EMBRYOLOGY OF ARISTIDA (POACEAE)

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Abstract: Embryology of *Aristida funiculata, A. hystrix, A. mutabilis* and *A. setacea* has been studied. The anthers are tetrasporangiate. The anther wall comprises of an epidermis, endothecium, middle layer and tapetum. The anther tapetum is of the Glandular type and its cells become 2-to 4-nucleate. Endothecium develops fibrous thickenings when the pollen grains are at 2-celled stage. Meiosis is normal and successive cytokinesis leads to the formation of isobilateral microspore tetrads. Pollen grains are monoporate and 3-celled at the time of shedding. Supernumerary nuclei have been observed in pollen grains of *A. hystrix*. The ovule is hemianatropous, bitegmic and pseudocrassinucellate. However in *A. funiculata* and *A. setacea* ovules are tenuinucellate. Meiosis in megaspore mother cell leads to the formation of either 'T'-shaped or linear tetrad of megaspores. The chalazal megaspore of the tetrad is functional and develops into Monosporic Polygonum type of embryo sac. The number of antipodals varies from 3 to 13. Endosperm development is of the Nuclear type and the embryo development conforms to the Asterad type.

INTRODUCTION

The genus *Aristida* belongs to the tribe Aristideae of the sub-family Pooideae of the family *Poaceae*. A perusal of literature revealed that very little information is available on the embryology of this genus. Embryology of *Aristida adscensionis* has been studied by Bhanwra et al. (1982) and Bhanwra (1988). Except this species other members of this genus have not been studied embryologically. Hence the present investigation has been undertaken to study the embryology of *Aristida funiculata* Trin. & Rupr. *A. hystrix* L. f., *A. mutabilis* Trin. & Rupr. and *A. setacea* Retz.

MATERIALS AND METHODS

The material for the present study was collected from Sri Krishnadevaraya University Campus, Anantapur and from nearby Places like Reddipalli farm during the months of July-September. Inflorescences at different stages of development were fixed in Formalin-Acetic Acid-Alcohol (F. A. A.). The florets were dehydrated in tertiary butyl alcohol series and embedded in Paraffin wax. Sections were cut at a thickness of 6 to 8 μ m and were stained in Delafield's haematoxylin. They were mounted in Canada balsam. Voucher specimen no. *GNVF* 5677, 7001, 5684 and 5698 have been deposited in the Herbarium of the department of Botany, Sri Krishnadevaraya University, Anantapur.

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RESULTS

Microsporangium, Microsporogenesis and Male gametophyte:

The anthers are tetrasporangiate. The anther wall consists of epidermis, endothecium, middle layer and tapetum (Figs. 1A, 2A, 4A, 6A). The endothecium develops fibrous thickenings by the time two-celled pollen grains are formed (Figs. 1Q, 2B, 4E, 6C). The middle layer is ephemeral and gets degenerated. The tapetum is of the Glandular type. Its cells are uninucleate at pollen mother cell stage (Figs. 1A, 2A, 4A, B, 6A). Later they become binucleate by the time pollen mother cells are at meiotic stage (Figs. 1B, 4C, 6B). Tapetal cells with three and four nuclei have also been observed in *Aristida funiculata* (Fig. 1C) and *A. mutabilis* (Fig. 4D).

The primary sporogenous cells directly function as pollen mother cells and undergo only transverse divisions to add up the number of pollen mother cells in *A. hystrix* (Fig. 2A) and *A. mutabilis* (Fig. 4A). In these species the pollen mother cells are arranged in a single row. Formation of two rows of pollen mother cells due to vertical and transverse divisions of the sporogenous cells has been observed in *A. funiculata* (Fig. 1A) and *A. setacea* (Fig. 6A). The pollen mother cells undergo normal meiosis and successive cytokinesis leads to the formation of isobilateral tetrads of microspores in all the four species (Figs. 1D-I, 4F-H, 6D-F). The divisions in the pollen mother cells of the anther lobe are synchronous.

The microspores seperate from the tetrads, become nearly spherical and increase in size (Figs. 1 J-L; 2 C-D; 4 I-M; 6 G-H). The microspores are richly cytoplasmic and possess a prominent centrally located nucleus. The microspore nucleus moves to the peripheral position near the wall due to the appearance of a large vacuole in its cytoplasm (Figs. 1M, 2E, 4N, 6I). The microspore nucleus divides to form a small generative cell and a large vegetative cell (Figs. 1N, 2F, 4O, 6J). The generative cell moves into the cytoplasm of the vegetative cell (Figs. 1O, 2G, 6K) and futher divides to form two male gametes. The mature pollen grains are monoporate with smooth exine and thin intine. They are three-celled at the time of anthesis (Figs. 1P, 2H, 4P, Q, 6L, M). Increase in the number of nuclei up to four in the mature pollen grains is observed in *A. hystrix* (Fig. 2I).

