STUDIES IN CYPERACEAE—VIII

Gametophytes and Fertilization in Two Members of Cyperaceae

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The present account deals with male and female gametophytes and fertilization in *Scirpus corymbosus* Heyne and *Fimbristylis monostachya* (Linn.) Hassk., two locally available species of the sedge family.

Material of both the species was collected locally and fixed in 70% formalin-acetic-alcohol. Dedydration and embedding were done according to routine methods. Sections were cut 8–10 microns thick. They were stained in Heidenhain's iron-alum haematoxylin and destained in picric acid. Light green was used as counterstain.

Observations

Microsporogenesis and male gametophyte.—The male archesporium develops hypodermally at 4 corners in the young anther. It consists of a plate of 2 or 3 cells that extends over the length of the anther. Anther development follows the monocotyledonous type of Davis (1966). A four-layered anther wall inclusive of epidermis is developed in both the species (Figs. 1, 2). Some cells of epidermis in mature anther are tannin-filled in *Scirpus corymbosus* while in *Fimbristylis monostachya* most cells are tannin-packed (Fig. 5). The hypodermal layer develops as endothecium with slightly thickened cells that possess spiral bands and globular markings (Fig. 5). Middle layer is ephemeral. Innermost wall layer develops into the glandular tapetum.

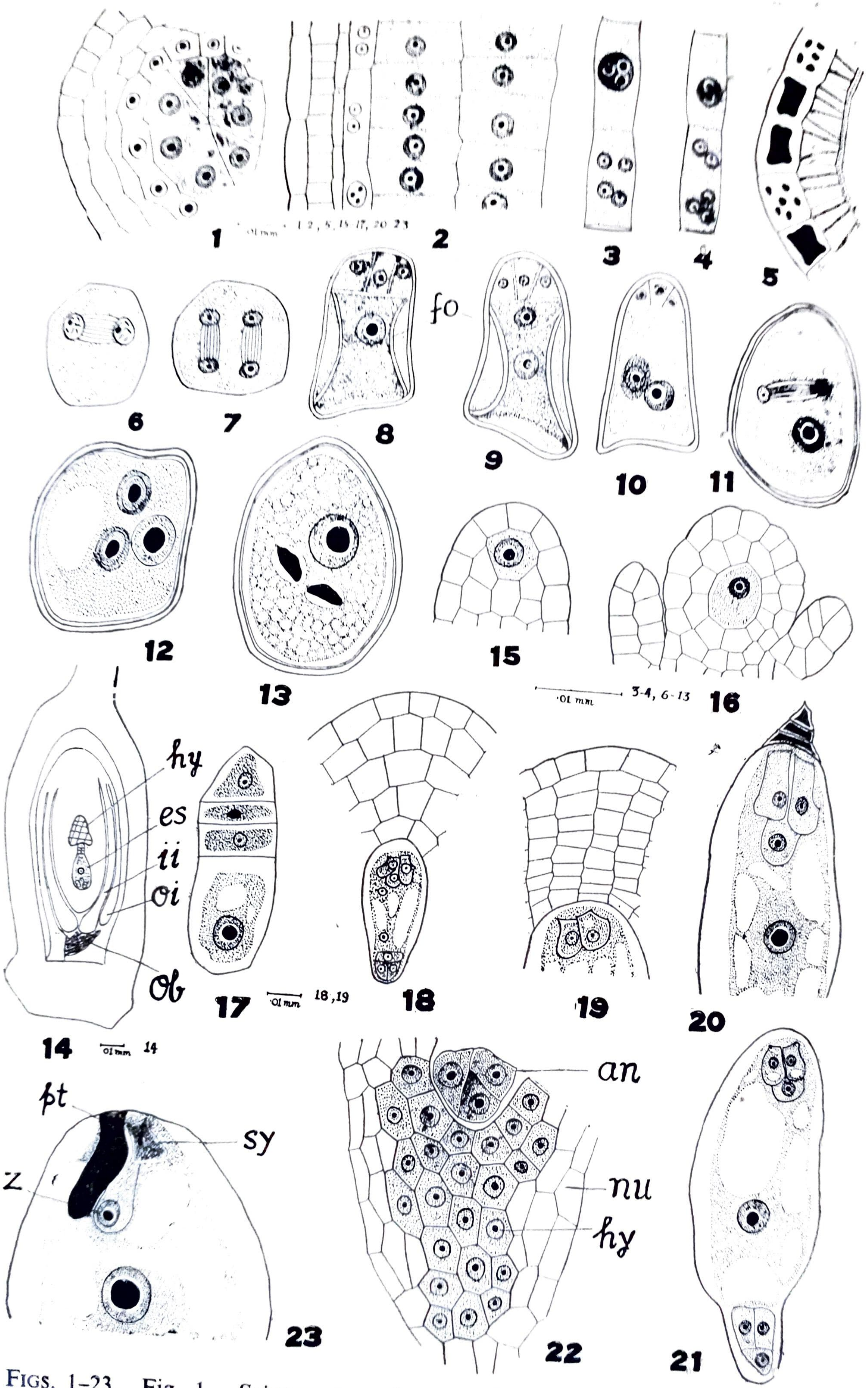
Its cells are uniformly uninucleate in *S. corymbosus*. In *F. monostachya*, however, many later become binucleate (Fig. 2) and some of them show as many as 3 to 5 nuclei (Figs. 3, 4). Subsequent nuclear fusion in some results in polyploid units (Fig. 3).

