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A CONTRIBUTION TO THE EMBRYOLOGY OF THE IRIDACEAE[1]

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ABSTRACT

The anther wall comprises the epidermis, endothecium, middle layer and the innermost glandular tapetum. The endothecium develops fibrous thickenings only in *Sisyrinchium stlanticum*. The development of anther conforms to the monocotyledonous type. The pollen grains when shed are 2-celled. The anther dehiscence in taxa with little fibrous thickenings occur by radial stretching and rupturing of the epidermal cells. The ovules are crassinucellate and bitegmic. The embryo sac development follows the Polygonum type.

INTRODUCTION

The earlier literature on the embryology of Iridaceae has been reviewed by Maheshwari (1950) and Davis (1966). The members of this family show high degree of sterility (Yasui and Sawada, 1940 and Lakshmanan and Philip, 1971). The present communication deals with some aspects of embryology in a few taxa of Iridaceae.

MATERIALS AND METHODS

The following taxa were used in the present study : *Belamcanda chinensis* DC., *Freesia armstrongii* W. Wats., *Iris decora* Wall., *I. Kumaonensis* Wall. ex D. Don and *Sisyrinchium atlanticum* Bickn. They were fixed in F. A. A. and after following the usual methods of dehydration, clearing and embedding, the materials were sectioned between 8-12 μm thickness. The slides were stained with either safranin-analin blue or safranin-fastgreen combinations.

OBSERVATIONS

Microsporangium, Microsporogenesis and *Male Gametophyte :* The pre-archesporial anther is a homogenous mass of parenchymatous cells surrounded by an epidermis (Figs. 1 A, D). Soon it becomes four lobed due to active cell divisions in four regions (Fig. 1 B, E). A plate of three or four hypodermal cells become distinct by their dense cytoplasmic contents and prominent nuclei in each of the four lobes (Fig. 1 E). These cells act as archesporial initals. A periclinal division in an archesporial initial results in an outer primary parietal cell and an inner primary sporogenous cell (Figs. 1, C, F). The former divides again by a periclinal wall to form an inner and an outer secondary parietal cell (Fig. 1 G). While the outer secondary parietal layer gives rise to endothecium, cells of the inner secondary parietal layer further divide periclinally forming middle layer and

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tapetum (Fig. 1 H). In a mature anther thus four wall layers, epidermis, endothecium, middle layer and tapetum become distinct (Fig. 1 I). The development of anther conforms to Monocotyledonous type (Davis, 1966). The epidermis is occasionally interrupted by stomata in *Iris decora* (Fig. 1 J).

Cells of the endothecium become radially elongated as pollen grains begin to mature (F8g. 1 K). In *Sisyrinchium atlanticvm* endothecial cells, which are comparatively larger (Fig. 1 K), develop typical fibrous thickenings at the time of the separation of pollen from tetrads (Fig. 1 L). The endothecial cells in other taxa investigated do not show any thickenings. The middle layer is ephemeral and disappears during the maturation of pollen (Fig. 1 T).

Whereas the peripheral part of the tapetal layer (outer face) is derived from the inner secondary parietal layer, the part facing inwards, towards the connective, is derived from cells of the connective adjoining the sporogenous tissue. These connective cells divide periclinally and arrange themselves in such a fashion as to form a layer that becomes continuous with the tapetum (Fig. 1 M). The tapetum thus shows dual origin and is also dimorphic (*See* Vijayaraghavan & Ratnaparkhi, 1973). The tapetal cells are glandular in nature and are uninucleate but may become binucleate at maturity (Fig. 1 I). The tapetum degenerates before the dehiscence of the anther.

The microspore mother cells undergo reduction division and simultaneously form either tetrahedral or rarely isobilateral or decussate tetrads (Fig. 1 N-R). Pollen grains are shed at the 2-celled stage (Fig. 1 S). A high degree of sterility has been observed in a few anthers where tapetal cells grow enormously in size and become multinucleate (Fig. 1 T). Proliferation of tapetal cells starts at sporogenous cell stage and by the time pollen grains are formed they almost fill the anther sac reducing greatly the sporogenous tissue. Some abnormal anthers with one, two or three lobes have also been observed (Fig. 1 U-X).

