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PLANT EMBRYOLOGY

A Symposium

Held under the auspices of the Biological Research Committee, CSIR: November 11-14, 1960 at the Department of Botany, University of Delhi

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Embryology of the Celastraceae

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The family Celastraceae shows several important morphological variations and the systematic position of this family has been a subject of controversy. While reviewing the earlier literature on the embryology of this family, Anderson (1931) described the development of ovule, embryo sac, embryo and endosperm in *Celastrus orbiculatus*, *C. scandens*, *Euonymus bungeanus*, *E. europaeus*, *E. latifolius* and *Tripterygium wilfordii*. Mauritzon (1936a) made some observations on the ovules of *Campylostemon*, *Hippocratea cinerascens*, *H. clematioides*, *H. grisebachii* and *Salacia* sp. He (1936b) further recorded some observations on the embryology and systematic limitations of Terebinthales and Celastrales.

The present paper deals with the embryology of *Celastrus paniculata* Willd., †*Elaeodendron glaucum* Wight & Arn., *Gymnosporia rothiana* Wight & Arn., *G. spinosa* Fiori. and ‡*Hippocratea grahamii* Wight.

MATERIAL AND METHODS

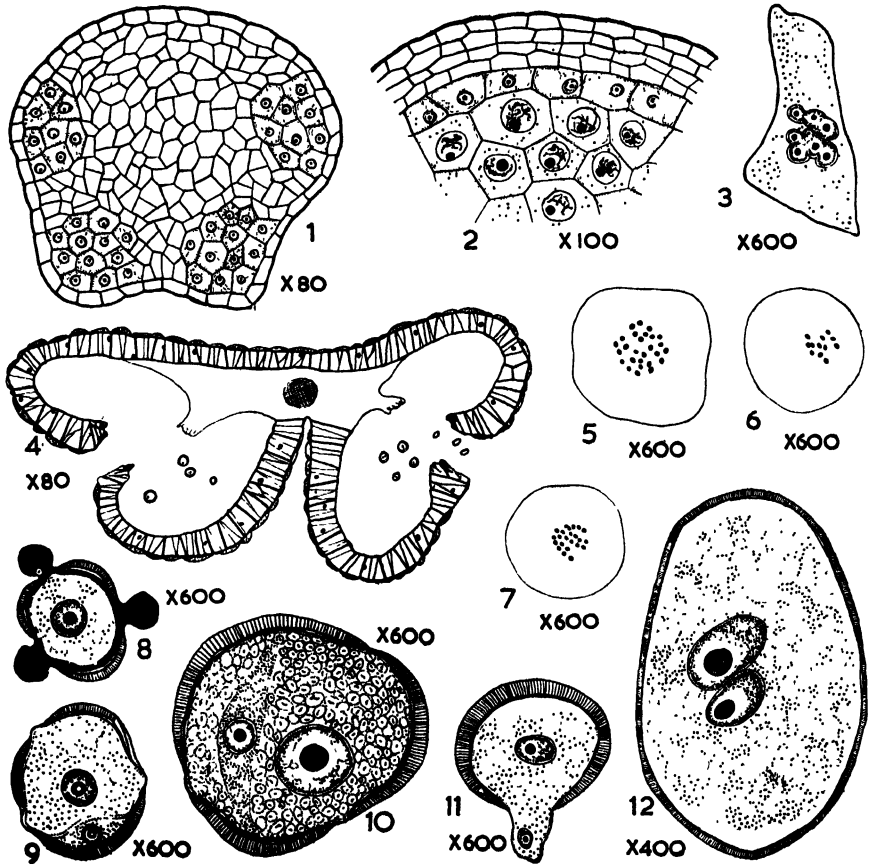
The material for the present study was collected from the suburbs of Bombay, Katriz Ghat, Poona, Khandala and Matheran and fixed in formalin-acetic acid-alcohol while some of the buds were fixed in acetic acid-alcohol (1:3). The customary methods of dehydration and imbedding were followed. Sections were cut 5 to 15μ thick and stained in iron-haematoxylin and counterstained with fast green or orange G. The former combination proved very satisfactory especially for post-fertilization stages.

OBSERVATIONS

Microsporangium. The hypodermal archesporium appears as two groups of cells in the anther of *Celastrus* and as four groups in *Gymnosporia*

*Present address : K. J. Somaiya College, Ghatkopar, Bombay † According to new nomenclature, *Elaeodendron roxburghii* Wight & Arn. ‡ According to new nomenclature, *Pristimera grahamii* Wight

spp. and *Elaeodendron glaucum*. Later, *Celastrus* also shows four groups (Fig. 1'). The number of parietal layers varies and in *Celastrus* the anther wall consists of five layers while the rest of the genera show only four layers (Fig. 2). The innermost parietal layer differentiates into tapetum. The tapetal cells are uninucleate to start with but become multinucleate when the microspore mother cells are undergoing reduction divisions. In *Celastrus paniculata* some of the cells showed as many as eight nuclei. During later stages the tapetal nuclei fuse producing a polyploid mass (Fig. 3). Towards



FIGS. 1-12—Figs. 1, 3, 7. *Celastrus paniculata*. Fig. 10. *Elaeodendron glaucum*. Figs. 2, 6. 9, 11, 12. *Gymnosporia rothiana*. Figs. 4, 5. *G. Spinosa*. Fig. 8. *Hippocratea grahamii;* Fig. 1. T. s. anther showing 4 groups of archesporial cells. Fig. 2. T. s. anther showing the 4-wall layers and microspore mother cells in early prophase of meiotic division. Fig. 3. Tapetal cell showing fusion of 8 nuclei. Fig. 4. T. s. anther at the time of dehiscence. Figs. 5-7. Metaphase I showing bivalents. Fig. 8. Pollen grain showing 'aspidote' pores. Fig. 9. Pollen grain with lenticular generative cell. Fig. 10. Pollen grain with spindle shaped generative cell. Fig. 11. Germination of pollen *in situ*. Fig. 12. Giant two-celled pollen grain

the close of meiotic divisions the tapetal cells begin to lose contact with one another and large vacuoles appear in their cytoplasm. Finally, the cells disorganize and disappear.

The outermost parietal layer differentiates into an endothecium. Its cells gradually enlarge and the walls get thickened. The inner and lateral walls are thicker in *Elaeodendron* and *Hippocratea* as compared to *Celastrus* and *Gymnosporia*. The fibrous thickenings appear in all the genera investigated (Fig. 4). The endothecium is usually one-layered but occasionally in both the species of *Gymnosporia* it becomes two-layered at some places.

At the time of dehiscence of anther the epidermis usually gets ruptured, but frequently in *Celastrus*, *Gymnosporia spinosa* and *Hippocratea* the epidermal cells may remain intact. In the latter the cells contain tannin and become papillate. Such papillae are also seen on the calyx, corolla, ovary wall and the disc in *Hippocratea*.

Microsporogenesis. The haploid number of chromosomes is 27 in *Gymnosporia spinosa* Fiori., 12 in *G. rothiana* Wight & Arn. and 23 in *Celastrus paniculata* Willd. (Figs. 5-7). All the mother cells in an anther do not divide simultaneously. Six secondary spindles were observed after the second meiotic division. Cytokinesis takes place by furrowing and the tetrads are either tetrahedral or decussate. Usually the microspores separate from one another but occasionally they remain together in *Gymnosporia spinosa*.

Male Gametophyte. The microspore nucleus migrates towards the wall and divides by an asymmetrical spindle. A small lenticular generative cell is separated from a large vegetative cell by a thin membrane (Fig. 9). Later the generative cell moves to a more central position in the pollen grain. The cytoplasmic sheath can be easily identified by its denser cytoplasm. The generative cell is usually spherical, rarely it assumes a crescent shape in *Gymnosporia rothiana* and in *Elaeodendron* it may be crescent, spindle (Fig. 10) or loop-shaped.

The generative cell does not divide prior to the shedding of pollen grains. In *Gymnosporia rothiana* the pollen grains may germinate *in situ* and the generative cell may migrate into the short pollen tube (Fig. 11).

The exine and intine develop in the normal manner and the former shows reticulate sculpturing. The intine protrudes out through the germ pore to a considerable extent in *Celastrus* and *Hippocratea* (Fig. 8) while in *Gymnosporia rothiana* only a small protrusion is seen. Erdtman (1952) described such pores as " aspidote pores ", while Hyde (1955) considered them to be definite morphological characters and designated them as " onci ". Such " onci " also occur in the Betulaceae, Campanulaceae, Corylaceae and Juglandaceae. The pollen grains are usually tricolpate but rarely tetracolpate grains were observed in *Celastrus* and *Hippocratea*.

The pollen grains are usually 10-12μ in diameter in *Hippocratea grahamii*, 12–14μ in *Gymnosporia spinosa*, 14–16μ in *G. rothiana*, 16–18μ in *Celastrus* and 28–30μ in *Elaeodendron*. In one loculus of *Gymnosporia rothiana* giant pollen grains with a diameter of 48μ were observed along with the normal pollen grains (Fig. 12).

Megasporangium. The ovules are orthotropous and bitegmic to start with but become anatropous at the megaspore tetrad or 2-nucleate stage of the embryo sac. Usually the raphe is ventral but sometimes in *Hippocratea* it may be dorsal. The inner integument makes its appearance at the time of differentiation of the archesporium. Both the integuments contribute to the formation of the micropyle in all the plants studied except in *Hippocratea grahamii* where only the inner integument takes part. However, Mauritzon (1936a) reports that the outer integument also takes part in the formation of the micropyle in the plants of the Hippocrateaceae studied by him. In most of the genera studied the outer integument is 3- to 8-layered while the inner integument is 3- to 4-layered. In *Hippocratea grahamii* the inner integument is 3-layered and outer integument is 3- to 4-layered. The integuments are swollen at the tips in all the plants except *H. grahamii*.

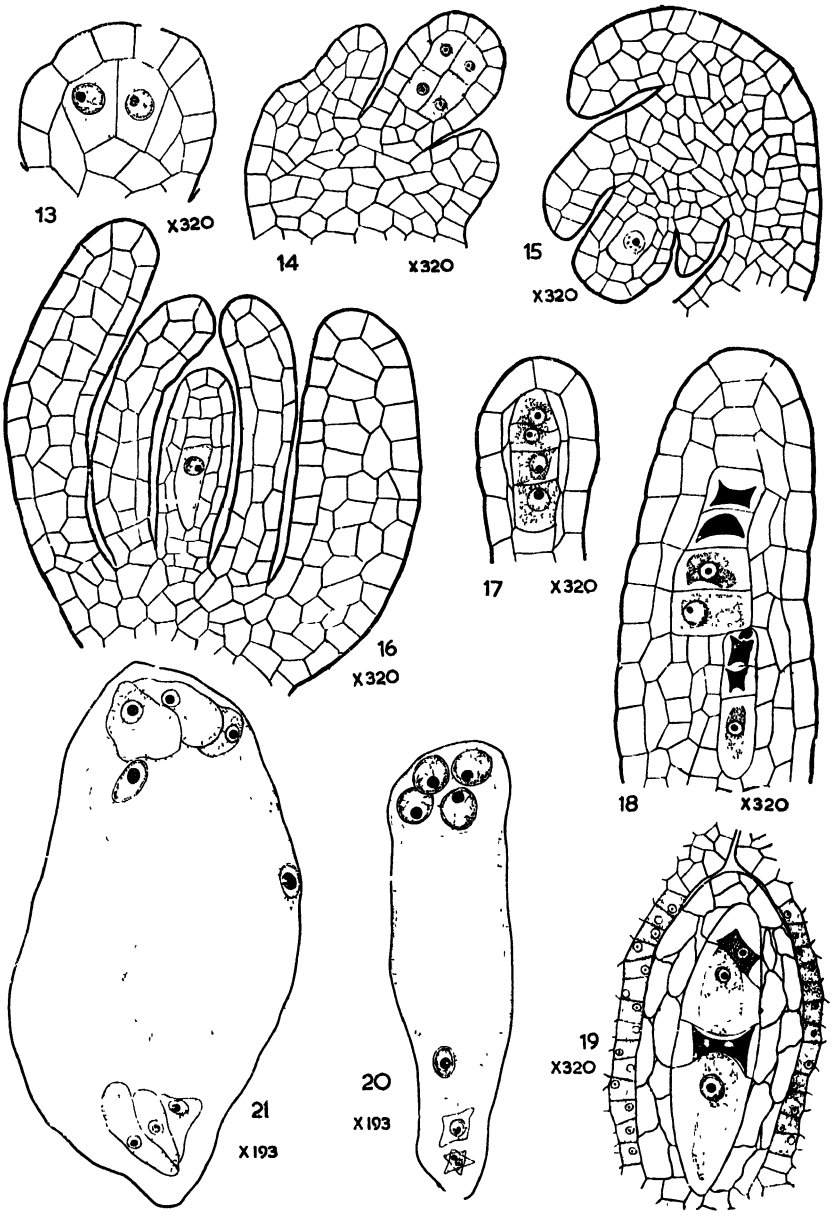
Usually a single hypodermal archesporial cell differentiates in the young ovule, as seen in *Celastrus scandens*, *Euonymus bungeanus*, *E. europaeus* and *E. latifolius* (Anderson, 1931). Occasionally two cells were observed in *Gymnosporia rothiana* (Fig. 13) and four in *Celastrus paniculata* (Fig. 14). Anderson (1931) has also reported occasional occurrence of two archesporial cells in *C. scandens*.

The archesporial cell directly functions as megaspore mother cell in *Hippocratea grahamii* and in both the species of *Gymnosporia*. But in *Celastrus* and *Elaeodendron* it divides periclinally forming the primary parietal cell and the primary sporogenous cell. In *Elaeodendron* the primary parietal cell rarely undergoes a longitudinal division (Fig. 15); but in *Celastrus paniculata* three to four parietal cells are formed (Fig. 16). Mauritzon (1936a) reported a single parietal cell in *Salacia* and further stated that the nucellus of *Hippocratea* species should be identical. However, this is not supported by the present study on *H. grahamii*.

The nucellar cells get crushed by the developing gametophyte and the latter comes in direct contact with the innermost layer of the inner integument which differentiates into an endothelium. This may happen even at the tetrad stage in *Celastrus paniculata* (Fig. 19). The cells of the endothelium elongate radially and their vacuolated cytoplasm stains deeply. The presence of endothelium in several plants of this family has also been reported by Anderson (1931).

Megasporogenesis. The ovule usually shows only one megaspore mother cell but occasionally in both the species of *Gymnosporia* two cells may differentiate. As a result of meiotic divisions a linear tetrad is formed in *Celastrus paniculata*, *Elaeodendron* and *Hippocratea* (Fig. 17). T-shaped and oblique tetrads were observed in both the species of *Gymnosporia*. Anderson (1931) reported linear tetrads in *Celastrus scandens*, *C. orbiculatus* and T-shaped condition in *Euonymus bungeanus* and *E. latifolius*. Occasionally twin tetrads were observed in *Celastrus paniculata* and *Gymnosporia rothiana* (Fig. 18).

Female Gametophyte. Usually the chalazal megaspore is functional, although occasionally in *Celastrus paniculata* any other megaspore may begin to enlarge simultaneously (Fig. 19). Even in such cases subsequently the



FIGS. 13-21—Figs. 14, 16, 18, 19, 21. *Celastrus paniculata*. Figs. 15, 20. *Elaeodendron glaucum*. Fig. 13. *Gymnosporia rothiana*. Fig. 17. *Hippocratea grahamii*. Fig. 13. L.s. ovule showing two archesporial cells. Fig. 14. L. s. ovule with multiple archesporium. Fig. 15. Same showing single parietal layer. Fig. 16. L. s. ovule with 4 parietal layers. Fig. 17. Same showing linear tetrad of megaspores. Fig. 18. Same with multiple tetrads. Fig. 19. L. s. ovule showing differentiation of endothelium. Fig. 20. Embryo sac showing the earlier differentiation of chalazal quartet into 3 antipodals and 1 polar nucleus. Fig. 21. Embryo sac with vacuolated antipodals

chalazal megaspore enlarges faster and ultimately develops into the female gametophyte.

The development of the embryo sac is of the Polygonum type. Jonsson' (1879-80) described bisporic development of the embryo sac in *Euonymus latifolius*. Anderson (1931) contradicted this and reported Polygonum type of development. This has been supported by the present study.

Occasionally the organization of the nuclei at chalazal end of the embryo sac may take place earlier than that at the micropylar end in *Elaeodendron* and *Gymnosporia rothiana* (Fig. 20).

The synergids are pear-shaped in all the plants studied but in *Hippocratea grahamii* they become hooked and develop filiform apparatus. Anderson (1931) observed hooked synergids in *Celastrus scandens*.

The polar nuclei usually meet at the centre of the embryo sac; but in *Hippocratea grahamii* they were found to lie very close or behind the egg apparatus. Jonsson* (1879-80), Anderson (1931) and Mauritzon (1936a) reported the fusion of the two polar nuclei in the centre of the embryo sac in the plants studied by them. The two-polar nuclei usually fuse before fertilization but this may be delayed in *Gymnosporia rothiana*.

