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Embryological Studies in Some Grasses

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The embryology of 7 species belonging to the tribes Chlorideae, Eragrosteae, Agrostideae, Sporoboleae, and Zoysieae has been studied. On the basis of the available data, the tribes Chlorideae, Eragrosteae, Sporoboleae and Zoysieae can be distinguished from the true festucoids (except the tribe Agrostideae) in certain embryological features, such as the absence of periclinal divisions in the nucellar epidermis in the region of the micropyle, narrow region of the ovular attachment to placenta, only 3–5 middle layers in the thicker distal region of the dorsal ovary wall and absence of a cytoplasmically dense layer subjacent to the locular epidermis of the ovary wall.

Key Words: Embryology, Grass, Chlorideae, Eragrosteae, Sporoboleae, Zoysieae

Introduction

Bor (1960) recognised 36 tribes in the subfamily Pooideae. Adequate embryological information is now available for some species belonging to the tribes Agrostideae, Aveneae, Brachypodieae, Bromeae, Chlorideae, Eragrosteae, Festuceae, Phalarideae, Stipeae and Triticeae (see Bhanwra & Choda 1980, Bhatnagar & Chandra 1975, 1976, Chandra 1963a, b, Kam & Maze 1974, Mahalingappa 1977, Maze et al. 1970, 1972, Maze & Bohm 1973, 1974, 1977, Mehlenbacher 1970, Muniyamma 1976, Narayanaswami 1952, 1955, Nygren 1967, Venkateswarlu & Devi 1964). The present investigation was undertaken with a view to further understanding the relationships between different festucoid grasses and deals with the embryology of some species belonging to the tribes Agrostideae, Chlorideae, Eragrosteae, Sporoboleae and Zoysieae. The investigated species are *Chloris barbata* Sw. (Chlorideae), *Leptochloa chinensis* L. Nees. and *Tripogon filiformis* Nees. Retz. (Eragrosteae), *Polypogon fugax* Nees. and *P. monspeliensis* (L.) Desf. (Agrostideae), *Sporobolus diander* Retz. (P.) Beauv. (Sporoboleae) and *Tragus biflorus* Schult. (Zoysieae).

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Materials and Methods

The material of *C. barbata*, *L. chinensis*, *S. diander*, *T. biflorus* and *P. monspeliensis* was collected from the Punjab University Campus in the months of August and September while that of *P. fugax* and *T. filiformis* was collected from Kalka and Kasauli hills in the months of March and April. The inflorescences at different stages of development were fixed in FAA. The spikelets were dehydrated in ethyl alcohol—tertiary butyl alcohol series and embedded in paraffin wax (Johansen 1940). Sections were cut varying in thickness from 6–10μ. The safranin—fast green combination and Heidenhain's haematoxyline were used for staining purposes. Voucher specimens have been deposited in the herbarium of the department of Botany, Punjab University, Chandigarh.

Observations

All the species studied show a close resemblance in the general developmental pattern and hence only *Chloris barbata* has been described in detail and reference has been made to other species wherever necessary.

