

ration of sporogenous tissue is often associated with aposporic mode of reproduction¹⁰ but in the present material, there was no such indication at any stage.

Inability of a species to set viable seed is limited not only to grasses but has also been recorded in members belonging to various other families. Recently Spooner¹¹ demonstrated sterility in one population of *Dentaria diphylla* via abortive embryo sac development which is identical to what has been noticed in *D. bipinnata*.

The production of viable seed is essential for survival and dispersal of a species. *D. bipinnata* reproduces vegetatively through the root stocks but this alone may not be able to account for its distribution over such wide geographical areas as India and Africa. Evidently more populations should be investigated to understand the nature of the reproductive system operative in *D. bipinnata*.

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CYTOCHEMICAL STUDY OF MUCILAGE SECRETING CELLS IN THE OVULES OF *NAJAS MARINA* L

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THE ovules of *Najas marina* L show two groups of secretory cells, one at the base of style and another at the funicle. From these cells secretion commences at the megaspore mother cell stage, reaching maximum just prior to fertilization. Secretory fluid manifests higher concentration of carbohydrates, lipids, total and -SH proteins, RNA and pectic substances gnarled with iron. The present investigation suggests a correlation between this secretion and the growth of pollen tube.

For the present study female flowers of *N. marina* L, containing different developmental stages of ovary, were collected from the greenhouse grown specimens and were fixed in ethanol acetic acid (3:1 v/v). The material was sectioned on a rotary microtome and the sections were stained for RNA (pyronin-G method), DNA (Feulgen reaction), total proteins (bromophenol blue method), -SH proteins (DDD reaction), insoluble polysaccharides (PAS reaction) and cuticle (aniline blue reagent) as suggested by Jensen¹, lipid (Sudan black blue method) and iron gnarled into the acid mucopolysaccharides (hematoxyline method) as mentioned by Pearse² and acid mucopolysaccharides (toluidine blue method) as suggested by Chayen *et al.*³. Squashes of secretory cells were also prepared for study of the above mentioned substances.

The developing ovules of *N. marina* L reveal two groups of secretory cells (figure 1A), one with 15-20 cells at the base of style and the second with 30-40 cells located at the funicle. The latter group is also known as funicular obturator. Each is narrow and elongated with a free broader end protruding into the ovarian cavity (figure 1C). A big elongated or spherical prominent nucleus in each cell shows deep staining reaction for DNA (figure 1B).

The cells are sessile, have a thick aniline blue positive cuticle, and vacuoles in the cytoplasm (figure 1C). Prior to fertilization, viscous colourless water-insoluble substances composed of fine fibrils ooze out. The fluid commences at megaspore mother cell stage and accumulates around the ovule

