EMBRYOLOGICAL INVESTIGATIONS IN *Utricularia aurea* LOUR (LENTIBULARIACEAE)

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ABSTRACT

In the present embryological investigations, the taxonomic consideration and affinities of the family Lentibulariaceae has been studied embryologically by studying the taxon Eutricularia aurea. Family resembles superficially with some members of the Scrophulariaceae. Hutchinson (1973), Stebbins (1974) and Takhtajan (1980, 1997) are of opinion that it is derived from Scrophulariaceae and is very close to it. The findings in the present investigation will help to justify the position of the families of the order Tubiflorae. The material for the present investigation was collected from localities in and around Nagpur District. The taxon Utricularia aurea shows some noteworthy features. The development of anther is of typical dicotyledonous type (Davis 1966) (Plate-XV, A). The anther is bisporangiate with a persistent epidermis, ten or more embryo sac developed concurrently and maximum reach maturity, the embryo sac behaves like the haustorium since its tip protruded out of the integument, the taxon is hydrophytic, the pollen germination is polysiphonous, endosperm formation is free nuclear, The embryogemy recognized as Onagrad type of Maheshwari (1950).

Keywords: *Embryology, Tubiflorae, Scrophulariaceae, Haustorium, Utricularia aurea, polysiphonous, Onagrad.*

Introduction

Angiosperm is a highly evolved group of Plant kingdom is widespread in its distribution and also exhibits the dominant vegetation in the world. Diversity in angiospermous taxa is at high level because of unique pollination and genetic mechanisms. The group is also showing its distinctness due to adaptive features both in external and internal characters.

The taxonomic consideration and affinities of the family Lentibulariaceae has been studied embryologically by studying the taxon *Eutricularia aurea*.

Family Lentibulariaceae

Family resembles superficially with some members of the Scrophulariaceae but distinguished from them by the presence of only 2 stamens. One celled anther lobe, free central placentation and insectivorous nature. Hutchinson (1973), Stebbins (1974) and Takhtajan (1980, 1997) are of opinion that it is derived from Scrophulariaceae and is very close to it. Khan (1970) also expressed the same view on the basis of morphological and embryological features. The findings in the present investigation will help to justify the position of the families of the order Tubiflorae.

Materials and Methods

The material for the present investigation was collected from localities in and around Nagpur District. The Nagpur District lies between 20^{0} 30'and 21^{0} 45' North latitude and 78^{0} 15' and 79^{0} 40' East longitude covering an area of about 9864 km^{2}. The maximum temperature during summer is 40.68º C and in winter 19.2º C. The tropical forest includes the dense vegetation and variations in the floristic composition are found (Nagpur District Gazetteer).

The embryological investigations were carried out on selected taxon of Lentibulariaceae. The taxonomic description of the taxon studied is given below.

Observations

Studies in the embryology of *Utricularia aurea* Lour (Lentibulariaceae)

The chapter includes the embryological finding in *Utricularia aurea*Lour. The study includes development of anther and male gametophyte, development of ovule and female gametophyte, embryo sac, fertilization, endosperm, embryogeny, seed and pericarp.

Microsporogenesis and male Gametophyte

The development of anther is of typical dicotyledonous type (Davis 1966) (Plate-XV, A). The anther is bisporangiate with a persistent epidermis. Single lobe of anther with centrally placed connective found (Plate-XV, B). At maturity two sporangia in a lobe become confluent due to the breakdown of the partition wall between them.

A young anther comprises a homogenous mass of cells almost rectangular bounded by well defined epidermis. During the development of anther it appears two lobed. A plate of archesporial cells comprising 4-5cells which are hypodermal becomes more prominent because of their larger size. These cells are characterized by conspicuous nuclei constitute the archesporium. In the transverse section the archesporium is composed of a single row of cells. It appears as a complete plate of cells. The broad sterile columella is present in the center (Plate-XV, B). The archesporial cells divide periclinally cutting of parietal cells towards the epidermis and primary sporogenous cells towards the inner side. The cells of primary parietal layer undergo anticlinal and periclinal division gives rise to endothecium, middle layer and tapetum.

The outermost layer epidermis below which singles layered endothecium, ephemeral middle layer and the uninucleate tapetum (Plate-XV, E) is present. The glandular tapetum seems to be uninucleate in the initial stages but later it becomes binucleate .

The single layered endothecium is originated from the parietal layer. The cells of endothecium show radial elongation and it develops to the maximum extent in the taxon studied here. Only few cells of endothecium show fibrous bands extended from the inner tangential wall towards the outer layer. This is because the species is hydrophytic. These endothecial cells are absent at the junction of the two sporangia. The middle layer is ephemeral. It degenerates during meiosis in the pollen mother.