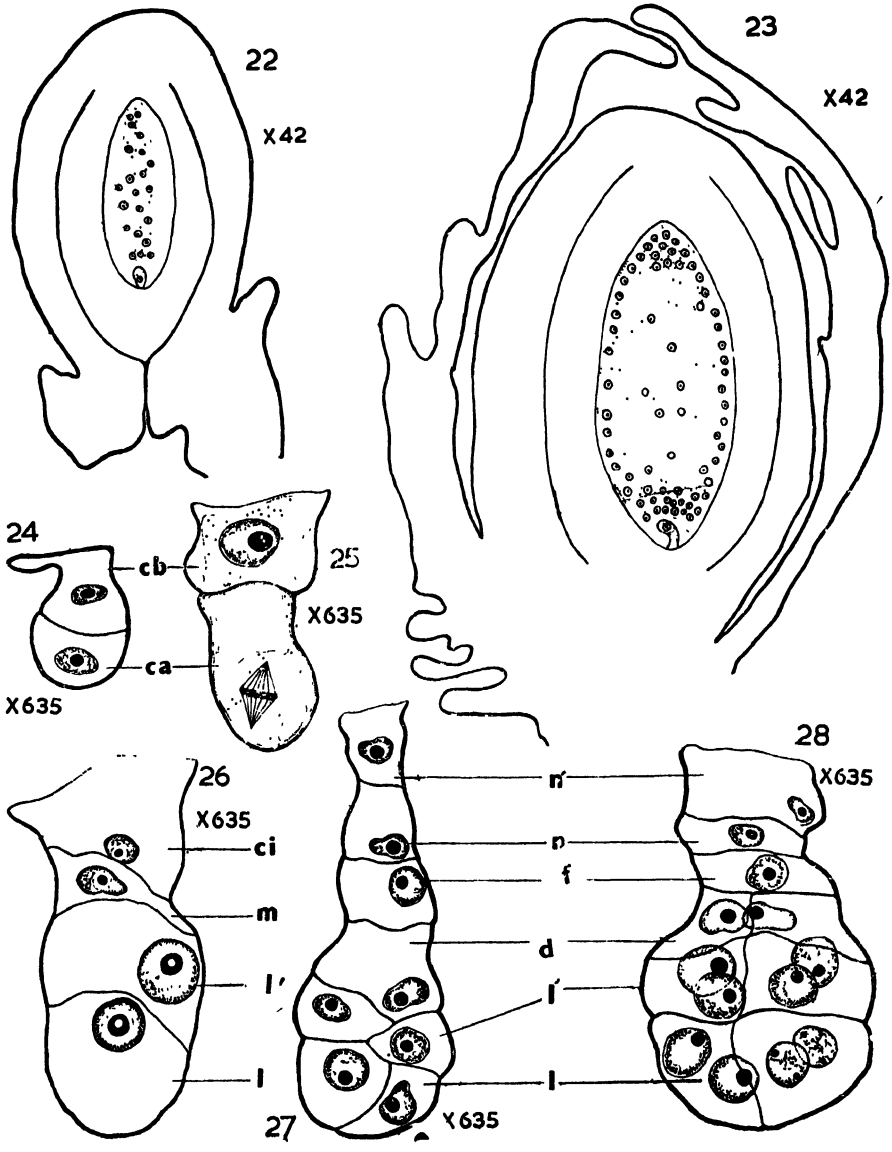
The antipodals are usually ephemeral. Occasionally, however, in *Hippocratea grahamii* and *Gymnosporia spinosa*, the antipodals persist till fertilization. In *Celastrus paniculata*, sometimes the antipodals vacuolate in such a manner as to simulate the egg apparatus (Fig. 21). Occurrence of such antipodals has not been recorded so far in this family. Occasionally multiple embryo sacs are observed in *Gymnosporia rothiana*.

Endosperm. Endosperm development has been studied only in *Celastrus paniculata* and *Gymnosporia spinosa*. The endosperm nucleus divides earlier than the zygote and the endosperm nuclei aggregate at the two ends of the embryo sac. After the production of about 200 endosperm nuclei, wall formation begins at the periphery and proceeds centripetally. Anderson (1931) has described a similar endosperm development in *Celastrus scandens*.

Embryo. In *Gymnosporia spinosa* the zygote undergoes a period of rest for one or two weeks. The zygote divides by a transverse wall to form the basal and the terminal cell (Fig. 24). Both the cells divide to form a four-celled proembryo (Figs. 25, 26). They can be designated as *ci*, *m*, *l'*, and *l*. The tiers *l'* and *l* divide transversely and vertically to form a globular mass (Figs. 27, 28). The cell *m* divides transversely to form *f* and *d*, while *ci* gives rise to *n* and *n'*. The tiers *l* and *l'* contribute to the formation of cotyledons, hypocotyl and radicle; *d* forms the root tip, while *n*, *n'* and *f* form the suspensor. According to Souèges' classification the development of the embryo is of Solanad type.

In *Celastrus paniculata* zygote undergoes a period of rest for 2 to 3 months. The development of the embryo is similar to that in *Gymnosporia spinosa*. Anderson (1931) has reported the embryo development as Sagina variation of Caryophyllad type in *Euonymus europaeus* and *Celastrus scandens*.

*Cited in Anderson (1931)



FIGS. 22-28 — Figs. 22-23. *Celastrus paniculata*. Figs. 24-28. *Gymnosporia spinosa*. Fig. 22. L. s. ovule showing the development of aril at the funicular side. Fig. 23. Fully differentiated aril surrounding the ovule. Fig. 24. Two-celled proembryo. Fig. 25. Terminal cell of the 2-celled proembryo in a division. Fig. 26. Four-celled proembryo. Fig. 27. Eight-celled embryo showing quartet formation at its tip. Fig. 28. Embryo showing the formation of octant

Polyembryony. Polyembryony has been observed in *Celastrus paniculata*. Occasionally two or three embryos were found overlapping one another. The occurrence of polyembryony has also been recorded in *Euonymus europaeus* by Jagar* (1814), Grabel* (1830) and Bailey* (1916) and in *E. americanus* by Braun* (1859).

False embryo-like structures similar to those reported by Anderson (1931) were also observed in *Celastrus paniculata*.

Seed Coat. Both the integuments take part in the formation of the seed coat. In *Gymnosporia spinosa* after fertilization, the inner integument becomes seven- to eight-layered while the outer becomes seven-layered with large epidermal cells.

At the six- to eight-celled stage of the embryo, the integuments thin out due to degeneration of some of the layers. Ultimately only four layers of the outer integument remain and the outer seed coat develops from these only.

Only the innermost layer of the inner integument, the endothelium, remains and forms the inner seed coat.

In *Celastrus paniculata* the development of the seed coats is essentially similar.

Aril. The development of the aril has been studied in *Celastrus paniculata* and *Gymnosporia spinosa*. In *Celastrus* the aril appears at the four nucleate stage of the embryo sac, in the form of a small outgrowth or hump on the outer side of the outer integument near funiculus. But further development is delayed and only after fertilization it starts growing. It rapidly enlarges and soon covers up the whole ovule. It also forms folds and wrinkles (Figs. 22, 23) and at maturity becomes bright scarlet in colour.

In *Gymnosporia spinosa* the aril arises in a similar manner but the entire growth takes place only after fertilization. It also develops folds and wrinkles but does neither completely cover the seed nor develop any colouration.

Systematic Position of Hippocratea. The genus *Hippocratea* has been included in the family Celastraceae by Hallier (1912) and Bentham & Hooker (1883) while Engler & Prantl (1889), Bessey (1915) and Hutchinson (1959) have separated the two families. Wettstein (1935) kept the genus *Hippocratea* in the order Celastrales. Smith (1940) pointed out numerous differences between the two families, Celastraceae and Hippocrateaceae, but still acknowledged the affinities of the two families. Smith & Bailey‡ (1941), while discussing the systematic position of the genus *Brassiantha* have pointed out that the division between the two families Hippocrateaceae and Celastraceae is artificial. Metcalfe & Chalk (1958) have pointed out a very close relation between the two families on anatomical grounds. Erdtman (1952) found great resemblance between the pollen grains of the two families.

The main similarities and differences in the morphology and embryology of *Hippocratea grahamii* and Celastraceae are shown in Tables 1 and 2 respectively.

On the basis of the above-mentioned dissimilarities it is suggested that

*Cited in Anderson (1931) ‡ Cited in Erdtman (1952)

TABLE 1— SIMILARITIES IN THE MORPHOLOGY AND EMBRYOLOGY OF *HIPPOCRATEA GRAHAMII* AND CELASTRACEAE

Character	*Hippocratea grahamii*	Celastraceae
Perianth	Two whorls of 5 each, imbricate	Two whorls of 5 each, imbricate
Tapetum	Glandular	Glandular
Pollen	Tricolpate, rarely tetracolpate; 'onci' present; exine reticulate	Tricolpate, rarely tetracolpate in *Celastrus;* 'onci' present in Celastrus; exine reticulate
Carpels	Tricarpellary, syncarpous	Tricarpellary, syncarpous
Ovule	Anatropous, bitegmic	Anatropous, bitegmic
Embryo sac	Polygonum type, endothelium present	Polygonum type, endothelium present
Endosperm	Nuclear	Nuclear

TABLE 2 — DIFFERENCES IN MORPHOLOGY AND EMBRYOLOGY OF *HIPPOCRATEA GRAHAMII* AND CELASTRACEAE

Character	*Hippocratea grahamii*	Celastraceae
Habit	Scandent shrub	Climbers, shrubs or trees
Inflorescence	Panicle	Dichotomously branched cymes, panicles or fascicles
Stamens	3, free, arising at the base of ovary within the disc, anthers dehisce transversely and extrosely	5, free, from the disc or fused with it, arising on the rim or outside the disc, anthers dehisce longitudinally and introsely
Ovule	Tenuinucellate, micropyle formed by inner integument	Tenuinucellate in *Gymnosporia*, weakly crassinucellate in *Celastrus* and *Elaeodendron*, micropyle formed by both integuments
Fruits	Schizocarpic	Capsules, drupes or follicles
Seeds	Exalbuminous, winged, exarillate	Albuminous, wings absent, arillate or exarillate

Hippocratea may be separated from the Celastraceae and placed in a separate family Hippocrateaceae close to the former.

SUMMARY

The archesporium in an anther appears as two or four hypodermal groups of cells. The tapetum is secretory.

The endothecium shows fibrous thickenings. Occasionally in both the species of *Gymnosporia* it becomes 2-layered. The epidermal cells are filled with tannin and develop papillae in *Hippocratea.*

The haploid chromosome numbers in *Gymnosporia spinosa* Fiori., *G. rothiana* Wight & Arn. and *Celastrus paniculata* Willd. are 27, 12 and 23 respectively.

The cytokinesis takes place by furrowing.

In *Celastrus* and *Hippocratea*, the pollen grains show " onci ".

Giant pollen grains are observed in *Gymnosporia rothiana.*

The archesporium is generally single-celled. The ovules are bitegmic. The micropyle is formed by both the integuments in all the plants, except in *Hippocratea grahamii* where it is formed by the inner integument only. An endothelium is present.

Occasionally multiple tetrads are observed in *Gymnosporia rothiana* and *Celastrus paniculata.*

Embryo sac development is of the Polygonum type.

Hippocratea grahamii shows hooked synergids with the filiform apparatus. It also shows the polar nuclei very close to the egg apparatus or behind it. Occasionally multiple embryo sacs have been observed in *Gymnosporia rothiana.*

The endosperm is free nuclear in *Celastrus paniculata* and *Gymnosporia spinosa.* The wall formation is centripetal.

The development of the embryo conforms to the Solanad type in *Celastrus paniculata* and *Gymnosporia spinosa.*

Polyembryony has been noted in *Celastrus paniculata.*

Both the integuments take part in the formation of the seed coats. Aril arises on the outer integument near the funiculus in *Celastrus paniculata* and *Gymnosporia spinosa.*

Separation of *Hippocratea* to a new family Hippocrateaceae seems to be justified from the morphological and embryological studies.

The authors are deeply indebted to Professor P. Maheshwari and Dr B. M. Johri of the Department of Botany, University of Delhi, for their valuable help.

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Morphological and Embryological Studies in *Dipcadi*

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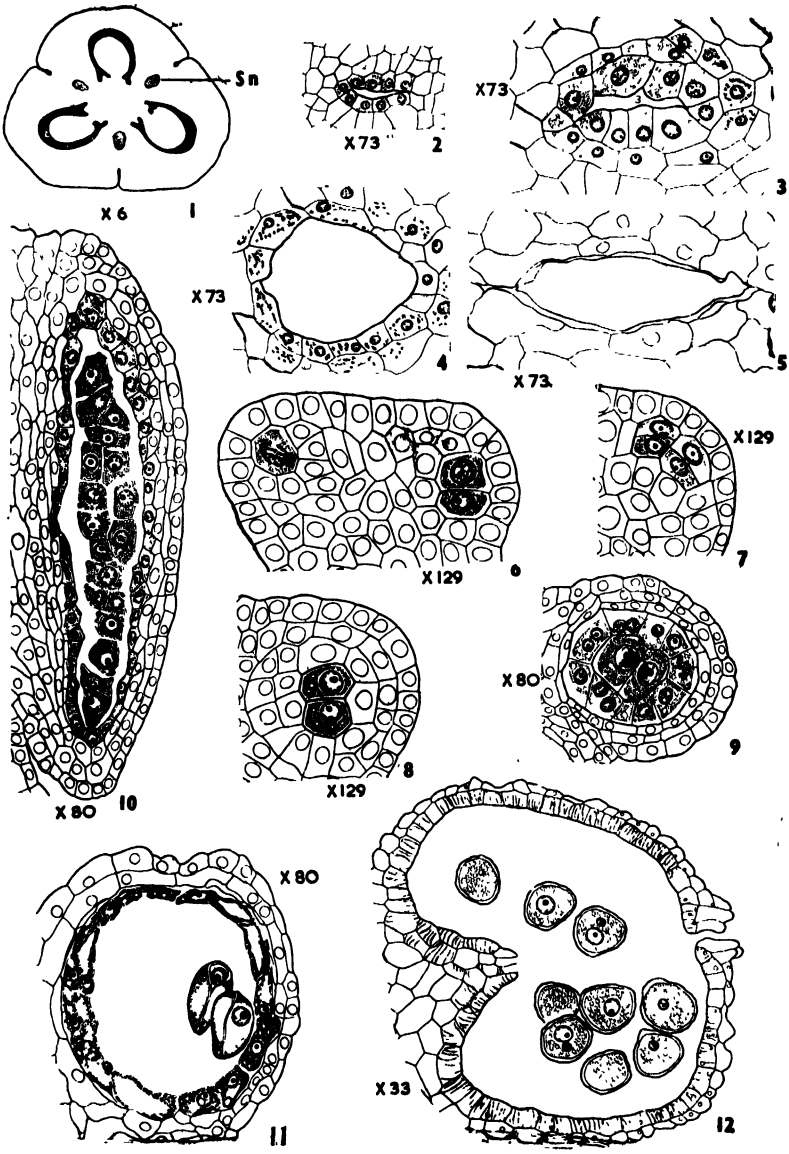
The genus *Dipcadi* is chiefly African. Eight species of it are reported to occur in India and some of them are endemic. Distinguished from the rest of the genera of Scilloideae, *Dipcadi* is characterized by tuberous scapigerous herbs with racemed flowers, cylindric perianth of six erect segments, the outer recurved from about the middle and the inner at the tips only. The stamens are included. The loculicidal capsule is many-seeded. The seeds are flat with black membranous testa.

The only embryological study on this genus is by Buchner (1948) and Chennaveeraiah & Mahabale (1959). Buchner's study was on the megasporogenesis, female gametophyte and other morphological aspects. Chennaveeraiah & Mahabale, while studying both microsporogenesis and megasporogenesis, have noted the occurrence of certain abnormalities in this species. But a detailed embryological work in this genus is wanting and the present paper deals with *D. saxorum* Blatter and *D. ursulae* Blatter.

The material of these two species was collected from their type localities, *D. saxorum* from the hills of Kanheri Caves, about 30 miles from Bombay at an altitude of 1,000 ft and *D. ursulae* from the tableland at Panchgani at an altitude of 4,400 ft. Formalin-acetic acid-alcohol was widely used as a fixing fluid. The customary paraffin method was employed and sections were cut at 8–20 microns. Iron-alum-haematoxylin staining was widely used. Permanent acetocarmin preparations of pollen mother cells were made by McClintock's method.

OBSERVATIONS

Flower. The inflorescence is 50 cm. long and 12- to 25-flowered in *D. saxorum*, and 15-30 cm. long and 8- to 15-flowered in *D. ursulae*. The flowers are slightly fragrant in the latter. The bracts are caducous, ovate and acuminate. The pedicels are long and stout. The six perianth lobes



FIGS. 1-12.—*Dipcadi saxorum*—SEPTAL NECTARY AND MICROSPORANGIUM (*Sn*, Septal Nectary): Fig. 1. T. s. ovary through the mid region showing three locules and three septal nectaries. Fig. 2. Septal nectary when the megaspore mother cell is being formed. Fig. 3. Same when the embryo sac is organizing. Fig. 4. Same, at the time of anthesis. Fig. 5. Same, when the embryo is two-celled. Fig. 6. T. s. anther lobes showing hypodermal archesporial cells. Figs. 7-9. T. s. anther lobe showing stages in the development of wall layers. Fig. 10. L. s. anther lobe showing one- to two-seriate sporogenous tissue. Fig. 11. T. s. anther lobe showing secretory tapetum, uninucleate microspores and degenerating middle layer. Fig. 12. Same, showing stomium and fibrous endothecium

are greenish white in *D. saxorum* whereas they are slightly pinkish brown on the lower surface and greenish white on the upper surface in *D. ursulae*. Their tips are glandular. The six stamens are attached to the perianth at the base. The superior tricarpellary ovary has a fairly long style which ends in three bifid stigmatic lobes. The epidermal hairs are found distributed on the stigmatic lobes.

