H. M. DEVI* & K. C. NAIDU*: **Embryological studies in Cucurbitaceae (1) Melothrieae**

H. M. デビ*・K. C. ナイヅ*: ウリ科の胚発生 (1) Melothrieae

The tribe Melothrieae comprises 31 genera and 495 species (Jeffrey 1962) and is distributed mostly in the new world. As compared to the size of the tribe the number of embryologically investigated species are few and the work is confined to the development of female gametophytes, endosperm and seed coat (Kirkwood 1904; Paliwal 1950; Singh 1955, 1956, 1957, 1961, 1964; Singh & Dathan 1972, 1974a, 1974b; Chopra 1963; Chopra & Agarwal 1958; Johri & Chowdhury 1957; Dzevaltovsky 1963). The present study incorporates the life history of *Cucumis trigonus* Roxb. and *Mukia scabrella* Hook.

Materials and Methods The material of *M. scabrella* was collected by Dr. A. G. S. M. Reddy at R. V. Nagar, Visakhapatnam District and *C. trigonus* was collected by the author at Peyanapalli (Vill), Chittoor Dist. of Andhra Pradesh and fixed in formalin-acetic-alcohol. Dehydration, embedding, sectioning and staining were followed adopting the procedure given by Johansen (1940). The sections were cut between 6 and 15 μm and stained in Delafield's haematoxylin.

Observations

Structure and development of the anther and pollen. The hypodermal archesporium is three-celled in transection. The development of the anther wall follows the dicotyledonous type (Davis 1966) and comprises the epidermis, fibrous endothecium, middle layer and the tapetum (Fig. 1, A–E). But in *M. scabrella* the middle layer undergoes periclinal division to produce two layers (Fig. 1, D). The tapetum is of the secretory type. The division of the microspore mother cells is of the simultaneous type and wall formation takes place by furrowing resulting in tetrahedral, isobilateral and decussate types of pollen tetrads (Fig. 1, F–I). The tetrahedral tetrads are more common. The pollen grains are tricolporate and shed at 3-celled stage (Fig. 1, J, K). In all the anthers of both the species, one, two or all the four pollen grains become degenerated from each pollen tetrad (Fig. 1, L).

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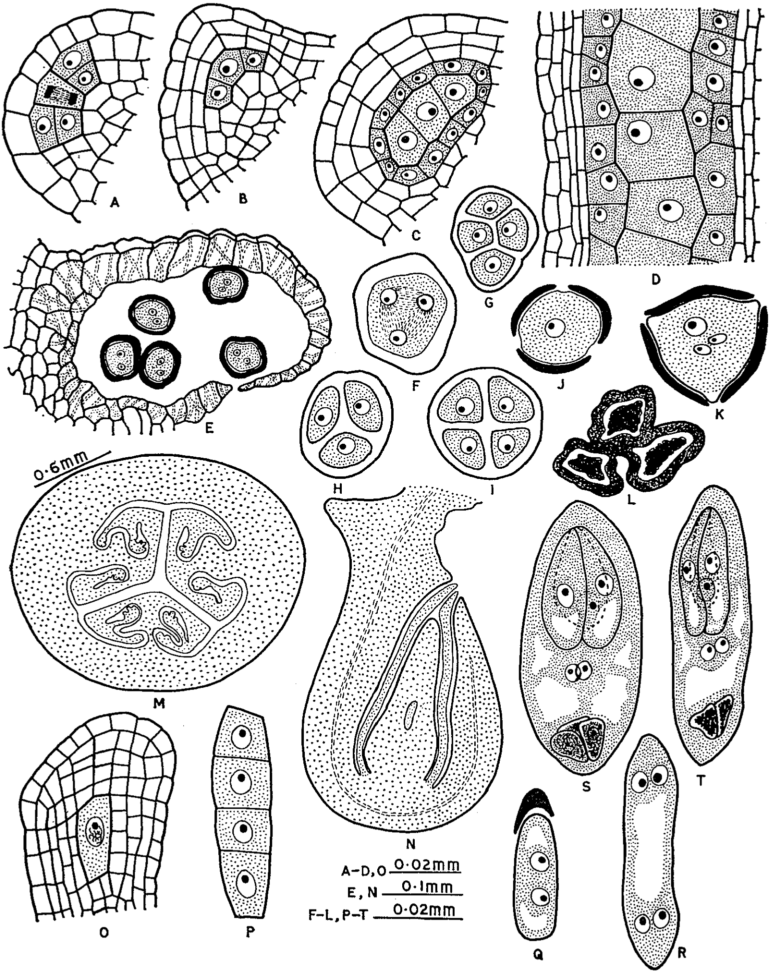

