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# Synergids and Antipodal Cells in *Ranunculus sceleratus* Linn. — A Histochemical Approach

M R VIJAYARAGHAVAN and USHA BHAT
*Department of Botany, University of Delhi, Delhi 110 007*

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In *Ranunculus sceleratus*, one of the synergids and all the three antipodal cells persist after fertilization. The antipodes enlarge in size; show considerable increase in the oncentration of proteins. histones, DNA and RNA; and are thus metabolically active in translocation of nutrients.

**Key Words: Synergids, Fertilization, Antipodal cells**

## Introduction

Various views have been put forth regarding the role of synergids and antipodal cells. Synergids are believed to be responsible for the secretion of chemotrophic substances which direct the growth of the pollen tube (Van Went 1940, Rifot 1975) and also for the absorption of metabolites from the nucellus (Jensen 1965, Schulz & Jensen 1968). Similarly, the antipodal cells have also been assigned various roles like absorption, secretion, and transfer of nutrients (*see* Kapil & Bhatnagar 1978). During a histochemical study of achene development in *Ranunculus sceleratus*, interesting observations were made regarding the synergids and the antipodal cells which have been discussed in the present communication.

## Material and Methods

Flowers and fruits (at various stages of development) of *Ranunculus sceleratus* Linn. were fixed in three fixatives: FAA (5 ml formalin, 5 ml glacial acetic acid, 90 ml 70% ethanol), AA (25 ml glacial acetic acid, 75 ml absolute alcohol) and 10% neutral formalin. The fixed material was dehydrated in tertiary butyl alcohol series and embedded in paraffin wax. Neutral formalin-fixed material was washed overnight in running water before dehydration. Material fixed in FAA was used for the localisation of insoluble polysaccharides and proteins; AA for nucleic acid; and neutral formalin for histones. Control slides were made to check the specificity of the various reactions. Sections were cut at 10 $\mu$m. Localisation of insoluble polysaccharides was done with periodic acid-Schiff's (PAS) technique (modified from Jensen 1962, incubation in periodic acid at 40°C), proteins with mercuric-bromphenol blue method (Mazia, Brewer & Alfert

1953) and histones, with alkaline fast green method (Alfert & Geschwind 1953). DNA was localised by Feulgen reaction after hydrolysis with 5N HCl at room temperature and RNA by pyronin Y method of Tepper and Gifford (1962).

## Observations

Each carpel has a single anatropous and unitegmic ovule. The mature embryo sac contains an egg, 2 synergids, 2 polar nuclei and 3 antipodal cells (figure 1D). The synergids show hooks and prominent filiform apparatus (figure 1 A). The persistent synergid (figure 1 B, E; figure 3 C) remains healthy up to 4-celled proembryo stage and its remnants are discernible even at the globular proembryo stage. After fertilization, the antipodal cells increase in size (figure 3 A) and persist up to the globular proembryo stage. A zone of thick-walled, elongated cells is seen beneath the antipodal cells. This zone, constitutes the nucellar-stalk (figure 2 C; figure 3 D), and disintegrates by the heart-shaped stage of the embryo.

In a mature embryo sac, the cytoplasm of the synergids and the antipodal cells is feebly PAS-positive, and is devoid of polysaccharide grains. The filiform apparatus, however, is intensely PAS-positive (figure 1 A). Both synergids and antipodal cells are rich in total proteins, histones and RNA. Feulgen staining, however, is more intense in the nuclei of antipodal cells (figure 2 D) than those of the synergids.

After fertilization, the persistent synergid remains rich in all the metabolites. Although no quantitation has been made, it is inferred by the staining reactions that the three antipodal cells show an enormous increase in the concentration of proteins (figure 1 E; figure 2 A), histones (figure 2 B, C), DNA (figure 2 E; figure 3 A) and RNA (figure 3 D). A few polysaccharide grains are seen in the antipodal cells at octant proembryo stage (figure 1C). At the globular proembryo stage, the antipodal cells show a faint staining for proteins, histones and RNA, but during later embryogenesis the antipodal nuclei stain well for DNA (figure 3 B) although disorted in outline.

