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MEGASPOROGENESIS AND DEVELOPMENT OF FEMALE GAMETOPHYTE, FERTILIZATION AND ENDOSPERM DEVELOPMENT IN *Aristida adscensionis Linn.*

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ABSTRACT:
The paper deals with the embryological investigations on *Aristida adscensionis.* The Voucher specimen: N-1deposited in Botany department of RTM Nagpur University. The female gametophyte, fertilization and endosperm development described in details. A mature ovule is the organ that forms seed of flowering plants. It is borne inside the ovary of the flower and consists of nucellus protected by integuments. The precursors of embryo/endosperm and seed coats gradually developed after fertilization. Fertilization is porogamous. A diploid zygote(2n) cell divide and redivide to form a multicellular embryo. A primary endosperm nucleus (3n) invariably divides and forms free nuclear stage, embryo sac increase in size considerably and cellular phase of endosperm sets in at 8-10 cell stage of embryo development. The ovule is Campylotropous, body of the ovule is curved this results in the micropyle and chalaza being not aligned in a straight line (Micropyle almost touching to the funiculus). During seed development the entire nucellus is consumed by developing embryo. During gradual growth of embryo, consumes earlier a part of cellular endosperm at micropyle region. The mature embryo is not embedded in the endosperm but lies peripheral to it. The seeds are endospermic in the present taxa.

Key words: Poaceae, Pooideae, Ovule, megasporogenesis, tenuinucellate, embryo sac, fertilization and endosperm development, antipodal complex, hypostase, endospermic seed.

INTRODUCTION:

The Poaceae is known for its structural diversity both in vegetative and reproductive morphology. The division of the Poaceae into two subfamilies viz, Pooideae and Panicoideae as proposed by Brown (1814) is maintained even today. Embryological investigations on this taxon indicate that embryological features at sub-family level follows more or less uniform pattern (Narayanswami,1955 a,b,c ; Koul, 1970 ; Raju , 1980 ; Bhanwra et. al. 1991; Deshpande and Makde, 1994 ; Nikhade and Makde, 1997; Febulaus, (T.N.V.& Pullaiah, 1992). The Present work deals with embryological investigation on *Aristida adscensionis* pointing out the characters of taxonomic value

MATERIAL AND METHODS

Materials was collected from Navargaon locality & fixed in F.A.A. (70% ethanol). Customary methods of dehydration, clearing & embedding were followed (Johanson ,1940).Sections were cut at 8-12micron thick & stained with Delafield hematoxylin. Erythrosin or fast green was used as counter stain. The sections were mounted in Canada balsam. Diagrams were drawn with the help of camera lucida.

MEGASPOROGENESIS AND DEVELOPMENT OF FEMALE GAMETOPHYTE

The family Poaceae is characterized by bicarpellary / tricarpellary, syncarpous pistil having a single ovule in unilocular ovary (Lawrence, 1951; Hutchinson, 1973; Campbell, 1985). The ovule is attached on overy wall with a short funiculus, laterally and almost in central position (Fig. 2C). The ovule is bitegmic and both the integuments are two layered for their major portion.

The female archesporium functions directly as megaspore mother cell. As such no parietal tissue is developed in the ovule, hence the ovule is tenuinucellate in the family (Fig. 2D, E). The nucellus is quite massive and continue to persist as a healthy tissue during the mature organization of embryo sac. The peripheral layer

persist till the embryonic development and its degeneration is gradual. The nucellus at the chalazal end persists and form the hypostase where the cells get tannin filled (Fig.2K-N). The ovule develops as a broad protuberance almost straight or slightly inclined protuberance from the short but broad funiculus (Fig.2.AB). It gradually turns inside the ovary and megaspore mother cell stage the ovule appears to be placed at right angles to the ovary wall, during further development it bends inside the ovarian cavity and finally assumes campylotropous form (Fig. 2C). The initials of the integuments develop almost simultaneously with the differentiation of megaspore mother cell. (Fig 2.B).

