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EMBRYOLOGY OF *ERAGROSTIS CILIENSIS* (ALL.) LINK[1]

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ABSTRACT

Eragrostis ciliensis, a forage weed, belongs to the family Gramineae. The anther wall is 4-layered and its development corresponds to the Monocot type of Davis. The endothecial cells at maturity acquire fibrous thickenings. The tapetum is single-layered, uninucleate and of secretory type. The meiosis of PMCs is normal and the mode of division is of successive type. The microspore tetrads are isobilateral. Pollen grains are 3-celled at anthesis. The ovule is bitegmic, tenuinucellate and campylotropous. The micropyle is formed by the inner integument. A single female archesporial cell acts directly as the megaspore mother cell. The tetrad of megaspores is linear and occasionally T-shaped. The chalazal megaspore develops into the Polygonum type of female gametophyte. The maximum number of antipodals found is 9.

The endosperm follows the Nuclear type of development. The zygote divides transversely forming *ca* and *cb*. *ca* divides vertically and *cb* horizontally into *m* and *ci* to form a T-shaped tetrad. The cotyledon is derived from *ca* derivatives. The derivatives of *l'* and *m* give rise to the stem apex, 1st leaf and coleoptile. *n* and *n'* are the derivatives of *ci*, the former engendering the root cap, coleorhiza and epiblast while the latter contribuetes to the suspensor.

INTRODUCTION

Eragrostis ciliensis (All.) Link. belongs to the family Gramineae (Poaceae). It grows wild in the grazing fields and has a potential to develop as a good forage crop. There has been no detailed embryological work on this plant. The work of Stover *et al* (1972), and Maheshwari and Swamy (1944) pertains only to the embryo sac. Hence a detailed study of the embryology of *Eragrostis ciliensis* was undertaken.

MATERIAL AND METHODS

Eragrostis ciliensis is an annual plant with a height 30 to 60 cm. The branches are slender and sprout mostly from the base. The stem is smooth and polished. Flowers are in panicles and well spread in acropetal succession. It flowers throughout the year.

The floral buds were collected from the plants growing in the fields of the Punjabrao Agricultural University, Akola and fixed in 70% FAA. It was then treated with 4% KOH at 60°C for one hour. The material was embeded after passing through the alcohol-xylol series. Sections were cut at the thickness of 8-12 microns. They were stained with Delafields' hematoxylin and destained in picric acid and finally, mounted in the DPX.

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Microsporogenesis:

The young anther consists of a homogeneous mass of parenchyma cells covered by a single layer of epidermis. It enlarges in size and assumes a rectangular shape. Soon after, the male archesporium is differentiated hypodermally in the 4 corners (Fig. 1). The archesporium is multicellular. Its cells divide pericilnally forming a primary parietal layer outside and a sporogenous layer inside. The cells of the primary parietal layer divide periclinally forming 2 layers. The outer one contributes endothecium. Cells of endothecium develop fibrous thickenings in the form of fingerlike bands (Fig.5). The inner one, however, divides to form a middle layer and the tapetum (Fig. 2). The latter is of the secretory type and its cells are uninucleate (Fig. 3). However, a few cells become binucleate. The developing anther wall conforms to the Monocot type (Davis, 1966).