Anther dehiscence : The middle layer and the tapetum degenerate as the pollen grains mature, whereas epidermis and endothecium are healthy until anther dehiscence. In young anther with well differentiated wall layers, cells of the stomium are alike and meristematic (Fig. 2 A, D). The septum is four to five cells wide in *Belamcanda chinensis*, *Freesia armstrongii* and *Iris decora* (Fig. 2 D) and two to three cells wide in *Sisyrinchium atlanticum* (Fig. 2 G, K).

The stomium develops in the shallow depression between the two pollen chambers of a theca (Figs. 2 B, E). As the stomial groove deepens, epidermal and endothecial cells divide anticlinally and stretch tangentially. The endothecial cells, except in stomial region, develop fibrous thickenings in *Sisyrinchium atlanticum* (Figs. 2 H, L-N). The two ather lobes become confluent due to breaking of septum below stomium (Figs. 2 I, J). The disorganised septum, however, remains attached to the connective. The mechanical action of the fibrous thickenings of the adjacent endothecial cells is responsible for separating the broken edges of the wall layers, thereby liberating the pollen grains (Fig. 2 I, J, M, N).

In *Belamcanda chinensis*, *Freesia armstrongii*, *Iris decora* and *Iris kumaonensis* where fibrous thickenings in the endothecium are lacking, cells are comparatively narrow (Fig. 2 C, F). In these taxa dehiscence is confined to the distal half of the anther. The epidermal cells