Ovary and Ovule:

The ovary as characteristic of Poaceae contains a single basal ovule. The ovule in all the four species of the present study is hemianatropous and bitegmic. The ovule is tenuinucellate in *A. funiculata* and *A. setacea* and pseudocrassinucellate in others. In these two species the nucellar epidermis undergoes one or two periclinal divisions to form 2 or 3 parietal layers (Figs. 2L, M, 4S). Both the integuments are well developed and the micropyle is formed by the inner integument alone (Figs. 2 L, 4S, 6O).

Megasporogenesis and Female gemetophyte:

The female archesporium is hypodermal and single-celled (Fig. 2J). The archesporal cell increases in size and directly functions as megaspore mother cell. The megaspore mother cell undergoes normal meiosis to give rise to the formation of either T-shaped or linear tetrad of megaspores. T-shaped megaspore tetrad has been observed in *A. setacea* (Fig. 6N) while linear megaspore tetrad is observed in *A. hystrix* (Fig. 2K) and in *A. mutabilis* (Fig. 4R). The chalazal megaspore of the tetrad is functional and develops into monosporic Polygonum type of embryo sac. The organised embryo sac is having an egg cell, two synergids and three antipodals. Later the two polar nuclei fuse to form the secondary nucleus and the three antipodals increase in number (Figs. 1 R, S; 2 L, M; 4 S-U; 6 O, P). The egg is pearshaped. The synergids are hooked in *A. funiculata* (Fig. 1R, S), *A. mutabilis* (Fig. 4 S, U), *A. setacea* (Fig. 6O, P) and pear-shaped in *A. hystrix* (Fig. 2L, M). Variation in the number of antipodals has