Septal Nectary. The septal nectaries are conspicuous (Fig. 1). They extend from the base of the ovary to half the length of the style. The nectary canals are surrounded by one- to three-layers of cells. They make their first appearance when the megaspore mother cell is being differentiated. The nectary secreting cells at this stage is one-layered consisting of only a few cells (Fig. 2). It remains in this condition till the two-nucleate embryo sac stage. Later, more layers are formed and the nectary becomes very conspicuous. It is fully developed when the mature embryo sac is formed (Fig. 3). When the flower opens, it begins to lose prominence (Fig. 4) and after fertilization, as the embryo becomes two-celled, it is completely disorganized (Fig. 5).

Microsporangium. The transection of a four-lobed young anther shows one or two hypodermal archesporial cells at each corner (Fig. 6). They divide periclinally resulting in an outer primary parietal layer and an inner primary sporogenous layer (Fig. 7). The primary parietal layer gives rise to three wall layers and the wall of the anther thus consists of the epidermis, endothecium, middle layer and the tapetum (Figs. 8, 9). Meanwhile the primary sporogenous cells divide and the sporogenous tissue, in a longitudinal section, shows one row at the ends and two rows in the middle (Fig. 10). To start with the tapetal cells are uninucleate. Later they become two-, four- or even six-nucleate. The nuclei may fuse to form large polyploid ones. The tapetum is of the secretory type and as the anther matures the tapetum and the middle layer degenerate (Fig. 11). The endothecial layer develops fibrous thickenings which extend even to some of the cells of the connective (Fig. 12). The anther dehisces by two longitudinal stomia.

Microsporogenesis. The microspore mother cells, as they prepare for division, undergo typical synizetic stage. Six and ten bivalents have been observed during Meiosis I in *D. saxorum* and *D. ursulae* respectively of which one is comparatively smaller in size (Figs. 13, 14). The larger bivalents in both the species have interstitial chiasmata. There is normal disjunction and at the end of the first division a wall is formed resulting in a dyad (Fig. 15). After a brief interphase the second division sets in, and tetrads are formed. These are mostly of the isobilateral type as is typical of most monocotyledons (Fig. 16). In addition, linear, obliquely linear and decussate tetrads are also formed (Figs. 17-19).

Male Gametophyte. The nucleus of the microspore moves towards the wall before it divides and a large vacuole is formed on the other side. The spindle formed during the division of the microspore nucleus is asymmetrical resulting in a small lenticular generative cell and a large vegetative cell.

Later the wall between the two cells dissolves and the generative nucleus gets into the cytoplasm of the vegetative cell. A sheath of cytoplasm remains quite distinct around the generative nucleus. The latter is almost spherical and much smaller than the vegetative nucleus. The pollen grains are shed at the two-celled stage. A rare instance was observed in *D. ursulae* where the generative nucleus had divided to form two male cells before shedding. The pollen grains are monocolpate as in species of *Massonia, Scilla, Ornithogalum, Veltheimia* of the group Scilloideae (Erdtman, 1952).

The division of the generative nucleus was studied from germination of the pollen grains *in vitro*. The pollen grains readily germinated on sugar-agar (8% sugar) medium and formed the male cells in about three hours. The germination is mostly monosiphonous. Usually the vegetative nucleus first gets into the pollen tube and the generative nucleus follows it (Fig. 20). Rarely the reverse may also happen (Fig. 21). Sometimes, the pollen grains produced two pollen tubes (Fig. 22). When there are two pollen tubes, it is observed that one of the tubes may contain both the vegetative and generative nuclei (Fig. 23), or one tube may contain the vegetative nucleus and the other the generative nucleus.

The spindle of the dividing generative nucleus seems to be a weak one as seen from the metaphase and anaphase configurations. At the end of this division there does not seem to be a wall formation, but a cleavage appears and two male cells are formed. They are mostly of the same size. In *D. ursulae*, some anthers were seen with germinated pollen.

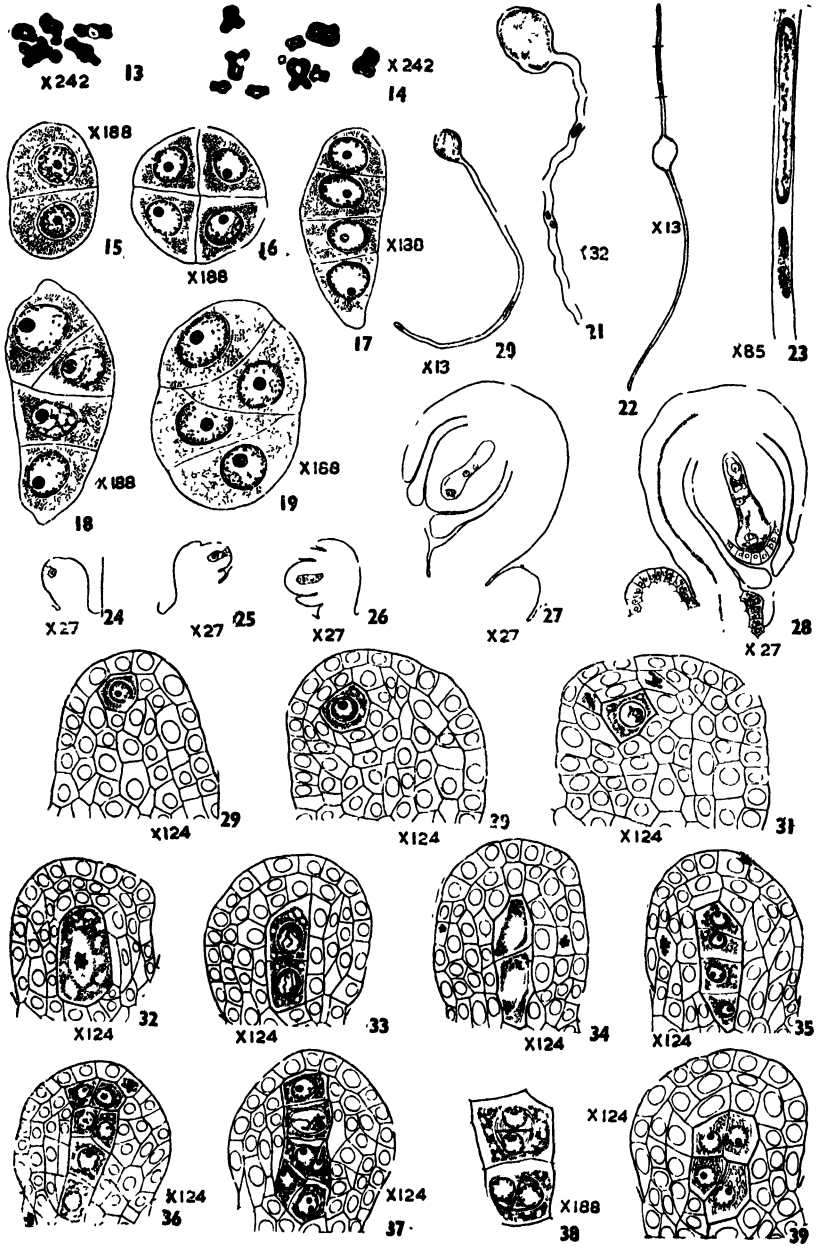
Megasporangium. Two rows of anatropous, bitegmic and crassinucellate ovules are borne on axile placenta in each locule. The ovule undergoes a curvature of 90° at the megaspore tetrad stage and becomes completely anatropous at the four-nucleate stage of the embryo sac (Figs. 24-28).

The inner integument makes its appearance when the megaspore mother cell is being formed, whereas the outer one differentiates when the megaspore mother cell is dividing. Both are epidermal in origin. The micropyle is formed by the inner integument. The latter is two-layered except in the region of the micropyle where it is three-layered (Fig. 50). A conspicuous annular swelling is formed at the base of the funiculus. Such a thing has also been observed by Eunus (1950) in *Albuca transvalensis*. It disorganizes soon after fertilization. The funicular strand extends upto the base of the nucellus where it connects to a group of nucellar cells having dense contents. The chalazal end of the embryo sac abuts on this tissue. Nutrition is conveyed to the embryo sac probably through this tissue.

Megasporogenesis. In the young nucellus, a hypodermal archesporial cell with a conspicuous nucleus and dense cytoplasm differentiates (Fig. 29). Sometimes two archesporial cells were noticed in both the species. The archesporial cell divides periclinally to cut off an outer parietal cell. The next division in the latter is either anticlinal or periclinal (Figs. 30, 31). The parietal tissue formed finally consists of two layers. Sometimes it may consist of three layers.

The megaspore mother cell, as it starts dividing, undergoes a typical

synizetic stage. The spindle during the first division of the megaspore mother cell is almost straight (Fig. 32) and a typical cell plate is formed resulting in a dyad (Fig. 33). The spindle during the second division (Fig. 34) is variously oriented resulting in different kinds of tetrads. Thus linear, obliquely linear and T-shaped tetrads have been noticed in both the species (Figs. 35, 36). In addition, an inverted T-shaped tetrad in *D. saxorum* (Fig. 38) and an isobilateral tetrad in *D. saxorum* (Fig. 39) have also been observed.



The lowermost megaspore of the tetrad is functional and the other three degenerate. In one ovule of *D. saxorum* the lowermost megaspore showed signs of degeneration (Fig. 40). Otherwise the degeneration of the megaspores in the linear tetrads of *D. saxorum* is basipetal (Figs. 41–43). In *D. ursulae*, the upper two megaspores degenerate first and the third one a little later.

Certain abnormal features have been noticed at the tetrad stage. In both the species some parietal cells with their large size, conspicuous nuclei and dense cytoplasm simulate the sporogenous cells (Figs. 36, 37). Their sporogenous nature is indicated by the fact that some of them are in syni-zesis · age. Such sporogenous cells have also been noticed in *Ophiopogon wallichianus* by Maheshwari (1934).

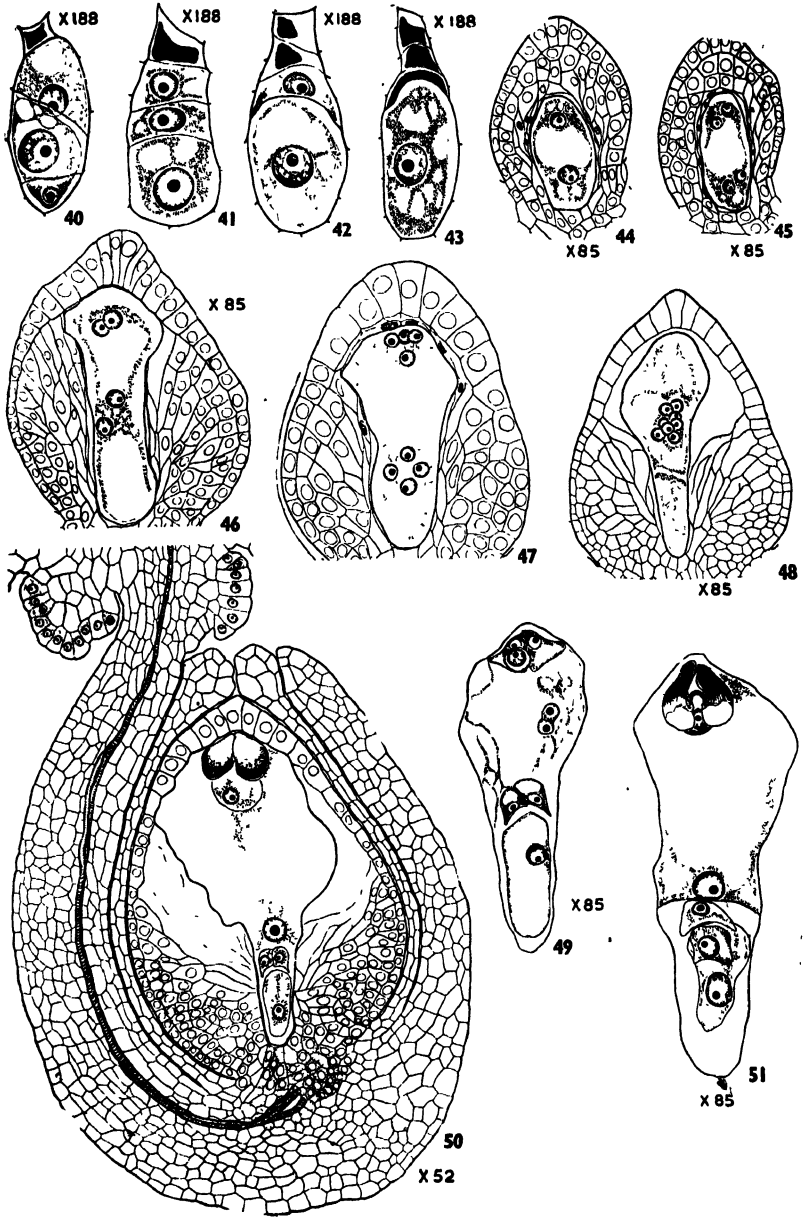
Female Gametophyte. The nucleus of the functional megaspore divides to give rise to a two-nucleate embryo sac (Fig. 44) followed by a four-nucleate stage (Fig. 45). At this stage, the chalazal end of the embryo sac grows considerably and develops a pouch. The chalazal nuclei, however, do not migrate downwards into this pouch, but remain *in situ*, occupying now a place in the centre of the embryo sac (Figs. 45, 46). After the next division of the nuclei, the chalazal group of four move down slightly towards the pouch (Fig. 47). No embryo sac has been observed to be without this pouch formation in both the species.

The polar nuclei meet in the centre and fuse (Fig. 49) and the secondary nucleus moves downwards and gets nearer to the antipodal cells (Fig. 50).

The egg apparatus consists of two synergids and an egg cell. The synergids have basal vacuoles (Fig. 49). Hooked synergids are also seen. Sometimes in *D. ursulae* the synergids simulate the egg (Fig. 50). The egg cell is usually ovoid having an apical vacuole.

Of the three antipodal cells, the lower one is invariably large and vacuolate. The other two are comparatively smaller and the three antipodal cells are arranged in a T-shaped manner. Sometimes the two smaller ones develop vacuoles at their apical ends (Fig. 49). All the antipodal cells may be lodged within the pouch or only the lower, larger one may occupy it. They persist for a long time till the embryo and endosperm are fairly well developed.

←FIGS. 13-15-18, 21, 24-38 — *D. saxorum;* Figs. 14, 19, 20, 22, 23. 39 — *D. ursulae* MICROSPOROGENESIS, MALE GAMETOPHYTE, OVULE AND MEGASPOROGENESIS : Figs. 13, 14. First division of pollen mother cell showing six and ten bivalents respectively in polar view. Fig. 15. Dyad. Figs. 16-19. Isobilateral, linear, obliquely linear and decussate tetrads Fig. 20. Monosiphonous germination of the pollen showing degenerating vegetative nucleus at the tip of the tube and the generative nucleus behind it. Fig. 21. Same, showing the male cells ahead of the vegetative nucleus in the tube. Fig. 22. Germinated pollen with two tubes, the shorter one containing both the vegetative and generative nuclei. Fig. 23. Enlargement of the portion marked in Fig. 22 showing the degenerating vegetative nucleus and the dividing generative nucleus. Figs. 24-28. Stages in the growth and curvature of the ovule. Fig. 29. L. s. nucellus showing hypodermal archesporial cell. Figs. 30,31. Same, showing parietal cells. Figs. 32-35. Same, showing stages in the formation of linear tetrad. Figs. 36,37. Same, showing T-shaped and slightly oblique isobilateral tetrads with two sporogenous parietal cells. Fig. 38. Meiosis II — orientation of spindles indicate formation of inverted T-shaped tetrad. Fig. 39. L. s. nucellus showing isobilateral tetrad ·



FIGS. 40-46, 48, 49, 51—*D. saxorum;* Figs. 47, 50—*D. ursulae.* Figs. 40-43. Stages in the degeneration of nonfunctional megaspores in the tetrads. Figs. 44, 45. L. s. nucellus showing two- and four-nucleate embryo sacs. Fig. 46. Same, showing late four-nucleate embryo sac with a pouch at the chalazal end. Fig. 47. Same, showing eight-nucleate embryo sac. Fig. 48. Same, showing all the eight nuclei in the centre of the embryo sac. Fig. 49. Embryo sac showing egg apparatus, polar nuclei fusing and two of the three antipodal cells having apical vacuoles. Fig. 50. L. s. ovule showing mature embryo sac with egg-like synergids and the funicular strand extending to the base of the nucellus. Fig. 51. Embryo sac showing a precocious partition above the antipodal cells

At the mature embryo sac stage, only the nucellar epidermis overarches it, as the parietal tissue gets crushed by the developing embryo sac.

In an abnormal embryo sac of *D. saxorum*, the chalazal pouch containing the antipodal cells was separated by a wall-like partition from the rest of the embryo sac even before fertilization (Fig. 51). This precocious partition simulates the formation of Helobial type of endosperm.

In both the species, some embryo sacs were observed with supernumerary nuclei whose origin, however, could not be traced. In one embryo sac of *D. saxorum* all the eight nuclei were grouped in the centre (Fig. 48).

Fertilization is porogamous and traces of the pollen tube are seen up to the two-celled stage of the embryo.

Endosperm. The development of the endosperm is of the Helobial type. The first division of the primary endosperm nucleus is followed by a wall resulting in a smaller chalazal chamber and a larger micropylar chamber. Further divisions in both the chambers are free nuclear, but the divisions in the micropylar chamber are more than those in the chalazal chamber. Both the chambers thus contain many nuclei. Wall formation starts when the embryo is fairly advanced in development. Finally the entire endosperm becomes cellular.