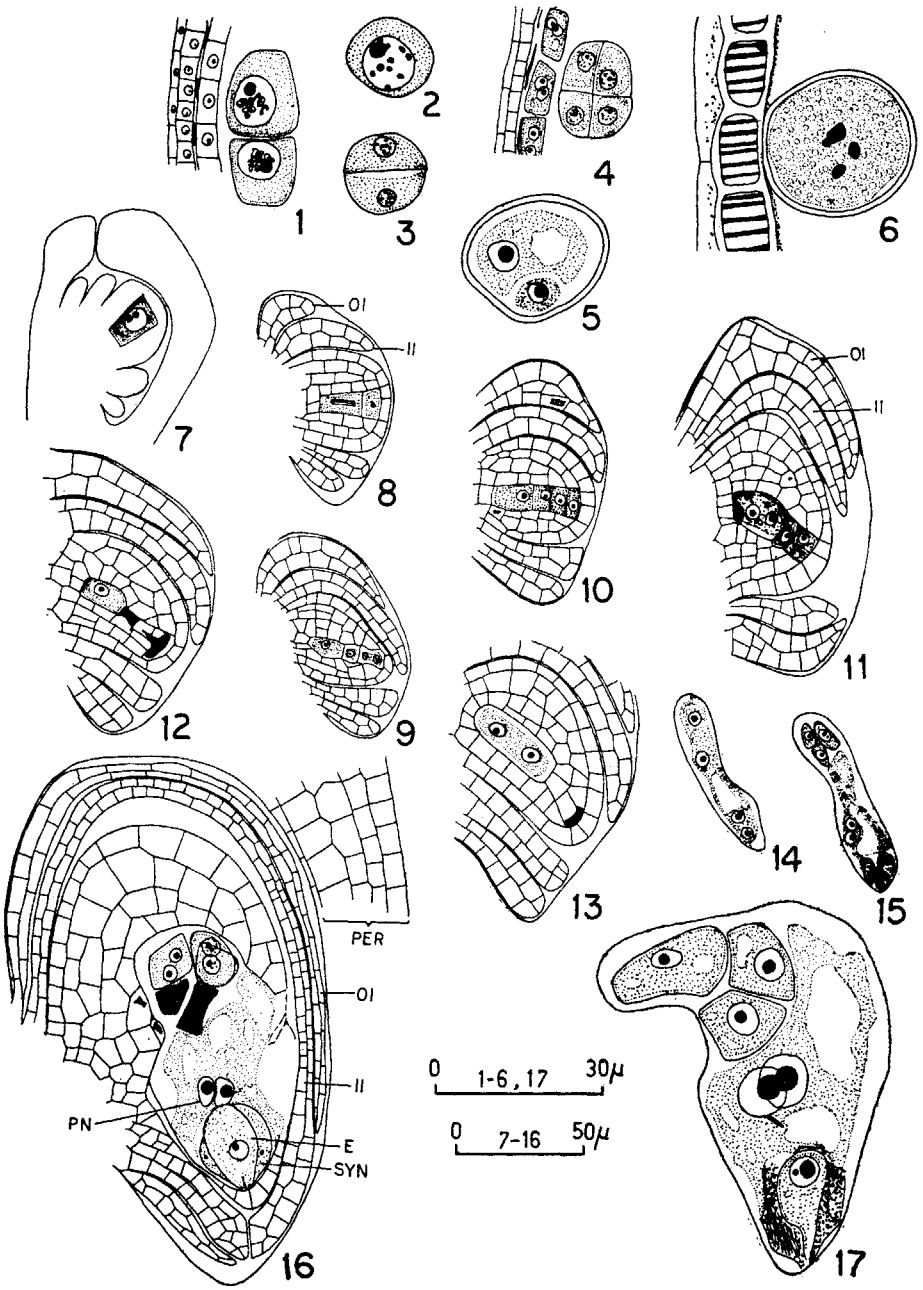
Microsporogenesis and male gametophyte

The anthers are tetrasporangiate. The anther wall is four layered, a thin-walled epidermis is followed by an endothecial layer, a single middle layer and tapetum which is of the glandular type (figure 1). The tapetal cells become binucleate. The tapetal cells however, remain uninucleate in *S. diander* and *T. filiformis*. The middle layer is ephemeral in nature and its cells start disorganizing at the oneset of meiosis in the microspore mother cells. The endothecial cells develop characteristic fibrous thickenings in mature anthers (figure 6). The sporogenous cells are compactly arranged in one or two rows as seen in a longitudinal section. The microspore mother cells separate from one another and become rounded. Meiosis is normal. Successive cytokinesis in microspore mother cells leads to the formation of isobilateral microspore tetrads (figures 1–4).

The microspores round off and increase in size. The microspore nucleus which lies near the wall divides to form a small lenticular generative cell and large vegetative cell (figure 5). The generative cell gives rise to two male gametes which are ovoid or spindle shaped (figure 6). The vegetative nucleus starts disorganizing while still within the pollen grain. The pollen grain is monocolpate with a thick smooth exine and slightly thinner intine and is three-nucleate at the time of shedding.

Ovule and ovary wall

The ovary contains a single ovule attached to its posterior wall (figures 16, 25). The ovule is sub-basal at the time of its inception but at megaspore tetrad stage it comes to lie at right angles to the longitudinal axis of the ovary (figures 8–10). During further growth the ovule undergoes a curvature towards the base of the ovary and finally becomes hemianatropous (figures 12, 13, 16). It is bitegmic and tenuinucellate. The inner integument is usually composed of two layers of cells and it alone forms the micropyle. The outer integument is slightly shorter than the inner integument (figure 16). It is composed of two layers of cells in *C. barbata*, *L. chinensis*, *S. diander*, *P. fugax* and *P. monspeliensis* but it is often three-layered near the chalaza in *T. filiformis* and *T. biflorus* (figure 11). The region



