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# A CONTRIBUTION TO THE EMBRYOLOGY OF *SCIRPUS ROYLEI* NEES.[1]

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## ABSTRACT

Aspects of embryology of *Scirpus roylei* Nees. are described. The anther wall is four layered. The endothecial cells develop spiral thickenings. The mature pollen grains are three-celled at anthesis and enclose starch grains. The female archesporium is unicellular and female gametophyte conforms to the Polygonum type. The obturator is funicular in origin. Embryo development follows the Juncus variation of Onagrad type. The development and structure of seed coat and pericarp are described.

## INTRODUCTION

The literature on the embryology of the Cyperaceae has been reviewed by Schnarf (1931) and Maheshwari (1950). The embryology of *Scirpus mucrinatus* was studied by Khanna (1965) who assigned the embryogeny to Asterad type, while Padhye (1967) studied the same species under the name *S. mucronatus* (which spelling as per Index Kewensis appears to be true) and described it under Juncus variation of Onagrad type (Johansen, 1950). The same is the situation in *Scirpus littoralis* (Shah and Neelakandan, 1971). Van der Veken (1965) after examining the embryology of many species of *Scirpus* concluded that the genus needs to be subdivided into at least 6 genera. The different aspects in the life history of *Scirpus roylei* have been discussed by the authors in the present paper.

## MATERIALS AND METHODS

Material was collected from Nasik (M. S.) and was fixed in F. A. A. having 70% ethanol during morning and evening hours. Customary methods of dehydration, clearing and embedding were followed. Sections were cut at 8-14 microns and stained with Heidenhain's iron-alum hematoxylin. Erythrosin was used as counterstain.

## OBSERVATIONS

*Microsporogenesis and Male gametophyte* : The male archesporium as seen in cross section is unicellular and hypodermal (Fig. 1). The anther wall consists of four layers (Figs. 2,3,4) including the epidermis. Its development conforms to the Monocotyledons type of Davis (1966). The epidermis which persists till anthesis has some of its cells

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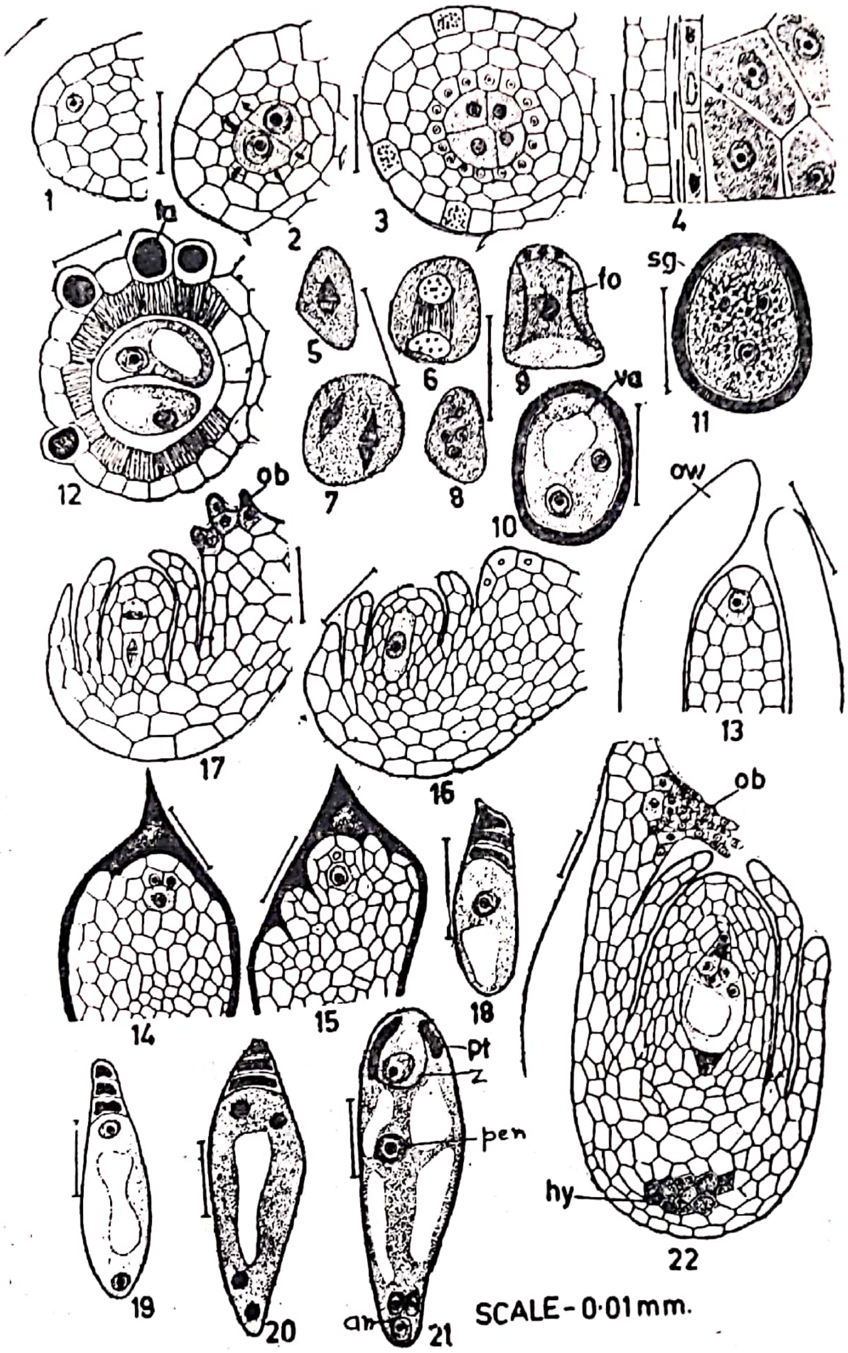