Embryo. The development of the embryo has not been studied in sufficient detail. On the whole the development is of the Caryophyllad type. Further work is in progress.

DISCUSSION

The successive type of division of the microspore mother cells is characteristic of most monocotyledonous plants, with certain exceptions, and likewise the formation of isobilateral tetrads. In addition to the isobilateral tetrads, the linear tetrads were frequent in the species of *Dipcadi* under investigation. Previously the linear tetrads were thought to be rare. They, however, have been noticed in *Habenaria* (Swamy, 1946), *Ottelia* (Islam, 1950) and others. Kausik & Rao (1942) have reported that they have always been linear in *Halophila ovata*. Vögl (1947) has noted them in many plants, such as *Sagittaria montevidensis*, *S. chinensis*, *Paris quadrifolia*, *Lilium menryi*, *Yucca filamentosa*, many species of *Allium*, *Tradescantia*, *Agapanthus*, *Anthericum*, *Pistia*, *Colocasia* and *Spathiphyllum*. The smear preparations of microspore mother cells in the species under investigation revealed that the isodiametric and rounded ones gave rise to isobilateral tetrads, while the elongated ones formed linear tetrads. Other types of tetrads were formed by microspore mother cells of different shapes. Thus it seems that the kind of tetrad formation depends more or less on the shape of the microspore mother cells.

The division of the generative nucleus is fairly uniform. A spindle is formed during this division, but it is not a prominent one. There is usually monosiphonous germination of the pollen grains, but sometimes two pollen tubes were also seen. Such a thing is not usually observed in families besides

Malvaceae, Cucurbitaceae and Campanulaceae. It will be of interest to know whether this phenomenon occurs in other genera of Liliaceae besides *Dipcadi*.

The megaspore tetrads in *Dipcadi* are usually linear, or obliquely linear or T-shaped. Other kinds of tetrads like the isobilateral and inverted T-shaped are also rarely met with. It has been observed that if the megaspore mother cell is narrowly elongated, both the spindles in the second division of it are straight and result in the formation of a linear tetrad. If the upper end of the megaspore mother cell is broader it results in an obliquely linear or T-shaped tetrad. Rarely when the lower end of the megaspore mother cell is broader, an inverted T-shaped tetrad is formed. If the megaspore mother cell is not elongated but is more or less isodiametric, an isobilateral tetrad is formed. Thus, it seems that the kind of tetrad formed depends on the availability of space in and the shape of the megaspore mother cell.

A constant feature noticed in the species of *Dipcadi* is the formation of a pouch at the chalazal end of the embryo sac at the four-nucleate stage. This has escaped the notice of Buchner (1948) who while studying *D. serotinum* did observe the increase in the size of the embryo sac at the four-nucleate stage. But she added that the noteworthy feature of it was the arrangement of the nuclei, the upper two situated normally side by side and the lower two mostly superimposed and shifted rather a little below the middle of the embryo sac. Contrary to her observations, however, a careful study made here showed the lower two nuclei do not shift their position; they rather remain where they were and only the chalazal end of the embryo sac gets elongated to form the pouch.

Eunus (1950) in his study on *Albuca transvalensis* figures a four-nucleate embryo sac similar to the one with the pouch in *Dipcadi*, but he interprets it as an abnormal embryo sac. His text figures 19 and 20 are both four-nucleate embryo sacs, the former being considered as a normal one and the latter an abnormal one. When these figures are compared with the corresponding stages in *Dipcadi*, it seems that what Eunus (1950) has interpreted as a case of abnormal four-nucleate embryo sac, is only a regular stage in the development of the embryo sac forming a pouch at the chalazal end. This may be the situation in *Albuca* also since the genus is closely allied to *Dipcadi*. A reinvestigation of *Albuca* is desirable in this respect.

The antipodals, of which one is considerably large, are conspicuous and mostly arranged in a T-shaped manner. The chalazal pouch containing these antipodals abuts on the nucellar tissue having dense contents connected to the funicular strand. They are persistent also. All this suggests their nutritive role. In this connection it is interesting to note that the antipodal cells figured by Eunus (1950) in *Albuca transvalensis* look similar to those in *Dipcadi*.

In many members of Scilloideae such as *Eucomis*, *Veltheimia*, *Ornithogalum*, *Muscari*, *Puschkinia*, *Heloniopsis* and *Veratrum*, the development of the endosperm is of the Helobial type (Cave, 1953). *Dipcadi* has also the Helobial type of endosperm. Considering on the whole, the embryological

data presented here for *Dipcadi* reveal that all such features as Polygonum type of embryo sac development, Helobial type of endosperm, the large embryo, spherical male cells, readily fall in line with those seen in the other genera of the Scilloideae.

Usually the embryological characters of the species within a genus are more or less constant. So also is the case in *Dipcadi*. There is but a little variation regarding the kinds of tetrads formed and the sequence in the degeneration of the nonfunctional megaspores among the species.

Regarding the systematic position of the genus in Scilloideae, *Dipcadi* is closely allied to *Albuca* in having the common characters such as compressed seeds, long embryo sac with three antipodal cells, the Helobial type of endosperm and three-radiate stylar canal. In other genera of Scilloideae the embryo sac may be broad or long and the endosperm nuclear or Helobial. *Dipcadi* and *Albuca* form a natural alliance with *Eucomis*, *Veltheimia*, *Ornithogalum*, *Muscari*, *Puschkinia*, *Heloniopsis* and *Veratrum* characterized by the Helobial endosperm, rather than with *Scilla*, *Hyacinthus*, *Camassia* and *Galtonia*, which have nuclear endosperm. All the genera in Scilloideae on the whole are well knit and distinct from Lilioideae, but within the subfamily Scilloideae there appear to be two distinct series, one with Helobial and the other with the Nuclear endosperms. This warrants, perhaps, further examination of the subfamily Scilloideae from the systematic point of view.

SUMMARY

The raceme in *D. saxorum* is 12- to 25-flowered, and in *D. ursulae* it is 8- to 15- flowered. The flowers are slightly fragrant in the latter.

Conspicuous septal nectaries are present.

In the young anther one- to two-celled hypodermal archesporium differentiates at each corner. The wall of the anther consists of four layers. The tapetum is of the secretory type.

The microspore mother cells divide successively. Six and ten bivalents are counted in *D. saxorum* and *D. ursulae* respectively.

The microspore tetrads are isobilateral, decussate, linear and obliquely linear.

The pollen grains are monocolpate and are shed at the two-celled stage. Their germination is mostly monosiphonous, but sometimes two pollen tubes per grain have been seen.

The ovule is anatropous, crassinucellate and bitegmic. The micropyle is formed by the inner integument. There is an annular swelling at the base of the funiculus.

The megaspore tetrads are linear, obliquely linear and T-shaped. A case of an inverted T-shaped and an isobilateral tetrad was met with in *D. saxorum* and *D. ursulae* respectively.

The degeneration of the nonfunctional megaspores is gradual in *D. saxorum*, but in *D. ursulae* it is not exactly so.

The embryo sac development is of the Polygonum type. At the four-

nucleate stage a pouch is formed at the chalazal end. Later this becomes lodged with the antipodal cells. The latter are arranged in a T-shaped manner and are persistent.

The endosperm formation is of the Helobial type. The embryo development conforms to the Caryophyllad type.

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Morphological and Embryological Studies in the Commelinaceae

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The members of the Commelinaceae have been favourite objects for cytological studies (*see* Darlington, 1929; Rau, 1930; Anderson & Sax, 1934; Yasui & Suita, 1939; Beatty & Alvin, 1953). In the field of experimental morphology, the work of Walkar (1938), Eigsti (1940) and Swanson *et al.*, (1949) may also be mentioned.

Dimorphic flowers, both cleistogamous (underground and aerial) and chasmogamous, occur in *Tradescantia erecta* (Henslow, 1879), *Commelinantia pringlei* (Tharp, 1927), *Commelina forskalaei* (Hagerup, 1932; Maheshwari & Maheshwari, 1955; Maheshwari & Baldev, 1958), *C. benghalensis* (Maheshwari & Singh, 1934; Maheshwari & Maheshwari, 1955) and *C. indehiscens* (Barnes, 1949). Some information is available regarding the number and the distribution of different kinds of flowers. The nature of cleistogamy is also discussed by various authors (Uphof, 1938; Maheshwari, 1960).

Earlier embryological work on the Commelinaceae has been reviewed by Schnarf (1931). Subsequently, considerable work has been done by Maheshwari & Singh (1934), Murthy (1934, 1938). Parks (1935), McCollum (1939), Tschermak-Woess (1947), Souèges (1958 a, b) and Maheshwari & Baldev (1958). As the information available on the morphology and the embryology is inadequate the study on *Commelina subulata* Roth, *Aneilema paniculatum* Wall., *Floscopa scandens* Lour., *Cyanotis axillaris* D. Don., and *Tinantia fugax* Scheidw, was undertaken to fill some of the gaps in the existing data.

MATERIAL AND METHOD

The material was collected from Belgaum, Londa, Castle-Rock and Jog Falls, Mysore State, during the months of July and August and fixed in formalin-acetic acid-alcohol. *Tinantia fugax*, collected from Mussoorie, was kindly passed on to the author by Professor P. Maheshwari.

The customary methods of dehydration and imbedding were followed. Sections were cut 8-18μ thick. Heidenhain's iron-haematoxylin with erythrosin as the counterstain was found satisfactory for staining. The male cells of *Cyanotis axillaris* were studied after germinating the pollen grains in distilled water at room temperature. The whole mounts of endosperm in acetocarmine-glycerine (1 : 1) were examined.

OBSERVATIONS

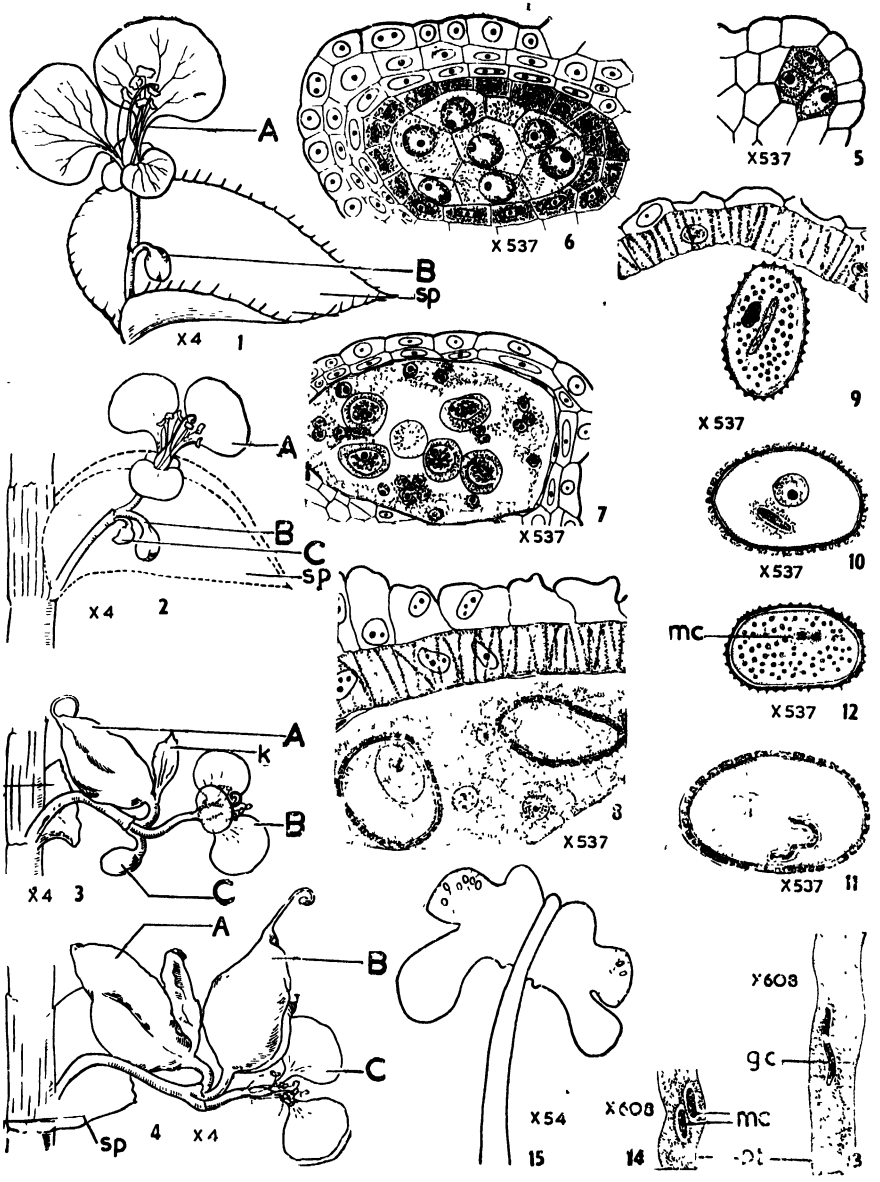
Flowers. All plants studied by the author bear only aerial flowers. The presence of underground flowers in *Tinantia fugax* could not be confirmed due to want of proper material. In *Commelina subulata* there are two to three chasmogamous flowers in the spathe (Figs. 1, 2, 3). The first flower is bisexual. The second flower may be bisexual (9 per cent), male with pistillode (64 per cent) or without pistillode (27 per cent). The third flower is male, 15 per cent of which possess a pistillode and it rarely opens if the first two flowers produce fruits (Fig. 4). In *Cyanotis axillaris* the first three flowers are bisexual and chasmogamous while the fourth one is cleistogamous and male, 36 per cent of which possess a pistillode.

The flowers are trimerous. All the six stamens are fertile in *Floscopa scandens*, *Tinantia fugax* and *Cyanotis axillaris*. In *Commelina subulata* and *Aneilema paniculatum* two to three stamens are reduced to staminodes which sometimes contain degenerating pollen grains in abortive microsporangia (Fig. 15).

Anther. The young anther is tetralocular. The hypodermal archesporial cells divide to produce the inner primary sporogenous and an outer primary parietal cells (Fig. 5). The latter by further divisions add to the wall of the anther which consists of a persistent epidermis, a fibrous endothecium, a degenerating middle layer and a tapetum (Fig. 6). A periplasmodium is formed in the early stage of microsporogenesis (Fig. 7) which is consumed by the developing pollen grains (Figs. 8, 9). Division of the microspore mother cells is of the successive type and the wall formation is by cell-plate method resulting in isobilateral and decussate tetrads.

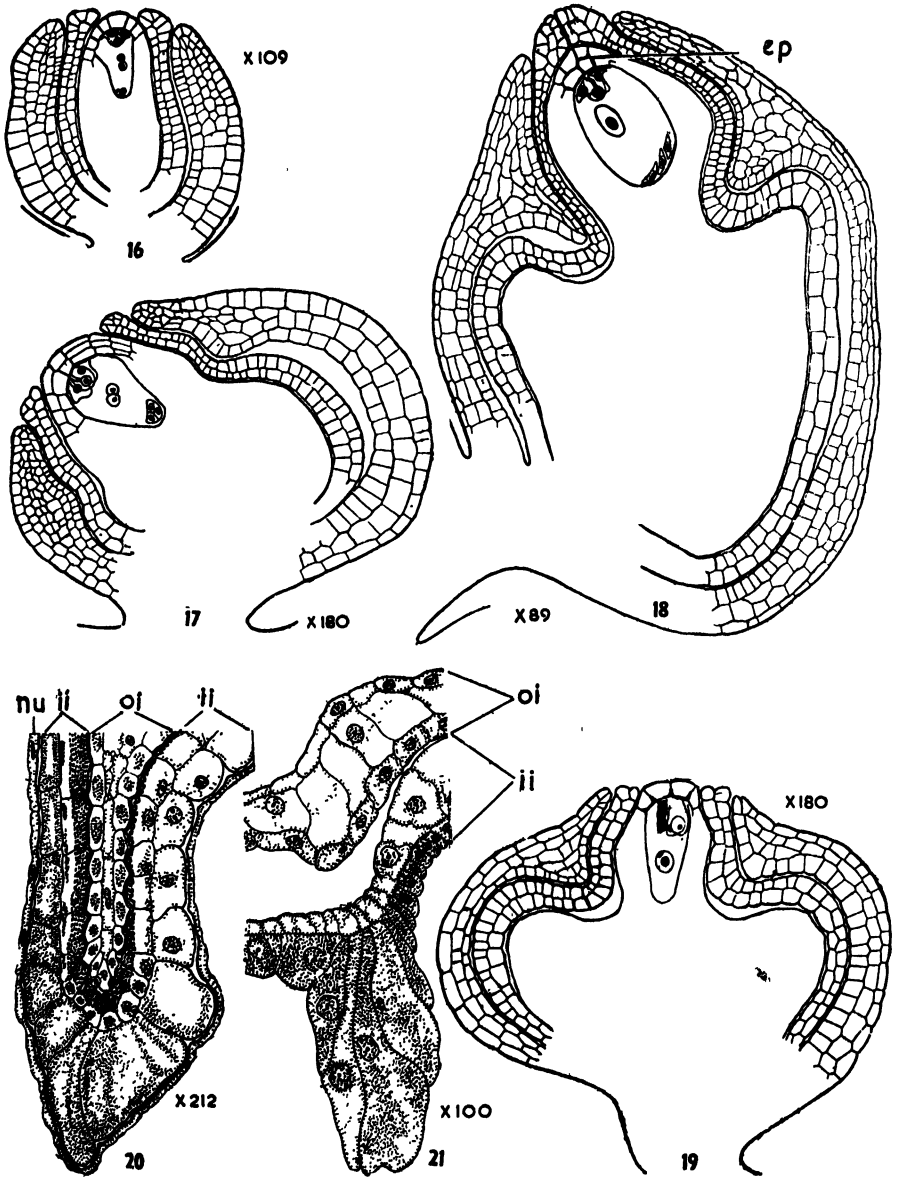
Male Gametophyte. The pollen grain is oval, unisulcate and the exine is spinulose in *Commelina subulata* and *Floscopa scandens* (Figs. 9, 12) and warty in others (Figs. 10, 11). The microspore divides to form a small generative cell and a large vegetative cell. There is a considerable variation in the form of the generative cell, elongated and slightly bent in *Commelina subulata* (Fig. 9), spindle-shaped in *Aneilema paniculatum* (Fig.10) as well as *Floscopa scandens* and worm-like in *Cyanotis axillaris* (Fig. 11). The pollen grain is shed at the three-celled stage in *Floscopa scandens* (Fig. 12) and at the two-celled stage in others. The generative cell divides in the pollen tube to form two male cells (Figs. 13-14).