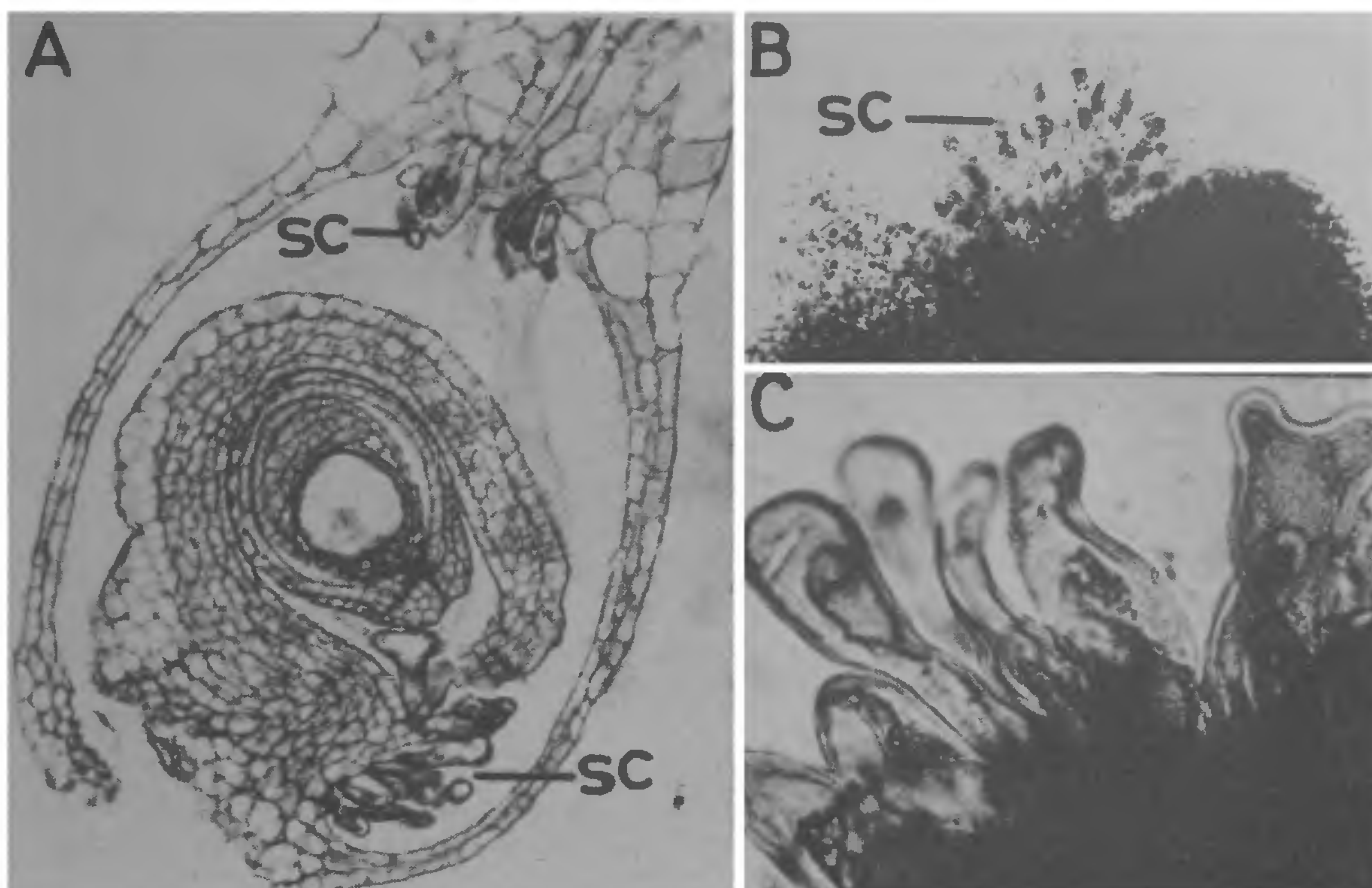


Figure 1 A–C. *Najas marina* L. **A.** L.S. of ovule showing two groups of secretory cells, one at the base of style while second located at the funicle, stained for insoluble polysaccharides ($\times 450$). **B.** Squash preparation of secretory cells of funicular region stained for DNA. Note deeply stained nuclei ($\times 100$). **C.** Secretory cells stained with aniline blue showing thick cuticle layers ($\times 450$).

reaching optimum just prior to fertilization. The secretion continues up to the maturity of the female gametophyte but ceases after fertilization.

The secretory fluid is periodic acid Schiff's (PAS) positive with dark uniform staining indicating the presence of higher amount of insoluble polysaccharides. Very high concentration of total lipids is detected in the secretory material using Sudan black B (saturated in 70% ethanol), while toluidine blue (0.1% pH 3.9–4.0) revealed the presence of acid polysaccharides. The mucilage manifests an intense staining reaction with alcian blue reagent which portrays the presence of alcianophilic mucopolysaccharides too. Secretion stained with 0.1% hematoxylin at pH 6.9 for 5–7 days shows a blue colour which points to pectic substances gnarled with iron. Pyronin-G reveals red-brown cytoplasm and deep brown secretion. The nucleoplasm is highly pyronin-G positive and contains 2–3 nucleoli. The total proteins manifest dark blue end product. Total and –SH proteins are more concentrated at the peripheral region of the cytoplasm in the cells.

