EMBRYOLOGY OF *CHLORIS ROXBURGHIANA* SCHULT. (POACEAE)

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Abstract: Anthers are tetrasporangiate. Anther wall develops in a Monocotyledonous manner, leading to the formation of three layers below the epidermis. Anther tapetum is of Glandular type and its cells become two-nucleate. Microspore mother cells are arranged in two rows and normal meiosis leads to the formation of isobilateral microspore tetrads. Cytokinesis is of the successive type. Pollen grain is 1-porate and 3-celled at the time of shedding. Some abnormal pollen grains with three to four sperms have been observed. The ovary contains a single bitegmic, hemianatropous and tenuinucellate ovule. Meiosis in megaspore mother cell leads to the formation of T-shaped tetrad of megaspores. The chalazal megaspore is functional and embryo sac development follows monosporic Polygonum type. Embryo sac after fertilization shows prominent caecum towards the chalazal end. Antipodal cells get displaced to this lateral caecum. Endosperm development is of the nuclear type and embryogeny follows Asterad type.

INTRODUCTION

The genus *Chloris* belongs to the tribe Chlorideae of the sub-family Pooideae of the family Poaceae. A total of 40 species have been recorded in this genus. Embryologically, only one species, *Chloris barbata* SW. has been investigated in this genus (Bhanwra, *et al.* 1981). *Chloris roxburghiana* Schult., being studied in this paper, is a perennial grass with purplish or yellow feathery head of numerous spike like inflorescence.

MATERIAL AND METHODS

The material of *Chloris roxburghiana* was collected from Reddipalli farm in Anantapur district during the months of July and 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 embeded in paraffin wax of melting point 58-60°C. Sections were cut at a thickness of 6-8 μm and were stained using Delafield's haematoxylin stain. They were mounted in Canada balsam. Voucher specimen No. GNVF 5678 has been deposited in the Herbarium of Botany department of Sri Krishnadevaraya University, Anantapur.

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OBSERVATIONS

Microsporangium, Microsporogenesis and Male gametophyte

The anthers are tetrasporangiate. The male archesporium is hypodermal. It divides periclinally giving rise to primary parietal layer and sporogenous layer (Fig. 1A). The development of anther wall follows the Monocotyledonous type. The anther wall consists of epidermis, endothecium, one middle layer and Glandular tapetum (Figs. 1B, C). The cells of epidermis become stretched in mature anther. The sub-epidermal layer develops into fibrous endothecium (Fig. 1D) The middle layer is ephemeral. The tapetum is of Glandular type and its cells are uni-nucleate at the beginning but become binucleate when the pollen mother cells are at meiosis stage (Fig. 1E, F).

Microspore mother cells are aligned in two rows in the longitudinal section (Fig. 1 B). The microspore mother cells undergo normal meiosis and successive cytokinesis leading to the formation of isobilateral microspore tetrads (Figs. 1G, H).

The microspores separate from the tetrad and become more or less spherical and increase in size (Figs. 1I-K). The microspore nucleus moves to a peripheral position near the wall (Fig. 1K). It divides to form a small generative cell and