Fig. 1. *Mukia scabrella* (A, D, E, F, I, K, L, Q, T) and *Cucumis trigonus* (B, C, G, H, J, M-P, R, S). A-D: Transverse section and longitudinal section of anther lobes showing development of wall layers and sporogenous tissue. E: Transverse section of mature anther lobe showing endothecium with persistent epidermis. F-I: Pollen tetrads. J, K: Pollen grains. L: Pollen tetrad (abortive). M: Transverse section of ovary. N: Longitudinal section of ovule. O: Nucellus showing megaspore mother cell. P: Linear megaspore tetrad. Q-T: 2, 4 and mature embryo sacs.

Ovary, ovule, megasporogenesis and female gametophyte. The ovary is inferior, tricarpellary syncarpous and unilocular with three massive T-shaped parietal placentae in *C. trigonus* (Fig. 1, M) and it is bicarpellary syncarpous in *M. scabrella*. The ovule is anatropous, bitegmic and crassinucellate (Fig. 1, N). The micropyle is formed by the inner integument alone (Fig. 1, N). A well developed nucellar beak is present. The single celled hypodermal archesporium undergoes a periclinal division cutting off a primary parietal cell and a megaspore mother cell. The former divides both by anti- and periclinal divisions (Fig. 1, O) giving rise to several-layered tissue above the megaspore mother cell making it deep seated. The parietal tissue together with the nucellus forms a prominant nucellar beak which extends up to the micropyle (Fig. 1, N). The megaspore mother cell undergoes meiotic divisions resulting in a linear tetrad of megaspores (Fig. 1, P). Sometimes in *C. trigonus* a triad is formed due to suppression of division in the micropylar cell of the dyad. The development of the female gametophyte is of the *Polygonum* type in *C. trigonus* (Fig. 1, R, S) and it is of *Allium* type in *M. scabrella* (Fig. 1, Q, T). The synergids are larger than the egg. The three antipodals are ephemeral.

Fertilization and endosperm. The broad pollen tube travels through the micropyle, crushing the cells of the inner integument at the apical region and also some cells of the nucellar beak and enters the embryo sac through one of the synergids. Syngamy and triple fusion take place more or less simultaneously. The pollen tube is persistent. It becomes dilated and swells to form 'Bulla' in *C. trigonus* (Fig. 2, A).

The endosperm development is of the nuclear type. A large number of free nuclei are at first distributed uniformly in the periphery of the embryo sac leaving a large central vacuole (Fig. 2, B). Simultaneously with the free nuclear divisions, the embryo sac enlarges considerably. The micropylar part of the embryo sac becomes broadened while the chalazal part remains narrow. Gradually the chalazal region elongates giving rise to a tubular process into which a few free endosperm nuclei migrate. Finally this acts as the chalazal endosperm haustorium (Fig. 2, C). Meanwhile wall formation sets in from the micropylar end and gradually extends towards the chalazal end (Fig. 2, C). It is restricted only to the vescicular part of the embryo sac (Fig. 2, D). The haustorium contains densely stained vacuolated cytoplasm and several free nuclei. The size of the haustorium is 500–700 μm in *C. trigonus* (Fig. 2, C) and 750–800 μm in *M.*