## Discussion

### *Synergids*

The synergids in *Ranunculus sceleratus* are rich in total proteins and RNA whereas those of *Stellaria media* have a high carbohydrate concentration (Pritchard 1964). The filiform apparatus is intensely PAS-positive. In *Allium cepa* one of the synergids is hypertrophied and rich in PAS-positive grains, proteins, DNA and RNA (Syamasundar & Panchaksharappa 1975). Malik and Vermani (1975) localised various enzymes in the synergids of *Zephyranthes rosea* and *Lagenaria vulgaris* and stated that these are metabolically active cells. The high metabolic activity of synergids is further evident by the presence of large number of mitochondria, dictyosomes, plastids, ribosomes and endoplasmic reticulum in them (*see* Schulz & Jensen 1968, van Went 1970, Vijayaraghavan, Jensen & Ashton 1972, Newcomb 1973a, Maze & Lin 1975).

Diverse views have been put forth regarding the function of synergids. Pritchard (1964) considers that the synergids act as "nurse cells". The synergids with convoluted filiform apparatus are considered as transfer cells (*see* Pate & Gunning 1972; Vijayaraghavan, Jensen & Ashton 1972). Maze and Lin (1975) hypothesized that in *Stipa elmeri*, the filiform apparatus in the two synergids

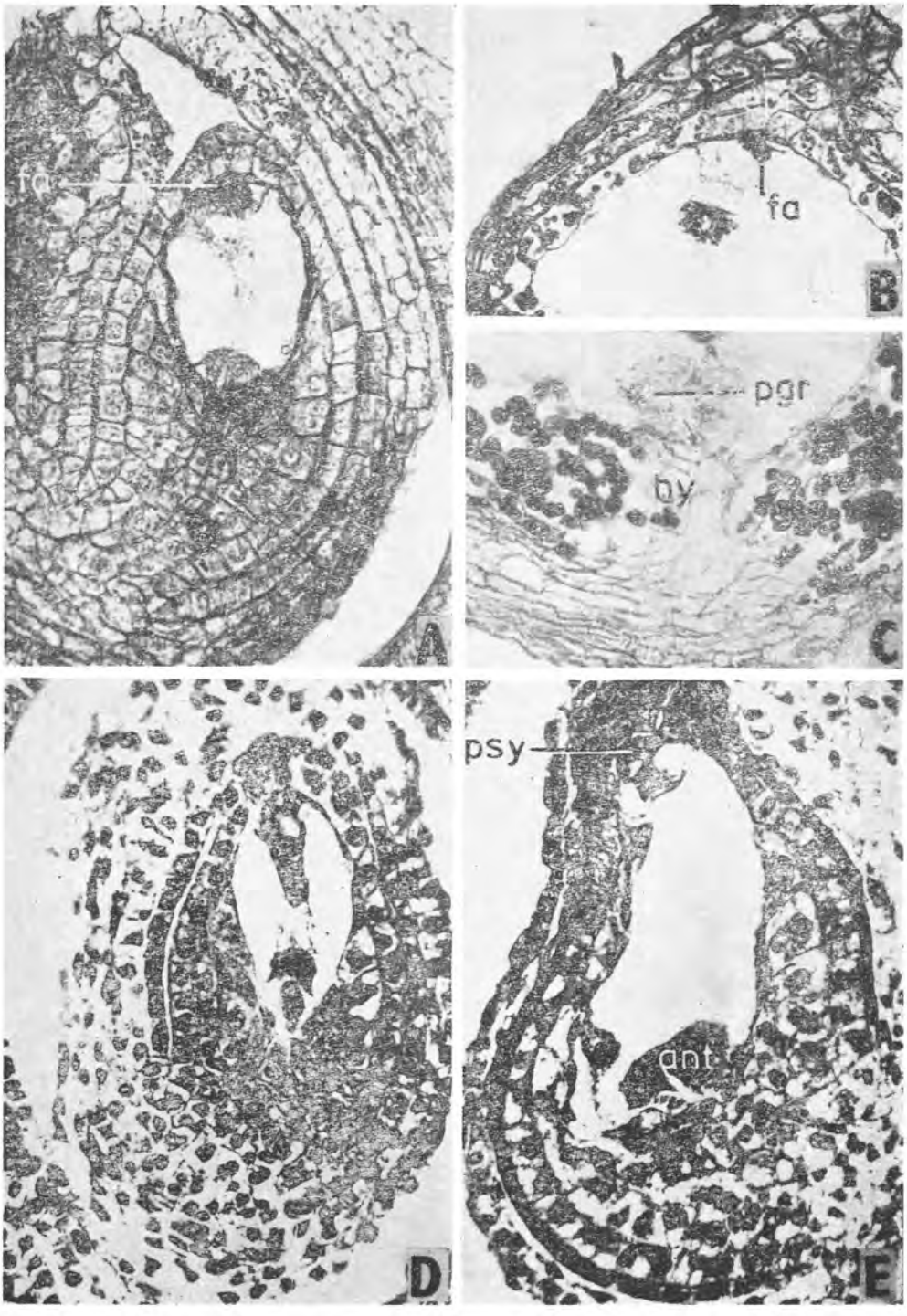


**Figure 1A-D** (**A-C**, *stained by PAS-technique for insoluble polysaccharides;* **D, E**, *stained with mercury-bromphenol blue for total proteins).* *A*, L. S. ovule showing mature embryo sac. Note the intensely-stained filiform apparatus (**fa**), *B*, portion of longitudinal section of seed at 3-celled proembryo stage to show PAS-positive filiform apparatus (**fa**) of the persistent **synergid**; *C*, chalazal portion of seed at **octant proembryo** stage to show a few **PAS-positive grains** (**pgr**) in the antipodal cells (**hy**,hypostase); *D*, L.S. **ovule** at mature embryo sac stage. Synergids and antipodal cells are rich in total proteins; *E*, L.S. seed showing protein-rich persistent synergid (**psy**) and antipodal cells (**ant**). **A-D** (× 482)