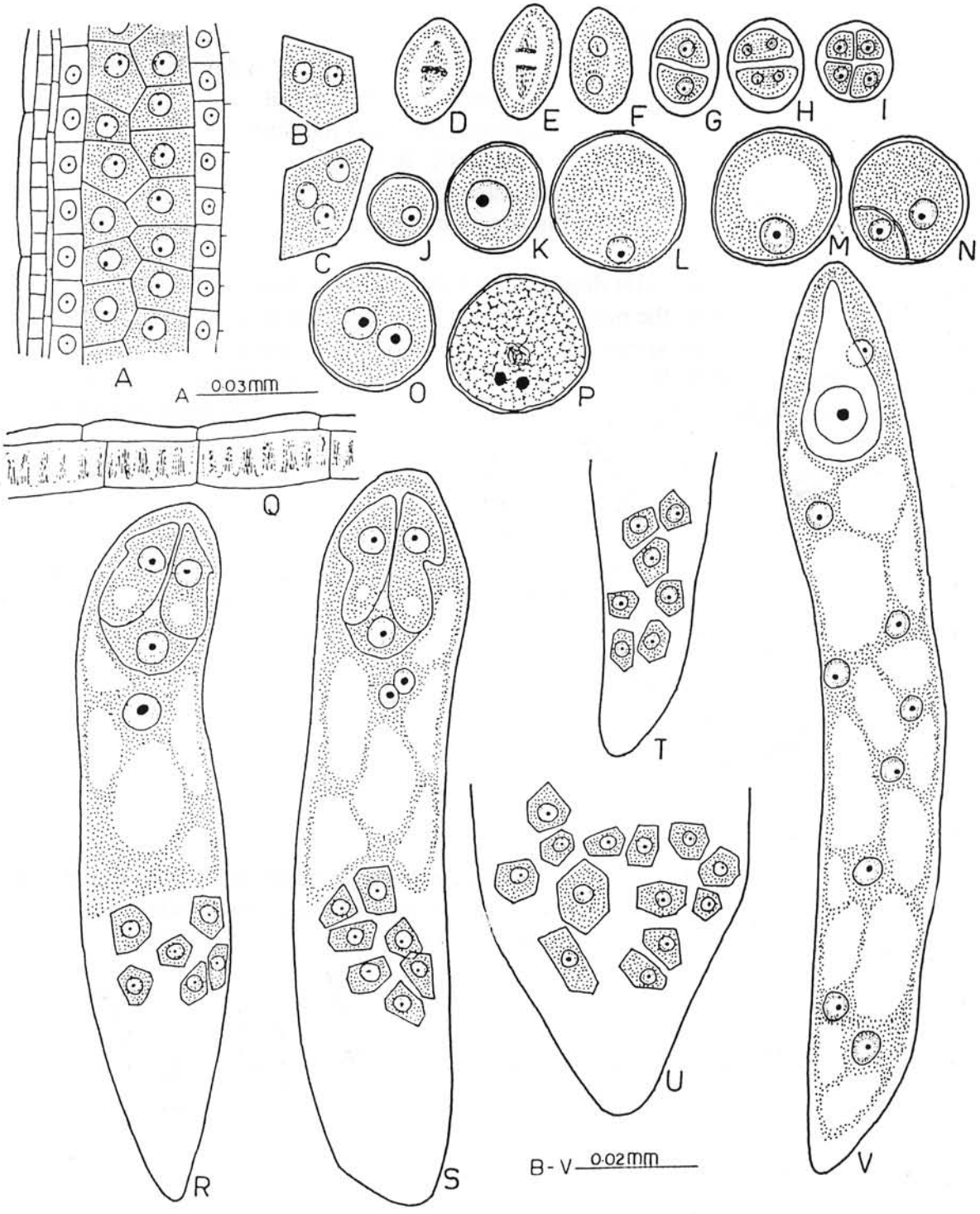

Fig. 1 A-V. *Aristida funiculata.* A. Ls. Part of anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cells; B, C. 2-and 3-nucleate tapetal cells; D-I. Pollen mother cells in meiosis; J-M. 1-nucleate pollen grain; N, O. 2-celled pollen grains; P. 3-celled pollen grain; Q. Fibrous endothecium; R. S. Organised embryo sacs; T, U. Chalazal part of embryo sac showing antipodals; V. Embryo sac showing zygote and endosperm nuclei.