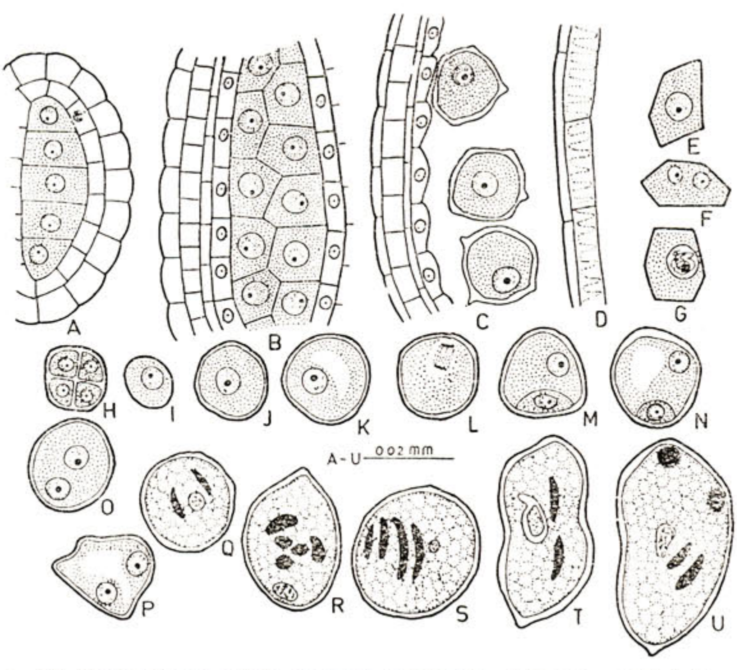

Fig. 1. LS. Part of anther lobe showing epidermis, primary parietal layer and sporogenous layer; B, C. LS of anther lobe showing epidermis, wall layers and pollen mother cells; D. Fibrous endothecium; E, F. One- and two-nucleate tapetal cells respectively; G. Pollen mother cell in meiosis; H. Isobilateral pollen tetrad; I-K. One-nucleate pollen grain; L. Pollen grain in mitosis; M-P. Two-cellen grains; Q. Three-celled pollen grain; R-U. Mature pollen grains of different sizes and with different numbers of sperms.

large vegetative cell (Figs. 1L, M). The generative cell moves into the cytoplasm of vegetative cell (Fig. 1O). Vacuole is observed in two-celled pollen grain (Figs. 1N, P). The generative cell further divides to give rise to two male gametes which are spindle-shaped (Fig. 1Q). Variations in the size of the pollen grains and the number of sperms were observed (Figs. 1R-U). Pollen grains with 3 or 4 sperms have been met with. Normal pollen grains are monocolpate with a thick smooth exine and a slightly thinner intine and are 3-celled at the time of shedding.

Ovary and Ovule

The ovary as characteristic of Poaceae contains a single basal ovule. The ovule is bitegmic, hemianatropous and tenuinucellate (Fig. 2A). The ovule at the beginning arises as a small protruberance. As the integuments develop, the ovule begins to curve and finally becomes hemianatropous. Both the integuments are two-celled in thickness. The micropyle is endostomic (Fig. 2A).

Megasporogenesis and Female gametophyte

The female archesporium is hypodermal and single-celled. It increases in size and directly functions as megaspore mother cell. The megaspore mother cell undergoes meiosis resulting in a T-shaped tetrad of megaspores (Fig. 2B). The chalazal megaspore of the tetrad becomes functional and it develops into an 8-nucleate monosporic Polygonum type of embryo sac (Figs. 2C-E). The egg

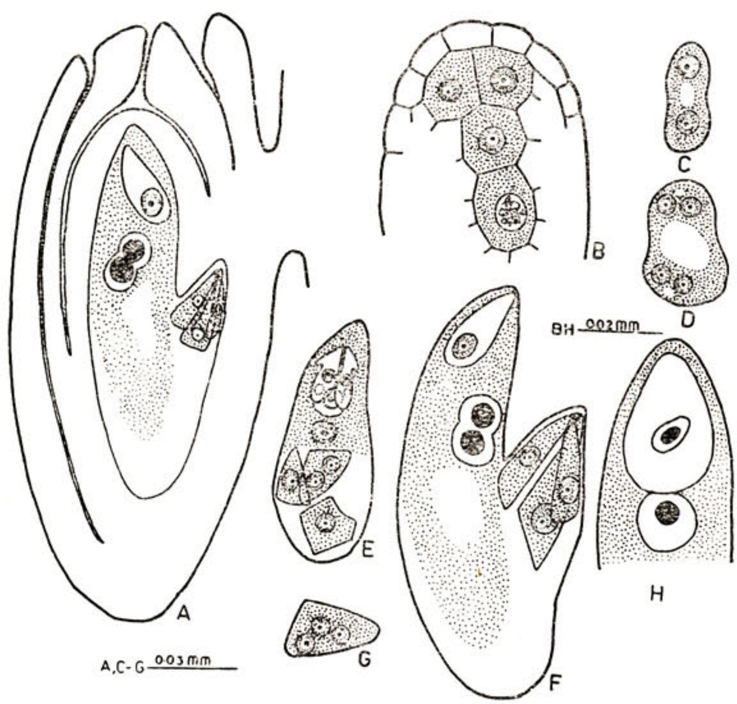

Fig. 2. A. Ovule; B. Megaspore tetrad; C, D. Two and four-nucleate embryo sacs respectively; E. Organized embryo sac; F. Embryo sac showing zygote, secondary nucleus and lateral antipodals; G. Three-nucleate antipodal cell; H. Zygote and primary endosperm nucleus.