The secretory cells of *N. marina* L are non-stalked, poreless and have a well-developed cuticle

like that in *Najas flexilis*⁴. Cuticle is however well-developed in nectaries of many plants through which secretion is discharged under hydrostatic pressure⁵. Mucilage secretion of *N. marina* contains two types of polysaccharides (1) neutral-positive to PAS reaction and (2) acidic-alcian blue positive as reported in the mucilage cells of the fruits in *Hibiscus esculentus*⁶. Secretion reveals blue colour fibrillar structure when stained with hematoxyline. According to Lillie and Fullmer⁷ acid mucopolysaccharides such as pectic acid contain traces of ferric ions which stained with hematoxyline. In *Paspalum orbicular* and *P. longifolium*, the fibrils, present in the PAS substances are composed of pectic acids⁸. Yamada⁹ observed the stylar canal of *Lilium longifolium* filled with a mucilaginous material following pollination. He analyzed the mucilage cytochemically and concluded that it also plays a nutritive role for the growing pollen tube. Similar observations were also made by Tilton and Horner¹⁰ on *Ornithogalum caudatum*. Rosen and Gawlik¹¹ represented the secretion, found in the canal cells of *L. longifolium* and the pollen tubes, as complementary halves sources and sink of a linked transfer system consist-

ing of an outward flux of secretion product from the former and an inward influx of the same material to the latter helping in the growth of pollen tubes.

The location of secretory cells in the ovules of *N. marina*, and the fact that secretion is maximum prior to fertilization and present in the entire ovarian cavity suggest that it not only serves as a suitable medium for the free suspension of the pollen tube in the ovarian cavity but also provides nutrition to growing pollen tubes, since mucilage is highly rich in lipids, carbohydrates and proteins. The copious secretion, that besieges the ovule also functions of protection in a taxon which lives in a particular ecological niche (saline water -pH 8.2).

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PLANTLET FORMATION IN EMBRYO CULTURES OF *CAPSICUM ANNUUM* L VAR G4

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THE techniques of plant tissue culture are increasingly being applied for the improvement of economically important crops. *In vitro* cultures used for propagation could be started either from existing meristems or from adventitious meristems in the form of shoot apices or embryos. Embryos of *Hordeum vulgare*¹, *Solanum melongena*² and *Capsicum annuum*³ have been successfully cultured for accelerating the rate of multiplication. George and Narayanaswamy⁴ and others^{5,6} reported the production of haploid plants through anther culture in *C. annuum*, and later Gunay and Rao⁷ and Saxena *et al*⁸ obtained plantlet regeneration from hypocotyl and cotyledon explants, and protoplasts, respectively.

Although red pepper cannot be considered as one of the world's major economic crop, it is one of the important cash crops of India with significant commercial value as a spice. Very little tissue culture work is being done in this crop. Our aim of experiment is to increase the multiplication rate through embryo culture in red pepper. We report here the formation of complete plantlets from excised mature embryos of *C. annuum* L var G4, a high yielding selection of a local cultivar.

Fresh seeds of *C. annuum*, var G4 were obtained from the Agricultural Research Station, Lam, Guntur, A.P. Soaked (24 hr) seeds were surface-sterilized with 0.1% mercuric chloride for 5 min and washed thoroughly in glass distilled water. The mature embryos were excised aseptically and cultured on modified Murashige and Skoog's (MS)⁹ medium consisting of various combinations of 2,4-dichloro phenoxyacetic acid (2,4-D), 3-Indole acetic acid (IAA), kinetin (Kn) and 6-benzylaminopurine (BAP). The pH of the medium was adjusted to 5.8 with 0.1% NaOH and solidified by 1% agar. Differentiating cultures were maintained under a 16 hr light and 8 hr dark cycle at 26±2°C.

In excised mature embryos (figure 1) cultured on modified MS medium supplemented with 2,4-D (0.5–1 mg/l) and Kn (0.5 mg/l), the cotyledons turned green and subsequently formed an actively