Figs. 1-22. Embryology of *Scirpus roylei* Nees.

Fig. 1. T. S. anther lobe showing unicellular anchesporium. Figs. 2-4. Stages in the development of anther wall. Figs. 5-8. Meiotic divisions in pollen mother cell. Fig. 9. Young pollen grain; note folds and non-functional nuclei. Fig. 10. Bicelled pollen grain. Fig. 11. Mature pollen grain; note starch grains. Fig. 12. T.S. of mature anther (in part). Fig. 13. L.S. ovule showing archesporium. Figs. 14, 15. L.S. ovule showing primary perietal cell and primary sporogenous cell. Figs. 16, 17 L.S. ovule showing megaspore mother cell and dyad respectively. Figs. 19, 20. 2-nucleate and 4 nucleate embryo sac respectively. Fig. 21. Mature Embryo sac soon after fertilization showing pollen tube, zygote, primary endosperm nucleus and degenerating antipodals. Fig. 22 L.S. ovule showing obturator, Mature embryo sac and hypostase.

(an, antipodals; fo, fold; hy, hypostase; ob, obturator; ow, ovary wall; pen, primary endosperm nucleus; pt, pollen tube; sg, starch grains; ta, tannin; va, vacuole; z, zygote).

papillose and filled with tannin (Fig. 1). The anther wall consists of four layers (Figs. 2, 3, 4) including the epidermis. Its development conforms to the Monocotyledons type of Davis (1966). The epidermis which persists till anthesis has some of its cells papillose and filled with tannin (Fig. 12). The hypodermal layer differentiates into endothecium with cells having spiral thickenings (Figs. 12). The middle layer is ephemeral (Fig. 4). The innermost wall layer constitutes the glandular tapetum (Figs. 3, 4) the cells of which remain uninucleate throughout.

The primary sporogenous cells divide mitotically. The microspore mother cells are wedge-shaped (Fig. 3). The microspore mother cells undergo meiosis (Figs. 5-8) followed by contraction because of which cytoplasm shows differential staining capacity (Fig. 9). The membrane develops between a functional and the non-functional nuclei (Fig. 9). The latter soon degenerate.

The centrally situated functional nucleus divides to form two unequal cells (Fig. 10). The smaller one situated at the distal end (Fig. 10). It is the generative cell which divides forming two elliptical male gametes (Fig. 11). The enlargement of the pollen grain is (Fig. 11) followed by vacuolation. The mature pollen grain is ovoid in shape with starch grains present around gametes and vegetative nucleus (Fig. 11). Exine of the pollen grains is thick and smooth while the intine is thin. The grains are shed at the 3-celled stage.

