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EMBRYOLOGY OF *LIPOCARPHA* WITH A BRIEF DISCUSSION ON ITS SYSTEMATIC POSITION[1]

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ABSTRACT

The paper describes embryology of *Lipocarpha sphacelata* Kunth. and *L. argentea* R. Br. The male archesporium is arranged in a single vertical row. A 4-layered anther wall develops according to the Monocot type of Davis (1966). Endothecial cells show spiral thickening. Mature pollen grains are 3-celled.

A sinlge anatropous ovule develops from basal placenta. Female archesporium is unicellular in *L. sphacelata* but, in *L. argentea* occasionally 2 cells are observed. Female gametophyte develops after the Polygonum type. A funicular obturator and hypostase are differentiated. Fertilization is porogamous and embryo development corresponds to the *Juncus* variation of Onagrad type.

INTRODUCTION

The Cyperaceae is cosmopolitan in distribution being represented by 4000 species (Koyama, 1961). Embryological work on the family dates as far back as 1879 and 1884 when Elfving and Strasburger studied its pollen development. Hicks (1929) reported normal embryo sac development in *Cyperus natalensis*. Earlier work on pollen development by Piech (1928) and Tanaka (1940, 1941) was reviewed by Maheshwari (1950) who indicated certain important points for further investigation. Padhye (1971a, b) studied embryology of *Cyperus iria* and *Kyllinga brevifolia*. Besides pollen development, pollen morphology, structure of seed coat and pericarp are quite interesting and require further study. The importance of pollen morphology for taxonomic consideration has been neglected. The present study incorporates pollen morphology also.

MATERIAL AND METHODS

The spikelets at various stages of development were fixed in 70% F. A. A. Customary methods were employed for dehydration clearing and embedding. Sections, 8-10 μm thick were stained in Heidenhain's iron-alum hematoxylin and counterstained with erythrosin. Pollen morphology was studied by acetolysis method of Erdtman (1952) after incorporating certain modifications suggested by Nair (1960).

OBSERVATIONS

Microsporogenisis and the male Gametophyte : The male archesporium is hypodermal and the cells are arranged in a single vertical row. (Fig. 1). It cuts off primary parietal and sporogenous layers. A 4-layered anther wall (Figs. 2, 3) develops according to 'Monocotyledonous' type of Davis (1966). At anthesis, the epidermis is persistent. In *L. sphacelata* most cells

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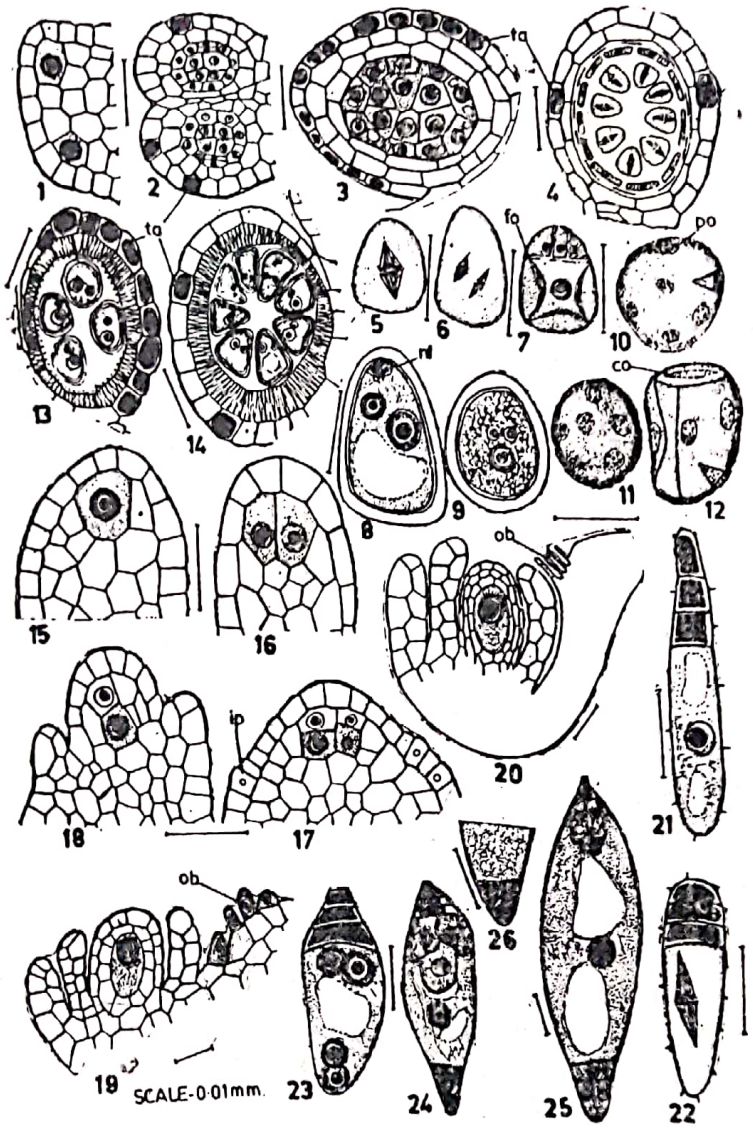
are filled with tannin (Fig. 13). The epidermal cells in the stomium region are small and flattened (Fig. 13). The cells of endothecium show distinct spiral thickenings (Figs. 13, 14). 'Ubisch' granules are present only in *L. sphacelata* (Fig. 13). The glandular tapetum has uninucleate cells (Fig. 3).