The megaspore mother cell is a large polygonal cell having distinct nucleus and cytoplasm. The MMCs undergoes Meiosis I followed by successive cytokinesis, Meiosis II in the dyad cells is synchronous. This division followed by wall formation. Thus linear megaspore tetrads are formed (Fig.2F).The chalazal megaspore alone functions & remaining three megaspore degenerate. The degenerating remains persist up to 2-4 nucleate embryo sac stage (Fig.2G.H). The functional megaspore enlarges in size and undergoes three successive mitotic division to produce eight nuclei. Two daughter nuclei produced at the end of first mitosis division migrate to opposite poles as their appears vacuole in between them .Each of these nuclei under second mitotic division to give rise to four nuclei, two at each pole (Fig. 2H).One more mitotic division in each nucleus results in 8-nucleate embryo sac. These nuclei are disposed in two group of four each with a large vacuole between them.

During organization of embryo sac, three nuclei from the Micropyle quartet organize to form an egg apparatus while three form the chalazal quartet give rise to three primary antipodals, the remaining two nuclei one each from either pole constitute the polars. The development of embryo sac, thus conforms to polygonum type (Maheshwari, 1950). The organized embryo sac is more or less cylindrical and its micropyle end remains slightly broader (Fig. 2 I,J).

The egg apparatus consists of a flask shaped egg and two synergids. The egg is characterized by basely placed nucleus and dense cytoplasm. Though the vacuole is absent in egg, but small vacuole is noticed in this taxon. Two polars are quite prominent and lie in close approximation to each other very near the egg.

Antipodals and Antipodal complex: At the time of organization of embryo sac there are only three antipodal cells located at the chalazal end. Mitotic divisions in them result in an increase in their number and form an antipodal complex of 8-10 cells (Fig. 2 I,J).

Hypostase : A group of nucellar cells at the chalazal end of the ovule becomes prominent and differ from the adjoining cells. This tissue constitute the hypostase are well developed and cylindrical (Fig. 2 K-N). After fertilization the cells constituting hypostase get filled with tanniniferous granular deposits (Fig 2MN).

Fertilization.

Pollination is anemophilous. The wind borne pollen grains germinate on the stigma to produce a pollen tube. The pollen tube grows through the style and on its way to ovary sac, first passes along the inner epidermis of the ovary wall, takes a bend comes over the outer integument and enter the micropyle passing over the inner integument. Thus fertilization is porogamous. During its passage in embryo sac, it invariably passes over or through one of the synergids. Two pollen tubes noticed. (Fig.3A). Pollen tube bursts and discharge its contents within the embryo sac. At the time of fertilization the two polar are in close approximation to each other but do not fuse. They lie invariably below and close to the egg. One male gamete is seen attached to one polar nucleus and those fuses with it (Fig. 3A).

Endosperm Development

The two polar nuclei fuse to form a secondary nucleus. This diploid nucleus fuses with one of

male gamete called triple fusion. Thus formation of primary endosperm nucleus 3n (contain 3 sets of chromosome per nucleus). It is a source of nutrition for developing embryo. The primary endosperm nucleus invariably divides soon after triple fusion and often earlier then the Zygote (Fig. 3B,C). The initially repeated divisions are of free nuclear type. A large number of nuclei thus formed & later on arranged in a peripheral cytoplasmic layer around a central vacuole (Fig. 3B). The fertilized egg remains undivided till 4-8 free endosperm nuclei are formed. At the bicelled/tetrad proembryonic stage the number of free endosperm nuclei is almost doubled (Fig.3C, D).