The tapetum provided nourishment to the developing spore mother cells and pollen grains. Finally it is disorganized. The tapetal cells possess the plastids, ‘pro ubisch’ bodies and peripherally associated vesicles. The ‘pro ubisch’ bodies have been extruded out of the tapetal cells, coated with the sporopollenin forming the ‘ubisch bodies’. After contributing to the developing sporogenous mass, the inner layer of the tapetum becomes thinner. After the deposition of sporopollenin, the tapetal cell wall degenerates *in situ*. The primary sporogenous cells directly functions as the microspore mother cells. The microspore mother cells appear as peripheral since the two anther locule separated by the broad columella. Each locule is considerably of smaller size. The cells of columella are parenchymatous and are continuous from the filament towards the stomium. The pollen mother cells undergo meiosis and appear to be hexagonal, enclosed in a thick callose wall. The callose walls are interconnected with the cytoplasmic channels but this connection lacks between the tapetal cells and the pollen mother cells. These massive cytoplasmic channels provide passage to move the cytoplasmic contents from one cell to other. The pollen mother cells undergo meiotic division followed by simultaneous cytokinesis results in the formation of microspore tetrads. The microspores are arranged mostly in a tetrahedral manner but few in opposite decussate manner .

The microspores are uninucleate at the time of separation. They get free in the anther locule by the breakdown of the common callose wall. The microspore is richly cytoplasmic with a prominent centrally located nucleus. The pollen grain expanded rapidly by the uptake of locular fluid and two celled when release. The nucleus of microspore divides to form vegetative nucleus and generative nucleus.The generative nucleus forms two male gametes. However, pollen grains are 2-celled when shed. The dehiscence of anther is by vertical slit. The presence of 8-10 celled columella pushes the pollen grains towards the peripheral portion of locule The septum slightly protrudes out and gradually absorbs followed by dehiscence.

Megasporogenesis and Development of Female Gametophyte

The taxon *Utricularia aurea* shows some noteworthy features. The gynoecium is swollen in the central region. The ovules arranged in a free central manner (Plate-XV, A). It has been observed that ten or more embryo sac developed concurrently and maximum reach maturity. The ovules are anatropous. They are unitegmic and tenuinucellate. The micropyle is absent and the pollen tube enters the embryo sac through the passage between the integument and the placenta. The lower narrow end is embedded in the chalaza surrounded by nutritive cells. The middle portion of the sac is lined by the endothelium. The upper bulbous region protrudes out beyond the ovule into the ovarian cavity.

The development of ovule initiates with the small nucellar epidermis. It undergoes curvature due to the rapid growth on one side. At this time the primordia of integument can be seen arising at the base of the nucellus. The massive integument surrounds the nucellus completely. The micropyle is not organized.

The inner wall of the integument is densely cytoplasmic forming the integumentary tapetum or endothelium. The cells of endothelium are thick, radially elongated with prominent nuclei in the center of each cell.The integument is 2-4 layered at micropylar end and forms the hood near the chalazal end. The integument close to the funiculus seems to be fused with it. In most of the ovule the integument is indistinguishable from the funiculus because of the congenital fusion.

The female archesporium directly functions as the megaspore mother cell. Before meiosis the megaspore mother cell undergoes slight elongation. The first meiotic division results into the formation of dyad which after second meiotic division forms linear megaspore tetrad. Out of the four megaspore the chalazal megaspore remains functional and the three micropylar megaspores degenerate. The degenerating megaspores can be clearly seen. The functional chalazal megaspore shows slight increase in the size with a conspicuous nucleus. By this time the flattened cells of endothelium cover almost the entire developing embryo sac. Densely cytoplasmic functional megaspore undergoes meiotic divisions to form two nucleate embryo sac. The nucellar epidermis degenerates at this stage. The two successive mitotic divisions resulting in the formation of four nucleate and eight nucleate embryo sac of Polygonum type (Maheshwari,1950).

The mature embryo sac comprises egg apparatus, central cell and antipodals. The tip of the embryo sac grows through the ovule and comes in direct contact with the placental tissues. It starts penetrating into the placental nutritive tissue and behaves like the haustorium. Prior to fertilization the two polars fuse to form secondary.