Figures 1–17 Microsporogenesis, male gametophyte, megasporogenesis and female gametophyte. **1–8, 12, 13–16,** *Chloris barbata*; **9,** *Leptochloa chinensis*; **10, 11,** *Tragus biflorus;* **17,** *Sporobolus diander* (**E,** egg, **II,** inner integument; **OI,** outer integument; **PN,** polar nucleus, **SYN,** synergid). *1,* V.S. portion of anther showing, epidermis, endothecial layer, degenerated middle layer, tapetal layer and microspore mother cells, *2,* Microspore mother cell at diakinesis stage, *3,* Dyad stage, *4,* V.S. portion of anther, showing wall layers and isobilateral microspore tetrad, *5,* Two-celled pollen grain; *6,* V. S. portion of anther showing stretched epidermal cells, fibrous endothecium, remnants of tapetum and a three-nucleate pollen grain; *7-11,* Megasporogenesis; *12,* Functioning megaspore; *13,* V.S. ovule at two-nucleate embryo sac stage; *14, 15,* Four-nucleate and organized embryo sac stages respectively; *16,* V.S. of mature ovule with embryo sac showing egg, two synergids, binucleate central cell and antipodal cells; *17,* One of the stages in fertilization

of ovular attachment to placenta is about half the length of the ovule in *L. chinensis*, *S. diander* and *T. biflorus* or even less than that in certain other species. The mature ovule is broad and round at the chalazal end but narrow at the micropylar end (figures 16, 25) except in *T. filiformis* where it is more or less ovoid in shape.

The dorsal ovary wall is thick near the style and it gradually becomes narrow towards the base of the ovary. The ovary wall has an outer epidermis, a locular epidermis and a variable number of middle layers. In *P. fugax*, *P. monspeliensis*, *T. filiformis*, and *T. biflorus* there are 4–5 middle layers at the top and a single middle layer in the region opposite to the micropyle. In other species, namely *C. barbata* (figure 16), *L. chinensis* and *S. diander* there are 3–4 middle layers in the thick distal part and 2 middle layers in the region close to the micropyle.

Megasporogenesis and female gametophyte

There is a single hypodermal archesporial cell in the nucellus which increases in size and acts as the megaspore mother cell (figure 7). It undergoes meiosis so as to form a linear or T-shaped tetrad of megaspores (figures 8–11). The chalazal megaspore functions and develops into an eight-nucleate Polygonum type of embryo sac having an egg, two synergids, a binucleate central cell and three antipodal cells (figures 12–15). The egg is pear-shaped and slightly larger than the two synergids which are also somewhat pear-shaped. The two polar nuclei occupy a position just above the egg apparatus in *C. barbata*, *L. chinensis*, *S. diander*, *T. filiformis* and *T. biflorus* (figure 16) while in *P. fugax*, *P. monspeliensis*, and *S. diander* they occur almost in the middle of the central cell (figure 17). Moreover, the egg apparatus and the antipodal cells lie very close to each other in *P. fugax* and *P. monspeliensis* as compared to other species.

The three antipodal cells proliferate further to form 5–7 cells in *C. barbata* and *L. chinensis* (figure 16). In other species the three antipodal cells do not divide further (figure 17). The antipodal cells contain one or two nuclei in *C. barbata* but they remain uninucleate in other species. The antipodal nuclei become abnormal in that they contain 4–8 nucleoli in *P. fugax* and *P. monspeliensis* (figure 22). This feature is lacking in all the other species. The antipodal cells also become hypertrophied in *P. fugax* and *P. monspeliensis*.