After the sufficient number of free nuclear endosperm nuclei are formed, cellular phase of endosperm initiate. In this taxon endosperm starts entering the cellular phase just after the embryo attains 10 celled stage (Fig.3F) . Initiation of the cellular phase in endosperm commences first in the micropyle region and gradually progress towards the chalazal end. The cell wall formation begins at the periphery and progress centripetally and entire endosperm becomes cellular. The cells of endosperm in the vicinity of embryo are full of cytoplasm and take a deep stain, elsewhere the cells are larger and highly vacuolated. The starch grains are spherical or elliptical with single aleurone layer (Fig. 3G). During seed development the entire nucellus is consumed by the growing endosperm which eventually fills the entire seed. The embryo consume only a part of cellular endosperm especially located on the sides & in the micropylar region. The seeds are thus endospermic in this taxon.

RESULT & DISCUSSION:

The Poaceae are characterized by Bi/ Tricarpellary syncarpous gynoecia with unilocular / uniovular ovary (Lawrence, 1951; Hutchinson, 1973; Campbell, 1985) . However, according to Aziz (1972) who studied *Triticum aestivum*, the gynoecium in its initiation and early histogenesis is essentially similar to single foliage leaf and does not reveal its tricarpellary condition. The attachment of the ovule to the ovary wall may be at lateral in *Aristida ascensions*. Diwanji (1976) and Gawali (1977) while working on different taxa at poaceae reported such variability. The attachment of ovule to ovary wall is variable in the family (Cronquist 1968, 1981).The present investigation corroborates this contention.

The ovule in its form is campylotropous in this taxa and this is true for *Poa pratensis & Poa compressa* (Anderson, 1927). The other forms of ovules have been described in the family by earlier embryologist. The archesporial cell enlarges and function as MMC thus parietal cell or tissue is not formed in the ovule. The ovule is thus, tenuinucellate. According to Davis (1966) this condition is found in the taxa belonging to pooideae while in panicoideae, nucellar epidermis at the tip in the micropylar region divides periclinally and forms a copy variable number of layers. The distinction between pooideae and panicoideae on the basis of this character is advocated by Bharwra (1988) who studied 59 species belonging to Bambusoideae, Arundinoideae, Pooideae, Chloridoideae and Panicoideae grasses together, the details regarding structure, origin and the development of nucellar cap are not given. The typical tenuinucellate condition is reported by Chandra (1963b), Venkateshwarlu & Devi (1964) , Diwanji (1976), Bhanwra (1985,1988) Bhanwra & Choda (1981) in the taxa studied by them.

The present paper also favour, the nucellar cap is the distinctive character of the two subfamilies and has considerable taxonomic value. The megaspore tetrads of varying pattern are described in the members belonging to poaceae and these pattern vary even within the limit of single species as reported by Raju (1980). Davis (1966) favours the statement the arrangement of megaspores is usually quite fortuitous and has no taxonomic significance.

The chalazal megaspore gives rise to embryo sac while the remaining megaspore degenerates as stated by Davis (1966). However, all or any one of the four megaspores functional may begin to enlarge and divide, give rise to an embryo sac (Raju, 1980). The contention that the embryo sac in all the sexually reproducing members of poaceae develops into polygonum type (Maheshwari, 1950). This is a very constant feature in the family as investigated so far. The egg apparatus shows an egg and 2 synergids. The synergids are invariably hooked and develop filiform apparatus prior to fertilization in this taxa.

According to Ambegaonkar and Johri (1977) in Triticale one synergid enlarges and persists up to 2 celled stage of proembryo. However, work of Cass and Jenson (1970) Chao (1971) and Maze and Lin (1975) emphasized that pollen tube penetrated to synergid is first to degenerate. This is also true in *Aristida adescensionis*. The antipodals in the family show considerable variation in the behaviour, nature and constitution which is very peculiar to this taxon. A tendency towards multiplication of antipodal tissues is a feature noticed in the Gramineae by Hector in 1936 and this is true in present taxon. The antipodals remain 3-celled and uninucleate in *Eleusine coracana* (Khosla, 1946; Narayanswami, 1955 a,c), *E. indica* (Chandra, 1963 a,b). The number of antipodals in *Melocanna bambusoides* has been recorded to be 40(Yakovlev, 1970), in *Sasa paniculata*) 300 (Yamaura 1933), in *Dendrocalamus hamilfoni* 40-60 (Hari Gopal & Manasi Ram, 1981), in *Dentrocalamus stricuts* 10-12 and in *Bambusa arundinacea* there are 6 antipodals (Bhuskute , 1990).

