary above the micropyle of each ovule. Each cell is spherical to elliptical and appears at 2-nucleate embryo sac stage and by the time of 8 nucleate embryo sac stage they start secreting mucilage, which eventually fills the entire ovarian chamber. The secretion consists of periodic acid Schiff's positive insoluble polysaccharides and periodate reactive and non reactive alcianophilic mucopolysaccharides. It is devoid of proteins and lipids. The probable involvement of secretion in embryo nourishment and protection of ovule has been reported in *Ottelia*. We suggest that the secretion provides a suitable medium for free movement of pollen tubes and their nourishment in the ovarian cavity.

Key Words - *Ottelia alismoides* Secretory cells Cytochemistry Pollen tubes ovary mucilage.

Few plants possess specialized intra-carpellary secretory cells inside the ovary. Several functions, such as protection of ovule (Vijayaraghavan & Kapoor, 1980) and nutrition to the developing embryo (Indra & Krishnamurthy, 1980) have been attributed to them. The present investigation describes the structure of intracarpellary secretory cells, histochemical composition and role of the secretion in *Ottelia alismoides*.

MATERIALS & METHODS Unanthesised floral buds and ovaries with ovules at various stages of ovule development of *Ottelia alismoides* Pers. were collected from the green house and fixed in ethanol acetic acid (3:1) and 4% formalin. The material was processed for microtomy through conventional methods using paraffin as infiltration medium. The cytochemical detection of RNA, total proteins, insoluble polysaccharides, mucopolysaccharides, starch and lipids was made as suggested by Tepper & Gifford (1962), Yasuma & Ichikawa (1953), Hotchkiss (1948), Scott & Darling (1965), and Jensen (1962), respectively. Suitable controls were kept. To study the development of secretory cells, a set of slides was stained in haematoxylin-tannic acid-ferric chloride. Secretory cells were scanned for their morphological details in a Cambridge Stereoscan S4-10. No specific stain was employed to detect pollen tubes, since they could be easily seen in the colourless mucilagenous secretion under a light microscope.

OBSERVATIONS A longitudinal section of the ovary of *O. alismoides* stained with Periodic acid-Schiff's reagent revealed the presence of colonies of secretory cells, scattered on the inner carpellary wall and protruding out from each carpellary wall. (one of them is seen between two ovules in Fig. 1A & B). Each colony consists of 10-90 secretory cells and lies just above the micropyle of the ovule (Fig. 1A, B; 2A). Each secretory cell is uninucleate and pear shaped with a free broader end protruding into the ovarian cavity and a thin tapering basal end by which it is attached to the ovary wall (Fig.1C). They are surrounded by a thick cuticular envelope. The scanning electron micrograph of these cells reveals neither a beak nor a pore-like opening on the cuticular envelope (Fig. 1D). Each cell

possesses dense cytoplasm and a large prominent nucleus in the centre (Fig.1C).

The secretory cells arise from the inner carpellary epidermis, above the site of ovule initiation and their presence can be noticed right from the stage of differentiation of the hypodermal archesporial cell in the ovule. They enlarge gradually in size, and at the stage of 2-nucleate embryo sac, they start secreting water insoluble mucilage which is released into the ovarian cavity. The rate of secretion is profuse at 8-nucleate stage of embryo sac. The ovarian cavity is completely filled up with mucilage prior to fertilization (Fig. 2B). The secretion is a thick colourless mucilage. It remains up to globular embryo stage and in the subsequent stages the prodnction of mucilage gradually stops.

The cytoplasm of secretory cells shows deep red colour with pyronin G and ninhydrin-Schiff's reagents at their free ends indicating the presence of RNA and total proteins respectively. The ovary wall and its cells which form a base to the colony of secretory cells show immense deposition of starch (Fig.1A) prior to secretion. Such an accumulation of starch is absent in the secretory cells. However a dense periodic acid-Schiff staining is seen in the cytoplasm of secretory cells. A gradual depletion of starch is seen in ovary wall cells as the rate of secretion increases (compare Figs. 1C & 1B). The cells of ovary wall forming the base to secretory cells show negative test with IKI reagent, thus revealing the absence of starch. The cytoplasm of secretory cells does not show any positive test with Sudan black B reagent.