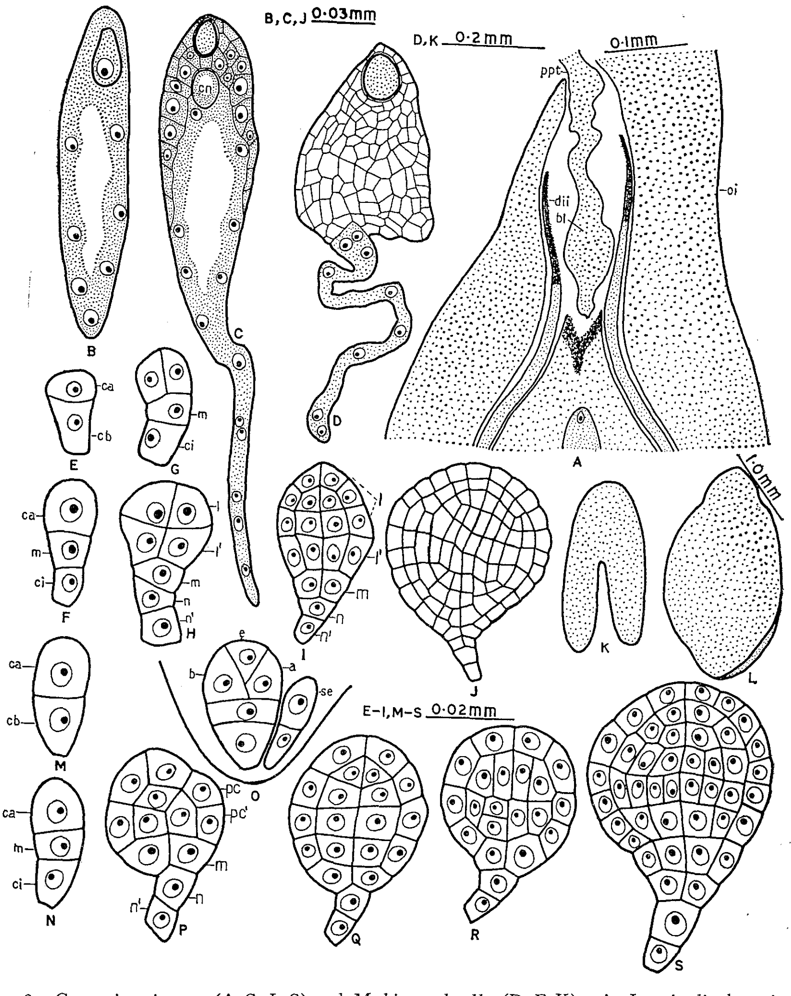

Fig. 2. *Cucumis trigonus* (A-C, L-S) and *Mukia scabrella* (D, E-K). A: Longitudinal section upper part of ovule showing persistent pollen tube and degeneration of nucellar beak. B-D: Development of endosperm and haustorium. E-S: Stages in the development of embryo. bl: 'Bulla'; cn: cytoplasmic nodule; dii: degenerating inner integument; oi: outer integument; ppt: persistent pollen tube; se: synergidembryo.

scabrella (Fig. 2, D). The coenocytic haustorium remains active till the embryo attains the heart-shaped stage. The endosperm is completely consumed in the mature seed. Cytoplasmic nodules have been observed only in the endosperm proper of the *C. trigonus* (Fig. 2, C).