Ovule. The bitegmic ovule is orthotropous in *Floscopa scandens* (Fig. 16), orthotropous with a slight bend in *Aneilema paniculatum* (Fig. 19) and hemianatropous in others (Figs. 17, 18). The ovule is tenuinucellate in *Aneilema paniculatum* and *Cyanotis axillaris* and crassinucellate in others. The



FIGS. 1-15—FLOWER, ANTHER AND MALE GAMETOPHYTE (A, B, C, flowers 1 to 3 in spathe; *k*, calyx; *gc*, generative cell; *mc*, male cells; *pt*, pollen tube; *sp*, spathe): Figs. 1-4, 9, 15. *Commelina subulata*. Figs. 5-8, 11, 13, 14. *Cyanotis axillaris*. Fig. 10. *Aneilema paniculatum*. Fig. 12. *Floscopa scandens*. Fig. 1. A spathe with an exserted flower (A) and bud (B). Fig. 2. Same with three flowers, (A) protruding and (B) and (C) in buds. Fig. 3. Inflorescence showing a fruit (A), a bisexual flower (B) and a bud (C). Fig. 4. An old inflorescence with two fruits (A, B) and a staminate flower (C). Figs. 5-9. T. s. microsporangium at different stages of development. Figs. 10-12. Pollen grain at the time of dehiscence. Figs. 13, 14. Pollen tubes showing generative and two male cells respectively. Fig. 15. Whole mount of a staminode showing the pollen grains in abortive anther lobes

inner integument is two-layered while the outer one is three- to seven-layered. A micropyle is formed only by the inner integument in *Tinantia fugax* (Fig. 18) and by both the integuments in *Cyanotis axillaris*, while in others it is want-



FIGS. 16-21—OVULE AND COLLAR FORMATION (*ep*, epistase; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument): Fig. 16. *Floscopa scandens*. Fig. 17. *Commelina subulata*. Fig. 18. *Tinantia fugax*. Figs. 19, 20. *Aneilema paniculatum*. Fig. 21. *Cyanotis axillaris*. Figs. 16-19. L. s. ovules showing the orientation. Figs. 20, 21. Enlargement of part of the ovules showing the collar formation in *Aneilema paniculatum* and *Floscopa scandens* respectively

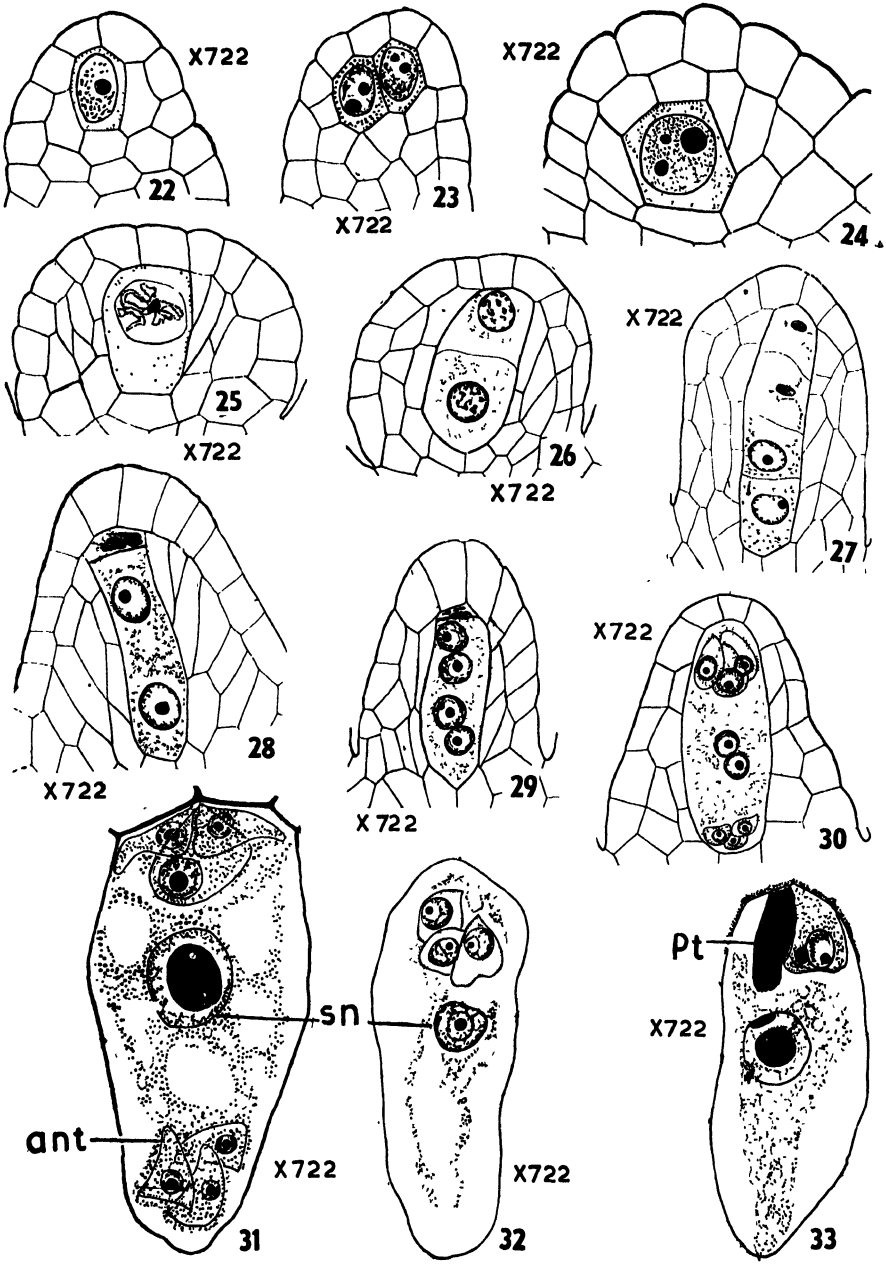
ing. The cells of the nucellus at the micropylar region enlarge slightly and after fertilization the integuments grow to cover the nucellar apex. The nucellar cells at the micropylar end become greatly thickened during later stages of seed development.

Usually a single hypodermal archesporial cell is present (Fig. 22). Rarely two such cells may be seen in *Commelina subulata* and *Cyanotis axillaris* (Fig. 23). But only one develops further. The archesporial cell directly functions as megaspore mother cell in *Aneilema paniculatum* and *Cyanotis axillaris* (Fig. 25) while in *Commelina subulata*, *Floscopa scandens* and *Tinantia fugax* (Fig. 24) a parietal cell is always cut off. The primary parietal cell may or may not divide further and is crushed by the developing megaspores. Meiotic divisions of megaspore mother cells result in linear tetrads (Figs. 26, 27). Rarely T-shaped and occasionally oblique tetrads are formed.

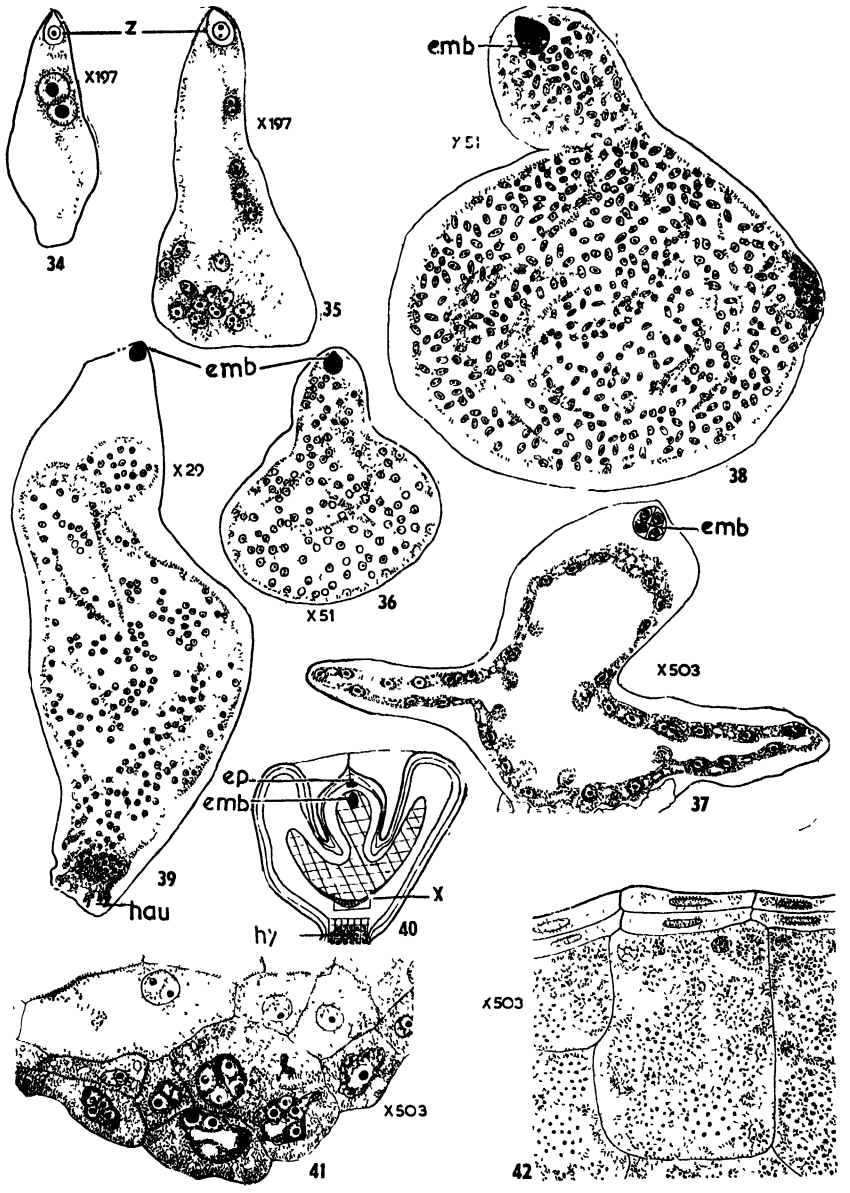
Female Gametophyte. The chalazal functional megaspore enlarges and the nucleus undergoes three successive divisions to form an 8-nucleate embryo sac (Figs. 28-30). The two polar nuclei fuse before the entrance of the pollen tube (Fig. 31). The antipodals are ephemeral (Fig. 32). However, in *Tinantia fugax* they are somewhat large and persistent (Fig. 31). Triple fusion occurs earlier than the syngamy (Fig. 33).

Endosperm. The endosperm is of the Nuclear type. After a few free nuclear divisions the endosperm becomes peripherally arranged (Figs. 34, 35). Later the endosperm extends basally beyond the collar and occupies the lower part of the developing seed. Thus, it gradually assumes an inverted top-shape (Fig. 36). At this stage in *Floscopa scandens* button-shaped enucleate vesicles appear on the inner side of the endosperm (Fig. 37). In *Tinantia fugax* there is only an aggregation of nuclei and cytoplasm at the chalazal end (Fig. 38) and in *Cyanotis axillaris* a persistent chalazal haustorium is formed (Fig. 39). The wall formation is centripetal, and starts from the micropylar end. The outermost layer of the endosperm cells are narrow and divide forming two similar layers (Fig. 42). The cells towards inside are large and irregular and usually multinucleate. The division and fusion of nuclei in these cells are common. In *Aneilema paniculatum* at the chalazal end the outer two layers of endosperm cells are large and contain big nuclei (Figs. 40, 41). Compound starch grains fill all the cells of the endosperm except outer two layers in *Commelina subulata* (Fig. 42) and outermost layer in others.

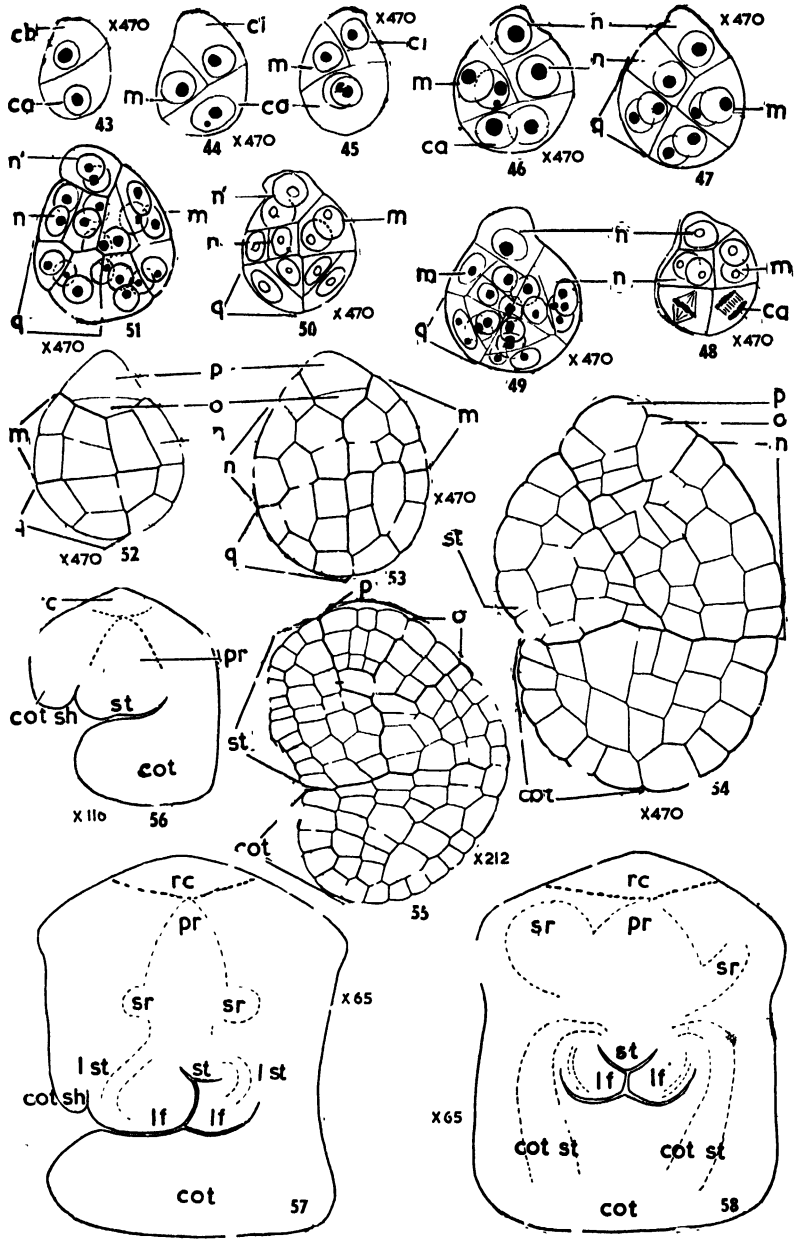
Embryo. The zygote divides usually by an oblique wall to form the cells *ca* and *cb* (Fig. 43). Both the cells divide vertically forming a four-celled proembryo (Figs. 44, 45). The two derivatives of *cb*, *m* and *ci* are unequal due to slightly oblique-vertical wall (Figs. 44, 45) and are juxtaposed. In *Aneilema paniculatum* the periclinal walls are laid down in *ca* cutting off the dermatogen initials (Fig. 48). But in others a second vertical division at right angles to the first in *ca* results in a quadrant *q* (Fig. 47). In *Commelina subulata*, *Floscopa scandens* and *Tinantia fugax* the dermatogen initials are cut off from *q* at this stage (Fig. 64). But in *Cyanotis axillaris* an octant is formed by the laying down of the diagonal walls in *q* (Fig. 51). In the mean-



FIGS. 22-33 — MEGASPOROGENESIS AND FEMALE GAMETOPHYTE (*ant*, antipodals; *pt*, pollen tube; *sn*, secondary nucleus) : Figs. 22, 23, 25-30, 32. *Cyanotis axillaris*. Figs. 24, 31. *Tinantia fugax*. Fig. 33. *Aneilema paniculatum*. Figs. 22, 23. L. s. nucelli showing one and two-celled archesporium respectively. Figs. 24, 25. Same with megaspore mother cells. Figs. 26, 27. Dyad and tetrad. Figs. 28-30. Stages in embryo sac development. Figs. 31, 32. Mature embryo sacs. Fig. 33. Embryo sac showing double fertilization



FIGS. 34-42 — ENDOSPERM (*emb*, embryo; *ep*, epistase; *hau*, haustorium; *hy*, hypostase; *z*, zygote): Figs. 34-36, 42. *Commelina subulata*. Fig. 73. *Floscopa scandens*. Fig. 38. *Tinantia fugax*. Fig. 39. *Cyanotis axillaris*. Figs. 40, 41. *Aneilema paniculatum*. Figs. 34, 35. Early stages in the development of endosperm. Fig. 36. Whole mount of endosperm at early globular embryo stage. Fig. 37. Endosperm showing button-shaped vesicles. Figs. 38, 39. Whole mounts of endosperm at later stages of development. Fig. 40. L. s. young seed showing cellular endosperm (diagrammatic). Fig. 41. Enlargement of part marked x in Fig. 40. Fig. 42. Enlargement of part marked G in Fig. 61



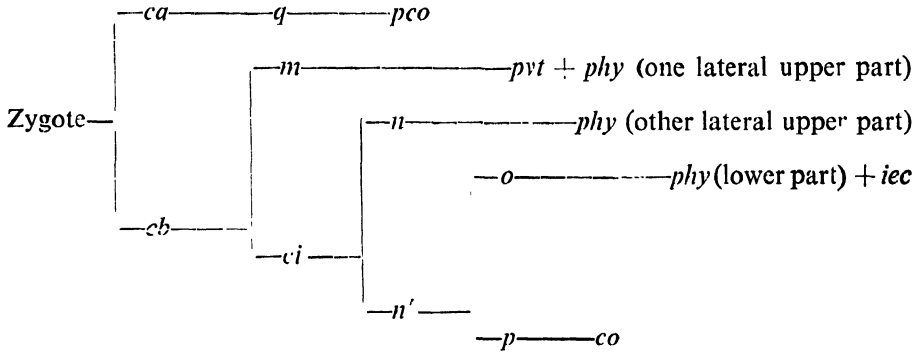
FIGS. 43-58 — EMBRYO (*cot*, cotyledon; *cot sh*, cotyledonary sheath; *cot st*, cotyledonary strands; *lf*, leaf primordium; *l st*, lateral traces for leaves; *pr*, primary root; *rc*, root cap; *sr*, secondary root; *st*, stem apex) : Figs. 43-47, 49, 52, 53, 55-58. *Commelina subulata*. Figs. 48, 50, 54. *Aneilema paniculatum*. Fig. 51. *Cyanotis axillaris*

time, *ci* divides transversely producing *n* and *n'* while *m* undergoes a vertical division (Figs. 46, 47). Dermatogen initials are cut off from all the segments except *n'* (Figs. 49, 50). Later the segment *n'* also divides transversely forming *o* and *p* (Figs. 52, 53).