Hoshikawa and Higuchi (1960-61) attribute haustorial role which has earlier been pointed by Rangaswamy (1935) and later accepted by Chikkannaiah and Mahalingappa (1976 a). The nutritive role is further confirmed by Bhuskute and Makde (1986) in *Briza minor* where they have described haustorium from the chalazal antipodals. The taxonomically interesting aspect of the antipodals is the position they occupy in the embryo sac. In the pooideae they are lateral supported by many embryologists. But taxa investigated by Nikhade and Makde (1995) show chalazal as well as lateral position of antipodals.

The fertilization in poaceae is porogamous and substantiated by the work on *Aristida adescensionsis*. The process of double fertilization are normal , Syngamy and triple fusion also occur in a normal manner (Bhanwra at. al. 1991). The primary endosperm nucleus invariably divides earlier than the zygote, this is reported in majority of the taxa investigated by Cannon (1900), Narayanswami (1953,1955 a); Luxova (1968); Yakovlev (1970); Deshpande (1976); Diwanji (1976); Gawali (1977); Raju(1998); Bhanwra and Choda (1981); Bhuskute (1990); Ghaisas (1991), Nikhade & Makde 1995.

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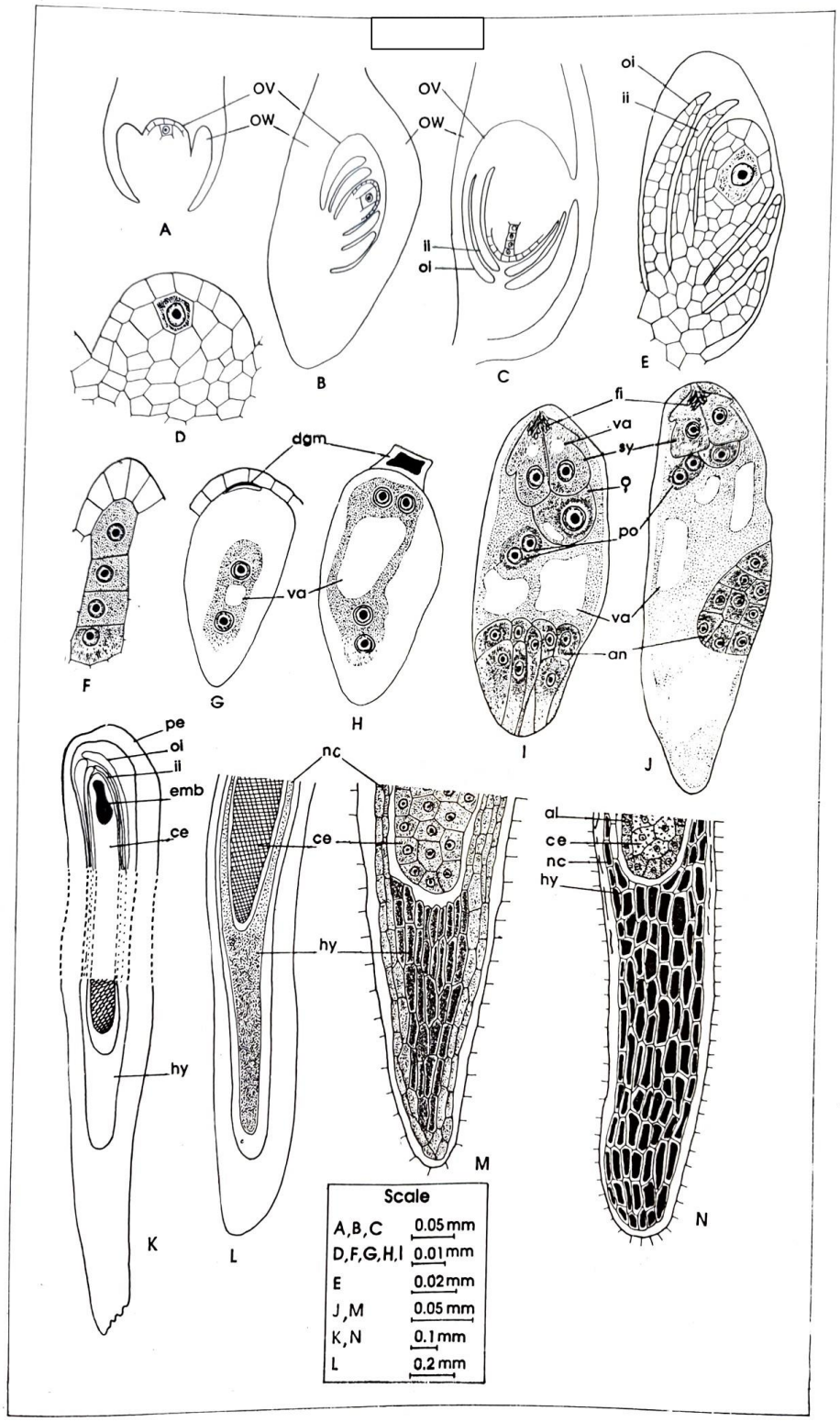
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Fig. 2