The primary sporogenous cells divide forming pollen mother cells that look triangular in outline with their apices meeting towards the centre of the anther loculus (Fig. 1). Meiosis (Figs. 6, 7) is not accompanied by wall formation. Consequently pollen mother cells show 4 free lying nuclei for some time. Later, three of these migrate to the narrow end and constitute the non-functional nuclei while the fourth remains in centre and represents the functional nucleus of the pseudomonad. Septum formation is regularly observed in both species (Figs. 8–10). During these post-meiotic stages the wall of the young microspore is thrown into folds and the central portion of the cytoplasm takes a comparatively deep stain (Fig. 8). The effete nuclei do not divide subsequently. They remain healthy till 2-celled stage (Fig. 10) and eventually degenerate.

The first division in young microspore takes place in a plane parallel to its long axis; the resultant generative cell rests on the effete nuclei (Fig. 9). Later on the pollen grain enlarges, vacuoles appear in it and the generative cell gets displaced from its original position (Fig. 10). It divides (Fig. 11) forming two male gametes. Mature pollen grains are thus 3-celled and vacuolate in *F. monostachya* (Fig. 12). In *S. corymbosus* they are highly vacuolate, the cytoplasm presenting alveolar appearance. The gametes here lose nuclear characters and are crescent-shaped bodies with hyaline areas around (Fig. 13).

Ovule.—A single ovule develops from basal placenta. It bends during development and becomes anatropous. It is bitegmic and crasinucellate. The growth of outer integument on the funicular side is suppressed during development and the micropyle is organised only by the inner integument (Fig. 14).

Megasporogenesis and embryo-sac.—The female archesporium develops as a single hypodermal cell (Fig. 15). A periclinal division in it sets apart the primary parietal cell and megaspore mother cell (Fig. 16). Later a parietal tissue of 3 or 4 layers is developed in *F. monostachya* (Fig. 18) while this is massive with 7 or 8 layers in *S. corymbosus* (Fig. 19). Consequently the embryo-sac is deep-seated. The megaspore mother cell enlarges considerably, undergoes meiosis and forms a linear tetrad of megaspores (Fig. 17). It is always the chalazal megaspore that functions and undergoes three successive mitotic divisions to form an octo nucleate megagametophyte of the Polygonum type. The micropylar megaspores eventually degenerate; their remains persisting for a long time (Fig. 20). Mature embryo-sac is broadly elongate with a narrow chalazal end (Figs. 18, 21). Egg is flask-shaped and synergids are hooked. The polars fuse in the centre of the embryo-sac (Figs. 20, 21). The ephemeral antipodals are lodged in a chalazal pouch in *S. corymbosus*. They are arranged variously.



FIGS. 1–23. Fig. 1. *Scirpus corymbosus.* T.s. anther lobe (in part) showing wall layers. Figs. 2–9. *Fimbristylis monostachya.* Fig. 2. L.s. anther lobe (portion) showing wall layers. Figs. 3, 4. Tapetal cells showing nuclear behaviour