Subsequent divisions are irregular resulting in a globular embryo (Fig. 54). Due to rapid divisions in the basal region the embryo becomes subspherical. At this stage a lateral depression appears separating the derivatives of *q* from those of *m* (Figs. 54, 55). The terminal cotyledon is formed from the segment *q*. The stem tip is derived from the lateral part of *m* just above the cleft. The cotyledonary sheath and a lateral upper part of the hypocotyl are contributed by *n*. The basal part of the hypocotyl and the root tip develop from *o*. Lastly, *p* gives rise to the root cap (Figs. 55, 56).

Procambial strands differentiate in hypocotyl, and the traces to the cotyledon and lateral roots are also formed. The leaf surrounding the stem apex is formed at a later stage (Figs. 57, 58).

Thus, the embryogeny of the Commelinaceae follows the Asterad type (Johansen, 1950) and may be represented as follows (*co*, root cap; *iec*, initials of central cylinder of root; *pco*, cotyledonary region; *phy*, hypocotyledonary region; *pvt*, shoot apex) :

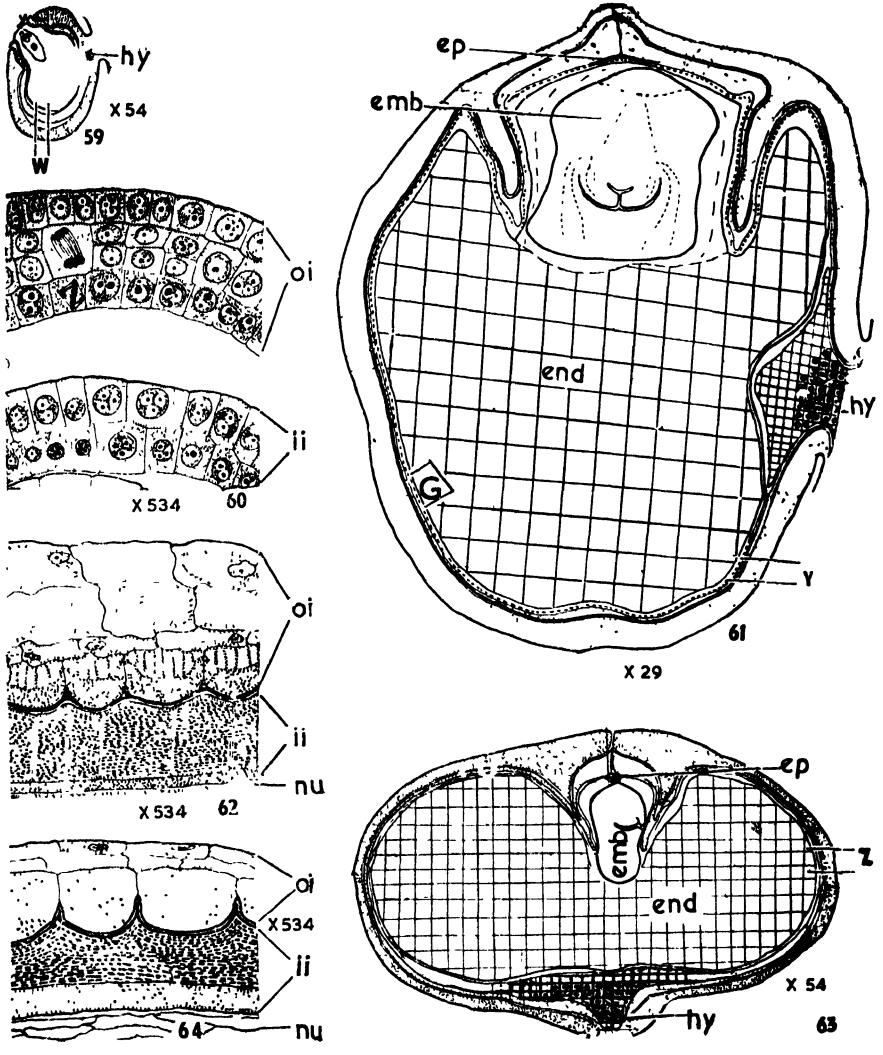


Seed and Seed Coat. After fertilization the nucellar cells at the chalazal region enlarge somewhat and become thickened forming the hypostase (Figs. 59, 61, 63). But in *Cyanotis axillaris* it is initiated before fertilization. Some of the cells of the hypostase are filled with dark brown material. The hypostase persists in mature seed. The epistase begins to form before fertilization and persists for some time during the development of the seed but is finally crushed.

Due to the differential growth in the nucellus between the chalazal end and the micropylar, a shallow depression appears a little below the micropylar end (Fig. 17). After fertilization the depression deepens into a narrow constriction and the inner integument is thrown into a fold. The cells of the outer integument lying opposite the groove divide forming a kind of ridge which fits into this groove (Figs. 19, 20). But in *Cyanotis axillaris* such a groove is not formed by the nucellus. As such the integuments do not take part in the formation of the collar. On the other hand, the cells of the inner

layer of the inner integument elongate and deeply protrude into the cavity of the seed (Fig. 21). Due to these changes the seed is divided into a narrow micropylar collar and a broad basal region.

In a mature seed the nucellus is reduced to a few layers of disintegrated cells except for a persistent hypostase and a crushed epistase. The basal portion of the seed is filled with mealy endosperm and the collar region is occupied by the embryo. The seed coat is hard and is formed by both the integuments.



FIGS. 59-64 — SEED AND SEED COAT (*emb*, embryo; *end*, endosperm; *ep*, epistase; *hy*, hypostase; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument): Figs. 59-62. *Commelina subulata*. Figs. 63, 64. *Floscopa scandens*. Fig 59. L.s. young seed. Fig. 60. Enlargement of portion marked W in Fig. 59. Figs. 61, 63. L.s. mature seeds. Figs. 62, 64. Enlargement of parts marked Y, Z, from Figs. 61 and 63 respectively

The inner layer of the inner integument gets stretched and their cells are filled with brown material (Figs. 59 – 64). On the other hand, the cells of the outer layer of this integument develop thickening. (Figs. 61 – 64). In *Commelina subulata* and *Aneilema paniculatum* the cells of the inner layer of the outer integument develop band-like and reticulate thickenings, and their inner tangential walls are partially thickened (Figs. 61, 62), whereas in other plants these cells are filled with refractive granules (Figs. 63, 64). Rest of the layers of this integument persist as a thin strip around the seed.

Similar changes take place in the collar region. The outer layers of the outer integument divide and enlarge filling the cleft around the collar.

DISCUSSION

Cleistogamy is constitutional in some, but in others it is controlled by the environmental factors [Uphof, 1838; Maheshwari, 1960. Summer School, Bot. (Darjeeling) : 9-10]. The presence of three chasmogamous flowers in the aerial spathe of *Commelina subulata* and a cleistogamous flower in the aerial spathe of *Cyanotis axillaris* indicate that cleistogamy is not determined by environmental factors alone.

Tetrahedral tetrads have been reported in *Commelina benghalensis* (Maheshwari & Singh, 1934) and *Cyanotis axillaris* (Murthy, 1934, 1938). However, the author did not observe tetrahedral tetrads either in *Cyanotis axillaris* or in any other plants investigated.

Some taxonomic books describe the ovules in this group as orthotropous although almost all types of ovules, orthotropous, hemianatropous, campylotropous have been described in this family. The present findings have revealed orthotropous in *Floscopa scandens*, orthotropous with a slight bend in *Aneilema paniculatum* and hemianatropous in others.

Presence of a parietal cell has been reported by some authors (Maheshwari & Singh, 1934; Murthy, 1938; Maheshwari & Baldev, 1958) and absence by others (Guignard, 1882; Murthy, 1938). The author has found the presence of parietal cell in *Commelina subulata*, *Floscopa scandens* and *Tinantia fugax*.

Occurrence of monosporic (Guignard, 1882; Maheshwari & Singh, 1934; Parks, 1935; Murthy, 1934, 1938; Maheshwari & Baldev, 1958), bisporic (Guignard, 1882; Walker, 1938) as well as tetrasporic (McCollum, 1939) types of embryo sac has been reported in this family. However, only the Polygonum type of embryo sac has been found to occur in the plants included in the present work.

Although the endosperm is of the Nuclear type (Maheshwari & Singh, 1934; Murthy, 1934, 1938; McCollum, 1939), no detailed account of its development has been given except in *Commelina forskalaei* (Maheshwari & Baldev, 1958). The present investigation indicates that the endosperm formation follows the conventional Nuclear type. In *Floscopa scandens* enucleate button-shaped vesicles appear on the inner side of the embryo sac. The aggregation of endosperm nuclei in the chalazal region (*Tinantia fugax*), the persistent chalazal haustorium (*Cyanotis axillaris*) and large

endosperm cells of the two outer layers at the chalazal region with hypertrophied nuclei (*Aneilema paniculatum*) suggest different degree of the nutritional activity of the endosperm at the chalazal region of the embryo sac.

The account of the development of the embryo in the Commelinaceae is incomplete (*see* Solms-Laubach, 1878; Süssenguth, 1921; Murthy, 1938; McCollum, 1939). However, Souèges (1958a, b) working on the embryology of *Commelina communis* and *Rhoeo discolor* has brought certain features such as (i) the juxtaposed orientations of *m* and *ci*, (ii) the division of these two segments in different planes, (iii) the derivatives of the embryonic part other than the cotyledon from the segments of *cb* and lastly (iv) the absence of suspensor. Though all these indicate a relationship with Muscari variation under the Asterad type they are so different that a special variation called Commelina variation under Asterad type is created by Souèges. Maheshwari & Baldev (1958) have described the development of the embryo in *Commelina forskalaei*. But they have failed to follow the segmentation correctly and have misinterpreted the findings of Souèges. The author's findings are in agreement with those of Souèges.

The hypostase and the epistase which are the characteristics of this group have escaped the notice of the previous workers. The hypostase persists as a pad like tissue in the mature seed. But the epistase gets crushed during the development of the seed.

There is a solitary report on the development of the seed coat (Maheshwari & Baldev, 1958). According to them the seed coat is formed mainly from the outer integument, the inner integument is transformed into a thick cuticular layer. On the other hand, the present work reveals that both layers of the inner integument and the inner epidermis of the outer integument contribute to the formation of the seed coat.

SUMMARY

Only aerial flowers are produced in all the plants with the exception of *Tinantia fugax*. Cleistogamous flowers are present in *Cyanotis axillaris*.

The anther wall comprises the epidermis, fibrous endothecium, a middle layer and an amoeboid tapetum. Only decussate and isobilateral tetrads are formed.

The pollen is shed at the two-celled stage in all the plants except in *Floscopa scandens* where it is three-celled.

The bitegmic ovules are crassinucellate excepting in those of *Aneilema paniculatum* and *Cyanotis axillaris*. The ovule is orthotropous in *Floscopa scandens*, orthotropous with a slight bend in *Aneilema paniculatum* and hemianatropous in others.

A hypodermal archesporial cell develops into a megaspore mother cell after cutting off the primary parietal cell in *Floscopa scandens*, *Commelina subulata* and *Tinantia fugax*. In others it develops directly. The development of the embryo sac is of the Polygonum type. The antipodals are ephemeral except in *Tinantia fugax*.

The endosperm is of the Nuclear type. A persistent chalazal haustorium is formed in *Cyanotis axillaris*.

The embryogeny follows the Asterad type and can be placed under the Commelina variation.

A hypostase, an epistase and a micropylar collar are present. The seed coat is thick and is contributed by both the integuments.

The author is indebted to Professor P. Maheshwari under whose guidance this work was taken up and to Dr C. S. Venkatesh for his interest and valuable suggestions during the course of this investigation. Thanks are also due to Dr N. S. Ranga Swamy for help rendered during the preparation of the manuscript.

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* Not seen in original

A Contribution to the Life-History of *Crinum defixum* Ker.

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About a century ago, Hofmeister (1861) investigated *Crinum variabile* and *C. capense*. He mentioned the presence of single integument for the ovules and found that the pollen tube branched in the micropyle of the latter species. Goebel (1889) described the ovules of *C. asiaticum* as devoid of integuments and found cork formation at the periphery of the endosperm. Schlimbach (1924) reported an Adoxa-type of embryo sac and nuclear endosperm in *C. asiaticum*.

Stenar (1925) investigated *C. latifolium*, *C. longifolium*, *C. powelli* and *C. amabile* and agreed with Goebel in considering the ovules as devoid of integuments. According to him the archesporial cell directly becomes the megaspore mother cell without cutting off a parietal cell and the embryo sac is probably of the Scilla (= Allium) type in *C. latifolium* and *C. longifolium*. Tomita (1931) considered the ovules of *C. latifolium* as ategumentary and tenuinucellate and confirmed the occurrence of an Allium type of embryo sac. He also described the formation of a nuclear endosperm. According to him, there are two types of embryos: (i) those that are surrounded by endosperm and (ii) others not accompanied by endosperm formation. Koshimizu (1930) made carpobiological studies of *C. asiaticum* var. *japonicum* and Merry (1937) studied the periderm formation in the wounded seeds of *C. asiaticum*.

Swamy (1946) studied the structure of the mature embryo sac of the *C. asiaticum* and reported the occurrence of " inverted polarity " in some of the embryo sacs, " the remaining aspects of its life-history being reserved for a later occasion. " Johansen (1950) considers the embryo of *C. capense* to correspond with the Anthericum Variation. Referring to *C. latifolium* he states : " it appears that the terminal cell of the two-celled proembryo divides first and transversely, and the basal cell then divides in the same plane. Apparently the daughters of the basal cell do not divide further but remain as a transitory two-celled suspensor." Writing on ategumentary ovules, Maheshwari (1950) said : " a complete absence of the integument is known only in some members of the Balanophoraceae and Loranthaceae but it seems

probable that it is a derived condition..... The case of *Crinum* (Amaryllidaceae), in which the nucellus is ephemeral and integuments are said to be absent (Tomita, 1931), deserves further study". Dutt (1957 a, b, 1959) showed the ovules of *C. defixum*, *C. asiaticum* and *C. latifolium* to be unitegmic and the embryo sac development to be of the polygonum type.

Many phases in the life-history of *Crinum* still remain unknown.

There are about 130 species of *Crinum* (Willis, 1948; Lawrence, 1951) but according to Koshimizu (1930) their number is 161.

The present investigation deals with *C. defixum* Ker., an elegant bulbous plant, which produces umbels of mild scented white flowers with bright red stamens.