is pear-shaped and larger than the two synergids. The synergids are hooked. The secondary nucleus lies near the egg apparatus. The antipodal cells are three in number, and show an increase in the number of nuclei up to three (Figs. 2 E, G).

Fertilization

The synergids start disorganising before the entry of pollen tube into the embryo sac. The pollen tube appears to enter through the micropyle into one of the synergids, where it discharges its contents. Syngamy and triple fusion

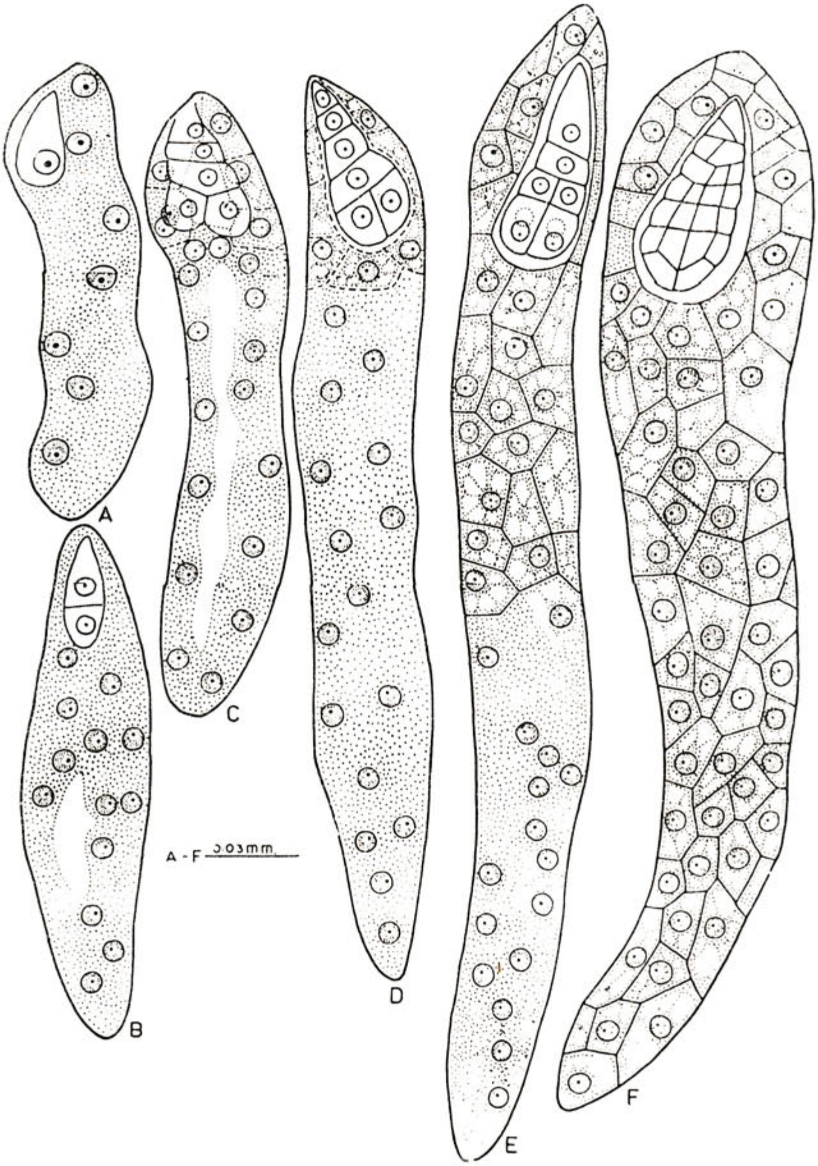

Fig. 3. A-F. Various stages in the development of endosperm.

occurs simultaneously and results in the formation of zygote and primary endosperm nucleus (Fig. 2H).