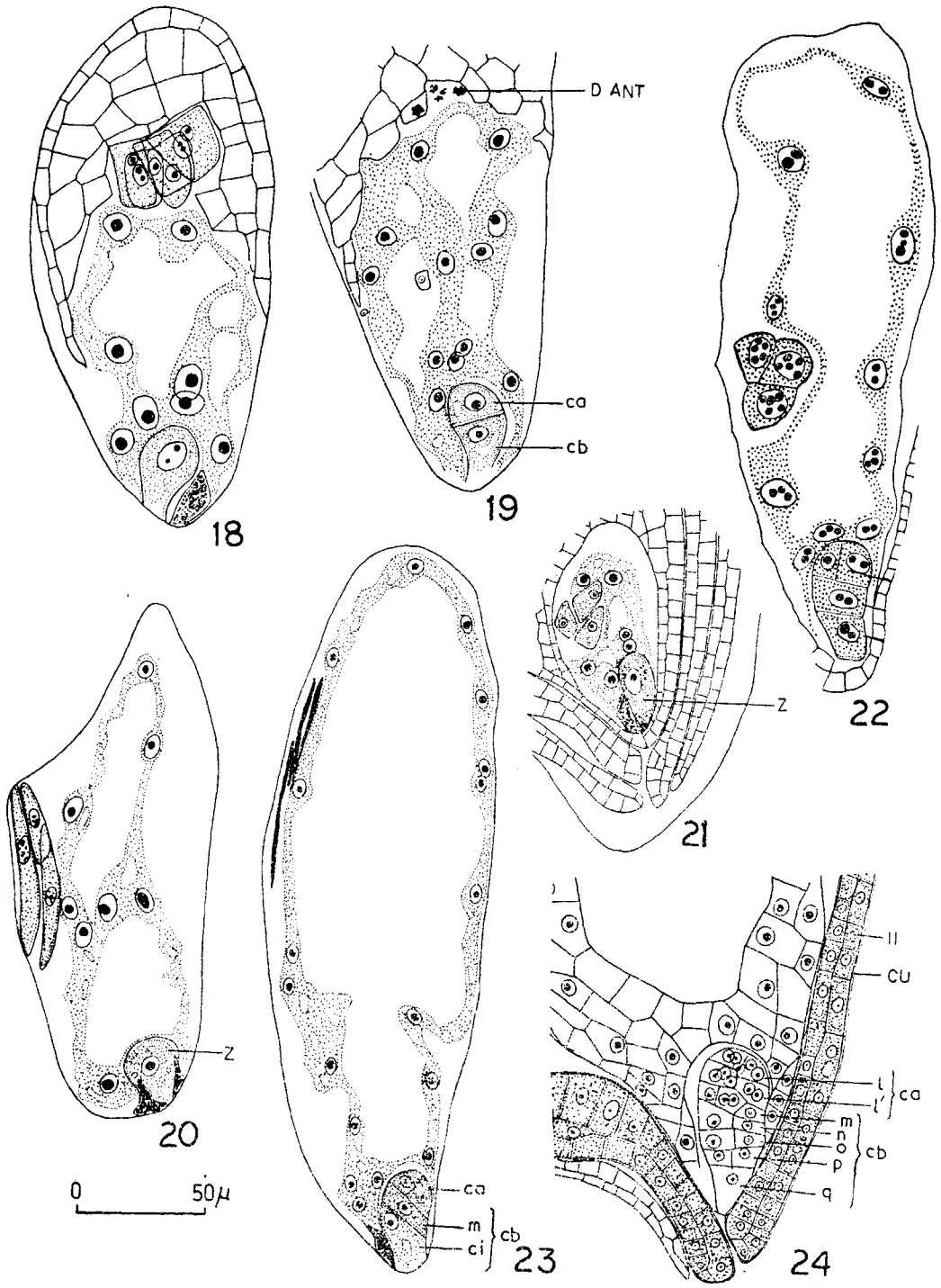
In mature ovule the embryo sac lies parallel to the longitudinal axis of the ovule in *C. barbata* (figure 16). The embryo sac is obliquely placed in *L. chinensis*, *T. filiformis* and *T. biflorus* while it shows a prominent curvature towards the chalaza in *P. fugax*, *P. monspeliensis* and *S. diander* (figures 17, 25).