Fig. 5. L.s. part of anther wall showing epidermis and endothecium. Figs. 6–7. Meiosis. Fig. 8. Young microspore showing functional and non-functional nuclei. Fig. 9. 2-celled pollen grain. Figs. 10, 11. *Scirpus corymbosus.* Fig. 10. 2-celled pollen grain. Fig. 11. Same with division of generative cell. Fig. 12. *Fimbristylis monostachya.* 3-celled pollen grain. FIGS. 13–14. *Scirpus corymbosus.* Fig. 13. 3-celled pollen grains. Fig. 14. L.S. ovary (Diagrammatic) showing anatropous ovule, obturator and hypostase. Fig. 15. *Fimbristylis monostachya* Female archesporium. Figs. 16, 17. *Scirpus corymbosus.* Fig. 16. Megaspore mother cell. Fig. 17. Linear megaspore tetrad. Fig. 18. *Fimbristylis monostachya.* L.s. apex of nucellus showing parietal layers and embryo-sac. Fig. 19. *Scirpus corymbosus.* Nucellus apex in L.s. showing parietal layer and embryo-sac. Fig. 20. *Fimbristylis monostachya.* Micropylar half of embryo-sac. FIGS. 21–23. *Scirpus corymbosus.* Fig. 21. Mature embryo-sac. Fig. 22. Hypostase and antipodals. Fig. 23. Micropylar half of embryo-sac showing pollen tube, synergid, zygote and primary endosperm nucleus.

an, antipodals; *es*, embryo-sac; *fo*, folds; *hy*, hypostase; *ii*, inner integument; *nu*, nucellus; *ob*, obturator; *oi*, outer integument; *pt*, pollen tube; *sy*, synergid; *z*, zygote.

Hypostase.—This is observed in *S. corymbosus* only. A group of nucellar cells just below the antipodals get marked out from the adjoining cells due to dense cytoplasm and prominent nuclei. It is deep saucer-shaped in appearance (Fig. 22). This hypostase persists up to fertilization and later degenerates.

Obturator.—This embryological character is noticed prominently in *S. corymbosus.* Here a few cells at the base of the funiculus divide repeatedly and form rows of elongated, flexuous cells that cover the micropylar region. This funicular obturator is well developed at the mature embryo-sac stage (Fig. 14). The tubular cells of funiculus are vacuolate and enclose elliptic nuclei. It is believed that obturator facilitates entry of pollen tube and also supplies nourishment to it. In *F. monostachya* obturator is rudimentary in nature.

Fertilization.—The wind-borne pollen grains are caught on the stigmatic surface and, after germination, the pollen tube travels down the stylar canal. It leaps over the ovarian cavity and reaches the ovule. It now enters the micropyle and passing over the nucellar cells finally enters the embryo-sac (Fig. 23). Here the male gametes are discharged. Triple fusion precedes syngamy.

DISCUSSION

In the Cyperaceae anther wall is uniformly four-layered; the epidermis with some tannin-filled cells and spiral endothecium with globular markings only persisting in mature condition. This is also true for the members investigated here. However, these globular markings are not reported by Gupta (1962) in *F. dichotoma* and needs reinvestigation. The glandular tapetum is reported to be uninucleate in a large number of sedges (Dnyansagar and Tiwari, 1956; Gupta, 1962; Shah, 1962; Padhye, 1967, 1968) as also in *S. corymbosus.* However, in *Cyperus tegetum* (Padhye and Moharir, 1958), *C. compressus*

(Padhye, 1959), *Kyllinga triceps* (Padhye, 1960) and *Scirpus mucronatus* (Padhye, 1967) there are reports that at least some cells of tapetum become binucleate prior to degeneration. In *F. monostachya* these cells not only show 3–5 nuclei but also subsequent fusion between them resulting in polyploid nuclei.

Septum formation in the pseudomonad is regularly observed in many members of the family. This is denied in species of *Scirpus* (Piech. 1924, 1928), *F. cericea* (Tanaka, 1939) and recently in *Scleria stocksiana* (Untawale and Padhye, 1969). Gupta (1962) reported an intermediate condition in *F. dichotoma* where a feeble septum develops between the functional and effete nuclei but there is none between the latter.

The young microspores present highly contracted appearance and look as if they are degenerating (Shah, 1962; Padhye, 1967, 1968). Likewise the cytoplasm in the central region of the grain is dense and takes a deep stain. Also folds developing in the pollen wall start from the broader end and converge on the narrow end where the non-functional nuclei are located. This is also true for the species investigated here. It is believed (Padhye, 1967, 1968) that these phenomena may be playing an important role in segregation of non-functional nuclei from the functional one.

Mature pollen grains are 3-celled. This is true for other investigated members of the family. However, 2-celled pollen grains are reported in *F. quinquangularis* (Dnyansagar and Tiwari, 1956) and *F. dichotoma* (Gupta, 1962). This needs reinvestigation.