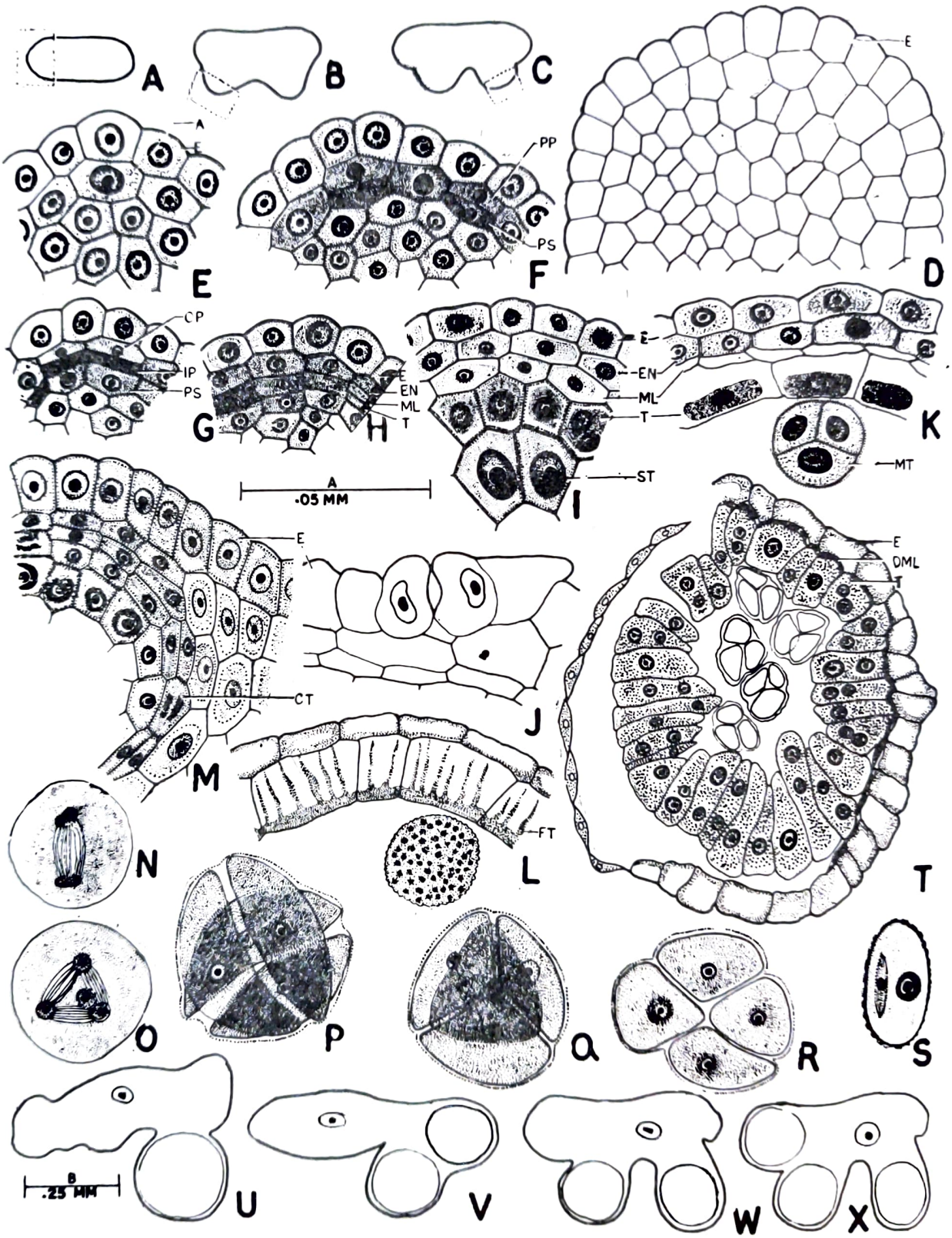

Fig. 1 A—X (*Microsporogenesis and the male gametophyte*) : A—C. Outline of transverse sections ofanthers of *Iris kumaonensis* at successive stages of development. D. A portion of young anther of *Iris kumaonensis* marked in figure A showing homogenous mass of parenchymatous cells. E—H. Parts of cross-sections of anthers of *I. kumaonensis* (figures E and F are the portions marked in figures B and C respectively) showing stages of development of anther wall layers. I. A part of transverse section of young anther of *I. kumaonensis* showing

of stomial region become radially stretched considerably. As the septum remains intact, the two adjacent pollen chambers retain their identity until the dehiscence. The endothecial cells also degenerate along with the middle layer of septum, resulting in the escape of pollen.

Megasporangium, Megasporogenesis and Female Gametophyte : The ovules are anatropous, bitegmic and crassinucellate. Each ovule develops as a small hemispherical projection on the placental tissue. Hypodermal archesporial cell soon differentiates in the apical region and is characterized by large size and prominent nucleus (Fig. 3 B). At this stage the primordium of the inner integument appears in the form of an annulus (Fig. 3C), followed by the initiation of outer integument (Fig. 3 D). Both the integuments are epidermal in origin. The inner integument grows much faster than the outer integument and extends beyond the nucellus and forms a long and slender micropyle. At this time ovules are anatropous (Fig. 3 L). The inner integument is 2-layered except in the micropyle region where it is 4-5 layered. The outer integument is two to three layered throughout. The base of the funiculus becomes slightly swollen and the epidermal cells in this region become enlarged and form the glandular obturator with the exception of *Belamcanda chinensis* (Fig. 3 L, M).

The hypodermal archesporial cell enlarges in size considerably and divides periclinally cutting off an outer parietal cell and an inner megaspore mother cell (Fig. 3 C). The former divides both anticlinally and periclinally forming a massive nucellus. The megaspore mother cell undergoes meiosis, first giving rise to a dyad and then a linear tetrad of megaspores (Fig. 3 E. F). Out of the four megaspores usually the chalazal one is functional.

The functional megaspore enlarges considerably and division of its nucleus results in two daughter nuclei, one of which lies at the micropylar end, the other at the chalazal (Fig. 3 G). Each of these two nuclei undergoes two successive divisions resulting into eight nuclei (Fig. 3 H, I). The development and arrangement in embryo sac conforms to the Polygonum type. The synergids are pyriform with the nucleus situated above the vacuole. The egg is pear shaped and the three antipodals are invariably of the same size (Fig. 3 I). The two polar nuclei vary slightly in their size.