*Megasporogenesis and Female gametophyte :* The unicellular female archesporium (Fig. 13) divides to form a primary parietal and a primary sporogenous cell (Fig. 15). Sometimes two parietal cells are formed (Fig. 14). The parietal cell by periclinical and anticlinal divisions produces a nucellar tissue of 4-5 layers (Fig. 22). A primary sporogenous cell functions as megaspore mother cell, elongates considerably (Fig. 16) and undergoes meiosis (Fig. 17). The megaspore tetrad produced is linear (Fig. 18) or "T" shaped (Fig. 17). The chalazal megaspore functions. The degenerating megaspores persist even after the mature organization of female gametophyte (Fig. 22).

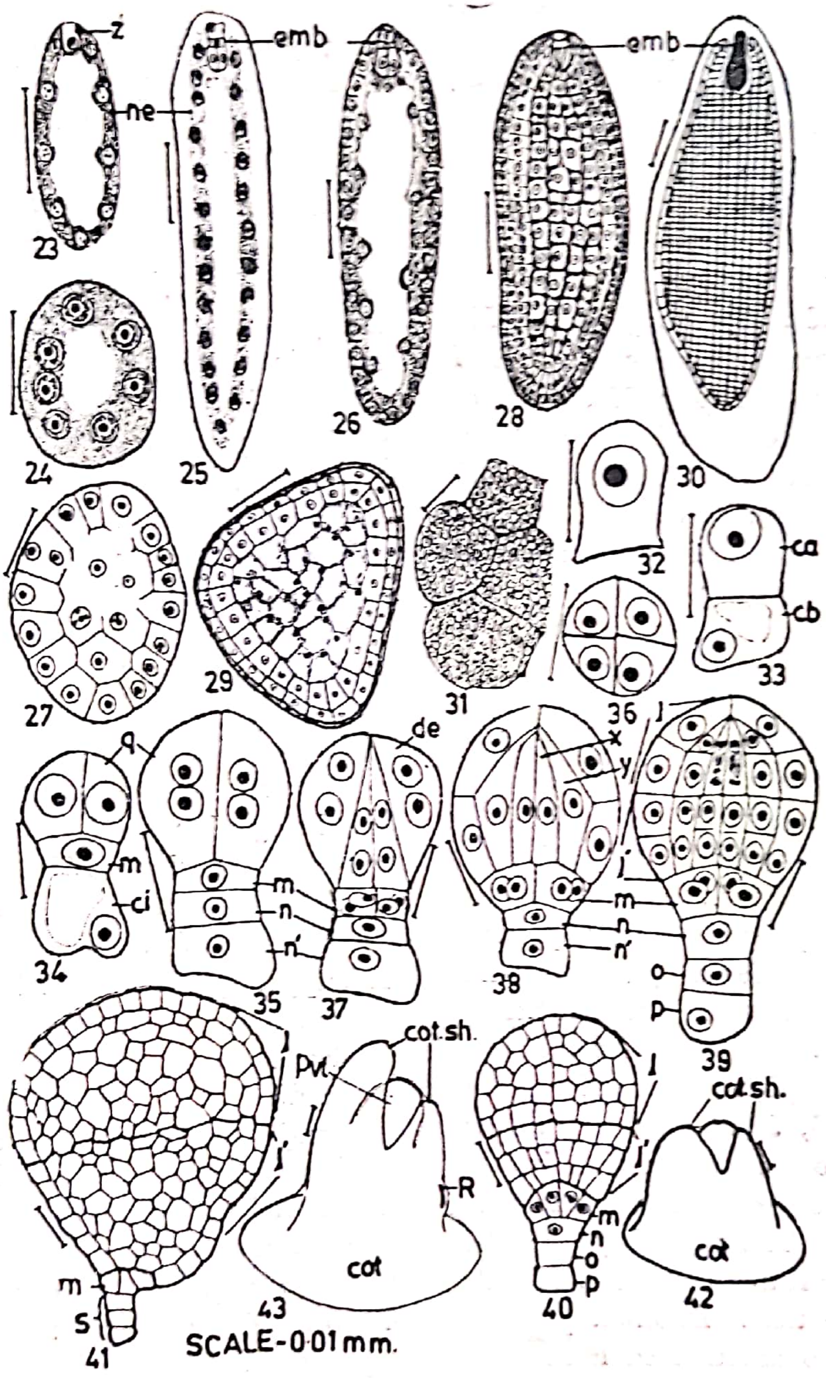
The functional megaspore nucleus by three successive divisions produces 2-(Fig. 19), 4-(Fig. 20) and finally 8-nucleate embryo sac (Fig. 22) and development conforms to Polygonum type. The mature embryo sac is broad at the micropylar end and tapers towards the chalaza. The antipodals disintegrate.