Embryogeny. The zygote divides transversely resulting in a terminal cell ca and a basal cell cb (Fig. 2, E, M). The basal cell cb divides transversely producing two superposed cells m and ci (Fig. 2, F, N). The terminal cell ca divides vertically and produces two juxtaposed cells. Thus a T-shaped four-celled embryo is formed (Fig. 2, G). From this stage onwards the sequence of development of embryo differs from one another and hence described separately. *M. scabrella*.— In two juxtaposed apical cells of the cell ca vertical division occur in each to engender quadrants. This tier is designated as q. In the tier q transverse division occurs resulting in the formation of octants arranged in two tiers of four cells each (Fig. 2, H). The apical tier is designated as l and the next one as l′. Then the cell ci divides transversely to produce two superposed cells n and n′. Later the cell m undergoes a vertical division resulting in two juxtaposed cells (Fig. 2, I). The tier 1 undergoes further divisions in all planes and gives rise to the cotyledons and stem tip. The derivatives of l′ contributes to the hypocotyledonary region and a part of the root. The derivatives of the tier m contribute to the hypocotyledonary region (Fig. 2, I–K). The two cells n and n′ directly function as the suspensor cells (Fig. 2, I, J). The mature embryo is straight with two leafy cotyledons (Fig. 3, G).

From the above it is clear that the four-celled proembryo is T-shaped and major part of the embryo is formed from the terminal cell ca alone. The mature embryo is dicotyledonous and the quadrant is formed regularly. Hypo- and epiphyseal initials are absent. Thus the embryogeny keys out to the *Lotus* variation of Onagrad type.

C. trigonus.— The vertical wall in the cell ca of the T-shaped tetrad is slightly oblique and two dissimilar cells are formed, of which the larger cell a divides by another oblique wall which is perpendicular to the first one and thus produces an epiphyseal initial e (Fig. 2, O). The cells a and b undergo transverse divisions to produce two tiers of cells, the upper pc and the lower pc′ (Fig. 2, P). The epiphyseal initial undergoes further divisions and gives rise to cortical initials of the stem. The cells of the tier pc undergo further transverse divisions followed by periclinal division producing an outer dermatogen layer and an inner layer of cells which after undergoing a few more divisions give rise to the central cylinder of the stem and the two cotyledons (Fig. 2, Q–S). The derivatives of pc′ contribute to the hypocotyledonary region. The activities of the cell m and ci are similar to *M. scabrella*. The mature embryo is straight (Fig. 2, L). Polyembryony is observed in *C. trigonus* (Fig. 2, O).

From the above it is clear that the terminal cell ca of the two celled proembryo contributes to a major part of the embryo proper. The presence of epiphysis, absence of hypophysis and well developed dicotyledonous embryo are characteristic of Trifolium variation of Onagard type.

Seed coat. The seed coat is formed by the outer integument alone. The inner integument which is two layered thick (Fig. 3. A–C) shows signs of disintegration with the entry of pollen tube into the micropyle. Finally by the time a globular embryo is formed no trace of the inner integument is seen.

The development of outer integument follows the usual cucurbitaceous pattern. The first division of the cells of the outer epidermis is at functional embryo sac stage. It divides periclinally producing two layers e and e_1 of which the outer one e again undergoes one more periclinal division resulting in two layers e_2 and e_3 (Fig. 3, C). The outer most layer e_3 functions directly as the seed coat epidermis. The cells of e_1 divide anticlinally to keep pace with the enlarging seed and at maturity it becomes single layered aerenchyma (Fig. 3, F).

The layer e_2 as a result of periclinal divisions becomes multilayered hypodermis. At the globular embryo stage the hypodermis is three layered in *C. trigonus* and six layered in *M. scabrella* (Fig. 3, D, F). By the time a mature embryo is formed the hypodermis becomes ten layered of which the inner two layers are sclerenchymatous and the outer eight layers remain parenchymatous in *C. trigonus* (Fig. 3, E). In *M. scabrella* the number of wall layers increased to eleven, the outer four layers are parenchymatous and inner seven layers are sclerenchymatous (Fig. 3, F).

The number of layers in the middle region vary from four to eight layers and remain parenchymatous throughout (Fig. 3, A, B, E–G). The inner epidermis of the integument remains parenchymatous and contributes to the formation of seed coat.

Structure and development of trichomes. The trichomes which are present on the floral parts can be divided into two categories viz. the glandular and non-glandular.