The pollen mother cells are wedge shaped (Fig. 3) with their apices meeting towards the centre of the anther loculus. Meiotic divisions in pollen mot her cells



Figs. 1-26. 1-3, 6, 7, 9-11, 13, 15, 17, 19, 22-24.-*Lipocarpha sphacelata* Kunth: Figs. 4, 5, 8, 12, 14, 16, 18, 20, 21, 25, 26.-*L. argentea* R. Br. *co*—colpus; *fo*—fold; ip—integumentary primordium; *ob*—obturator; *po*—pore; *ta*—tannin. Figs. 1, 2, 3 T. S. anther (in part) showing development of anther wall. Fig. 4. T. S. anther lobe showing meiosis I in pollen mother cells; note degenerating middle layer. Fig. 5, 6. Meiosis I & II. Fig. 7. Young microspore showing central functional nucleus and 3-non functional nuclei; note folds. Fig. 8. 2-celled pollen grain; note 4 nonfunctional nuclei. Fig. 9. Mature pollen grain; note starch grains. Figs. 10-12. Acetolysed mature pollen grains; note crustate aperatures and folds. Figs. 13, 14. T. S. anther lobe showing epidermis, endothecium with Spiral thickenings and 2-celled pollen grains. Figs. 15, 16. L. S. ovule showing primary parietal, primary sporogenous and developing integumentary primordia. Figs. 19, 20. L. S. ovule showing megaspore mother cell and developing obturator. Figs. 21, 22. Linear tetrad of megaspores; note division of functional megspore in fig. 21. Fig. 23. 4-nucleate embryo sac. Fig. 24, 25. Mature embryo sac. Fig. 26. Antipodals.

are synchronous and the spindles lie parallel to the long axis (Figs. 4-6). No cell plates are formed and the resulting four nuclei lie free in the mother cell. Soon, three of the four nuclei migrate to the narrow end of the 'pseudomonad' and eventually degenerate. These constitute the non-functional nuclei (Fig. 7). At this time the young microspore enters a phase of contraction with the development of folds on the wall. The cytoplasm around functional nucleus is dense. Probably this results in relegation of the non-functional nuclei to the proximal end (Fig. 7.). In *L. argentea* at times four non-functional nuclei are observed (Fig. 8).

The functional nucleus divides forming a small generative and large vegetative cell. Soon the pollen grains enlarge and develop large vacuole (Fig. 8). The generative cell divides to from spherical male gametes (Fig. 9). Mature pollen grains are 3-celled when shed. The cytoplasm is alveolar and it shows starch grains (Fig. 9).

Pollen morphology : Pollen in *L. sphacelata* are ovoid, pantoporate; pore membrane is distinctly crustate. The diameter ranges from 13-17 μm. Exine is 0.5 μm thick and ectine is thicker than intine. Exine surface is faintly granulose (Figs. 10, 11). In contrast pollen grains of *L. argentea* are 4-aperturate with one distal colpus and 3-zonal pores. The zonal apertures are crustate. Grains are ellipsoidal and diameter ranges from 18-23 μm. Exine thickness is 1.5 μm, ectine being thicker than intine. Grains are somewhat folded and the surface is granular (Fig. 12).