Fertilization

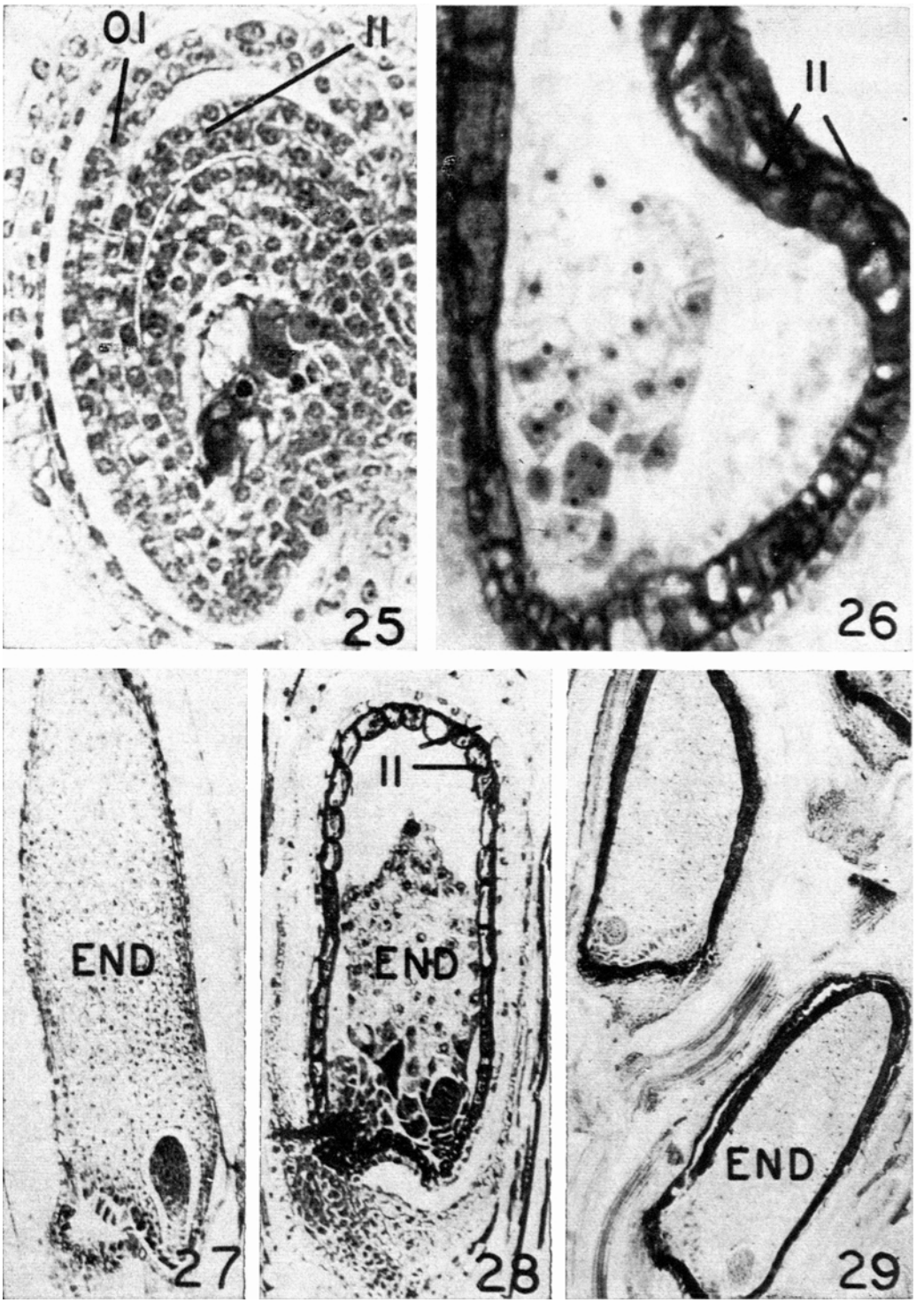
The synergids start disorganising before the entry of the pollen tube into the embryo sac. The pollen tube appears to enter through the micropyle into one of the synergids where it discharges its contents. Syngamy and triple fusion occurs almost simultaneously in *P. monspeliensis* but in *S. diander*, syngamy seems to precede triple fusion (figure 17).

Post-fertilization changes

The primary endosperm nucleus undergoes free nuclear divisions and the



Figures 18–24 Development of embryo and endosperm; **18, 19, 24,** *Chloris barbata,* **20, 23,** *Tragus biflorus;* **21,** *Leptochloa chinensis,* **22,** *Polypogon monspeliensis* (**Ca** terminal cell, **Cb**, basal cell, **Cu**, cuticle, **DANT**, degenerating antipodals, **II**, inner integument, **Z**, zygote); *18*, Zygote, endosperm nuclei and five antipodal cells; *19*, Two-celled proembryo, nuclear endosperm and disorganized antipodal cells; *20, 21*, Zygote, endosperm nuclei and laterally displaced antipodal cells; *22*, Four-celled proembryo, nuclear endosperm and three laterally placed antipodal cells; *23*, Four-celled proembryo, nuclear endosperm and disorganized antipodal cells; *24*, Early globular proembryo stage and cellular endosperm