The secretion is Periodic acid Schiff positive (Fig.2B) ninhydrin-Schiff negative and shows nonspecific pyroninophilia as evidenced from the altogether different colour of RNA-pyronin end product. The secretion is strongly alcian blue positive (Fig. 1C & 2C) and does not reveal the presence of lipids. The secretion only encircles the ovules but does not enter into them (Fig. 2C). A cross section of the ovary at the instant of fertilization reveals the presence of many pollen tubes which are suspended freely in the secretion (Figs. 2D & 2E). The secretory cells disorganise and become flaccid at later stages of ovule development.

DISCUSSION The secretion within the carpels of *O. alismoides* is Periodic acid Schiff's and alcian blue positive. It does not show the presence of proteins or lipids. Two types of polysaccharides are thus envisaged in the secretion, one being neutral and the other, acidic.

The source of carbohydrate for the observed PAS positive secretion is linked with the disappearance of starch from the cells of ovary wall forming a base to secretory cells and no secretory cell reacts with iodine to give a starch test during the active period of secretion. Thus, the starch digestion is an overall reaction within the secretory cells.

Indra & Krishnamurthy (1980) opined that the polysaccharide rich secretion in *O. alismoides* is utilized for the nourishment of the young and developing embryo in the ovule. But a transverse section through the pistil during fertilization shows the presence of several pollen tubes freely suspended in the secretion within the ovarian cavity. This observation leads us to believe that the secretion serves in the first instance as a suitable medium for lodging and free movement of pollen tubes in the ovarian cavity, which facilitates the entry of pollen tubes into the ovules with least resistance.

Secondly, the secretion also helps in the nourishment and growth of the pollen tubes in the ovarian cavity. A similar observation was made