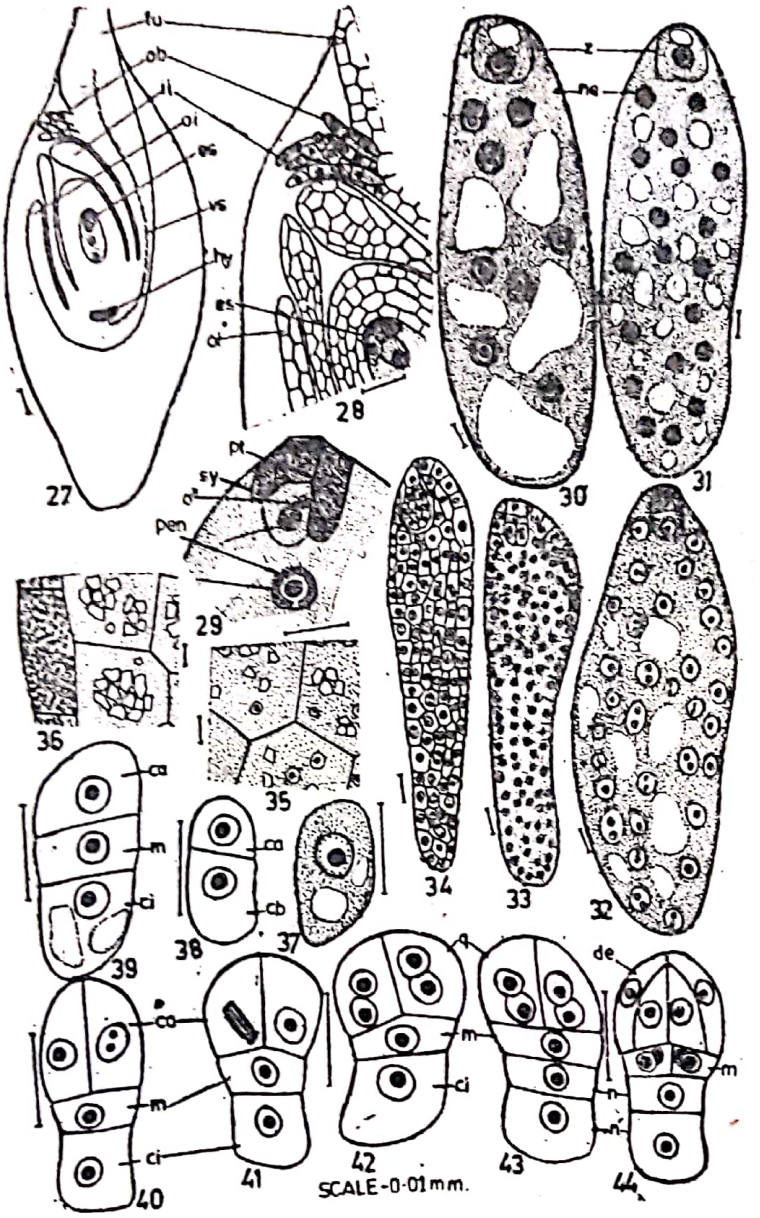
Megasporogenesis and female gametophyte: A single ovule is anatropous, bitegmic and crassinucellate. The integuments arise in succession (Figs. 17, 18). The development of outer integument on the funicular side is arrested (Figs. 19, 20, 27). Both the integuments are two layered, however, in the micropylar region, inner integument becomes 3-4 layered (Fig. 28).

A single archesporial cell differentiates hypodermally in the nucellus (Fig. 15). In *L. argentea* rarely two cells are noticed (Fig. 16). A parietal cell is cut off (Figs. 17, 18) which later forms a 3-layered parietal tissue (Fig. 28). The megaspore mother cell enlarges (Fig. 19) and after meiosis results in a linear tetrad of megaspores (Fig. 21) and undergoes three mitotic divisions to form the Polygonum type of embryo sac (Figs. 22-25). The degenerating remains of the micropylar megaspores persist till embryo sac stage. The egg and synergids show normal structure. The 3 antipodals housed in a chalazal pouch, are linear in *L. sphacelata* (Fig. 24) but T or inverted T shaped in *L. argentea* (Figs. 25, 26). They degenerate after fertilization. At the megaspore mother cell stage, a group of superficial cells at the base of the funiculus elongate (Figs. 19) divide and redivide to form filamentous rows of cells that loosely cover the micropyle. This constitutes the funicular obturator (Figs. 20, 27, 28).

A pad of nucellar cells in chalazal region becomes cutinised and later gets filled with tannin. This constitutes the hypostase. It persists in mature seed (Fig. 27).

Fertilization : It is porogamous. The pollen tube enters the embryo sac through the micropyle. During its passage, it destroys one of the synergids and lies close to egg. One male gamete fuses with secondary nucleus while the other enters the egg (Fig. 29) and later fuses with its nucleus to complete syngamy.