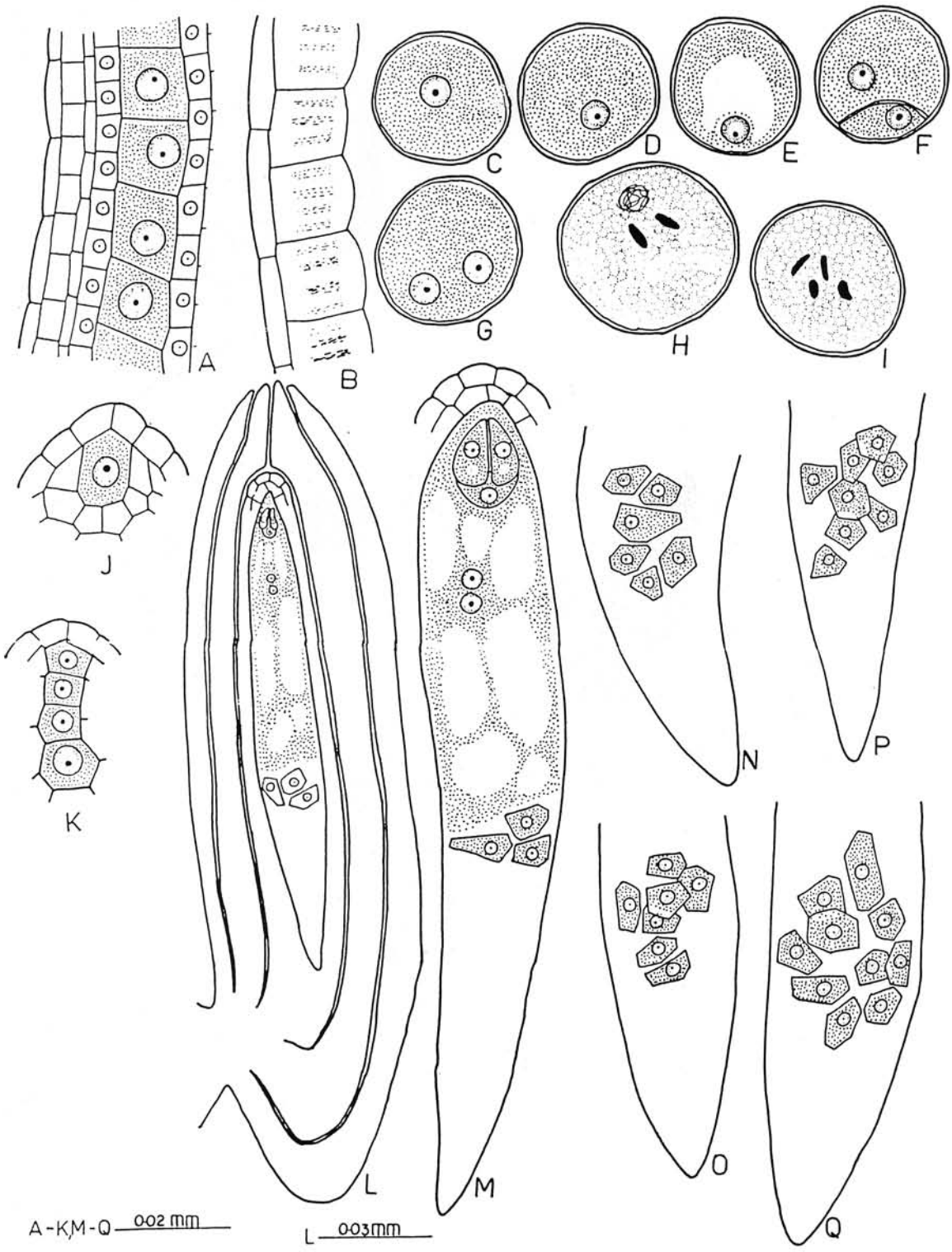

Fig. 2 A-Q. *Aristida hystrix*. A. Ls. Part of the anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cells; B. Fibrous endothecium; C-E. 1-nucleate pollen grains; F, G. 2-celled pollen grains; H. 3-celled Pollen grain; I. Pollen grain with supernumerary nuclei; J. Female archesporium; K. Megaspore tetrad; L. Ls of Ovule at organized embryo sac stage; M. Organized embryo sac; N-Q. Chalazal part of embryo sac showing antipodals.

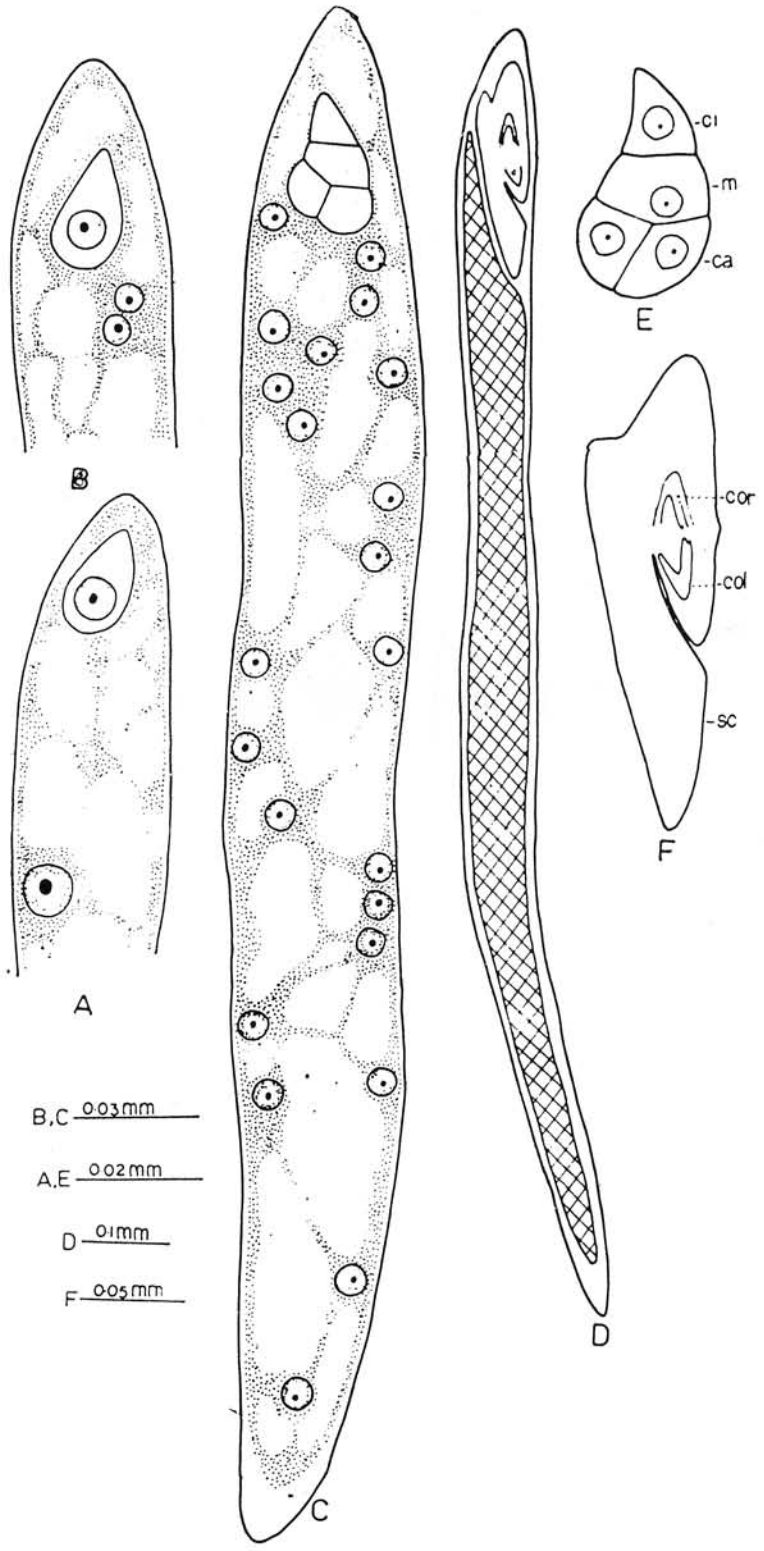

Fig.3 A-F. *Aristida hystrix.* A, Micropylar part of embryo sac showing zygote and primary endosperm nucleus. B. Micropylar part of embryo sac showing zygote and endosperm nuclei; C, D. Stages in the development of endosperm; E. 4-celled embryo; F. Mature embryo. (Sc. Scutellum; Col-Loleoptile Cor-Colecrhiza).