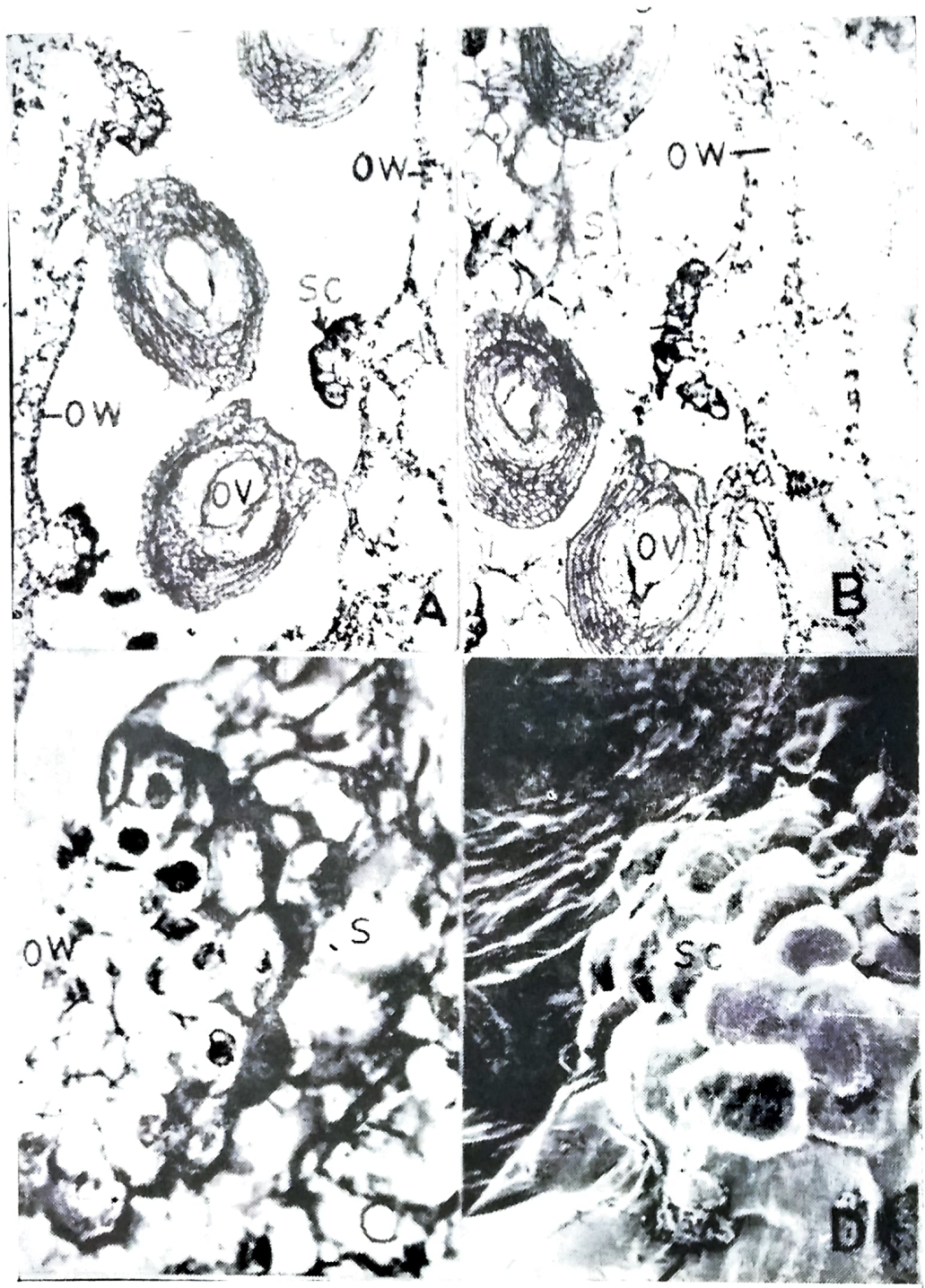

Fig. 1 A. Longitudinal section of Ovary showing the presence of secretory cells above the ovules. (x100) B. Secretory cells at mature embryo sac stage showing PAS positive secretion in the ovarian cavity. A and B stained with PAS reagent (x100) C. Secretory cells during the active period of secretion, stanined with alcian blue and Schiff's reagents (x450) D. Scanning electron micrograph of secretory cells (x650). (OV = Ovule; OW = ovary wall; S = secretion; SC = secretory cells).