*Hypostase :* A few cutinised cells from chalazal region filled with tannin constitutes hypostase (Fig. 22) which persists in the mature seed.

*Obturator :* The cells at the base of funiculus become filamentous at megaspore mother cell stage (Fig. 17). They loosely cover the micropyle forming obturator (Fig. 22) which degenerate after fertilization.

*Fertilization :* It is porogamous. The pollen tube penetrates through one of the synergids and comes to lie by the side of egg (Fig. 21). It bursts open to discharge male gametes. Triple fusion precedes syngamy.

*Endosperm :* The primary endosperm nucleus divides in a free nuclear manner. The free nuclei produced after successive mitotic divisions get themselves arranged in peripheral layer of cytoplasm (Fig. 23-25). The wall formation initiates at peripheral layer and proceeds towards the centre (Figs. 26, 27). The uninucleate cells of endosperm are smaller at the periphery than those towards the centre (Fig. 28). The meristimatic acti-



Figs. 23-43. Embryology of *Scirpus roylei* Nees.

Figs. 23-27. Stages in the development of free nuclear endosperm; note cellularization in fig. 26. Figs. 28-30. Cellular endosperm; note meristematic cells at the periphery in fig. 28. Fig. 31. Endosperm cells showing starch. Figs. 32-41. Stages in embryogeny. Figs. 42-43. Whole mounts of mature embryo. (cot, cotyledon; cot.sh, cotyledonary sheath; emb, embryo; ne, nuclear endosperm; pvt, stem tip; R, radicle; S, suspenser; z. zygote).

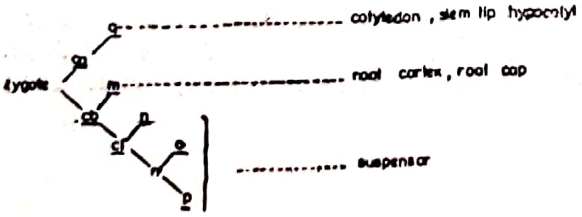
vity in part of peripheral cells increases the bulk of endosperm (Fig. 30).

The starch which begins to accumulate in cells of endosperm (Fig. 31) shadow the presence of nuclei from cells. The entire endosperm is not consumed by by the developing embryo, thus,the seeds are endospermous.

*Embryo* : The divisions in primary endosperm nucleus precedes division in zygote (Fig. 23). The zygote (Fig. 32) divides transversely forming two cells of equal size *ca* and *cb* (Fig. 33). Cell *ca* next divides vertically and *cb* transversely forming cells *m* and *ci*. Thus at the end of second cell generation 'T' shaped proembryonic tetrad of three tiers (*ca, m* and *ci*) is formed (Fig. 34).

The juxtaposed cells of *ca* next divides vertically to form a quadrant *q* (Fig. 35). Transverse division in *ci* results in two tiers *n* and *n'*. (Fig. 35). Thus, at the end of 3rd cell generation the proembryo has 7 cells disposed in 4 tiers, viz., *g, m, n* and *n'*. (Fig. 35).

During the next cell generation embryo development follows a pattern unique to the families the Juncaceae and the Cyperaceae. Each cell of tier *q* divides periclinally to cut off the dermatogen inidials (*de*) and the 4 central cells (Figs. 36, 37). The tier *m* now undergoes two vertical divisions but at right angles to each tier to produce 4-cells (Fig 37). The proembryo at the end of this genration consists of 14 cells disposed in 4 tiers. Different parts of mature embryo are derived from these tiers as per schematic representation given below :



The details of development is described in following paragraphs. The dermatogen initials divide only anticlinally (Fig. 38). The transverse segmentation in each of the four central cell gives rise to tiers *l* and *l'* (Fig. 39). Thus, as in other members of the Cyperaceae, here too there is not only precocious differentiation of dermatogen initials (on the basis of which Johansen (1950) created the Juncus variation under Onagrad type) but also the precocious development of histogenic layers of plerome and periblem prior to the organization of tiers *l* and *l'*.