Endosperm : It is *ab inimio* nuclear and as many as 8 nuclei are formed before the first division in zygote in *L. argentea* (Fig.



Figs. 27-44. 27-29, 31-34, 37-39, 41-43. *L. sphacelata* Kunth; 30, 35, 36, 40, 44.-*L. argentea* R. Br. *es*—embryo sac; *fu*—funiculus; *ii*—inner integument; *ne*—nuclear endosperm; *ob*—obturator, *oi*—outer integument; *pen*—primary endosperm nucleus; *pt*—pollen tube; *sy*—synergids; *vs*—vascular supply; *z*—zygote. Fig. 27. L. S. ovule showing obturator, hypostase and embryo sac. Fig. 28. L. S. ovule (micropylar half) showing obturator, 3-layered parietal tissue and egg apparatus of the embryo sac. Fig. 29. L. S. micropylar half of embryo sac showing pollen tube, degenerating synergids, primary endosperm nucleus and second male gamete inside the egg. Figs. 30-33. Stages in the development of free nuclear endosperm; note cellularization in fig. 33. Fig. 34. cellular endosperm. Fig. 35. 36. Endosperm cells with starch grains; note oil sheath in fig. 36. Figs. 37-44. Various stages in the development of embryo.

30) and 16-24 nuclei in *L. sphacelata* (Fig. 31).

At the bi-celled stage of embryo there are about 32-48 nuclei in *L. sphacelata* (Fig. 32). After about 128-256 endosperm nuclei are formed, cellularisation of endosperm sets in around the embryo in a centripetal manner (Fig. 33). Ultimately the endosperm becomes cellular (Fig. 34). The superficial layer of endos-

perm shows feeble meristimatic activity (Fig. 34).

After globular stage of embryo, cells of endosperm show deposition of starch (Fig. 35). Starch grains are polygonal and crowded (Fig. 36). The superficial layer of endosperm is converted into the oil sheath and it is studded with innumerable oil globules (Fig. 36). Mature seeds are endospermous.

Embryogeny : The zygote (Fig. 37) divides transversely forming a bi-celled proembryo where the basal cell *cb* is invariably larger than the terminal cell *ca* (Fig. 38). A transverse division in *cb* forms cells *m* and *ci* (Fig. 39) while a vertical wall in *ca* results into 2 juxtaposed cells (Fig. 40). The proembryonic tetrad is 'T'-shaped.

During third cell generation, cells of *ca* again divide vertically forming a quadrant *q* (Figs. 42, 43). In *L. sphacelata* divisions in *ca* may not be synchronous (Fig. 41). Cell *ci* divides transversely into *n* and *n'* while *m* remains undivided (Fig. 43). In *L. argentea* the division of *ci* is also delayed (Fig. 42). At close of this generation, the proembryo shows 7-cells dispose off in 4 tiers in *L. sphacelata* (Fig. 43) but 6 cells in 3 tires in *L. argentea* (Fig. 42).