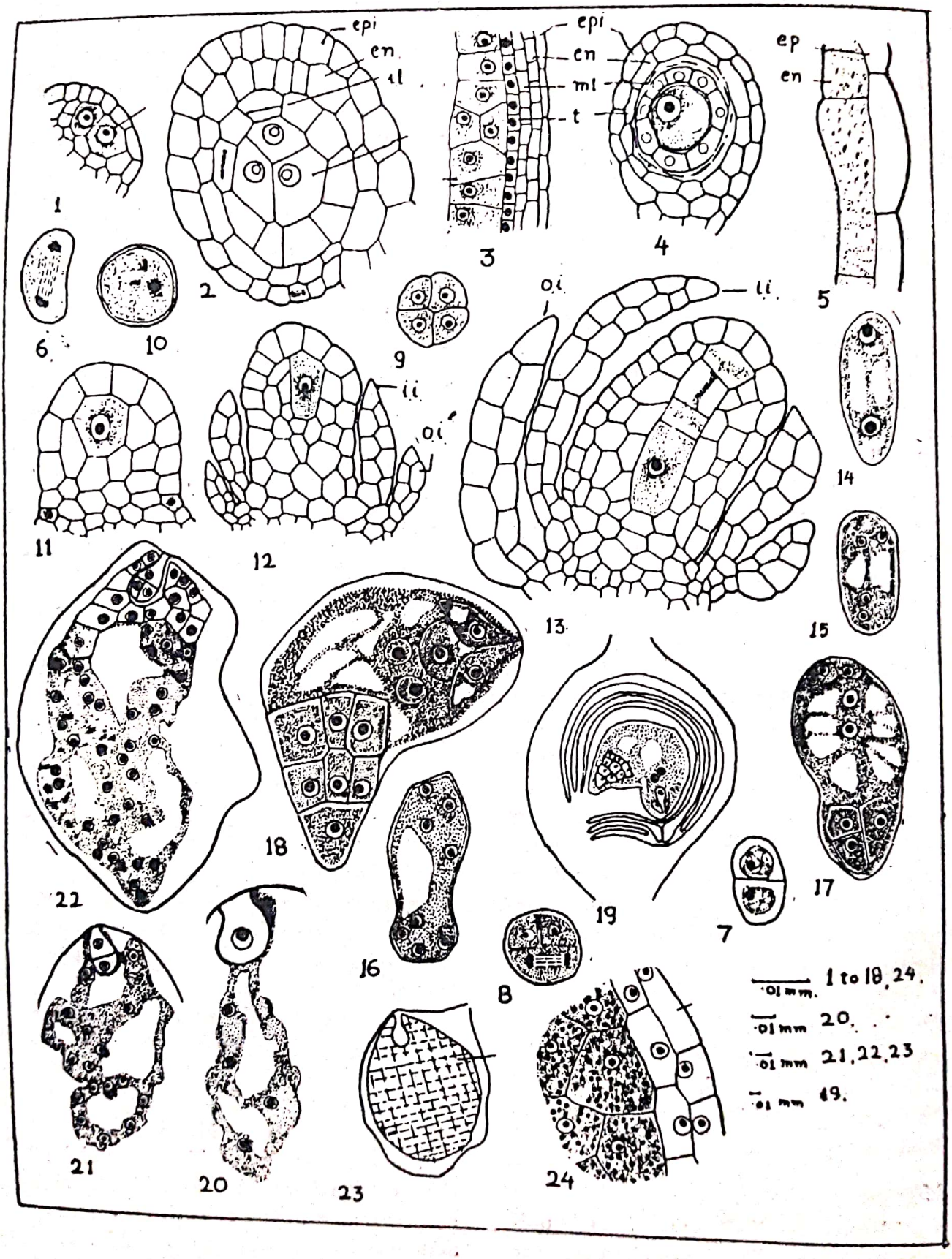
The sporogenous cells divide mitotically, and increase their number and ultimately form microspore mother cells. A longitudinal section of the anther shows 2-3 rows of microspore mother cells in each microsporangium (Figs. 3, 4). The microspore mother cells which are polygonal in outline become round at the time of meiosis. The meiosis I is accompanied by wall formation, and two dyads are formed. The second meiotic division gives rise to microspore tetrads (Figs. 6, 7, 8, 9, 10). Thus the cytokinesis is of the successive type. The tetrads are of isobilateral type.

Megasporogenesis:

There is a single ovule in the unilocular ovary. It is attached to the ovary wall in a lateral position. The ovule is tenuinucellate and bitegmic. The young ovule gradually curves due to the unilateral growth of the integuments. The curvature of the ovule also affects the embryo sac making it curved (Fig. 19). Thus the ovule becomes amphitropous.