A parietal tissue is regularly developed in the ovule. But its extent varies considerably in different species. Normally it consists of 2 layers. In *Eleocharis geniculata* (Padhye, 1968) it has 3 layers, in *F. monostachya* 3 or 4 layers while in *F. quinquangularis* (Dnyansagar and Tiwari, 1956), *S. mucronatus* (Padhye, 1967) and *S. corymbosus* it is made up of 6–8 layers. This renders the megagametophyte in these taxa deep-seated. Embryo-sac development in the family is always of the Polygonum type (Maheshwari, 1950).

The funicular obturator is well developed in *S. corymbosus*. This feature is fairly constant in the family. It is reported previously in *Kyllinga triceps* (Padhye, 1960), *C. niveus* (Patel and Shah, 1962) and species of *Kyllinga*, *Cyperus*, *Eleocharis*, *Scirpus* and *Fuirena* studied by Padhye (1967). Only in species of *Fimbristylis* (Padhye, 1967) it is poorly developed and rudimentary. Fertilization is porogamous and triple fusion precedes syngamy.

SUMMARY

Anther wall is four-layered. Epidermis with tannin-filled cells and spiral endothecium with globular markings persist in mature

anther. Middle layer is short-lived. The glandular tapetum has uninucleate cells in *Scirpus corymbosus*. In *Fimbristylis monostachya* these later become 2–5-nucleate. Subsequent nuclear fusion is noticed in some tapetal cells. Pollen mother cells are wedge-shaped and their apices meet towards the centre of anther loculus. Septum formation is regular in both taxa. Mature pollen grains are 3-celled. In *S. corymbosus* the crescent-shaped male gametes are enclosed in alveolar cytoplasm.

Female archesporium is a single hypodermal cell. Parietal tissue of 3–4 cells is developed in *F. monostachya*; in *S. corymbosus* it has 6–8 layers. Megaspore tetrads are linear. Embryo-sac develops after the Polygonum type. Egg apparatus shows normal structure. Secondary nucleus is centrally situated and the antipodals are house in a chalazal pouch in *S. corymbosus*.

A deep saucer-shaped hypostase is developed in *S. corymbosus*. The obturator is funicular in origin and well-developed in *S. corymbosus* while in the other species it is rudimentary. Fertilization is porogamous and triple fusion precedes syngamy.

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REFERENEES

DNYANSAGAR, V. R., AND D. K. TIWARI. 1956. Sporogenesis and gametophytes of *Fimbristylis quinquangularis* Kunth. *Bull. bot. Soc. Univ. Saugar* **8**: 3–6.

GUPTA, M. N. 1962. Morphological studies in Cyperaceae. I. Development of the ovule and gametophytes in *Fimbristylis dichotoma* Vahl. *Agra Univ. J. Res.* **11**: 59–67.

MAHESHWARI, P. 1950. *An Introduction to the Embryology of Angiosperms.* McGraw-Hill Co., New York.

PADHYE, M. D. 1959. Microsporogenesis and male gametophyte of *Cyperus compressus* Linn. *J. biol. Sci.* **2**: 116–117.

———. 1960. A contribution to the life-history of *Kyllinga triceps* Rottb. *Bull. bot. Soc. Coll. Sci. Nagpur* **1**: 1–15.

———. 1967. *Ph.D. Thesis.* Nagpur University.

———. 1968. Male and female gametophytes of *Eleocharis geniculata* Roem et Schult. *Proc. Indian Acad. Sci.* B **67**: 54–60.

———, AND S. K. MOHARIR. 1958. Studies in embryology of *Cyperus tegetum* Roxb. *Ibid.* B **48**: 89–95.

PIECH, K. 1924. Zur Entwicklung der Pöllenkörner bei *Scirpus lacustris* L. *Bull. Acad. Polon. Sci. Lettr.* 113–123.

———. 1928. Zytologische Studien an der Gattung *Scirpus*. *Ibid.* B: 1–43.

PATEL, C. M., AND S. K. SHAH. 1962. Life-history of *Cyperus niveus* Retz. *J. Gujarath Univ.* **5**: 148–158.

SHAH, C. K. 1962. Pollen development in some members of the Cyperaceae. In *Plant Embryology.* A Symposium C.S.I.R., New Delhi, 81–93.

TANAKA, N. 1939. Chromosome studies in Cyperaceae. VII. Chromosome number and pollen development of *Fimbristylis. Bot. Mag. Tokyo* **53**: 480–488.

UNTAWALE, A. G., AND M. D. PADHYE. 1969. Embryological studies in the Cyperaceae. 3. Sporogenesis and gametophytes in *Scleria stocksiana* Boeck. *Proc.* 56th *Indian Sci Congr. Abst*, 377.