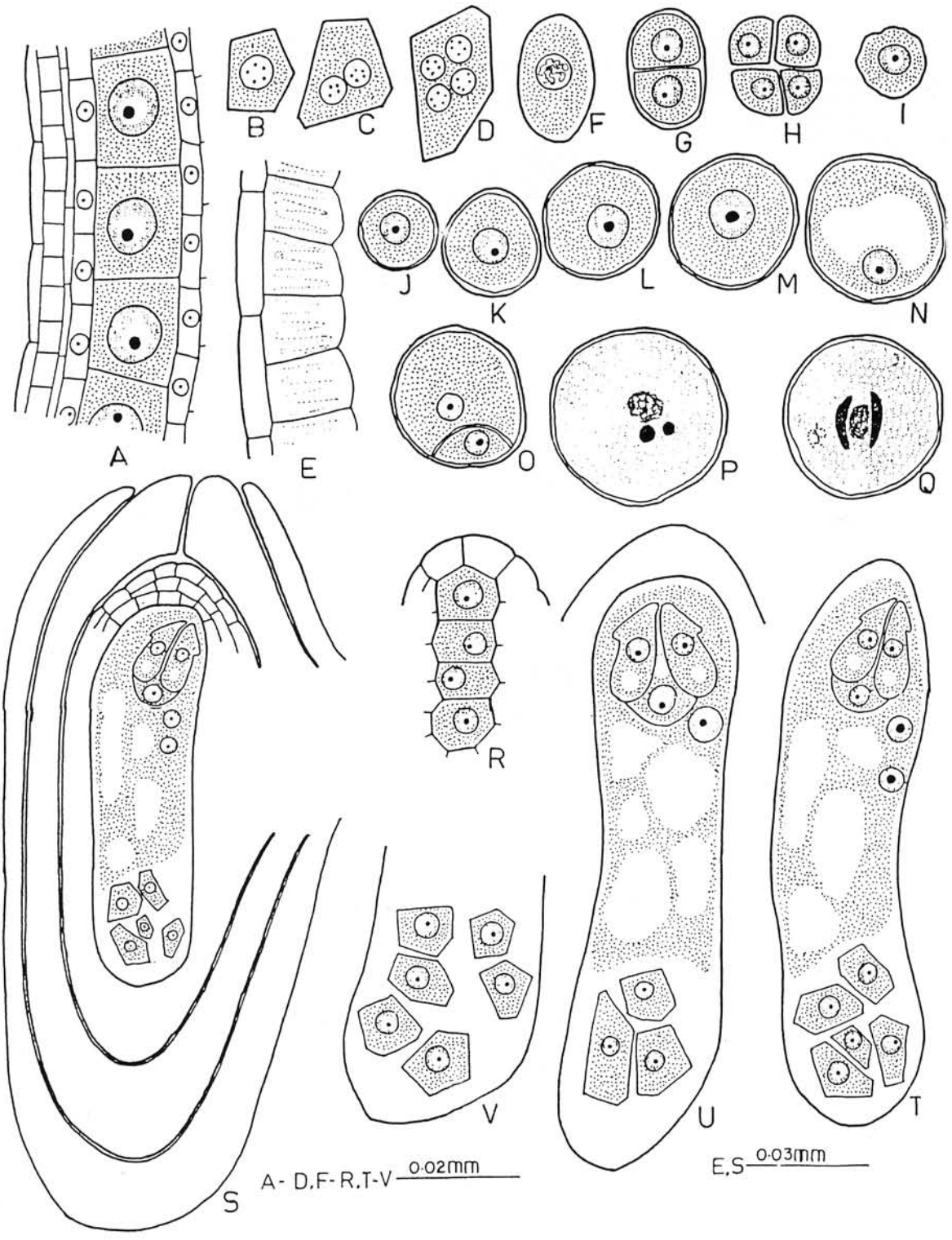

Fig. 4 A-V. *Aristida mutabilis.* A. Ls. Part of anther lobe showing epidermis, endothesium, middle layer, tapetum and pollen mother cells; B. D. Tapetal cells with 2, 3 and 4-nuclei; E. Fibrous endothecium; F-H. Pollen mother cells in meiosis; I-N. 1-Nucleate pollen grains; 0.2-celled pollen grain; P, Q. 3-celled pollen grains; R. Megaspore tetrad; S. Ovule at organised embryo sac stage; T-U. Organized embryo sac. V. Chalazal part of embryo sac showing antipodals.