Fertilization

Fertilization initiates with the landing of pollen grains on the stigma. It has been observed that the pollen tube can easily reach the egg because of short style and swollen ovary .Moreover, the taxon is hydrophytic therefore the water contents on the stigmatic surface favours the pollen germination. The pollen tube pierces through the stigmatic papillae and enters the ovular surface. Many pollen tubes enter the stigmatic papillae and fuses with the egg (Plate-XV, D). Thus, the germination is polysiphonous. The pollen tubes travels through the stylar canal and enters the embryo sac through the passage between the integument and the placenta. One of the sperm fuses with the egg brings about syngamy while the other fuses with the secondary nucleus forming primary endosperm nucleus. During the act of fertilization the embryo sac is very much rich in starch contents. The endothelium is also densely cytoplasmic.

Endosperm

The primary endosperm nucleus lies in the middle of the embryo sac immediately after its formation. The membrane of the primary endosperm nucleus is contributed by the secondary nucleus and the male nucleus. This dual contribution increases its metabolic activity and initiates the cellular differentiation. The special details regarding endosperm formation has been observed. The endosperm formation is free nuclear. The division takes place soon after syngamy forming two daughter nuclei. When free nuclei are form wall formation takes place. In this taxon few interesting features have been observed by the author. Firstly the degeneration of the nucellar epidermis and the protrusion of the embryo sac through the so called micropyle and secondly the micropylar haustorium comes to lie in direct contact with the placental nutritional tissue.) (Plate-XVII, B). The walls of the placental cells frequently breakdown and their nuclei become scattered in a common mass of cytoplasm along with the nuclei of the haustorium.

Embryo

Syngamy precedes the triple fusion. The zygote is pear shaped with dense cytoplasmic contents. It divides by the transverse wall to form apical cell *ca* and basal cell *cb* (Plate-XVII, C). Both these cells soon divide transversely to form four cells which are arranged in a superposed manner. The four cells are *l,l'* and *m* and *ci*. Thus the pro-embryo at the end of second cell generation comprising four cells arranged in superposed manner. The transverse division in the derivatives of *ca* continues (Plate-XVII, E, F).

The morphological and embryological characters are taken into consideration to correlate the taxon. On the basis of embryological characters, one can prepare the dendrogram which shows affinities amongst families studied.

Discussion and Conclusions

In order to resolve the issue of the relationship of different families included in the Tubiflorae, the embryological characters analysis have been carried out to discuss the relationship between the taxa. The family studied in the present investigation is Lentibulariaceae. In the same way if One or two taxa have been studied belonging to each family amongst Tubiflorae, and accordingly taxonomic relationship has been discussed among the families.

Family: Lentibulariaceae

The genus *Utricularia aurea* falls under the group of carnivorous plants commonly called Bladderworts. Embryological studies on several Indian speices of *Utricularia* have been carried out. In the present study on *U. aurea*, the development of anther is of typical dicotyledenous type, the anther is tetrasporangiate with a persistent epidermis. The plate of archesporial cells comprising 4-5 cells, hypodermal in origin is radially elongated. The same is observed in *Utricularia stellaris* (Farooq, 1958); *Utricularia wallichiana* (Khan et al 1964); *Utricularia scandens* (Farooq and Bilquis, 1966); *Utricularia flexuosa* (Khan, 1954).

The single layered endothecium is originated from parietal layer but only few cells shows fibrous thickening. The endothecial cells are absent at the junction of the two sporangia (present study). A prominent fibrillar endothecium is shown in *Utricularia flexuosa* (Khan, 1954); *Utricularia scandens* (Farooq and Bilquis, 1966); *Utricularia exoleta* (Shivaramaiah, 1964a); *Utricularia caerulea* (Siddiqui, 1965a).

The presence of broad columella in between two anther locule is another feature of the present study, the cells of which are parenchymatous. This is the unusual feature not found in all the members of Lentibulariaceae. The pollen grains are 3-celled when shed in *Utricularia flexuosa* (Khan, 1963a). In Utricularia *aurea* (Present study), the pollen grains liberate at 2-celled stage. The *in situ* pollen germination is common in *U. flexuosa* and *U. stellaris* (Khan, 1952; Farooq, 1964a). Shedding at 3-celled stage and *in situ* germination, enclosed starch grains also resulted in *U. scandens* (Shivramaiah et al, 1975).

The ovule assumes the anatropous form (Present study). The same is noticed in *U.stellaris* var *inflexa* (Farooq, 1964a, b). Primordium of integument appears very early and surrounds the nucellus completely, micropyle has not been observed (present study)

The variations in the form of the ovules described are interesting because they range from orthotropous to anatropous forms throughout the taxa of entire family. Occasional presence of hemianatropous ovules has been reported in *U. flexuosa* (Khan, 1954). In *U. flexuosa* (Khan, 1954) and *U. stellaris* var *inflexa* (Farooq, 1965) the entire spherical surface of the placenta is covered with numerous ovules as in present study. In *U. coerulea* (Kausik, 1938); *U. reticulata* (Kausik and Raju, 1955) the apical part of the placenta bears no ovules.