A-N : *Aristida adescensionis* Linn.

(Megasporogenesis and female gametophyte)

A,B,C : L.S. Ovary (diagrammatic) showing stages in the development of campylotropous ovule.

D : L.S. ovule at megaspore mother cell stage.

E : L.S. young ovule showing megaspore mother cell.

F : Megaspore tetrad.

G,H : 2 and 4-Nucleate embryo sacs with degenerated megaspores.

I : Mature embryo sac; note antipodal complex at the chalazal end.

J : Mature embryo sac; note antipodal complex on the lateral side.

K : L.S. developing grain (diagrammatic) showing hypostase.

L : Basal part of developing grain magnified (diagrammatic) to show the hypostase.

M : Same as in L but with cellular details; note the initiation of tannin deposition.

N : L.S. grain at mature embryo stage; note tannin filled cells of hypostase.

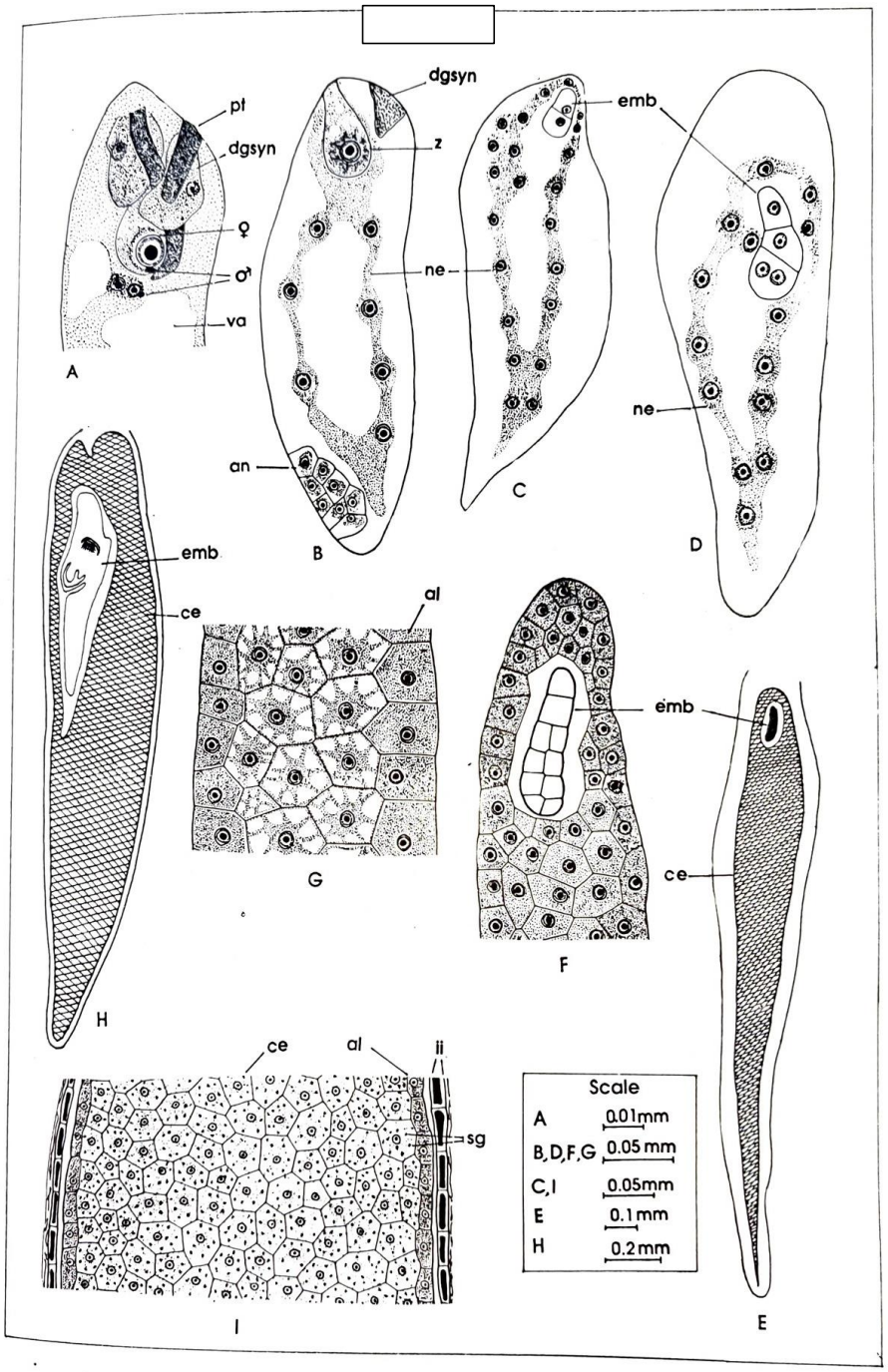

Figure 3

Abbrivations: ov ovule,-ov-ovary wall,oi-outer integument, ii-inner integument, va-vacuole, fi-fillform apparatus, sy-synergid, po-polar, an-antipodal, pe-pericarp, emb-embryo, ce-cellular endosperm, nc-nucellus, hy-hypostase, al-aleuron, pt-pollen tube, dgsyn-degenerating synergid, z-zygote, sg-starch grain.

EXPLANATION OF FIGURE - 3

A-I : Aristida adescensionis Linn.

(Fertilization and of endosperm development)

A : Embryo sac (micropylar region) showing syngamy and triple fusion; note two pollen tubes.

B : Embryo sac; note zygote, degenerating synergid, free nuclear endosperm and persisting degenerating antipodals.

C,D : Stages in the development of free nuclear endosperm.

E : L.S. young grain (diagrammatic); note cellular endosperm and globular embryo.

F : Micropylar part of grain (E); note densely cytoplasmic endosperm cells surrounding embryo.

G : Lower part of cellular endosperm at stage F magnified; note aleurone layer.

H : L.S. mature grain (diagrammatic) showing endosperm and mature embryo.

I : Part of cellular endosperm as shown in H; note starch grains, aleurone layer, persistent nuclei in the endosperm cells and persistent inner layer of inner integument.