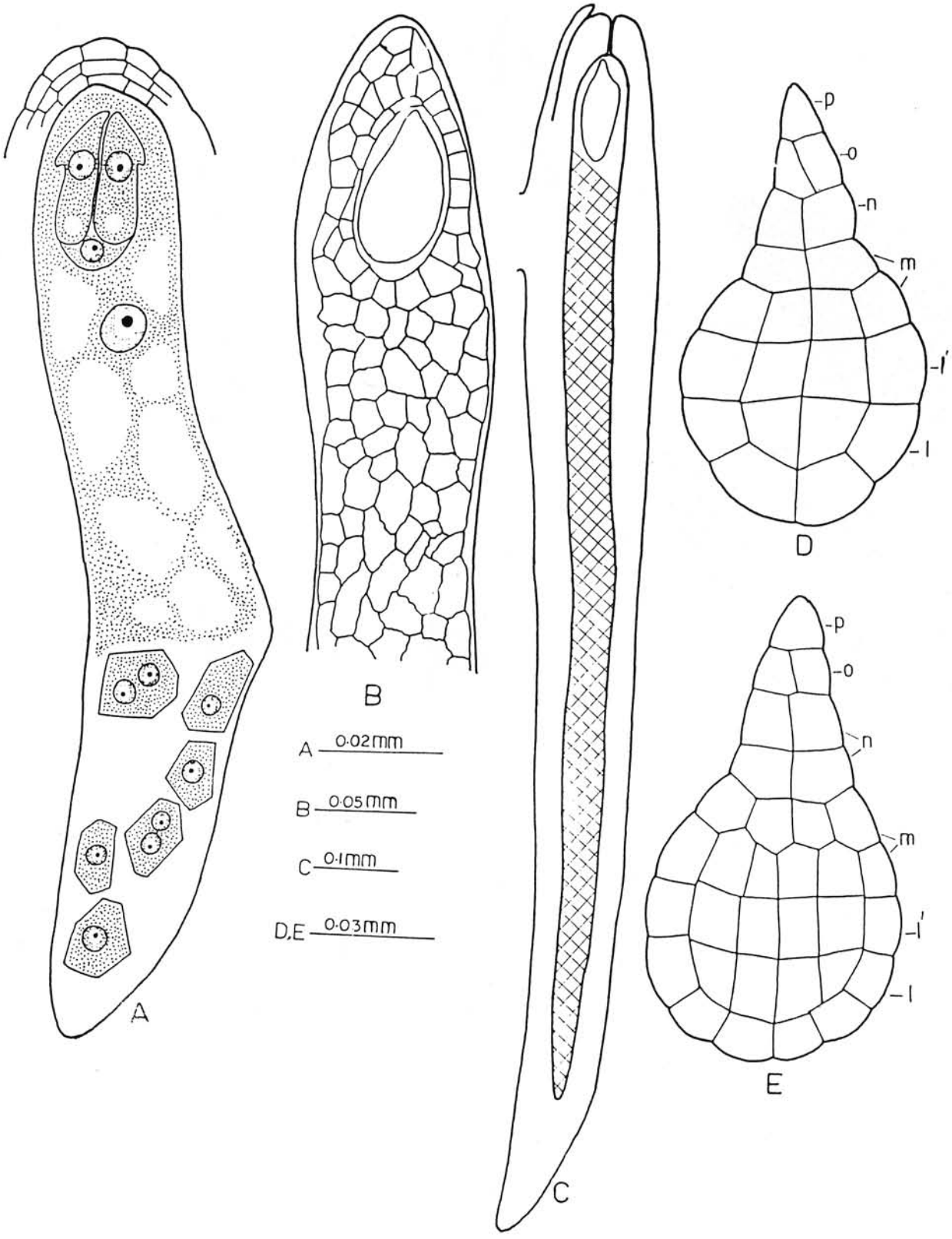

Fig. 5 A-E. *Aristida mutabilis.* A. Organized embryo sac with lateral antipodals; B, C. Stages in the development of endosperm; D, E. Stages in the development of embryo.