Post-fertilization changes

The antipodal cells get displaced to a lateral position towards the chalaza after fertilization. They do not proliferate but they project into a caecum which is developed at the chalazal end (Fig. 2F). The antipodal cells are disorganised at 2-celled embryo stage. The primary endosperm nucleus undergoes free unclear divisions and the nuclei are arranged themselves along the periphery of the embryo sac (Figs. 3A-C). Well formation in the endosperm takes place first around the proembryo and progresses towards the chalazal end (Fig. 3C). Cellularization starts at about 4-celled embryo stage and the embryo becomes completely cellular at globular stage of embryo (Figs. 3D-F). Thus endosperm development is of the Nuclear type.

Embryo

The first division in the zygote takes place in transverse plane resulating in the formation of the terminal cell *ca* and the basal call *cb* (Figs, 4A. B). The terminal cell undergoes a vertical division while the basal cell divides transversely so as to form cells *m* and *ci* (Fig. 4C). Another vertical division in *ca* gives rise to a quadrant (Fig. 4F). The cell *m* undergoes vertical division and *ci* divides transversely to form the cells *n* and *n′*, the latter further forming the

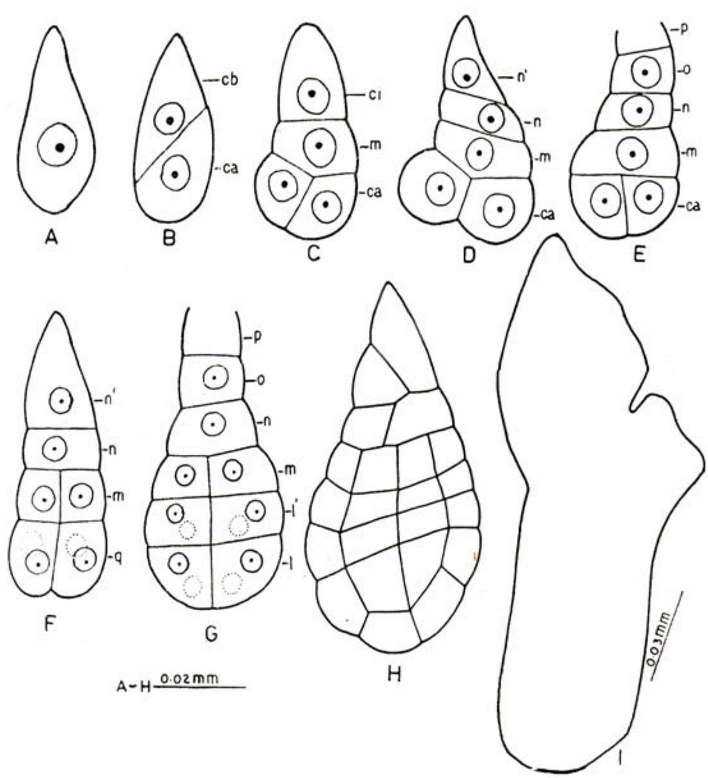

Fig. 4. A-I. Various stages in the development of embryo.

cells *o* and *p* (Figs. 4D-G). Afterwards the cells of *ca* divide transversely to form an octant proembryo with tiers *l*, *l′*. Further transverse and vertical divisions in tiers *l*, *l′* *m* and *n* form a globular embryo (Fig. 4H). The organogenesis is essentially like in other grasses (Fig. 4I). The schematic representation of the embryo development is as follows:

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         / ca·········q············Cotyledon & stem tip
Zygote <      / m··················Hypocotyl
         \ cb <      / n ··············Initials of root
              \ ci <
                     \ n′-o············Root cap
                        \ p············Suspensor
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The development of embryo thus conforms to the Asterad type of Johansen (1950).