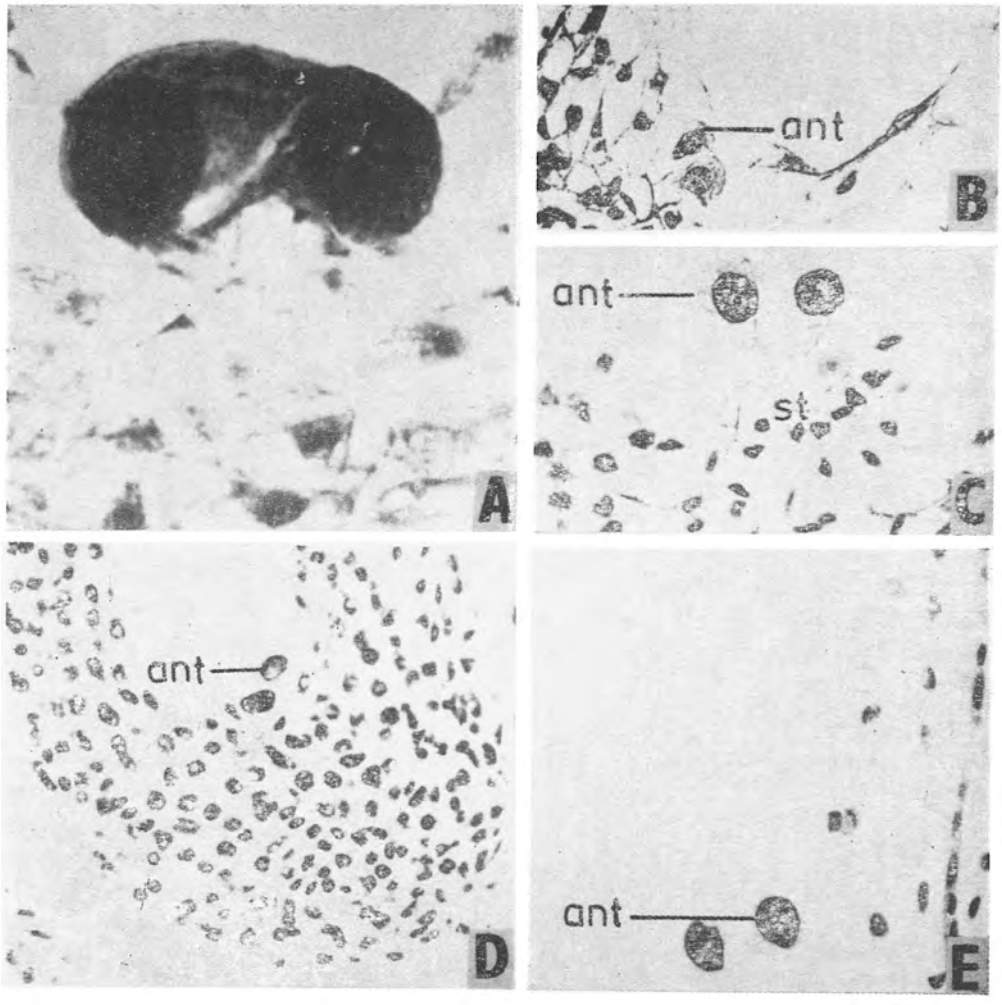


**Figure 2A-E** (**A**, *stained with mercury-bromphenol blue for total proteins;* **B, C**, *stained with alkaline fast green for histones;* **D, E**, *stained for DNA with Feulgen reaction).* *A*, large protein-rich antipodal cells at 2-celled proembryo stage (×1124); *B, C*, chalazal portions of seeds at zygote and 2-celled proembryo stages respectively, showing deeply-stained nuclei of antipodal cells (**ant**). (**st**, nucellar stalk) (× 400 and 482 respectively); *D, E*, parts of longisections of ovules before and after fertilization. In *E* the DNA content in the antipodal nuclei (**ant**) has increased (× 482)

perform different functions. In the degenerated synergid—the filiform apparatus increases the surface area of the plasma membrane—and thereby offers a large area for pollen tube-growth-directing compounds to diffuse out of the synergid. In the persistent synergid, the filiform apparatus is involved in the transference of metabolites into the megagametophyte (*see also* Newcomb, 1973b). The persistent synergid of ***R. sceleratus*** (present work) suggests that it may have a role in the translocation of metabolites to the proembryo.