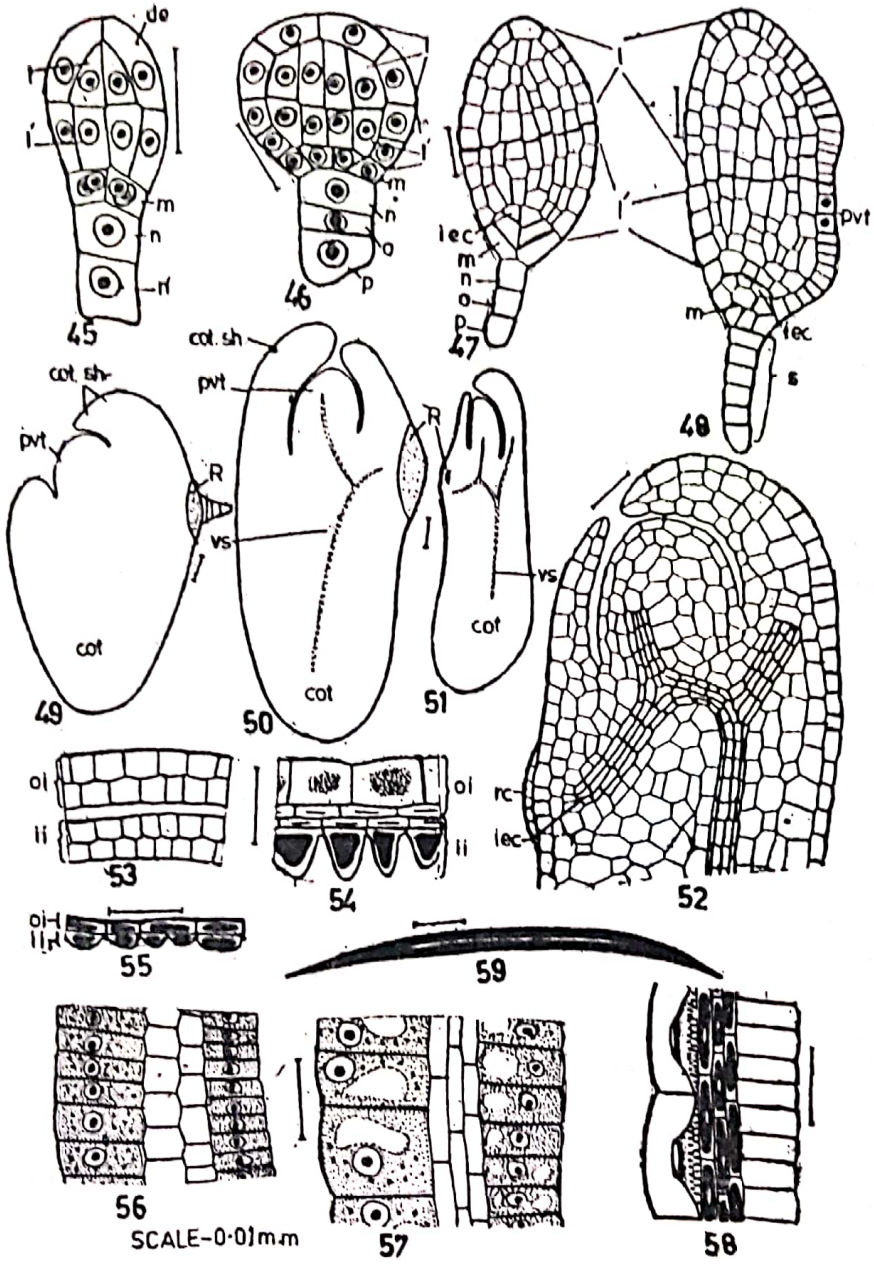
During 4th cell generation cells of *q* divide periclinally (Fig. 44) cutting off dermatogen initials *de* and four central cells in a manner characteristic of the *Juncus* variation of the Onagrad type of Johansen (1950). The cell *m* divides vertically. The two cells of *m* undergo one more vertical division to form a group of 4-cells (Figs. 44, 45). Cells in other tiers do not divide. Thus at close of this generation proembryo has 14 cells disposed in 4 tiers (Fig. 45).

The dermatogen initials *de* divide only anticlinally and form the epidermis of future embryo (Fig. 45). The four central cells divide transversely thus tiers 1 and 1' are demarcated (Fig. 45). Initially the cells of 1 and 1' divide vertically (Fig. 46) and later transversely thus resulting in the elongation of the proembryo (Fig. 47). The cells of *n'* divide first to form *o* and p (Figs. 46, 47) while that of *n* form *r* and *t* (Fig. 48). Thus derivatives of *n* and *n'* form a short suspensor of 4-6 cells (Figs. 47, 48). The cells of tier *q* form cotyledon, stem tip and hypocotyl while *m* gives rise to the radicular portion.

The rudiments of cotyledonary sheath (upper lip form 1 and lower lip form 1') and stem tip (from the tier 1') appear along one side of the embryo (Fig. 48). This disturbs the radial symmetry of the embryo. The epidermal cells of 1 at its base and 1' situated near tier *m* divide anticlinally. The adjascent cells in these regions are comparatively bigger, this results in development of bulges (Fig. 48). The divisions in two flaps of the sheath are more rapid than in the stem tip region, so that the cotyledonary sheath gradually encircles the stem tip at its base (Figs. 49, 51). The radicular portion and suspensor get laterally displaced while plumule and cotyledonary sheath face the micropyle (Figs. 50, 51).

The four cells of tier *m* divide periclinally, thus delimiting 4 outer and 4 inner cells (Fig. 47). The former form the epidermis and root cap of the radicular portion. The latter again divide obliquely forming four cells near the embryonal mass and four cells to the outside (Figs. 47 48). The cells adjacent to the embryonal mass constitute *iec* elements.