The single hypodermal archesporial cell in the ovular primordium directly functions as the megaspore mother cell (Farooq, 1965; Kausik and Raju, 1955; Kausik, 1938; Khan, 1954; Present study). The first transverse division in the megaspore mother cell gives rise to a dyad cell and a second division to a linear tetrad. (Present study). In *U. flexuosa* (Khan, 1954) and *U. stellaris*, var. *inflexa* (Farooq, 1965), the division in dyad cells is simultaneous but in the latter the division in the micropylar dyad cell may be slightly delayed. In *U. coerulea* (Kausik, 1938), the micropylar dyad cell divides after the chalazal one. Moreover, the megaspore tetrads with variable forms have been reported in *U. flexuosa* (Khan, 1954), but inverted (┴) shaped and L-shaped tetrads observed in *U. stellaris* var. *inflexa* (Farooq, 1965) appears to be the first record in the Lentibulariaceae.

The chalazal megaspores of a linear tetrad develop into a Polygonum type of embryo sac (present study). Occasionally one or all the megaspores of an old tetrad may be healthy as in *U. stellaris* var. *inflexa* (Farooq, 1965).

In *Utricularia aurea* (Present study), the tip of the embryo sac grows through the ovule and comes in direct contact with the placental tissues. It penetrates in to it and behaves like the haustorium. In *U. coerulea*, the egg apparatus is situated at the placental surface, completely outside the ovule (Kausik, 1938). Moreover, presence of an egg-like synergid has been reported in *U. flexuosa* by Khan (1954) as an occasional feature. But its occurrence is frequent in *U. stellaris* var. *inflexa* (Farooq, 1965). Merz (1897) also reported the apical part of embryo sac in *Utricularia* extends beyond the limits of the ovule and is haustorial. In *Genlisea* (Merl, 1915) nutritive tissue is not organized outside the ovule and in *Pinguicula* (Merz, 1897; Stolt, 1936) the nutritive tissue is totally wanting and thus in these two genera the embryo sac remains within the ovule.

The two polars fuses before fertilization forming the secondary nucleus (Present study). But the occurrence of more than two polars in an embryo sac, due to the migration of more than one nucleus of the chalazal quartet is not a rare phenomenon according to Maheshwari (1950). The members *U. flexuosa* (Khan, 1954) and *U. stellaris* var. *inflexa* in which two or even three nuclei of the micropylar quartet of the embryo sac behaves as polars (Farooq, 1964a, b).

The embryo sac with reverse polarity is of the usual occurrence in *Utricularia* though it is not the usual organization of a normal sac (Maheshwari, 1950). The situation in *U. stellaris* var. *inflexa* (Farooq, 1964a, b) and *U. flexuosa* (Khan, 1954) is somewhat different. Here the embryo sac instead of growing on its micropylar end, consumes the chalazal tissue and develops at the chalaza. In *U. flexuosa*, 6-nucleate matare embryo sac and reverse polarity have been reported by Khan (1953, 1954). Moreover, multiple embryo sac has also been reported in *U. flexuosa* (Khan, 1954).

The inner wall of the integument is densely cytoplasmic forming integumentary tapetum. The cells are thick and radially elongated with prominent nuclei. (Present study). However, in a large number of ovules of *U. aurea* (Syn. *U. flexuosa*) and *U. stellaris* (Syn. *U. inflexa* spp. *stellaris*), a ususual behaviour of the endothelium on the funicular side has been observed (Khan, 1963a; Farooq, 1964a). The walls of the endothecial cells break down and their nuclei come to lie in a common cavity. An endothelium is differentiated and usually encloses the lower portion of the embryo sac while in *Utricularia* sp. including present taxon the embryo sac becomes extraovular invade in placenta and behaves like haustoria.

The endosperm formation is *ab initio* cellular in *U. aurea* (present study) where the aggressive micropylar haustorium comes in direct contact with the nutritive tissue of the placenta. The placental nuclei and the nuclei of haustorium lie in the common mass of cytoplasm due to the breakdown of the placental walls. (present study). The two-nucleate micropylar haustoria has been reported in *U. scandens* where chalazal haustorium may extend into the funicle and show nuclear divisons (Shivaramaiah et al 1967). The same has been noted in *U. stellaris* (Farooq, 1964 a,b).