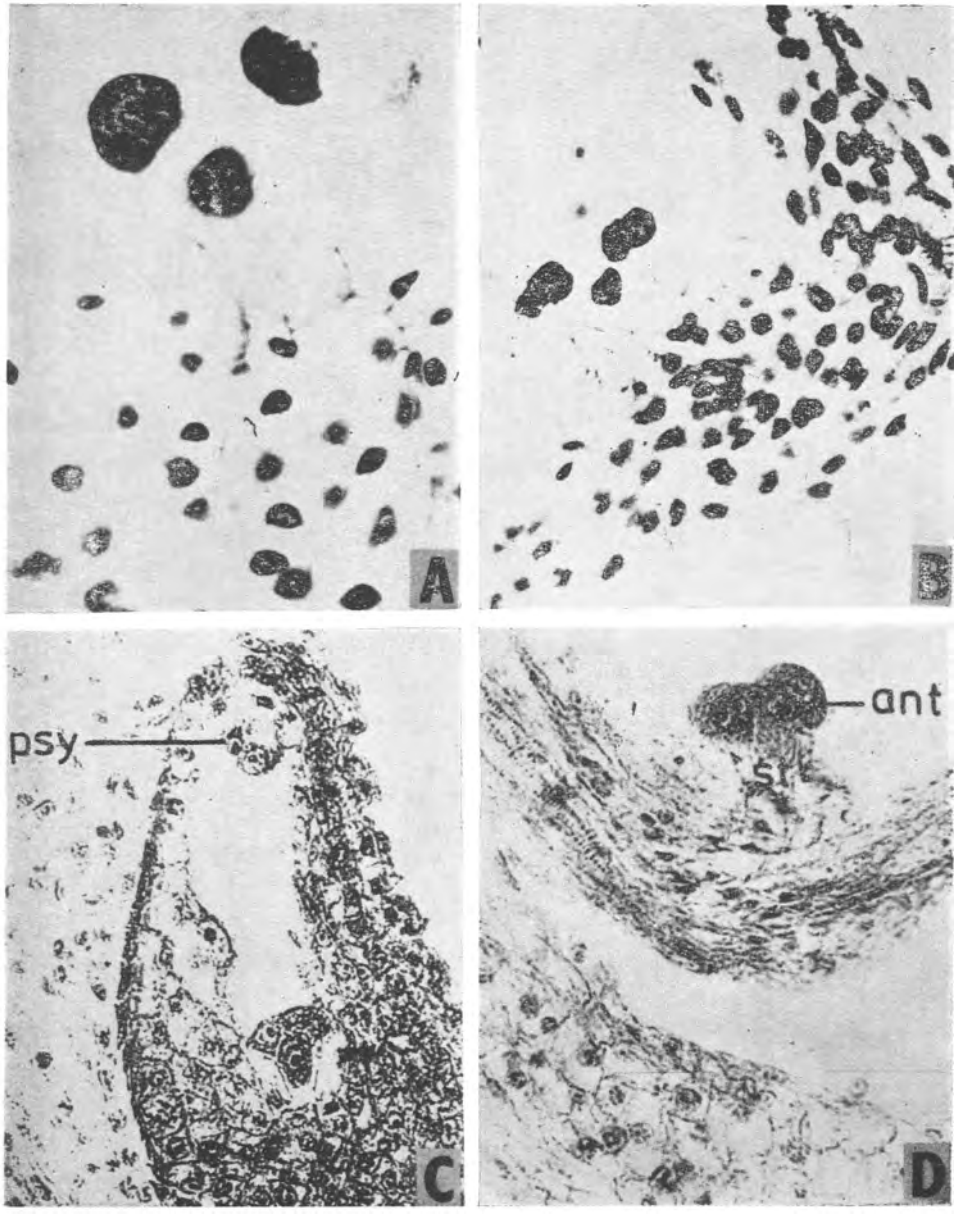


**Figure 3** (**A**, **B**, *stained for DNA with Feulgen reaction;* **C**, **D**, *stained for RNA with pyronin Y*). *A*, antipodal cells at 2-celled proembryo stage. DNA content in the antipodal nuclei has increased considerably (× 710); *B*, chalazal portion of seed at young globular proembryo stage to show intensely-stained antipodal nuclei (×640); *C*, L.S. seeds showing fertilized embryo sac. The persistent synergid (**psy**) and the antipodals are well-stained (× 482); *D*, intensely-stained antipodal cells (**ant**) at 6-celled proembryo stage (**st**, nucellar stalk) (× 482)

### *Antipodal cells*

In *Ranunculus sceleratus* (present work) the antipodal cells persist after fertilization. The presence of a few polysaccharide grains in these cells at about the octant proembryo stage indicates that the antipodal cells are involved in storage activity. In *Ranunculus tripartitus*, the nuclei of persistent antipodal cells show up to 40-fold increase in volume and an enormous increase in DNA content (Patel & Cook 1972). A high DNA content in the antipodal nuclei has been observed in *Zephyranthes rosea* and *Lagenaria vulgaris* (Malik & Vermani 1975) and *Dipcadi montanum* (Panchaksharappa & Syamsundar 1975). Kaltsikes (1973) noted DNA content of 256 C in the antipodal cells of hexaploid triticale and presumed that the persistence of the large antipodal cells is essential for the production of full seeds. It is concluded that in *R. sceleratus* active metabolic synthesis in specialized cells—like antipodes—is associated with high level of polyploidy.

Papillate extensions of the antipodal wall adjacent to the nucellus have been observed in *Linum usitatissimum* (Vazart 1968), *Zea mays* (Diboll 1968) and *Aquilegia vulgaris* (Rifot 1973). Wall projections of this type occur in cells which are actively engaged in absorption and secretion (Gunning & Pate 1969). In *R. sceleratus* (present work), the antipodal cells may have a role in the transfer of nutrients during early stage of embryo development. Function of storage of polysaccharides during embryo maturity.

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