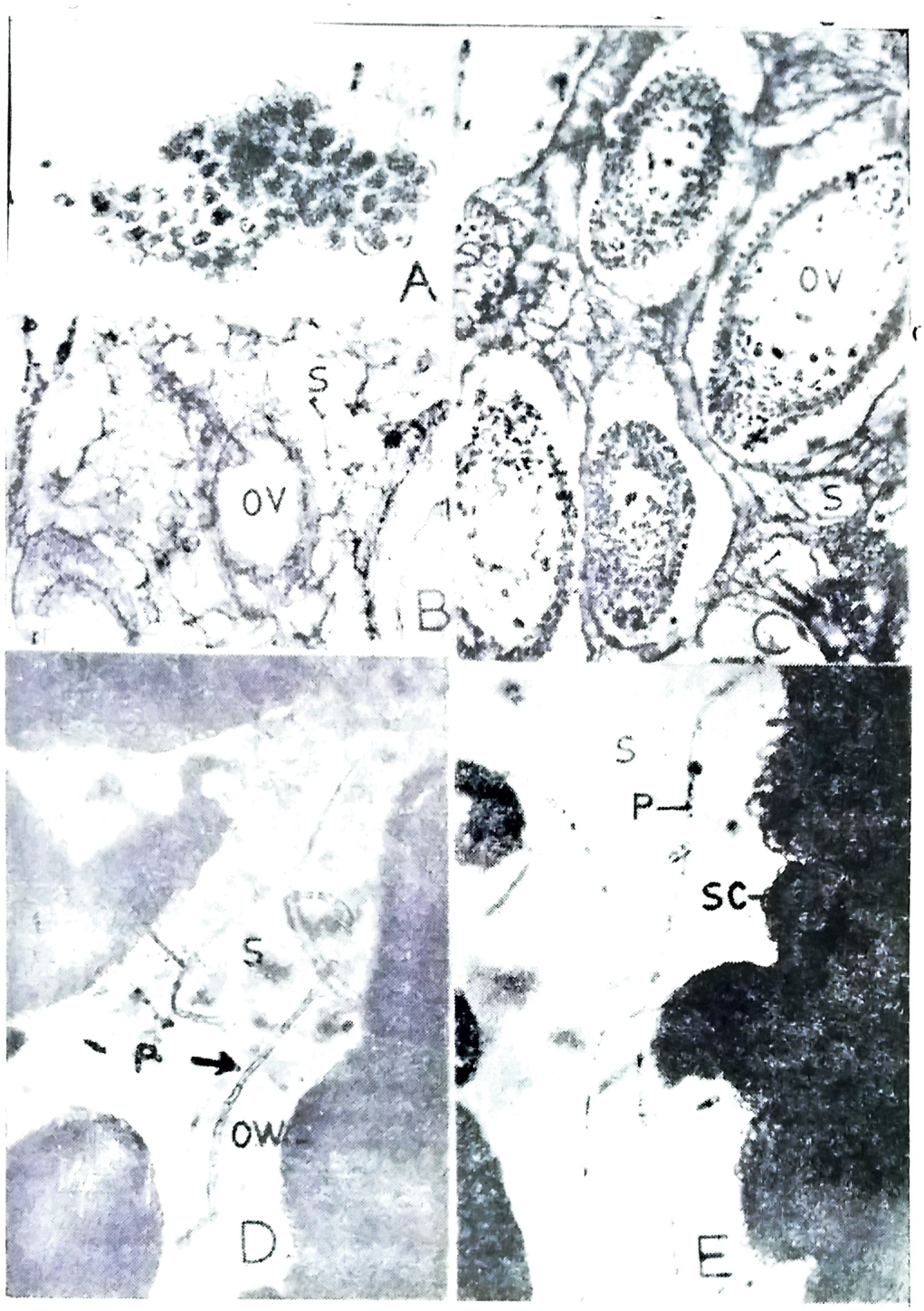

Fig. 2 A. A colony of secretory cells (unstained) (x100). B. Secretion showing PAS positive nature (x450). C. Longitudinal section of ovary showing the mucilagious secretion encircling the ovules. Stained with Schiff's and alcian blue combination (x450). D. Cross section of ovary at the time of fertilization (unstained). Note the presence of the pollen tubes in the secretion (x100). (OV = ovule; OW = ovary wall; P = Pollen tubes; S = Secretion; SC = secretory cells).

by Yamada (1965) in *Lilium longiflorum*. Rosen & Gwalik (1966) represented the secretion found in the canal cells of *L. longiflorum* and the pollen tubes as complementary halves (source and sink) of a linked transfer system consisting of an outward flux of secretion product from the former and an inward flux of the same material to the latter helping in the growth of the pollen tube. A similar mechanism also holds true between the pollen tubes and the secretion found in the ovarian cavity of *O. alismoides*.

What is the fate of secretion, soon after its role in fertilization is fulfilled ? Since the secretion remains in the ovarian cavity until late embryo development, it is likely that it is used for some other function such as nourishment of young and developing embryos as suggested by Indra & Krishnamurthy (1980).

Acknowledgement The financial assistance by UGC, New Delhi to BJA is gratefully acknowledged.

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