MATERIAL AND METHODS

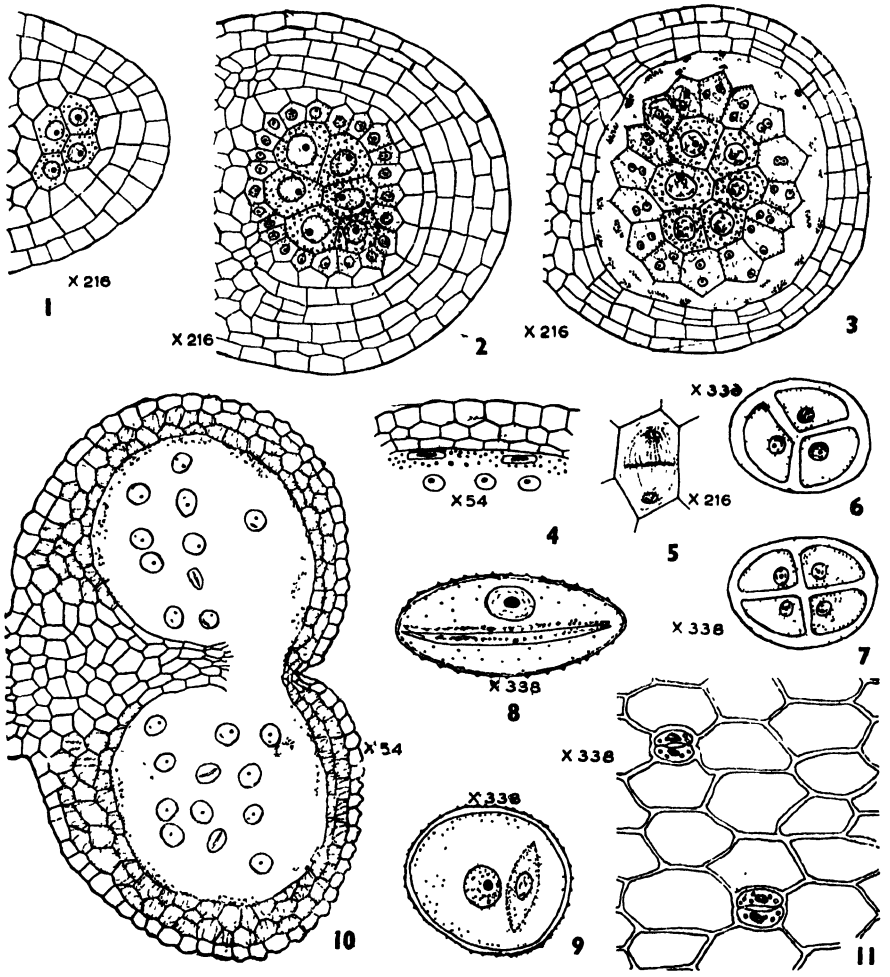
The material for the present study was collected at Gudivada (Andhra State) and fixations were made in formalin-acetic acid-alcohol. The usual methods of dehydration and infiltration were followed. Sections were cut 8 – 12μ thick for younger stages, 16 – 20μ for older stages and stained in Delafields Haematoxylin or safranin and fast green.

OBSERVATIONS

Microsporogenesis and Male Gametophyte. There are six stamens in two whorls of three each. The anthers are four lobed and the youngest stage in which differentiation could be clearly made out, showed sporogenous cells and two wall layers beneath the epidermis (Fig. 1). The sporogenous cells undergo a few divisions to form the microspore mother cells. The parietal cells divide further to form six wall layers below the epidermis (Fig. 2). The innermost of these forms the tapetum with radially elongated cells, and the outermost the fibrous endothecium. Of the four middle layers the inner two or three become crushed during the enlargement of the tapetal cells and the microspore mother cells. The tapetal cells have dense cytoplasm and prominent nuclei. They later become 2-nucleate and develop vacuoles in the cytoplasm. In some tapetal cells the nuclei were found to fuse with each other (Fig. 3). As the secretory tapetum is gradually absorbed, droplets corresponding to the granules of "Ubisch" (1927) and Kosmath (1927) were seen lining the locule of the anther (Fig. 4). The mature anther wall consists of the epidermis, the fibrous endothecium and the remaining middle layer with fibrous thickenings (Fig. 10). A point of interest is the presence of stomata on the anthers (Fig. 11). The divisions of the microspore mother cells are successive. During Meiosis I, a cell plate is laid down separating the two nuclei (Fig. 5). The tetrads are tetrahedral or isobilateral (Figs. 6, 7). The pollen grains are 2 sulculate and the exine shows characteristic spinules (Figs. 8, 9) and are shed at the 2-celled stage. Some of the pollen grains may degenerate.

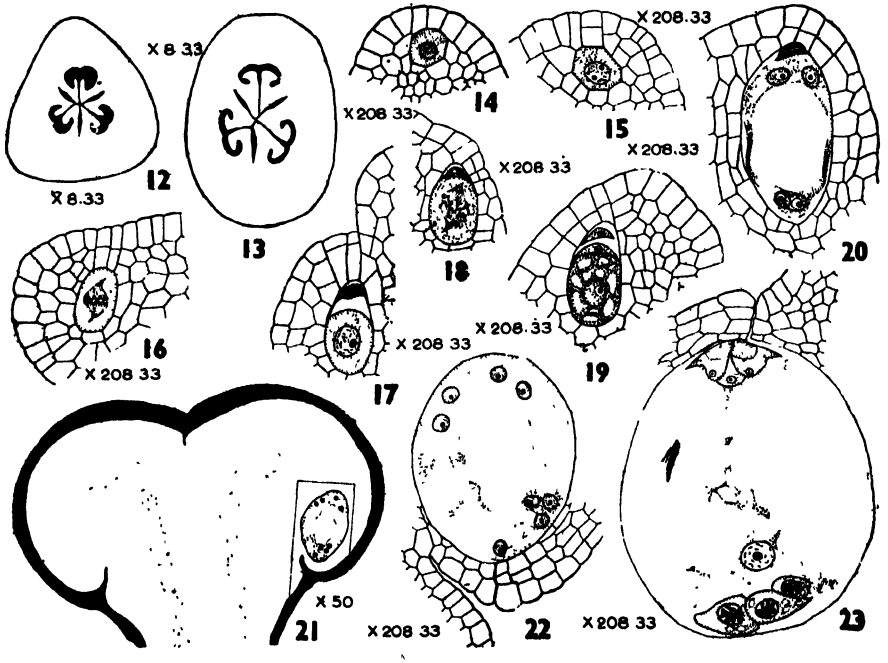
Ovary, Ovule and Embryo Sac. The ovary is inferior, tricarpellary and

trilocular with six pairs of collateral ovules borne on axile placentae, in two superposed rows. The ovule starts as a small protuberance on the inner margin of the carpellary wall (Fig. 12). By the time the archesporium is differentiated, the ovule begins to curve and assumes a hemianatropous form (Fig. 13). There is a single hypodermal archesporial cell (Fig. 14). The epidermal cells are radially elongated and the one above the archesporium divides periclinally (Fig. 15). Subsequently the division also extends to



FIGS. 1-11 — Fig. 1. T. s. anther lobe showing sporogenous cells and two wall layers beneath the epidermis. Fig. 2. Same showing microspore mother cells and 6 wall layers. Fig. 3. Same showing epidermis, endothecium, middle layers and microspore mother cells surrounded by tapetal cells; note the degenerated middle layers. Fig. 4. Portion of the anther showing droplets on the inner walls. Fig. 5. Cell plate formation after Meiosis I in a microspore mother cell. Figs. 6 & 7. Tetrahedral and decussate tetrads. Figs. 8, 9, 1- and 2-celled pollen grains. Fig. 10. T. s. mature anther lobe. Fig. 11. Anther epidermis showing stomata

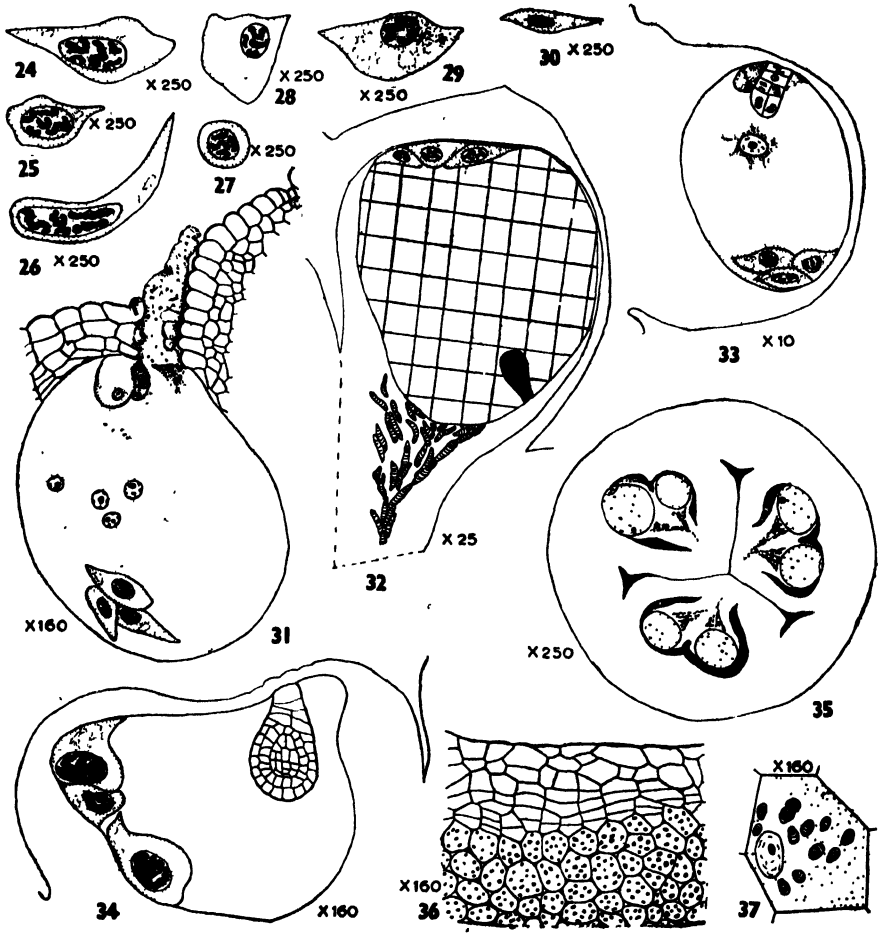
two or three adjacent cells. When only one epidermal cell which is situated directly above the archesporium divides periclinally, the inner cell may be mistaken for a parietal cell. However, it is the archesporial cell itself which enters the synizesis stage proving that it becomes the megaspore mother cell without cutting off a parietal cell. The megaspore mother cell undergoes the first meiotic division forming two dyad cells of which the lower enlarges considerably while the upper promptly disintegrates (Figs. 16, 17). The lower dyad cell divides transversely (Fig. 18) giving rise to two megaspores situated below the disintegrating dyad cell. Therefore, a row of three cells is formed, the uppermost being an undivided dyad cell (Fig. 19). The remnants of the disintegrating dyad and the upper megaspore could be recognized even at the 4-nucleate stage of the embryo sac (Fig. 20). The lower megaspore is functional and it becomes vacuolated. Three nuclear divisions result in an 8-nucleate embryo sac (Figs. 21, 22). The 4-nucleate embryo sac undergoes a pronounced enlargement during the course of which one or two layers of cells surrounding it become destroyed. By the time the 8-nucleate stage is reached, the ovule curves further and attains the ana-



FIGS. 12-23—Fig. 12. T. s. ovary showing ovular primordia. Fig. 13. Same showing ovules at the time of the differentiation of the archesporial cell. Fig. 15. Same showing periclinal division in an epidermal cell. Fig. 16. Meiosis I in the megaspore mother cell. Fig. 17. L. s. apical part of the ovule showing the degenerating upper dyad cell. Fig. 18. Lower dyad cell in Meiosis II. Fig. 19. Triad. Fig. 20. 4-Nucleate embryo sac showing disintegrating dyad cell and the upper megaspore. Fig. 21. Two collateral ovules at 8-nucleate embryo sac stage. Fig. 22. Portion marked in Fig. 21 magnified to show the details. Fig. 23. Mature embryo sac

tropous condition. The two collateral ovules of a loculus are very closely appressed to each other. Each funicle develops a knee-shaped bend at this stage (Fig. 21).

The 8 nuclei of the embryo sac organize themselves into the egg-apparatus, the three antipodal cells and two polar nuclei. The synergids are hooked, with filiform apparatus, non-vacuolate and the nucleus is situated in the basal part. The apical parts may persist for some time. The polar nuclei fuse in the vicinity of the antipodals and form the secondary nucleus (Fig. 23). The antipodal cells have large nuclei with very rich chromatin. They assume various shapes, develop beak-like projections and become



FIGS. 24-37 — Figs. 24-30. Antipodal cells assuming different shapes. Fig. 31. Embryo sac showing pollen tube and endosperm nuclei. Fig. 32. L. s. ovule in advanced stage of endosperm development (diagrammatic). Fig. 35. T. s. ovary showing the ovules with the endosperm abutting on the ovary wall. Fig. 36. Part of the endosperm showing phellogen and cork; note chloroplasts in the inner cells. Fig. 37. A cell of the endosperm showing starch grains. Figs. 33, 34. Embryo sacs having embryos but no endosperm

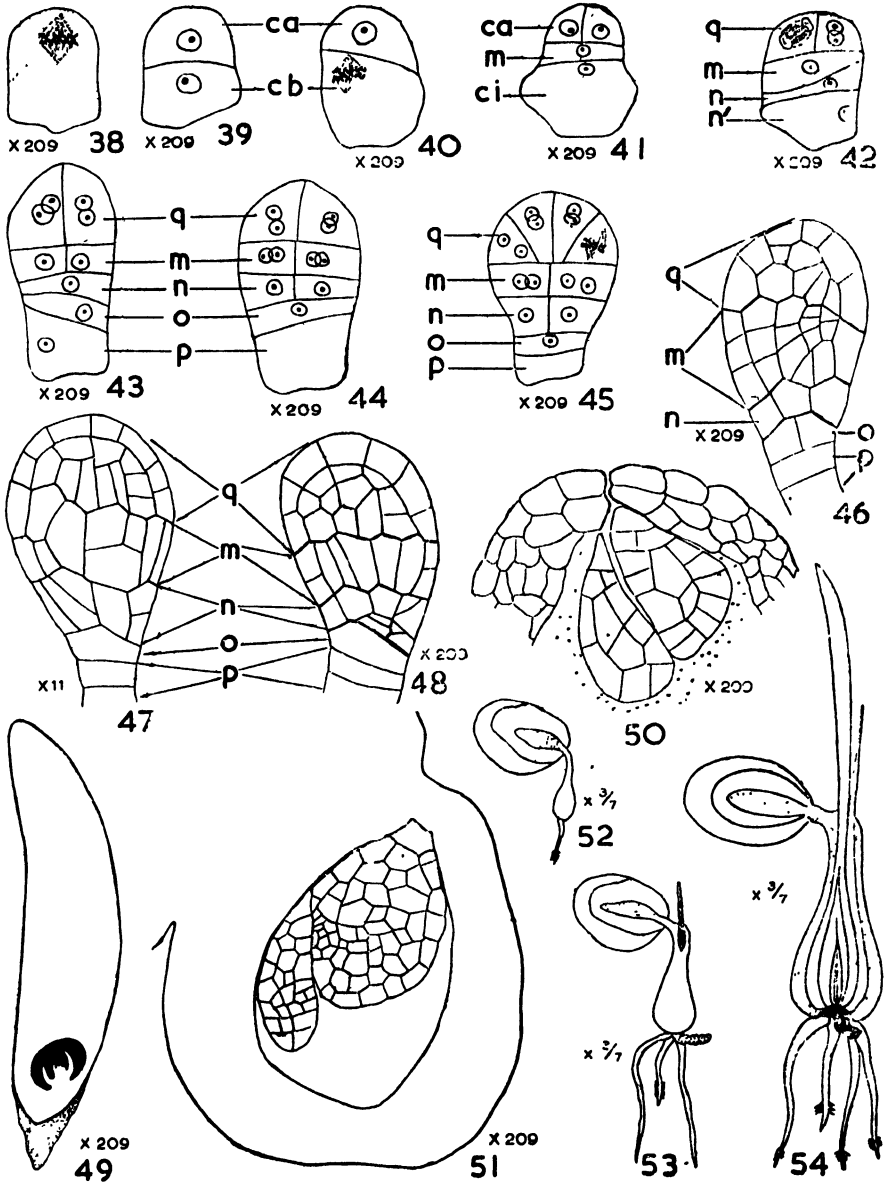
vacuolated (Figs. 24–30). Even in advanced stages of endosperm formation they persist in the hypertrophied condition (Fig. 32). The vascular bundle supplying the ovule branches extensively in the chalazal part of the ovule. The xylem elements usually reach the base of the embryo sac. Septal nectaries are present in the ovary and have the pockets with glandular lining.

Fertilization. About 60 hr. after the opening of the flower the pollen tube reaches the embryo sac and enters the ovule through the micropyle (Fig. 31). It is probable that the knee-shaped bend of the funicle functions as an obturator. In the entry of the pollen tube one of the synergids is destroyed. Triple fusion does not seem to take place in every case, since in some ovules endosperm is never formed and the embryos ultimately degenerate (Figs. 33, 34).

Endosperm. Division of the primary endosperm nucleus precedes the zygote (Fig. 31). In the earlier stages the nuclei are distributed in the peripheral cytoplasm. Later the endosperm becomes completely cellular and destroys the ovular cells around the embryo sac. The peripheral cells of the endosperm, especially those near the chalaza, stain deeply and they come to lie practically in direct contact with the tracheids at the chalazal region (Fig. 32). In more advanced stages the endosperm abuts directly on the ovary wall destroying some of its inner layers of the cells (Fig. 35). Finally the peripheral cells of the endosperm even behave like a phellogen by undergoing tangential divisions and producing a few layers of cork tissue surrounded by the thin pericarp. By this time all the cells of the ovule have been consumed. The 3- or 4-layered cork cells are lignosuberized (Fig. 36). If a portion of the endosperm is cut away, the exposed cells behave like a phellogen and produce fresh cork tissue. Another feature of special interest is that chloroplasts arise in abundance in the peripheral cells lying below the phellogen even when the endosperm is covered by the thin pericarp. Starch grains accumulate in the cells of the endosperm (Fig. 37). In rare instances, chloroplasts extend even to the inner layers of the endosperm.