Figures 25-29 Mature ovule, development of embryo, endosperm, seedcoat and pericarp; **25, 28,** *Sporobolus diander*; **26, 29,** *Leptochloa chinensis*. **27,** *Chloris barbata*. **END,** endosperm, **II,** inner integument, **OI,** outer integument; *25*, V.S. mature ovule × 550; *26*, Quadrant Proembryo, wall, formation in the endosperm and deposition of a darkly staining substance in the cells of the inner integument × 550; *27*, *29*, V.S. Caryopsis at globular proembryo stage × 120

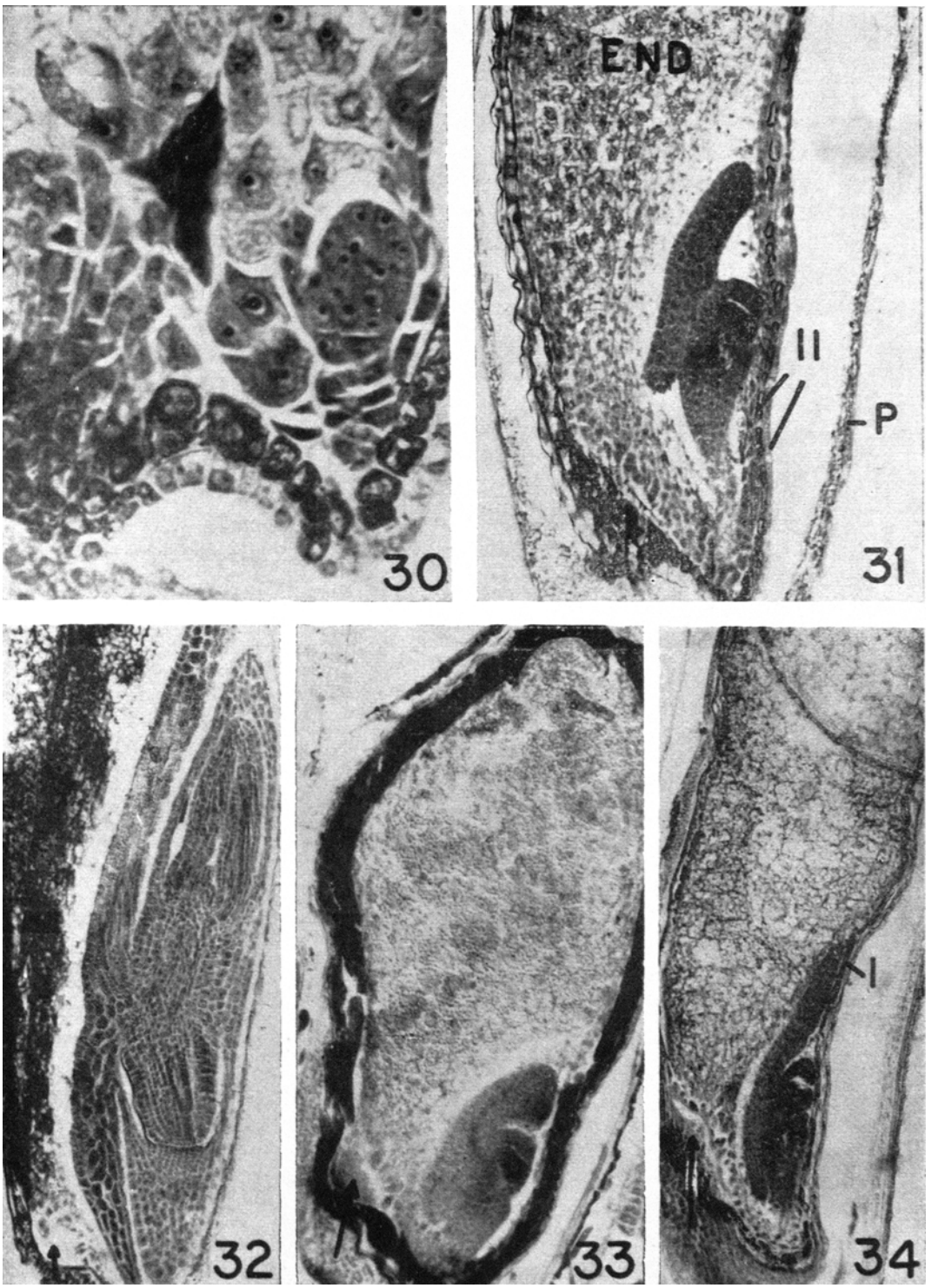
daughter nuclei arrange themselves along the periphery of the embryo sac (figures 18–23). Wall formation in the endosperm takes place first around the proembryo and progresses towards the chalazal end (figure 26). The endosperm cells multiply further till the entire cavity of the embryo sac gets filled (figures 27–29). There is a single aleurone layer and aleurone transfer cells also get differentiated near the chalazal region (figure 27). In mature caryopsis the endosperm cells which are full of starch grains develop thick walls in all the species except in *L. chinensis*.