In *Sisyrinchium atlanticum* the cells of the inner integument adjacent to the mature embryo sac become radially elongated and densely cytoplasmic. These

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four wall layers. J. Epidermis of the anther of *Iris decora* showing stomata. K. A part of the transverse section of the anther wall of *Sisyrinchium atlanticum*. Note the absence of thickness in the cells of the endothecium. L. A part of the transverse section of a comparatively mature anther of *S. atlanticum* showing fibrous thickenings in the endothecium. M. A part of the transverse section of anther of *Iris kumaonensis* showing dual origin of tapetum. N—O. Successive stages of the development of microspores in *I. kumaonensis*. P—Q. Decussate and tetrahedral tetrads of *Belamcanda chinensis*. R. Isobilateral tetrad of *Iris kumaonensis*. S. Two celled stage of microspore of *I. decora*. T. Transverse section of an anther lobe of *I. kumaonensis* showing degenerating middle layer and exceptionally large tapetal cells. U—X. Anthers of *I. kumaonensis* with three, two and one sterile lobes respectively.

A—Archesporial cell; CT—Connective tissue; DML—Degenerating middle layer; E—Epidermis; EN—Endothecium; FT—Fibrous thickenings; ISP—Inner secondary parietal layer; IP—Inner parietal cell; ML—Middle layer; MT—Microspores tetrad; OP—Outer parietal layer; OSP—Outer secondary parietal layer; PP—Primary parietal cell; PS—Primary sporogenous cell; ST—Sporogenous tissue; T—Tapetum.