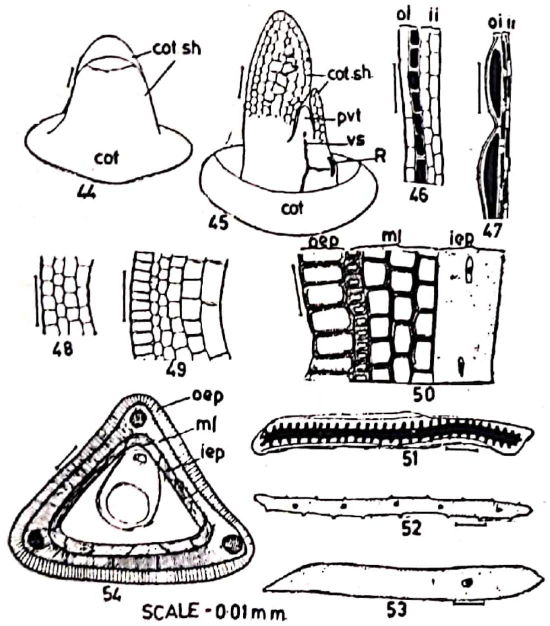
The cells of tier l divide both transversely and longitudinally to form a single cotyledon. The derivatives of tier *l'* differentiate into hypocotyl and radicular region (Fig. 40). Untill this time the embryo follows a radial symmetry (Fig. 40).

Later development more prominent in *l'* brings about a change in the radial symmetry of embryo. The rudiments of cotyledonary sheath and stem tip appears on one side of the embryo (Fig. 41). The upper lip made up of 4 to 5 cells in thickness originates from tier *l* while the lower lip derived from tier *l'* near the radicle is 2-cells thick (Fig. 45). The major derivatives of tier *l'* form a single massive disc shaped cotyledon (Figs. 42-45). Because of its vigorous growth, suspensor and the radicular portion are displaced to one side (Figs. 42-45). The stem apex in fully developed embryo is practically covered by the flaps of the cetyledonary sheath, leaving a narrow slit (Fig. 45).

The oblique walls laid down in elements of tier *m* form a group of 4 outer cells around the 4 central cells (Fig 40). The former differentiates into root epdermis and root cap of the radicular portion while the derivatives of latter engenders, plerome and periblem of the radicle.

The cell *n*' divides transversely to give rise to *o* and *p* (Fig. 39). These along with cell *n* forms uniseriate suspensor of 3-cells which is displaced to one side. During subsequent development it degenerates.During development the plumule gets gradually oriented towards the micropyle, while the radicle occupying the micropylar position to begin with, shifts to the lateral side (Fig. 41).

*Mature Embryo :* The single cotyledon forming bulk of mature embryo is terminal to the plumule-radicle axis which is curved with radicle lateral in position and plumule enclosed by unequal cotyledonary sheath facing the micropyle (Fig. 45). The three histogenic layers are well differentiated in mature embryo (Fig. 45). A vascular strand which connects plumule-radicle axis descends downwards into the cotyledon (Fig. 45). The whole mature embryo is surrounded by endosperm.



Figs. 44-54. Embryology of *Scirpus roylei* Nees. Figs. 44, 45. Whole mounts of mature embryo. Figs. 46, 47. Development of seed coat. Figs. 48-50. Stages in the development of pericarp. Figs. 51, 52. Sclerides from outer epidermis and middle layers. Fig. 53. Parenchymatous cell from inner epidermis. Fig. 54. T. S. of mature fruit.

(cot. cotyledon; cot.sh. cotyledonary sheath; oi, outer integument; oep, outer epidermis; ii, inner integument; i ep, inner epidermis; ml, middle layers; pvt, stem tip; R, raditcle;, vs, vascular supply).

*Seed Coat :* Both the integuments take part in the organisation of mature testa. The outer layer of testa is derived from the outer epidermis of the outer integument and inner one from the inner epidermis of the inner integument. This layer has larger tannin packed cells (Figs. 46, 47). The interveing layers get crushed during development.