The development of ovule starts as a protuberance of parenchymatous cells in the ovarian cavity and soon after that, the female archesporium gets differentiated hypodermally (Fig. 11). It is usually unicellular but occasionally there may be 2 cells. The archesporial cell does not divide to give rise to a primary parietal cell and a megaspore mother cell. It itself enlarges and directly acts as the megaspore mother cell. The primordia of both the integuments are formed, after the division of the archesporial cell (Fig. 12). The inner integument grows faster than the outer (Fig. 13). The nucleus of the megaspore mother cell enlarges in size and undergoes meiosis, giving rise to the linear tetrad of megaspores (Fig. 13). By this time, the inner integument reaches the top of the ovule. It grows faster during the curvature of ovule and forms the micropyle. Both the integuments are 2-layered (Fig. 13). The chalazal megaspore functions and other 3 megaspores degenerate (Fig. 13). The functional megaspore enlarges considerably at the cost of the nucellulr tissue.

The functional megaspore undergoes mitosis and ultimately 8 nuclei are formed (Figs. 14-16). Of the micropylar quartet, 3 contribute to the egg apparatus and remaining one acts as the polar nucleus. The 3 nuclei of the chalazal quartet form antipodal cells and one moves towards the centre and acts as a polar nucleus (Fig. 17). Thus the development of the embryo



sac is of the *Polygonum*—type. The original antipodal cells divide further and ultimately form the antipodal complex of 9 to 11 cells. This complex soon occupies lateral position in the embryo sac (Fig. 18).

Fertilization:

The pollination is anemophilous and the fertilization is porogamous. The pollen tube passes through the stylar canal and reaches the ovarian cavity. It then crowls towards the micropyle and finally reaches the embryo sac. It appears that the pollen tube passes over one of the synergids. The fusion of one of the male gametes with a polar nucleus results into a larger fusion product which merges with the other polar nucleus and the primary endosperm necleus is formed. Thus the triple fusions precedes syngamy. The embryo sac as well as zyote contain abundant starch grains.

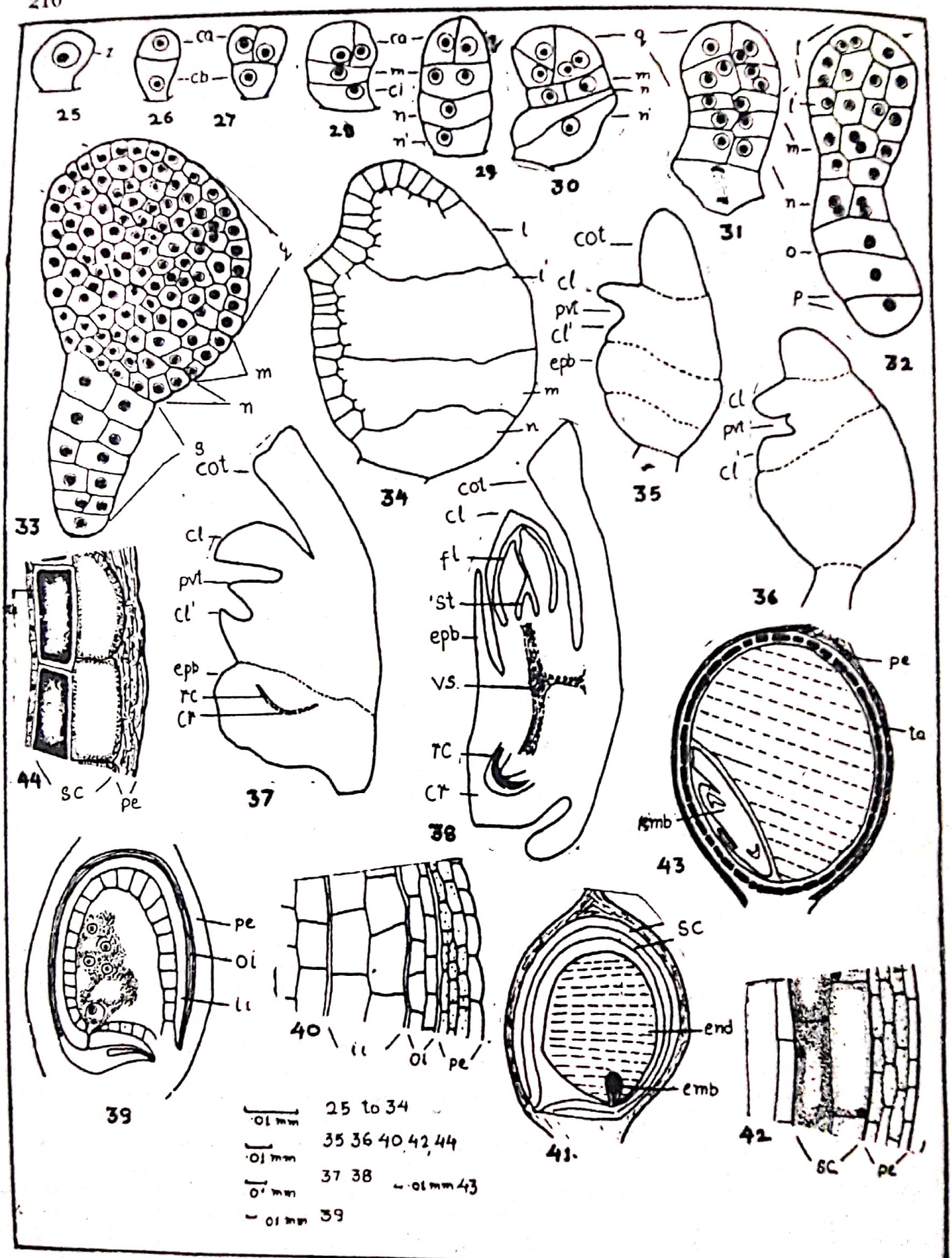
Endosperm:

The endosperm development is of the Nuclear type. The primary endosperm nucleus divides before the division of the zygote (Fig. 20). At the zytote stage, more than 4 nuclei are seen. The initial divisions are mostly synchronous (Fig. 21). Nuclei arrange at the periphery around centrally placed one or two vacuoles. Some of them aggregate around the proembryo. The cellularization of the endosperm begins as early as 2-to 4-celled embryo stage (Fig. 22). It begins around the embryo and proceeds towards the chalazal region. At the globular embryo stage, the embryo sac becomes completely filled up with the cellular endosperm (Fig. 23). The cells around the embryo are smaller in size. The cells of the two peripheral layers of the endosperm are brick-shaped and stand out clear differentiated from rest of the cells which are polygonal in outline (Fig. 24).

Embryo:

The division of the zygote (Fig. 25) is transverse and unequal, resulting in the formation of the smaller terminal cell, *ca* and larger basal cell,

Figs. 1-24. Fig. 1—L.S. part of young anther showing hypodermal archesporial cells, *arch*. Fig. 2—T.S. part of anther showing development of parietal tissue and microspore mother cells, *mmc; epi*, epidermis; *en*, endothecium; *il*; inner layer of which some cells dividing to form ultimately middle layer and tapetum. Fig. 3—L.S. part of anther showing wall layers and microspore mother cells, *mmc. epi*, epidermis; *en*, endothecium; *ml*, middle layer; *t*, tapetum. Fig. 4—T.S. part of anther showing absorption of middle layer, *ml*. Fig. 5—epidermis, *epi* and endothecium with fibrous thickenings from the mature anther. Fig. 6 to 9—Stages in meiosis of microspore mother cells. Fig. 10—Mature 3-nucleated pollen grain. Fig. 11—L.S. young ovule showing differentiation of hypodermal archesporium and primordia of integuments. Fig. 12—L.S. ovule showing megaspore mother cell and developing integuments. *oi*, outer integument; ii, inner integument. Fig. 13—L.S. ovule showing tetrad of megaspore, the upper 3 are degenerated and the chalazal one is functional and has become enlarged. The inner integument *i* has grown faster than outer integument, *oi*. Fig. 14—2-nucleated embryo sac. Fig. 15—4-nucleated embryo sac. Fig. 16—8-nucleated embryo sac. Fig. 17—8-nucleated embryo sac showing organisation of 2 polar nuclei and 3 antipodal cells. Fig. 18—Mature embryo sac showing egg apparatus, 2 polar nuclei and 7 antipodal cells. Fig. 19—L.S. mature ovule showing amphitropous ovule with micropyle and curved embryo sac. Fig. 20—L.S. part of embryo sac showing zygote and endosperm of 4 nuclei. Fig. 21—Embryo sac with 2-celled proembryo and free nuclear vacuolated endosperm. Fig. 22—Embryo sac with 3-celled proembryo surrounded by cellular endosperm and lower below free nuclei of the endosperm. Fig. 23—Embryo sac at globular embryo stage showing mature endosperm. Fig. 24—Part of mature endosperm with 2 brick-shaped outer layers and the inner cells laden with starch grains.