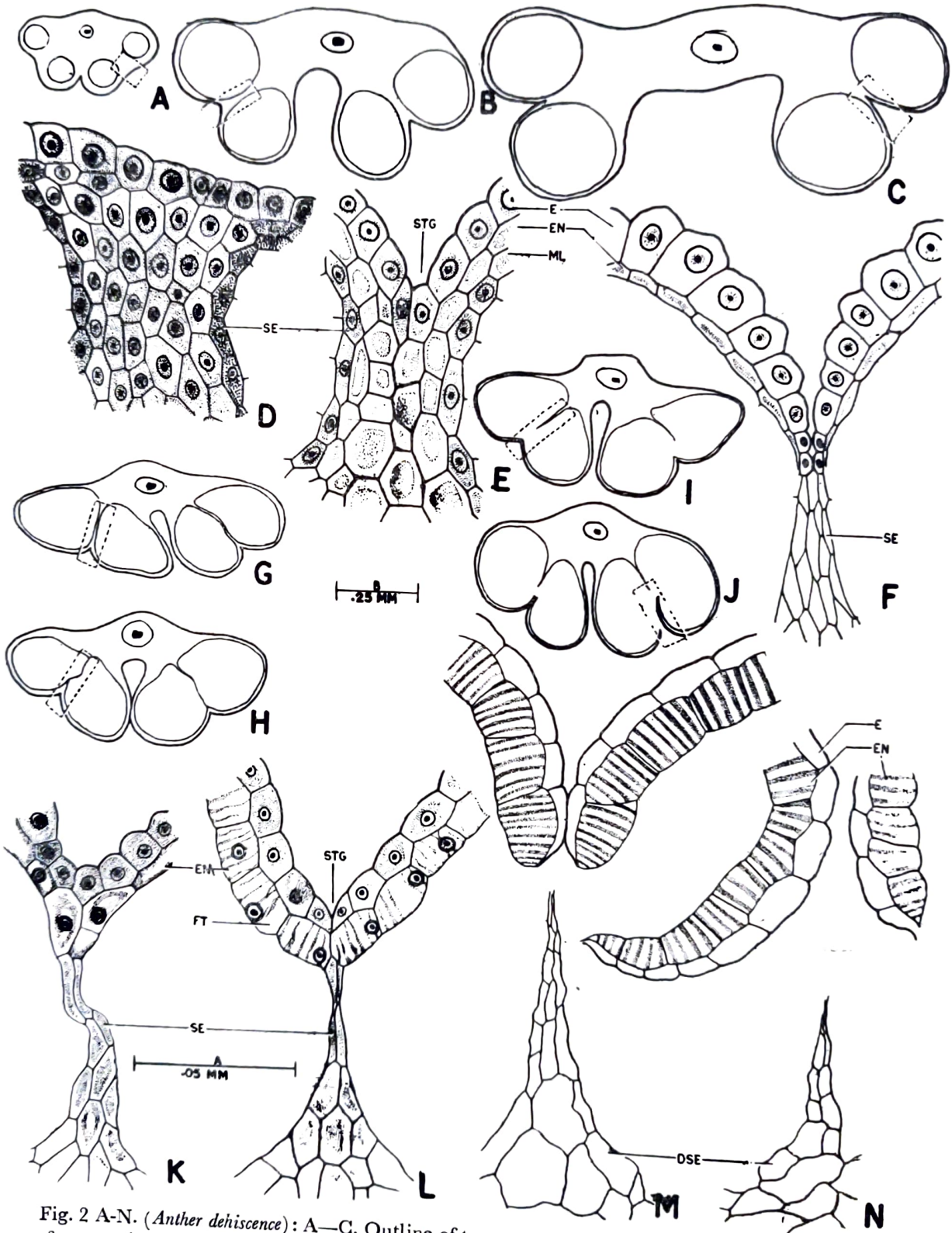

Fig. 2 A-N. (*Anther dehiscence*): A—C. Outline of transverse sections of anthers of ;*ris decora* at successive stages of maturation. D—F. Portions marked in figures A to C respectively magnified. G—J. Cross-sections of anthers of *Sisyrinchium atlanticum* at successive stages of anther dehiscence. K-N. Portions marked in figures G to J respectively magnified.

DSE—Detached septum; E—Epidermis; EN—Endothecium; FT—Fibrous thickenings; ML—Middle ayer; SE—Septum; STG—Stomial groove.

cells form the integumentary tapetum (Fig. 3 J).

The members of Iridaceae studied show a very high degree of sterility. The degeneration of embryo sac starts very early in the development. In most of the ovules the embryosacs start degenerating at the four nucleate stage (Fig. 3 K) and only in a very few ovules organized mature embryo sacs could be observed.

DISCUSSION

It is interesting to note that the anther epidermis is interrupted by stomata in *Iris decora* and *I. kumaonensis*. Chikkannaiah (1962) has also reported stomata on the anther walls of some Commelinaceae. The endothecium develops fibrous thickenings only in *Sisyrinchium atlanticum*. Such thickenings have also been reported in some other species of the genus, e.g. *S. striantum* and *S. californicum* by Lakshmanan and Philip (1971). They have also reported two middle layers in these species but we have observed only one middle layer not only in *Sisyrinchium atlanticum* but also in other taxa of the family investigated. The tapetum is glandular and although homogenous, it has a dual origin. It is partly derived from the inner secondary parietal layer and partly from the cells of the connective. Hence the present observations support the contention of Periasamy and Swamy (1966) that the anther tapetum of angiosperms, although physiologically homogenous in most cases, is morphologically heterogenous.

The divisions of microspore mother cell are simultaneous, forming generally tetrahedral or rarely decussate or isobilateral tetrads of microspores. The pollen grains are shed at two celled stage although the shedding of pollen grains at three celled stages has also been reported in Iridaceae (Lakshmanan and Philip, 1971) and some other monocotyledonous families (Narayana and Lodha, 1972).

A very high degree of anther sterility has been observed in Iridaceae. Yasui and Sawada (1940) have reported 63.4% sterility in the anthers of *Iris japonica* and Lakshmanan and Philip (1971) have also observed a significant percentage of sterility in the species of *Sisyrinchium*. But they did not attribute any cause for the sterility. We have, however, observed that in those anthers where pollen grains are few, the tapetal cells show hypertrophy thus greatly reducing the sporogenous tissue causing sterility.