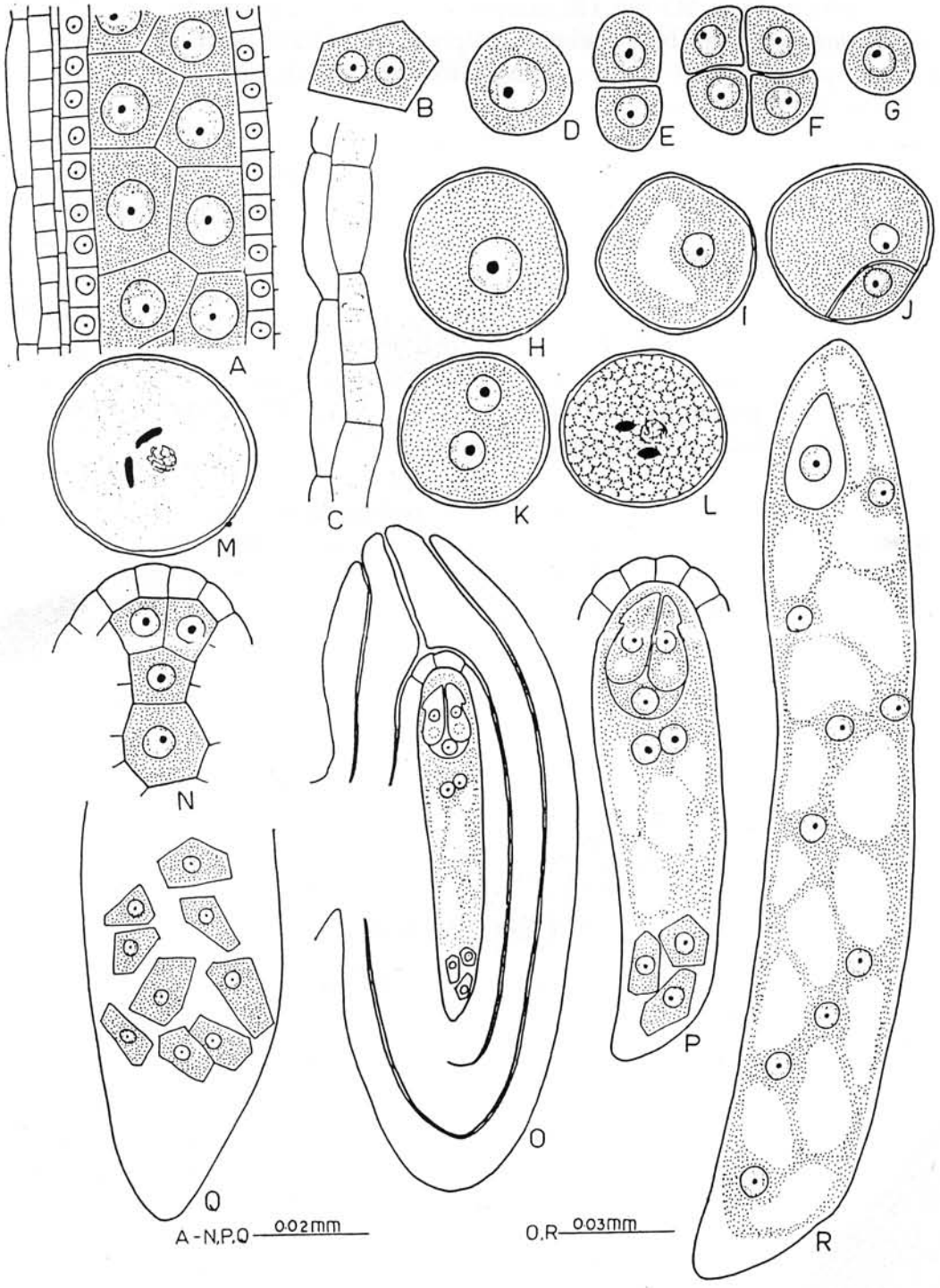

Fig. 6 A-R. *Aristida setaceae.* A. Ls. Part of anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cells; B. 2-nucleate tapetal cell; C. Fibrous endothecium; D. Pollen mother cell. E. Microspore dyad. F. Pollen tetrad G-I. 1-Nucleate pollen grains; J, K. 2-celled pollen grains; L, M. 3-celled pollen grains; N. Megaspore tetrad; O. Ovule at organized embryo sac stage; P. Organized embryo sac; Q. Chalazal part of embryo sac showing nine antipodals; R. Embryo sac showing zygote and nuclear endosperm.

been observed in all the four species of this genus. The number of antipodals varies from 6-13 in *A. funiculata* (Fig. 1R-U), 3-10 in *A. hystrix* (Fig. 2L-Q), 3-6 in *A. mutabilis* (Fig. 4 S-V) and 3-9 in *A. setacea* (Fig. 6O-Q). The antipodal cells in all the species are uninucleate except in *Aristida mutabilis* where two-nucleate antipodal cells have been met with (Fig. 5A). The organised embryo sac shows slight curvature towards the chalaza. After fertilisation the antipodal cells occupy lateral position.

Fertilization:

The actual course of pollen tube is not traced. But it appears to enter through the micropyle into one of the synergids. Syngamy and triple fusion occur simultaneously and results in the formation of zygote and primary endosperm nucleus (Fig. 3A).

Development of endosperm and embryo:

The primary endosperm nucleus divides earlier than the zygote (Figs. 1V, 3B, 6R). The primary endosperm nucleus undergoes repeated free nuclear divisions resulting in the formation of free nuclear endosperm (Figs. 1V, 3B, C, 6R). Wall formation in the endosperm takes place first around the proembryo and then progresses towards the chalazal end of the embryo sac (Fig. 5B). The endosperm becomes completely cellular at later stages of embryo development (Figs. 3D, 5C).