DISCUSSION

Microsporogensis and male gametophyte in *Chloris roxburghiana* is similar to those examined species of the subfamily Pooideae. The anther tapetum is of Glandular type and its cells are uninucleate at the beginning and binucleate or trinucleate later on. Tapetal cells remain uninucleate in *Eleusine compressa* (Mahalingappa, 1977), *Sporobolus diander* and *Tripogon filiformis* (Bhanwra, *et al.*, 1981). Binucleate tapetal cells are reported in *Eleusine indica, Dactyloctenium aegyptium* (Chandra, 1963 a), *Chloris barbata, Tragus biflorus, Leptochloa chinensis* (Bhanwra *et al.*, 1981). Successive cytokinesis in microspore mother cells and formation of isobilateral microspore tetrads are features reported in almost all examined, species of the subfamily Pooideae. However T-shaped and linear microspore tetrads have been reported in *Eleusine coracana* (Narayanaswami, 1952) and *E. africana* (Chikkannaiah & Mahalingappa, 1976), respectively.

The ovule in the subfamily Pooideae is bitegmic and tenuinucellate (Bhanwra 1988; Chandra 1963 a, b; Kulkarni & Dnyansagar, 1984; Satyamurty 1985; Venkateswarlu & Devi 1964). Ovules are anatropus or heminatropous in *Eragrostis poaeoides, E. coarctata, E. uniolodes* and *E. pilosa* (Chandra 1976), camylotropous in *Elytrophorus spicata, Dinebra retrofexa* (Satyamurthy 1985 a, b); *Desmostachya bipinnata* (Bhanwra, 1986), and nearly orthotropous in *Poa annua*, (Bhanwra, *et al.*, 1985), Hemianatropous ovules are reported in *Dactyloctenium sindicum* (Sharma *et al.*, 1981). In *Chloris roxburghiana* also the ovule is hemianatropous. Formation of linear or T-shaped megaspore tetrads from a single hypodermal archesporial cell (Venkateswarlu and Devi 1964) lack of periclinal divisions in the nucellar epidermis are common in almost all examined species of the sub-family Pooideae. (Chandra 1963 b; Bhanwra *et al.*, 1986). In *Chloris roxburghiana* T-shaped megaspore tetrad is observed.

In the sub-family Pooideae antipodal cells come to occupy a lateral position in the embryo sac prior to their degeneration (Shadowsky 1926; Chandra 1963 b). The antipodal cells proliferate further to form 5–7 cells in *Chloris barbata* and *Leptochloa chinensis* (Bhanwra, *et al.*, 1982), or a group of cells in species of *Eragrostis* (Chandra, 1976). However, they do not proliferate in *Poa annua* (Bhanwra, *et al.*, 1985) or in *Chloris roxburghiana* (Present study). Antipodals are uninucleate in *Dactyloctenium aegyptium, Eleusine indica* (Chandra, 1963 a), but coenocytic in *Cynodon dactylon* (Shadowsky, 1926). In *Chloris roxburghirna* one, two and even three nuclei are observed in antipodal cells.

In possessing bitegmic, hemianatropous ovules, lack of periclinal divisions in nucellar epidermis, lateral position of antipodals prior to their degeneration, slightly shorter outer integument, *Chloris roxburghiana* is closely related to the other tribes of the subfamily Pooideae.

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Chloris roxburghiana Schult.（禾本科）之胚胎形成過程

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摘　　要

Chloris roxburghiana 花藥爲四孢子囊型。花藥壁的發育屬單子葉植物型，在表皮細胞下面有三層花藥壁。花藥的營養層爲腺質型，其細胞具二核。小孢子母細胞成二行排列，經由一般的減數分裂後形成兩邊相同的小孢子四分粒。細胞質分裂屬連續型。花粉粒被釋出時具單孔及三核。具三至四個精子的不正常花粉粒亦被觀察到。子房內含一橫生胚珠，胚珠具一雙珠被及薄珠心。大孢子母細胞經減數分裂後形成T字型的大孢子四分粒。遠離珠孔的大孢子發育爲胚囊，胚囊的發育屬單孢子蓼型。授精後，胚囊在朝遠離珠孔的一端呈盲腸狀。反足細胞被此側盲腸取代。胚乳的發育爲核型，而胚的發育爲紫苑型。