Figs. 25-44. Figs. 25 to 38. Various stages of development of embryo. Fig. 39—L.S. developing seed at 2 –celled embryo stage. Fig. 40—A part magnified of ovary wall, *pe*, pericarp. Fig. 41—L.S. developing seed at globular embryo state. Fig. 42—A part magnified of fruit wall. *sc*, seed coat; *pe*, pericarp. *end*, endosperm; *emb*, embryo. Fig. 43—L.S. mature grain, *ta*, tannin; *emb*, embryo. Fig. 44—A part magnified of mature grain.

cb (Fig. 26). In the second cell generation, *ca* divides vertically (Fig. 27). and *cb* transversely into *m* and *ci*, forming a T-shaped tetrad of proembryo (Fig. 28). In the third cell generation, *m* divides vertically to form 2 juxtaposed cell, and *ci* divides transversely into *n* and *n'* (Figs. 29, 30). The two derivatives of the terminal cell designated as *q* divide vertically in such a way that the walls are oriented perpendicularly to the first wall, and thus the quadrants are formed from *ca* (Figs. 30, 31). In the next cell generation, quadrants divide more or less transversely or horizontally by curved walls to give distinct layers *l* and *l'*. Afterwards, simultaneously the derivatives of *m* and *n* divide by many vertical walls and then by transverse walls while *n'* divides transversely into *o* and *p* (Figs. 31, 32). Now the embryo becomes club-shaped (Fig. 33). The destinations of various embryonal cells are given below :

	ca.....*q*.....*l*.....*pco*	
Zygote	*l'*.....*pvt*	
	cb.....*m*.....*phy* + *icc* + *iec*	
	ci.....*n*.....	Radicle
	n.....*o*.....*co*	
	p.....*s*	

The young embryo which was club-shaped now shows lateral symmetry. A depression is produced at the limit of tiers, *l* and *l'* (Fig. 33, 34). This depression marks the separation of the cotlyedonary part, *pco* and the hypocotyledonary part, *hyp*. The former grows faster, becomes curved and develops into the single scutellum (Fig. 35). The epidermal cells of the scutellum in contact with the endosperm form the epithelial layer.

The cells of epiderms of *l'* situated just below the scutellum contribute to the upper lip of the coleoptile, *cl'* (Figs. 35, 36, 37). The stem apex, *pvt*, the first leaf, *fl* and the lower lip of the coleoptile, *cl'* are also differentiated from *l'* in the region below the upper lip of the coleoptile (Fig. 36, 37). The primordia of *pvt* and *l'* first appear as rudimentary protuberance . *cl.* and *cl'* grow towards each other and finally enclose the stem apex togther with the primordium of the first eleaf (Fig. 38).

The derivatives of *m* give rise to the hypocotyledonary axis, *phy* while those of *n* form the radicle and those of *o* contribute to the root- cap, coleorhiza and epiblast. The last organ (epiblast) is an outgrowth formed as a result of activity of the epidermal cells of *n*. It is situated opposite to the scutellum. The suspensor, *s* is short and formed by the derivatives of *p*.