The zygote undergoes a period of rest and undergoes division. The early stages in the development of embryo are not traced. But the observations of the later stages reveals that the first division takes place transversely resulting in the formation of the terminal cell *ca* and the basal cell *cb*. The next division in the cell *ca* is vertical and in *cb* it is transverse resulting in the formation of cells *m* and *ci* (Fig. 3E). Further transverse and vertical divisions in cells *ca, m* and *ci* results in the formation of tiers *1, 1', m, n, o* and *p* (Fig. 5 D, E). Further divisions in these tiers are irregular and from the globular embryo, which later develops into mature embryo (Fig. 3F). Embryo development conforms to the Aslerad type of Johansen (1950). The mature embryo consists of scutllum, coleoptile, coleorhiza and epiblast. The lower part of the scutellum is absent (Fig. 3F). According to Reeder (1957) this type of embryo is known as Festucoid type.

DISCUSSION

The microsporogenesis and the development of male gametophyte is almost similar in all the members of the subfamily Pooideae including the present study. The anther tapetum is of the Glandular type and its cells become twonucleate in all the four species of the present investigation. However tapetal cells with three to four nuclei have been observed in *A. funiculata* and *A. setacea* (present study). The pollen grains are monoporate and three-celled as reported in other members of the sub-family (Chandra, 1976; Bhanwra, 1988). However Chikkannaiah and Mahalingappa (1976) reported 2-celled pollen grains at the time of shedding. This observation need confirmation. Supernumerary nuclei have been observed in *A. hystrix* (present study). Supernumerary nuclei have earlier been reported in *Eleusine coracana* (Narayanaswami 1952), *Eragrostis poaeoides* (Chandra, 1976) and in *Chloris roxburghiana* (Febulaus and Pullaiah, 1991).

The ovule in the subfamily Pooideae is hemianatropous, bitegmic and tenuinucellate (Bhanwra et al. 1981; Chandra, 1963 a, b; 1976; Febulaus and Pullaiah, 1990, 1991). Such ovules have been reported in *Stipa tortilis* (Maze et al., 1970), *Tragus biflorus, Chloris barbata* (Bhanwra et al., 1981), *Aristida adscensionis* (Bhanwra et al., 1982) and the present study *A. funiculata* and *A. setacea.* However in *A. hystrix* and *A. mutabilis* two or three parietal layers are observed occassionally. Bhanwra et al. (1982) also reported the occurrence of

one or rarely two periclinal divisions in *A. adscensionis.*

Proliferation of antipodal cells is a common feature reported in several species of the subfamily Pooideae (Santos, 1933; Venkateswarlu and Devi, 1964; Chandra, 1976; Bhanwra, 1986). The present study is also in conformity with this condition. Increase in the number of antipodal nuclei has been reported by Maze et al. (1970) in *Oryzopsis* and *Stipa.* Three nucleate antipodal cells have been reported in *Chloris roxburghiana* (Febulaus and Pullaiah, 1991). In the present investigation also 2-nucleate antipodal cells are observed in *Aristida funiculata* and *A. mutabilis.*

In possessing bitegmic, hemianatropous ovules, lateral position of antipodals prior to degeneration, mature embryo sac showing curvature towards the chalaza, absence of enlarged nucellar cells below micropyle, well developed outer and inner integument presence of epiblast and absence of lower part of scutellum to four species of *Aristida* of the present study are closely related to the other members of the subfamily *Poolideae.*

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三芒草屬（禾本科）之胚胎發育

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摘要

本文研究四種三芒草屬(*Aristida*)植物之胚胎發育，其種類爲珠柄三芒草(*A. funiculata*)，瓶刷三芒草(*A. hystrix*)，多型三芒草(*A. mutabilis*)及剛三芒草(*A. setacea*)。其花藥具有四囊，花藥壁由表皮(epidermis)，內皮層(endothecium)，中間層及營養層(tapetum)組成。營養層屬於腺體型(glandular type)，其細胞最後發育成二核到四核。內皮層於花粉粒是二細胞的階段，發育出纖維狀加厚，減數分裂後，連續的質分裂形成等二側的四分體小孢子。成熟花粉粒爲單孔，具三個細胞。在瓶刷三芒草曾觀察到具有更多的細胞核。胚珠是半倒生型(hemisnatropous)，具二層珠被(bitegmic)及擬厚珠心(pseudocrassicellate)，但是珠柄三芒草及剛三芒草則爲薄珠心型，大孢子母細胞經減數分裂後形成型或線型之四分體大孢子。四分體大孢子的合點(chalazal)是有功能的，並發育成蓼型(polygonum type)的胚囊。反足細胞(antipodals)具3到13個。胚乳之發育屬於幼壁式(nuclear type)。胚胎之發育屬於紫菀型(Asterad type)。