Mature Embryo : The single terminal cotyledon forms the bulk of mature embryo. The plumule-radicle axis is curved. The vascular system of the embryo axis is at right angles to that of the cotyle-



Figs. 45-59. 45, 48-50, 53-59.-*L. sphacelata* Kunth; 46, 47, 51, 52.-*L. argentea*.. *cot*—cotyledon; *cot. sh.* —cotyledonary sheath: *iec*—plerome initials; *ii*—inner integument; *oi*—outer integument; *pvt*—stem apex; *rc*—root cap; *s*—suspensor. Figs. 45-48. Various stages in the development of embryo. Fig. 49. Embryo showing cotyledon, stem tip being enveloped by cotyledonary sheath. Fig. 50, 51. Mature embryo. Fig. 52. Upper part of fig. 51 magnified to show cellular details. Figs. 53-58. Various stages in the development of pericarp ; note thickening in the outer epidermis. Fig. 59. Sclereid.

don. The plumule faces the micropyle while the radicle is lateral (Fig. 57). The three histogens are well marked (Fig. 52). The dermatogen is uniseriate the peribelm shows 5-8 layers and the plerome 4-5 layers (Fig. 52).

Seed Coat and Pericarp : Both the integuments are two layered (Fig. 53). During development the inner layer of outer integument and outer layer of inner integument get crushed (Fig. 54). The other layers form the seed coat which is

thus derived from both the integuments. The outer layer of seed coat is flattened while the inner has comparatively larger cells with wavy outline. Both cell layers are filled with tannin (Fig. 55).

The young ovary wall is 4-layered, the outer and inner epidermal covers enclosing 2 layers (Fig. 56). One of the middle layers divides and pericarp becomes 5-layered (Fig. 57). All these layers persist in mature fruit wall. The outer epidermis consists of enlarged, more or less rectangular cells. From the inner tangential wall are developed blunt rod-like projections around a mound of siliceous matter (Fig. 58). The cell wall in the remaining part become thick. The cells of the inner epidermis remain thin walled and enlarge radially (Fig. 58) while those of middle layers are converted into sclereids (Fig. 59).

DISCUSSION

This study reveals that *Lipocarpha* resembles other investigated members of the family in development of pollen grains, embryo sac, endosperm, embryo, and seed coat (Padhye and Moharir 1958; Shah, 1962, 1965; Khanna, 1965; Padhye, 1960, 1967, 1968, 1971a, b; Tiwari, 1969).

The systematic position of *Lipocarpha* appears controversial. Most taxonomists treats it as a separate genus under Cyperaceae (Clarke, 1894; Hutchinson, 1959). Koyama (1961) merged *Lipocarpha*, *Kyllinga* and *Remirea* under *Cyperus* on the basis of prophyll and spikelet characters. Kern (1962), however, believes that the genus is allied to *Scirpus*. It is thus grouped under both the Cypereae and Scirpeae.

The pollen grains of *Lipocarpha* are 4-6 aperturate; the apertures being crustate. Most species of *Cyperus* studied so far, however, possess pollen grains that are characterised by a single colpus at the broad end (Sharma, 1967; Padhye, 1967; Tiwari, 1969). Padhye (1967) opines that this appears a dominant type of aperture condition in the family. Earlier, Erdtman (1952) has stated that the *Carex* type of pollen, characterised by one ulceroid aperture at the distal end and 3 lateral faintly marked poroid or elongate apertures, is the commonest type in the family. Pollen morphology thus does not support merger of *Lipocarpha* with *Cyperus*.

The structure of mature embryo of *Lipocarpha* is similar to that of *Cyperus*. Van der Veken (1965) also found *Lipocarpha* embryo to be of the *Cyperus* type but considered that the evidence for including it under *Cyperus* is insufficient.

According to Metcalfe (1971) anatomical evidence indicate affinity of *Lipocarpha* with *Cyperus*, since the bundle sheath of *Lipocarpha* is often similar to that of genera belonging to Cypereae. Such type of sheath is unheard of in the Scirpeae.

The structure of pericarp in *Lipocarpha* is like that in most species of *Cyperus* (Padhye, 1967, 1971b; Shah, 1962, 1965; Khanna, 1965) especially with respect to outer epidermal cover of pericarp. Such type is unusual in the Scirpeae.

It is thus evident that *Lipocarpha* be attributed to the Cypereae rather than Scirpeae. The embryo type, bundle sheath and pericarp structure favours affinity with *Cyperus* but pollen morphology militates against this. More work on hitherto uninvestigated taxa of *Lipocarpha* is required.

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