*Pericarp :* The ovary wall consists of 4 to 6 layer of parenchyma cells (Figs. 48, 49). The outer epidermis consists of thick walled elongated cells (Fig. 50). The middle layers made up of fibre which show pits with some projections on their walls (Figs. 51, 52). The parenchymatous cells of inner epidermis become elongated and form the spongy layer (Fig. 53). In transection only thick walled and inner parenchymatous region is well marked (Fig. 54).

## DISCUSSION

The spiral thickenings in the endothecium are reported in the family (Padhye, 1971a, b; Deshpande and Untawale, 1972 ; Untawale and Makde, 1971 Makde, 1981). However, report of fibrous endothecium in *Scirpus mucrinatus* (Khanna, 1965) needs confirmation. The microspore mother cells in the family invariably appear triangular in outline with their acute apices almost meeting towards the centre with the exceptions of *Scirpus palustris* (Piech, 1928) and *Cyperus iria* (Padhye, 1971a). The meiosis in microspore mother cell as in *Juncus filiformis* (Wulff, 1939) is not followed by cytokinesis and it is delayed until the two-nucleate stage of pollen grain. Khanna (1965) indicated the derivation of this condition in the Cyperaceae through Juncaceae.

The pollen grains are 3-celled when shed and full of starch. The sperm cells possess distinct nuclei in most cases. However, in *S. corymbosus* sperm cells having crescent shaped bodies with hyaline areas have been reported (Padhye *et al.*, 1970).

The parietal tissue is of five layers (present work) but the same is reported to be two layered in *S. mucrinatus* (Khanna, 1965). The number of layers is variable (Dnyansagar and Tiwari, 1956); Gupta, 1962; Padhye, 1968 and Tiwari, 1969). The obturator present in *S. roylei* (present work) and *S. mucrŏnatus* (Padhye, 1967), is absent in *S. mucrinatus* (Khanna, 1965).

Embryogeny conforms to Juncus variation under Onagrad type (Johansen, 1950) which is in conformity with the earlier work (Shah, 1965; Padhye, 1960, 1971a; Patel and Shah, 1962). In Justification for the Juncus variation Johansen (1950) writes "the extreme precocity of the differentiation of the epidermal initials characterises this variaiton. This feature occurs in no other angiosperms except possibly in *Orobanche hederaceae* and may be considered as the most highly evolved one under the Onagrad type. The only exception being *Scirpus mucrinatus* where development is described to follow Asterad type (Khanna, 1965).

Shah and Neelakandan (1971) working on *Scirpus littoralis* reported that development is not uniform in one and the same species as a vertical section in *ca* is followed by a diagonal, periclinal or transverse division to result in a octant in *Scirpus*. Their figure 45 seems to be non-median. Likewise figure 47 appears to be non-median while figure 46 only where dermatogen initials are shown represents the correct situation in the family.

In present species there is not only the precocious differentiation of dermatogen initials but also the precocious differentiation of histogenic layers. Such a condition is present in *Bulbostylis barbata* (Shah, 1965) and *Kyllinga brevifolia* (Padhye, 1971a). It is interesting to note that the related family Juncaceae also exhibits these patterns (Souéges, 1923, 1933; Shah, 1963).

These two tendencies appears to have arisen in the Juncaceae continued in the Cyperaceae. Such an evidence is additional proof for the contention that the Juncaceae and the Cyperaceae are closely related and sedges are derived from rushes (Hutchinson, 1959; Shah, 1967; Padhye, 1967; Takhatajan, 1967 and Cronquist, 1968).

In the present investigation the cells of tier *m* contributes only twowards the radicular region. However, Shah and Neelakandan (1971) have visualised the origin of lower lip of the cotyledonary sheath from tier *m*. Do Shah and Neelakandan (1971) advocate endogenous origin of cotyledonrary sheath ? Shah and Neelakandan (1971) though in schematic representation have shown that tier *n* gives rise to a part of the root cap their figures do not indicate mode of division of this cell. Van der Veken (1965) advocated the splitting of *Scirpus* into six different genera on the basis of embryographic studies.

The mature test in the present taxon corresponds to the outer epidermis of outer integuments and inner epidermis of inner integuments, which corroborate the findings in other members of family

(Khanna, 1965; Padhye, 1971a).

The pericarp in the mature fruit consists of outer layers of starch containing cells. The cells from the remaining layers transformed into sclereids. In *Kyllinga melanospora* and *Scirpus mucrinatus* (Khanna, 1965) no starch grains have been reported.

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