The ovules are anatropous, bitegmic and crassinucellate. The micropyle is formed by the inner integument. The base of the funiculus is conspicuously swollen and is marked with glandular obtruator. Such glandular cells are also known to occur in Liliaceae (Eunus, 1950; Chennaveeraiah and Mahabale, 1962) and Aizoaceae (Narayana and Lodha, 1972) and are known to facilitate entry of pollen tube into the ovule (Maheshwari, 1950).

There is only one megaspore mother cell in all the taxa studied. Haeckel (1931) and Yasui and Sawada (1940) have, however, reported two to three megaspore mother cells in *Iris* spp., but only one megaspore develops into embryo sac. All the taxa of this family studied so far by earlier workers are also characterised by the presence of Polygonum type of embryo sac (Davis, 1966). Haeckel (1931) did not observe antipodal cells in *Sisyrinchium anceps* but Lakshmanan and Philip's (1971) study on *Sisyrinchium striatum* and *S. californicum* and our work on *Sisyrinchium atlanticum* indicate the presence of antipodal cells. There are two

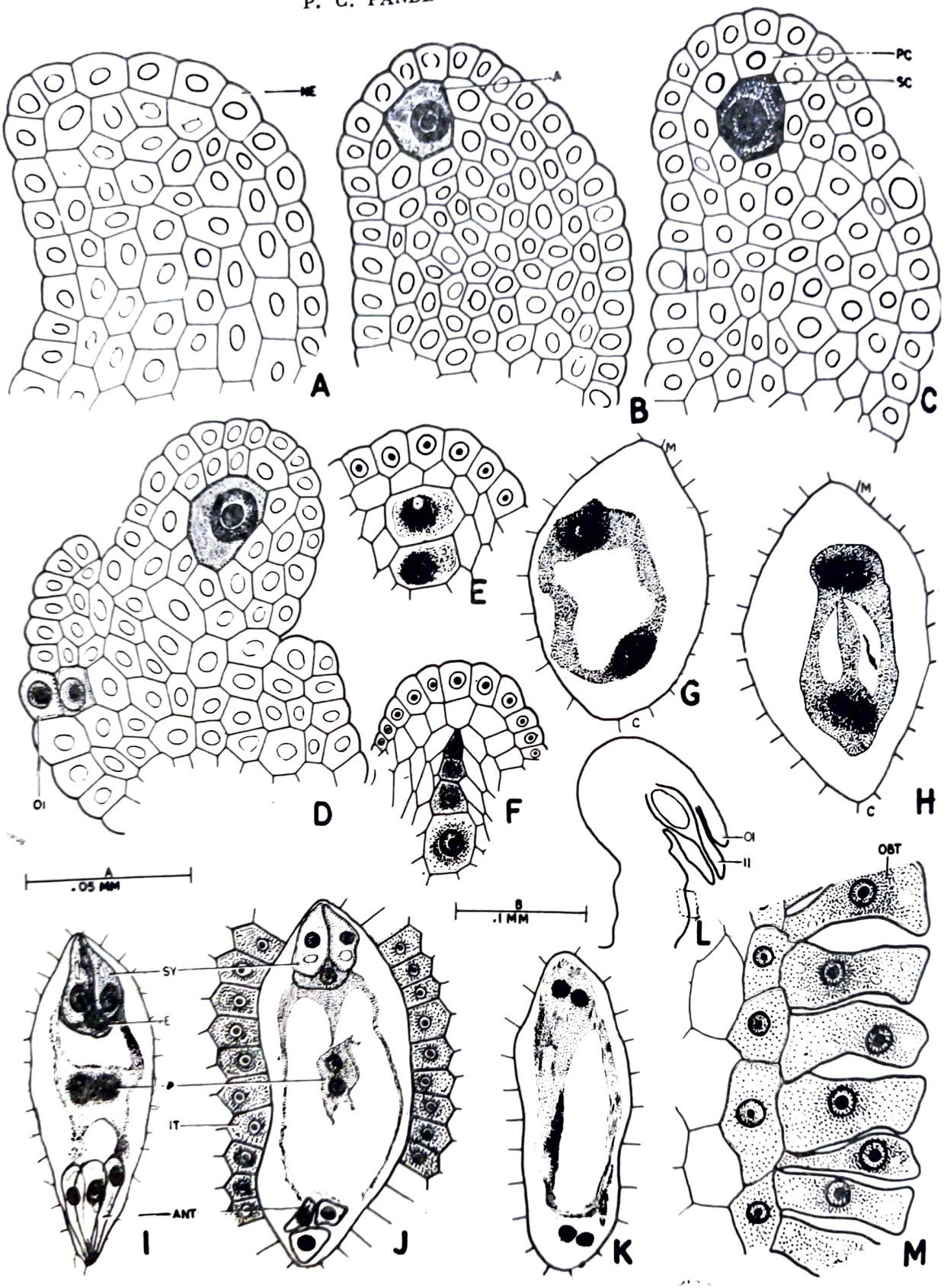

Fig. A—M. (*Megasporogenesis and the Female Gametophyte*): A. L. S. of a very young ovule of *Iris decora* showing undifferentiated homogenous tissue. B. L. S. of an ovule of *I. decora* showing hyodermal archesporial cell. C. L. S. of an ovule of *I. kumaonensis* showing parietal cell, sporogenous cell and initiation of inner integument. D. L. S. of an ovule of *I. decora* showing initiation of outer integument. E-F. Megaspore dyad and tetrad of *Iris kumaonensis*. G. H. Successive stages of megasporogenesis of *I. kumaonensis*. I. Organised embryo sac of *I.*

polar nuclei of unequal size in *Iris kumaonensis* (present work) but Karagyozova (1963) has reported up to four polar nuclei in *Iris pseudocorus* with two cells each in the egg apparatus and the antipodal groups.