The antipodal cells get displaced to a lateral position towards the chalaza after fertilization except in *Chloris barbata* (figures 18, 21–23). The antipodal cells disorganize at two-celled stage of the proembryo in *C. barbata*, *L. chinensis*, *T. filiformis* and *S. diander* (figure 19), at four-celled proembryo stage in *T. biflorus* (figure 23) but at globular proembryo stage in *P. fugax* and *P. monspeliensis*

The embryo sac is oblong, spindle-shaped or curved as in *S. diander* (figures 16, 17) at the time of fertilization. After fertilization the embryo sac grows at the expense of the nucellus and is usually narrow at the micropylar end but much wider in the chalazal half (figures 18–23) except in *T. filiformis*, where it is more or less oblong. In mature caryopsis only a patch of nucellar cells opposite to the region of ovular attachment survives while it gets completely absorbed by the endosperm elsewhere (figures 32–34, arrow mark).

The outer integument degenerates after fertilization. The cells of inner layer of the inner integument enlarge and show deposition of a darkly staining substance in its cytoplasm and the cell walls become thick after the globular proembryo stage (figures 30–32, 34). In *L. chinensis*, however, the deposition occurs at an early stage, it is in both the layers and it is more profuse as compared to other species (figures 26, 29, 33). The outer layer of the inner integument becomes cutinized and this layer along with the safraninophilic layer forms the seed-coat in *T. filiformis* but in other species only the compressed layer is usually discernible between the safraninophilic inner layer and compressed remains of the pericarp.

The first division in the zygote takes place in a transverse plane forming the terminal cell ca and the basal cell cb (figure 19). The terminal cell undergoes a vertical division while the basal cell divides transversely so as to form the cells m and ci (figures 22, 23). Another vertical division but at right angles to the previous one in ca results in a quadrant proembryo. At the same time the tier m undergoes a vertical division but ci divides transversely to form the cells n and n′, the latter further forming the cells O and P (figure 24). All the cells of ca divide transversely to form an octant proembryo with tiers 1 and 1′. Further vertical and transverse divisions in tiers 1, 1′, m and n form a globular proembryo while the products of n, o and p form a suspensor (figures 27, 30). The globular proembryo increases in length and assumes a club-shaped form. The organogenesis is essentially like in other grasses (figures 31–34). The mature embryo is more than half the length of the caryopsis in *C. barbata* (figure 32) but in all other species it is less than half the length of the caryopsis (figures 33, 34). The embryo has a shield like scutellum, the lower part of which is free from the coleorhiza in *C. barbata*, *L. chinensis*, *T. filiformis*, *S. diander* and *T. biflorus* (figures 32-34) but it is lacking in



Figures 30–34 Embryo endosperm, seed-coat and pericarp; **30,** *Sporobolus diander*, **31, 32,** *Chloris barbata*, **33,** *Leptochloa chinensis*. **34,** *Tragus biflorus*. (**END** endosperm, Inner layer of inner integument, **II** inner integument, **P,** pericarp); *30*, V.S. of caryopsis showing and darkly staining inner layer of the integument × 550; *31*, V.s. of caryopsis showing organogenesis in the embryo×160; *32-34*, V.S. of the inner mature caryopsis; *32*, 34×120; 33, ×160

Table 1 *Comparison of embryological features of various Tribes*

Features	Chlo.	Era.	Sporo.	Zoys.	Agrost.	Aven.	Brach.	Brom.	Fest.	Phal.	Trit.	Stip.
Differentiation in inner layer of inner integument	+	+	+	+	+ or —	+ or —	+	+	+	—	+	+ or —
Outer integument slightly shorter than the inner integument	+	+	+	+	+	+	+	+	+	+	+	+
Periclinal division in the nucellar epidermis near the micropyle	—	— or rarely +	—	—	—	—	+	+	+ or —	+	—	+ or —
Region of ovule attachment to placenta narrow in relation to ovule length	+	+	+	+	+	—	—	—	—	—	—	—
Mature embryo sac showing a prominent curvature towards the chalaza	+ or —	+ or —	—	—	+	—	—	—	+	+	+	—
Displacement of antipodal cells to a lateral position after fertilization	+ or rarely —	+	+	+	+	+	+	+	+	+	+	+
Thick dorsal ovary wall at the top (8-10 middle layers)	—	—	—	—	—	+	+	+	+	+	+	+
Cytoplasmically dense layer subjacent to the locular epidermis	—	mostly —	—	—	—	+	+	+	+	+	+	+