The zygote consists of maximum concentration of the starch grains. The first division produces *ca* towards chalazal end and *cb* towards the micropylar end. Here, in *U. aurea* (present study), it is observed that basal cell plays only a minor role in the development of embryo proper. The embryogemy thus recognized as Onagrad type of Maheshwari (1950).

These conditions throw light on the ancestral types of the Lentibulariaceae with a bilocular ovary and axite placentation as found in the Scroplulariaceae. There are similarities in some of the embryological features of these two families. Even. In some members of both the families, the apex of the embryo sac becomes extra ovular.

References

1. Davis GL. 1966.Systematic Embryology of the Angiosperms. New York: John Wiley and Sons.
2. Farooq M. 1958. The development of the embryo in Utricularia stellaris Linn. f. var. inflexa. Sci. Cult.23: 479-480.
3. Farooq M. 1964a. Studies in the Lentibulariaceae I. The embryology of Utricularia stellaris Linn. f. var. inflexa Clarke. Part I. Flower, organogeny, ovary, megasporogenesis and female gametophyte. Proc. Natn. Inst. Sci. India30b: 263-279.
4. Farooq M. 1964b. Studies in the Lentibulariaceae I. The embryology of Utricularia stellaris Linn. f. var. inflexa Clarke. Part II. Microsporangium male gametophyte fertilization, endosperm embryo and seed. Proc. Natn. Inst. Sci. India30b: 280-299.
5. Farooq M. 1965a. Studies in the Lentibularuaceae. II. The embryology of Utricularia arcuata Wt. J. Indian Bot. Soc.44: 326-346.
6. Farooq M. 1965b. Studies in the Lentibularuaceae. III. The embryology of Utricularia uliginosa Vahl. Phytomorphylogy15: 123-131.
7. Farooq M. 1966. Studies on the Lentibularuaceae. IV. The embryology IV The embryology of Utricularia striatula Sm. J. Indian Bot. Soc.45: 1-13.
8. Farooq M, Bilquis S. 1966. Studies in the Lentibulariceae. VII. The embryology in Utricularia scandens Benj. Beitr. Biol. PA.42: 127-131.
9. Hutchinson J.1973.The Families of Flowering Plants (2 Vols). 3rd Edition Macmillan London
10. Khan R. 1952. Morphology and embryology of Utricularia and some other plants. Ph. D. Thesis, Univ. Delhi.
11. Khan R. 1953 Haustorial behaviour of the chalazal end of embryo sac and reversion of polarity in Utricularia flexuosa Vahl. Curr. Sci22: 179-180.
12. Khan R. 1954. A contribution to the embryology of Utricularia flexuosa Vahl. Phytomorphology4: 80-117.
13. Khan R. 1963a. The phenomenon of degeneration in the ovules of Utricularia flexuosa Vahl. Proc. 50th Indian Sci. Cong. Assoc. Delhi 382-383.
14. Khan R. 1963b. The behaviour of the integumentary tapetum in the ovules containing degenerating gametophytes in ovules containing degenerating gametophytes in Utricularia flexuosa Vahl. Proc. Natn. Acad. Sci. India.33b: 651-655.
15. Khan R. 1970. Lentibulariaceae Its: Symposium on comparative embryology of angiosperms. Indian Natn. Sci. Acad. 290-297.
16. Khan R, Siddiqui SA, ZaidiZH1964. Development of the ovule, the anther and the gametophytes in Utricularia wallichiana Wt. Proc. 51st and 52nd Indian Sci. Cong. Assoc. Calcutta pt. III. Abstr. 340.
17. Maheshwari P. 1950.An introduction to the embryology of angiosperms. McGraw – Hill Company. Toronto, New York.
18. Shivaramaiah G. 1964a. A contribution to the embryology of Utricularia exoleta R. Br. Curr. Sci.33: 501-503.
19. Shivaramaiah G. 1964b. A contribution to the embryology of Utricularia wallichiana Wt. Curr. Sci.33: 657-658.
20. Shivaramaiah G. 1967. Observations on the floral morphology and embryology of Utricularia stricticaulis Stapf. Proc. Indian Acad. Sci.65B: 56-62.
21. Shivaramaiah G, Rajan S, Rao KS. 1975. A contribution to the embryology of Utricularia scandens Oliv. Curr. Sci.44:327-328
22. Stebbins GL. 1974.Flowering Plants: Evolution at Above the Species Level. Belknap, Cambridge MA
23. Takhatajan A. 1967.A System and Phylogeny of Flowering Plants (in Russian). Moscow and Leningrad.
24. Takhatajan A. 1980.Outline of the classification of flowering plants (Magnoliophya). Botanical Review 46 (3): 225–359