In *Sisyrinchium atlanticum* an integumentary tapetum is present around the embryo sac, cells of which become radially elongated and even assigned nutritive functions (*see* Maheshwari, 1950). The formation of such layer has been reported only in *Sisyrinchium* sp. (Lakshmanan and Philip, 1971).

Present study shows high degree of sterility in ovules and only a few ovules with organized embryo sac could be observed. Yasui and Sawads (1940) have also reported about 87% sterile ovules in *Iris japonica*.

The members of Iridaceae studied show two different modes of anther dehiscence. In *Sisyrinchium atlanticum*, where endothecium develops typical fibrous thickenings, the anthers dehisce by a longitudinal slit whereas in *Belamcanda chinensis*, *Freesia armstrongii*, *Iris decora* and *I. kumaonensis* fibrous thickenings are absent and dehiscence is confined to the upper half of anther (*see* also Venkatesh, 1955). In Iridaceae, there appears a gradual transition from longitudinal and complete dehiscence (e.g. *Sisyrinchium*) to a more specialized apical dehiscence through intermediate stages (e.g. *Belamcanda chinensis*, *Freesia armstrongii*, *Iris decora*, *I. kumaonensis*) where lack of fibrous thickenings on endothecial cells lead to incomplete and partial dehiscence.

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kumaonensis. J. J. Organised embryosac of *Sisyrinchium atlanticum* showing integumentary tapetum. K. Degenerating embryosac of *Iris decora* at the four nucleate stage. L. Longitudinal section of ovule of *I. decora* showing obturator. M. Enlarged view of the portion emblocked in figure L to show glandular hairs of the obturator of *I. decora*.

A—Archesporial cell; ANT—Antipodals; E—Egg; II—Inner integument; IT ϕ Intetumentary tapetum; NE—Nucellar epidermis; OBT—Obturator; OI—Outer integument; P—Polar nuclei; PC—Parietal cell; SC—Sporogenous cell; SY—Synergids.