—, absent; +, present; *Chlo.*, Chlorideae; *Era*, Eragrosteae; *Sporo*, Sporoboleae; *Zoys*, Zoysieae; *Agrost*, Agrostideae; *Aven.*, Aveneae; *Brach*, Brachypodieae; *Brom.*, Bromeae; *Fest.*, Festuceae; *Phal.*, Phalarideae; *Trit.*, Triticeae; *Stip.*, Stipeae.

P. fugax and *P. monspeliensis*. Epiblast, a lateral extension of coleorhiza is present in all the species.

The locular epidermis of the pericarp degenerates soon after fertilization followwed by other middle layers. The pericarp is free from the seed-coat in *C. barbata*, *L. chinensis*, *T. filiformis*, *S. diander* and *T. biflorus* (figures 27, 28, 31) but it is in close contact with the seed-coat in *P. fugax* and *P. monspeliensis*. The middle layer next to the locular epidermis shows some meristematic activity only in *L. chinensis*. In mature caryopsis, the partly disorganised epidermis and one or two middle layers form a loose covering around the seed-coat (figures 33, 34).

Discussion

The microsporangium, microsporogenesis and male gametophyte in the species reported here is like in most other festucoid grasses in having tetrasporangiate anthers, four-layered anther wall, glandular tapetum, successive cytokinesis in microspore mother cells resulting in the formation of isobilateral spore tetrads and three-nucleate pollen grains at the time of shedding. The notable exceptions to some of the above features include the bisporangiate anthers in *Bromus unioloides* (Bhanwra & Choda 1980) and the formation of linear or T-shaped tetrads besides the isobilateral ones in *Eleusine coracana* (Narayanaswami 1952), *E. africana* (Chikkannaiah & Mahalingappa 1976), *Hordeum hexastichon* (Vogl 1947), *Stipa ischu* (Gould 1970) and *Zizania aquatica* (Weir & Dale 1960).

Table 1 presents a comparison of embryological features of the tribes Chlorideae, Eragrosteae, Sporoboleae, Zoysieae and Agrostideae, Aveneae, Brachypodieae, Bromeae, Festuceae, Phalarideae, Triticeae (often designated as the true festucoids) and Stipeae. As is evident from the available data, the three common features to the above tribes are, the well-developed outer integument, displacement of antipodal cells to a lateral position after fertilization and the presence of epiblast in the embryo (see Reader 1957).

The tribes Chlorideae, Eragrosteae, Sporoboleae and Zoysieae differ from the true festucoids (except the tribe Agrostideae) in (1) the absence of periclinal divisions in the nucellar epidermis close to the micropyle, (2) narrow region of the ovular attachment to placenta, (3) the thick distal region of dorsal ovary wall having only 3–5 middle layers, and (4) the absence of a cytoplasmically dense layer subjacent to the locular epidermis. Moreover, these tribes i.e., Chlorideae, Eragrosteae, Sporoboleae and Zoysieae have small or medium size chromosomes with 10 as the most common basic number (Mehra et al. 1968, Stebbins 1956), Chloridoid leaf-anatomy (Brown 1958), Chloridoid-Eragrostoid embryo (Reeder 1957), and Eragrostoid underground organs of the seedlings (Hoshikawa 1969). The true festucoids on the other hand have large chromosomes with 7 as the most common basic number, Festucoid leaf-anatomy, Festucoid embryo and Festucoid underground organs of the seedlings. Although the tribe Agrostideae has all the above features, the available embryological data suggests that it is closely related to the tribes Chlorideae, Eragrosteae, Sporoboleae and Zoysieae.

The tribe Stipeae has most of the embryological features met within the

true festucoids as are the Festucoid embryo and underground organs of the seedlings. The Stipeae, however, differ from the true festucoids in having small chromosomes with basic number 11 or 12 and Bambusoid leaf-anatomy.

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