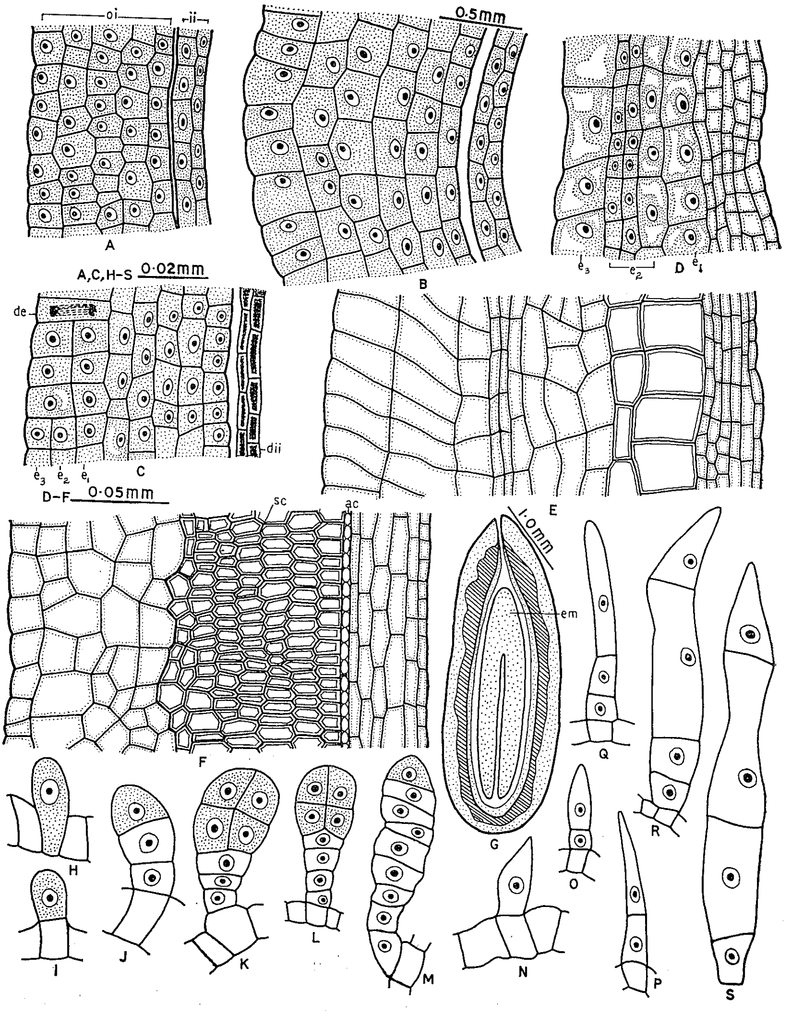

Fig. 3. *Cucumis trigonus* (A, D, E, H-J, L, M, R, S) and *Mukia scabrella* (B, C, F, G, K, N-Q). A-F: Stages in the development of seed coat. G: Longitudinal section of mature seed. H-S: Trichomes. ac: aerenchyma; de: dividing epidermis; dii: degenerating inner integument; em: embryo; ii: inner integument; oi: outer integument; sc: sclerenchyma.

Glandular trichomes. They are initiated from single protoderm cells which are readily distinguished due to their dense cytoplasm (Fig. 3, H). Soon they divide by a transverse division when the trichome appears projection towards the distal end of the part on which it is borne (Fig. 3, I). The upper cell divides periclinally twice or thrice and gives rise to the stalk (Fig. 3, J, K). The terminal cell either directly functions as the single celled head or divide to give a multicellular head (Fig. 3, L, M).

Non-glandular trichomes. The development of these trichomes is identical to those of the glandular trichomes, the only difference being that the head cell directly functions as pointed head (Fig. 3, N–S).