The development of embryo follows the Megarchetype II, Series A of the First Period of Soueges or the Asterad type of Johansen.

Seed coat & Fruit wall:

After fertilization, the cells of the outer integument become compressed (Figs. 39, 40) and after the division of the zygote they start degenerating. The inner layer of the inner integument becomes conspicuous (Figs. 41, 42) and its cells become brown coloured probably because of deposition of tannin (Figs. 43, 44).

The wall of the ovary at the time of the mature embryo sac is of 4 to 8 layers thick. The deposition of starch grains in the cells of the wall can be seen even when the embryo sac is mature. After fertilization, all the layers of the wall except the outermost are filled with starch grains. It is quite likely that these starch grains act as food storage and might be utilized either

during the development of the embryo, since some of the layers are absorbed as indicated by lessening of the thickness of the wall (Fig. 44). It is not possible to count the layers of the cells which are masked by starch grains. When the embryo becomes mature (Fig. 44), the pericarp is very thin. It is fused with the seed coat which consists of 2 layers of the inner integument. The cells of the inner layer of the inner integument become very conspicuous and are filled with the tannin, while the cells of outer layer are vacuolate.

DISCUSSION

Eragrostis ciliensis shows several embryological characters which are common to the Gramineae. These are : (1) 4-layered anther well including epidermis, (2) secretory type of tapetum, (3) bitegmic ovule, (4) single-celled female archesporium, (5) Polygonum type of embryo sac, (6) Nuclear type of endosperm development, (7) Asterad type of embryo development, and (8) seed coat exclusively coming from the inner integument (Johansen, 1950, Davis, 1966).

In *Eragrostis ciliensis,* the endothecial cells develop thickenings in the shape of U-or V-shaped bands. A similar condition has been reported by Gawli (1977) in *Brachyaria, Chloris, Eragrostis, Paspalum* and *Tripogon* in the Gramineae. However, finger-like thickenings have been observed by Untawale (1969), Batygena (1974) and Diwanji (1976) in several members of the family. Although the tapetal cells in *E. ciliensis* are usually uninucleate, occasionally a few cells become binucleate. Such a condition appears to be of a wider occurrence in grasses, since it has been reported by many authors. It seems that *E. ciliensis* indicates an intermediate condition where some of the tapteal cells show a tendency to become binucleate. The ovule is amphianatropous and the micropyle is organized by the inner integument. The grass ovule is described as tenuinucellate. In the present case, the nucellus is present in the form of a few-celled layers around and below the embryo sac. So it is clear that the nucellus is not thin in *E. ciliensis.* Such a condition has been reported by Gawli (1977) in the taxa studied by him. In the Panicoideae also the nucellus develops a few-layered cap. So it is not justifiable to term the grass ovule as tenuinucellate. Davis (1966) prefers the term pseudocraassinucellate in such cases. The present authors also favour this term.

Although there are 3 antipodals in the embryo sac of *Eragrostis ciliensis* in the beginning, a complex of 9-12 antipodals is formed afterwards. The presence of such a complex seems to be more prevalent in the species of the Gramineae than the occurrence of only 3 antipodals.

In *Eragrostis ciliensis,* the embryo development follows the Asterad type of Johansen or the Megaarchetype II, Series A of the First period of Souèges. Similar is the condition in *E. uniloides* (Deshpande, 1976), *Eleusine indica, Brachyaria* and *Paspalidium* (Gawli, 1977). In *E. ciliensis,* the development of embryo is similar to *Poa* variation as for as the destinations are concerned but differs from it in the derivation of the stem tip from *ca* and not from *m.* In *Poa ca* forms the cotyledon and coleoptile and *cb* gives rise to the remaining part.

In *Eragrostis ciliensis,* the outer integument is not involved in the seed development and organization of the seed coat exclusively comes from the inner integument. This is a common

feature in the majority of grasses. However, Anderson (1927) records formation of the seed coat by the outer integument in *Poa compressa* and *P. pratensis*.

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