Discussion In the tribe Melothrieae the previous studies on the development of anther and male gametophyte are restricted to only a few members like *Cucumis sativus* (Heimlich 1929, Passmore 1930), *Cucumis melo* (Passmore 1930), *Melothria madaraspatana* (Singh 1957) and *Melothria scabra* (Dzevaltovsky & Zhalalov 1976). The development of anther wall in all these cases, including the present investigated members is of the Dicotyledonous type (Davis 1966). However, in *M. scabrella* the additional middle layer is formed as a result of periclinal division of the original layer.

The micropyle is formed by the inner integument in all the hitherto investigated taxa including the present investigation. However, Singh (1955) did not mention about the formation of the micropyle in *Cucumis melo* var. *pubescens*, the figures given by him show the involvement of both the integuments. The single celled female archesporium is reported in *Cucumis melo* var. *pubescens* (Paliwal 1950, Singh 1955), *Melothria madaraspatana* (Singh 1956), *Cucumis metuliferus* (Singh & Dathan 1972) and present study. Multicellular archesporium is reported by Dzevaltovsky (1963) in *Cucumis melo* and *Cucumis sativus*. The development of the female gametophyte in all the genera is of the *Polygonum* type except in *Mukia scabrella* where it is *Allium* type. Mihov and Zagorcheva (1966) reported generative apomictic embryo sac in *C. sativus*. Recently, Singh and Dathan (1972) also reported aposporic embryo sac in *C. metuliferus*. The persistence of pollen tube is a characteristic feature of this tribe (Longo 1901, Singh 1955, 1956, 1963, 1967, 1970 and present investigation). The 'Bulla' formation occurs near the tip of the nucellar beak in *C. trigonus*. The development of nuclear endosperm and formation of chalazal endosperm haustorium is common to all the hitherto investigated species including the present report.

Although the tribe is characterised by Onagrad type of embryo development, it is interesting to note Nicotiana variation of Solanad type in *C. melo* var. *pubescens* (Singh 1955). The seed coat development follows the typical cucurbitaceous pattern in the taxa of the tribe so far investigated (Singh 1956; Singh & Dathan 1974a, b and present study).

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ウリ科の *Cucumis trigonus* と *Mukia scabrella* の花粉形成と種子形成とを報告した。胚珠は2枚の珠皮をもち厚層珠心である。*Cucumis trigonus* の胚嚢形成は *Polygonum* typeで，*Mukia scabrella* では *Allium* type である。胚乳形成は nuclear type で，形成の途中に珠孔側に細胞膜が形成され，それが次第にカラザ側に及び，カラザ側には多核の細長い吸収器が作られる。胚形成は onagrad type である。種皮は外珠皮のみから作られ，内珠皮は消失する。外珠皮の外側の層は柔細胞からなる外種皮を作り，内側は厚膜細胞からなる内果皮を作る。

○高等植物分布資料 (107) Materials for the distribution of vascular plants in Japan (107).

○キブネダイオウ *Rumex nepalensis* Sprengel var. *Andreanus* (Makino) Kitamura newly found in Okayama Pref. of western Honshu キブネダイオウは京都北郊の貴船で見出され，これまで京都附近にしか知られておらず，大陸の *R. nepalensis* に最も近いことから，北村博士は植物分類地理 **16**: 194 (1956)；**26**: 141 (1975) で果して天然の野生であるか疑が残ると記されている。ところが本年 (1983) 6月5日，古瀬義氏が岡山県備中町布瀬（フセ）でキブネダイオウの果実をつけた標本を採集された。同氏によれば，本年4月杉林の中でその若葉をみて奇異に思い，その後二度同地を訪れようやく他のギシギシと異ることが分ったという。同地域では岩窟渓の渓流の辺りや上布瀬の杉の植林や田畦に群生していて，ヤマトレンギョウ，チョウジガマズミ，キクガラクサ，アズマガヤ，クロタキカズラなども採集された由である。このことからキブネダイオウも，他のアジア大陸と関連の深い植物と共にこの地域に遺存したものと推察され大変興味深い発見である。今回その標本を送って下った古瀬氏にお礼を申し述べると同時に，同氏の炯眼と熱意に敬意を表する次第である。

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