Embryo. The fertilized egg enlarges considerably and divides by a transverse wall (Figs. 38, 39). The basal cell, *cb*, divides transversely (Fig. 40) forming the middle cell, *m*, and the inner cell *ci* (Fig. 41) while the terminal cell, *ca*, divides vertically. The two cells of *ca* again divide vertically at right angles to the first division resulting in a quadrant *q* (Fig. 42). Transverse division of *ci* results in two superposed cells, *n* and *n'*. The middle cell, *m*, later divides longitudinally to form two juxtaposed cells, and *n'* divides transversely producing two superposed cells *o* and *p*. The cells of tier *q* divide longitudinally and an octant is produced. The derivatives of *m* again divide vertically at right angles to the first producing four circumaxially arranged cells. The cell *n* divides by longitudinal wall (Figs. 43-45). At this stage the proembryo is 16-celled, disposed in 5 tiers. Dermatogen becomes differentiated first in the tier *q*. The derivatives of cells of tier *q* give rise to the single cotyledon, those of *m* to the hypocotyl and stem tip, while the derivatives of *n* give rise to the initials of the root. The root-cap is contributed by the derivatives of *o* and the suspensor is formed by the derivatives of *p*

(Figs. 46–48). The mature embryo is monocotyledonous with lateral plumule and terminal cotyledon (Fig. 49). From the above it is clear that the embryo proper is formed from the derivatives of both the terminal and



FIGS. 38-54 — Figs. 38-48. Stages in the development of the embryo. Fig. 49. L. s. mature embryo. Figs. 50, 51. Polyembryony. Fig. 52. Germination of seed showing the developing bulb; note root hairs on the radicle and the enlarged part of the cotyledonary apex. Fig. 53. The first plumular leaf piercing through the cotyledonary sheath; note the disintegrating primary root. Fig. 54. The bases of plumular leaves enlarging within the cotyledonary sheath (Figs. 52-54 slightly diagrammatic)

basal cells (*ca* and *cb*) of the two celled proembryo. Therefore, the development conforms to Asterad Type (Johansen, 1950) and since the mature embryo is monocotyledonous and *n* contributes to root initials, it keys out to the Muscari variation. The developing embryos are either spherical or pear-shaped. It may be pointed out that the embryo becomes deep seated before the differentiation of the cotyledon, radicle and plumule and comes to lie in the endosperm cells by the growth of the endosperm beyond the embryo. Starch grains are present in the cotyledon and amylase could be detected in the cell sap as indicated by the hydrolysis of starch solution at 38° C.

Polyembryony. Two ovules with twin embryos have been met with. In one the second embryo is presumably developed from the synergid (Fig. 50). In the second the exact origin of the smaller embryo was difficult to decide (Fig. 51). These ovules did not contain any endosperm.

Seed. It has been mentioned earlier that all the cells of the ovule get csushed and absorbed by the developing endosperm. The 'seed' therefore comprises the endosperm and the embryo. It is interesting to note that about 65 per cent of the seeds have a specific gravity of about 0·95 while the rest of the seeds sink in tap water (specific gravity, 1·001 at 27° C.).

Germination of the seed and bulb formation. The seeds begin to germinate in about 15 to 20 days when put in moistened saw-dust. Those that are left in tap water germinated after 4 months while those that are cooled to a temperature of 8° C. for 8 days germinated in about 20 to 25 days. In the early stage of germination the radicle grows out of the endosperm. The single cotyledon enlarges considerably and its apical part takes up a haustorial function within the endosperm. The lower part of the cotyledon forms a sheath around the plumule when it emerges out of the seed (Fig. 52). During the early stages of germination the lower part of the cotyledonary sheath becomes bulbous and forms the outermost bulb scale. As the process of germination continues, the radicle develops root hairs, becomes thicker and grows in length, while the first plumular leaf in its rolled condition pierces through the sheath of the cotyledon (Fig. 53). During later stages, the starch grains previously accumulated in the seed disappear and the basal parts of the plumular leaves also become thickened within the enlarging cotyledonary sheath in which accumulate starch grains and raphides abundantly. The bases of the various leaves are arranged in concentric manner so that the bulb is of the tunicated type. The earliest root shows a contracted nature and begins to disintegrate while adventitious roots arise from the abbreviated axis (Fig. 54). The germination of the seed is hypogeal.

DISCUSSION

During the development of the anther, the secretory tapetum becomes gradually used up and 'Ubisch' granule-like droplets appear on the inner walls of the anther. Such bodies have not been reported by Stenar (1925), who studied the anther structure of *C. latifolium*. The divisions of the

microspore mother cells are successive and the tapetal cells are binucleate as in *C. latifolium* (Stenar, 1925).

Stenar (1925) found two carpels in *C. latifolium* and considered it to be an exceptional feature in the Amaryllidaceae. Tomita (1931) has, however, figured the usual three carpels and three locules. The ovules of *C. capense* and *C. amabile* have been described as unitegmic (Hofmeister, 1861). Stenar (1925) and Tomita (1931) considered the ovule of *C. latifolium* as tenuinucellate, while Wunderlich (1959) treated it as crassinucellate. In the present investigation the ovule is found to be an undifferentiated structure with a narrow micropyle. It seems best to describe the ovule of *C. defixum* as anatropous as its curvature during development is typical of an anatropous ovule. Two to three nucellar epidermal cells above the archesporium divide periclinally and produce a layer of variable thickness. The cells lying in this position are characterized by periclinal divisions during development of the ovule and seem to correspond to nucellar epidermal cells of plants like *Rubic.* though in *C. defixum* these cells are distinguished from the integument tissue which constitutes the bulk of the ovule only by the periclinal divisions. Therefore, the ovule is tenuinucellate. The pollen tube creeps along the cells of the knee-shaped bend of the funicle and enters the embryo sac through the narrow micropyle in the region of the egg apparatus. According to Fagerlind (1937) undifferentiated ovules can arise in two ways : (i) The cells of the epidermis divide periclinally; and (ii) No such division takes place. While reviewing the observations made by the earlier investigators, he assigned the ovule of *Crinum* to the latter type. Since periclinal divisions do occur in cells which correspond to the nucellus, Fagerlind's assignment of *Crinum* to the latter category becomes untenable. The description of the ovule by Goebel (1889), Schlimbach (1924), Stenar (1925), and Tomita (1931) as ategumentary and naked by Koshimizu (1930) and Rendle (1950) is not appropriate. Merry (1937) mentioned the " usual seed coats " in *C. asiaticum* but did not clarify as to what is meant by him by ' usual '.

Swamy (1946) described "a non-stainable hyaline apical region" for the synergids of *C. asiaticum*. In *C. latifolium* (Stenar, 1925) and *C. defixum* studied by the author the synergids show a filiform apparatus. A re-examination of *C. asiaticum* (Dutt, 1957b) has also revealed the presence of a filiform apparatus in the synergids. Swamy (1946) stated that the unorganized 8-nucleate embryo sac of *Crinum asiaticum* may organize in three different methods and that the antipodal polar nucleus shows no movement upwards, remaining stationary; "the micropylar nucleus moves downwards to the antipodal polar nucleus and fusion takes place near the antipodals (Fig. 1)". The figure, given by him, however, shows only secondary nucleus but no polar nuclei. Further his Figs. 2 and 2a indicate movements of both the polar nuclei. According to Swamy (1946) " both groups of 4 nuclei develop an egg apparatus and there is thus one at each end. " It follows that the two quartets form a polar nucleus and an ' egg apparatus ' at each end. According to him : " The synergids of the ' antipodal ' end showed great similarity to typical synergids in their shape, vacuolation and

position of the nucleus (Figs. 3, 3a and 5); they even developed beak-like projections (Figs. 3a and 4). However, the only difference between the synergids of the fertilizable egg apparatus (developed at the micropylar end) and those of the egg apparatus organized from the antipodal group of 4 nuclei is that the apical non-stainable, hyaline region of the former is absent in the latter." In addition to the 'only difference' (mentioned by him), the so-called 'synergids' at the antipodal end differ from those of the opposite end in that the nucleus is situated in the upper part in the former and in the lower part in the latter. Further, his Fig. 1a relating to *C. asiaticum* shows no vacuoles for the synergids. Incidentally, nonvacuolated synergids are also found in *C. latifolium* (Stenar, 1925; Tomita, 1931; Dutt, 1959) and *C. defixum* studied by the author. The "antipodal synergids" do not, therefore, resemble the normal nonvacuolated synergids with a basally placed nucleus but are antipodals themselves. In the 'inverted' embryo sac representing the 'egg apparatus' at the 'Chalazal region' (Fig. 2a of Swamy), there is nothing to support the conclusion since the 'synergids' are without hooks and are devoid of even the "hyaline apical part". On the other hand, Swamy's interpretation of the three cells as the 'egg-apparatus' goes against his earlier observation, "that the secondary embryo sac nucleus is always nearer to the antipodals and not the egg apparatus" as the polar nuclei are represented to be fusing with each other nearer to 'egg apparatus'.

While describing the endosperm of *Haemanthus katherinae*, Stenar (1951) remarked that the Helobial endosperm might also occur in *Crinum*. The endosperm formation of *C. asiaticum* (Schlimbach, 1924), *C. latifolium* (Tomita, 1931) and *C. defixum* is of the free nuclear type and as such Stenar's surmise is not substantiated by actual observation.

According to Johansen (1950) the embryo development of *C. capense* follows the "Anthercium variation of the Asterad type" and from his description of *C. latifolium* it appears to follow Solanad type. In *C. defixum* it follows the Asterad type. In view of the variation seen in the embryo development, further work on the embryogeny of other species of *Crinum* is desirable. Tomita (1931) stated that the embryo unaccompanied by the endosperm formation differentiates into radicle, plumule and cotyledon but did not give any illustration in support of his findings. It may be pointed out that in *C. defixum* the ovules with embryos and no endosperm fail to develop and become shrivel up, sooner or later. Koshimizu (1930) states that the specific gravity of the seed of *C. asiaticum* var. *Japonicum* is always less than one but this is not true of about 35 per cent of the seeds of *C. asiaticum* and *C. defixum*. He states "starch grains are not detected at all in the endosperm even at the dormant stage of the seed", a feature already reported by Schlimbach (1924). He also remarks "it is very interesting that starch is not reserved in the endosperm but only in the embryo". The author's observations on the endosperm of *C. asiaticum* and *C. defixum* reveal the presence of starch grains in the endosperm. Therefore, the findings of Schlimbach (1924) and Koshimizu (1930) seem to be incorrect. It may be pointed out that starch grains disappear during the germination of the seeds presumably

due to the hydrolysis brought about by amylase present in the embryo. The rootlet of *C. defixum* has a distinct root-hair region and as such Koshimizu's observation that " the root of *Crinum* has no root-hair " needs modification. The presence of starch in the endosperm characterizes Juncineae of Liliflorae and in this respect *Crinum* resembles the members of the Juncineae and differs from Lilineae in having starch in the endosperm.

SUMMARY

The anther shows six wall layers under the epidermis of which the innermost forms the secretory tapetum. The divisions of the microspore mother cells are successive. Pollen tetrads are isobilateral or tetrahedral. The pollen grain is 2-sulculate and two-celled.

Usually there are twelve anatropous ovules. A micropyle is organized. Two or three nucellar epidermal cells above the archesporium divide periclinally. The ovule is unitegmic.

The ovule shows a single hypodermal archesporial cell. A parietal cell is not cut off. The embryo sac conforms to the Polygonum type.

The pollen tube enters the ovule through the narrow micropyle. Fertilization takes place normally.

The endosperm is of the Nuclear type. In later stages cell formation takes place. The endosperm becomes green and at the peripheral region a phellogen is formed which gives rise to cork.

The embryo development conforms to the Muscari variation in the Asterad type of Johansen. Two cases of polyembryony are described.

The germination of the seed is hypogeal. During the course of germination the cotyledon enlarges considerably and its apical part plays a haustorial role.

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Embryological Studies in Relation to Interspecific Hybridization in Jute

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Plant Embryology is no longer an isolated discipline of biology. This science after having passed through the two developmental phases, namely, the descriptive and the phylogenetic, has now entered the threshold of the experimental phase. This last phase represents the confluence of embryology with a galaxy of other sister sciences such as physiology, biochemistry, cytogenetics and plant breeding.

The importance of interspecific hybridization for crop improvement needs no emphasis, but this method often presents problems to the plant breeder, owing to the existence of barriers to crossability, which may appear at any stage between pollination and seed maturity. To overcome this obstacle, it is essential to locate the site of the incompatibility reaction which a critical study of the embryological process in the cross alone can reveal. There is evidence to show that in members of the Gramineae (Wakakuwa, 1934; Boyes & Thompson, 1937; Reusch, 1959), Leguminosae (Ledingham, 1940; Greenshields, 1954), Malvaceae (Weaver, 1957) and Solanaceae (Brink & Cooper, 1947; Sachet, 1948) the embryological data have proved immensely useful in understanding problems of incompatibility. The present work on jute is an additional proof of the positive value of such studies in interspecific hybridization.

Each of the two cultivated jute species, *Corchorus olitorius* L. and *C. capsularis* L. possess several desirable qualities (Kundu, 1959) and jute breeders have long been looking for a variety that yields a strong white fibre, is early-maturing, has a tall and unbranched stem, is able to grow on all types of soils and shows resistance to diseases and pests. Attempts have hence been made to hybridize *C. olitorius* and *C. capsularis* (Finlow, 1911 cited from Kundu, 1959) but only recently it has been possible to achieve this objective (Islam & Rashid, 1960; Swaminathan *et al.*, 1961). In the present paper, the results of embryological studies made in connection with the successful hybridization of the two jute species achieved at the Indian Agricultural Research Institute (IARI) are summarized.

MATERIAL AND METHODS

Some varieties of both the species of *Corchorus* obtained from the Jute Agricultural Research Institute (JARI), Barrackpore, were used in the present study. Two sowings were carried out during May, at a fortnightly interval. Material for grafting was sown in pans and later transplanted to pots. Actively growing young plants 1-1·5 ft. high, were chosen for reciprocal cleft-grafting and in every case, all foliage from the stock and most of it from the scion was removed in order to promote active meristematic growth at the region of the union.

Buds for crossing were emasculated on the previous evening and covered by means of a hood of non-absorbent cotton which was replaced after dusting pollen of selected parent on the following morning. Over 2000 reciprocal crosses were made during 1959-60, using ordinary, X-rayed as well as pollen from grafted parents. Abscission of flowers following cross-pollination was effectively prevented by the application of a 10 ppm solution of ∝-NAA to the pedicel with a brush. Smearing of ovary with 10 ppm each of IAA, IBA, β-NOA, and of 5 ppm casein hydrolysate in 2·5 per cent agar, was also tried in order to improve seed-setting in the crosses.

Pollen irradiation was carried out by exposing freshly dehisced anthers to doses varying between 600r to 2500r of X-rays from a 50 KvP Philips unit, at a distance of 15 cm. from the source.

Fixations for embryological studies were made in formalin-acetic acid-alcohol at the following intervals after self- and cross-pollinations: 12 hr, 24 hr, 2, 3, 4, 5, 6, 7, 9, 11, 13, 16, 21, 25 and 30 days. Material for microtomy was processed in the customary way and sectioned at 8 – 16μ. Heidenhain's iron-haematoxylin staining procedure was adopted, and wherever possible dissected whole mounts stained in acetocarmine were also prepared.

EXPERIMENTAL RESULTS

A schematic comparison of the embryological sequences in selfed *C. olitorius* and the cross between *C. olitorius* (♀) and *C. capsularis* (♂) is presented in Fig. 1. The developmental stages in selfed *C. capsularis* (not shown in figure) are essentially similar to those of *C. olitorius* with the only difference that, in the former, the embryo attains maturity quicker by about a week or ten days and is somewhat bigger in size. The data reveal that fertilization and early embryogeny follow a similar course both in selfed and hybrid material. The hybrid proembryo, however, does not develop beyond the 8- to 10-celled stage. Vacuolation occurs in the cells of embryo on the eleventh day following cross-pollination, while the nuclear endosperm shows shrinkage and signs of degeneration on the fifteenth day. Eventually we get from these crosses only shrivelled seeds with aborted embryo and dead remains of the endosperm. A close examination of the nucellus and surrounding tissues in the hybrid ovules showed no evidence of hyperplasia or other abnormality.

The reciprocal cross between *C. capsularis* (♀) and *C. olitorius* (♂) resulted only in the abscission or drying up of flowers. A study of the ovules from this cross shows that the pollen tube reaches the micropylar end in many cases, but fertilization does not take place. This premature abscission could be prevented if the *C. capsularis* parent was grafted on *C. olitorius* rootstock. As a result, there was an increase in the percentage of fruit-set, accompanied by a marked development of the hybrid embryo and endosperm. The globular proembryos obtained in these crosses were much larger than those in the straight crosses, but they failed to undergo differentiation and finally aborted. The endosperm too remained healthy for three weeks but was free-nuclear until its degeneration.

On the other hand, in crosses where *C. olitorius* (♀) is pollinated by *C. capsularis* grafted on the former, occasionally, late stages of heart-shaped embryos with cellular endosperm have been observed. From crosses made

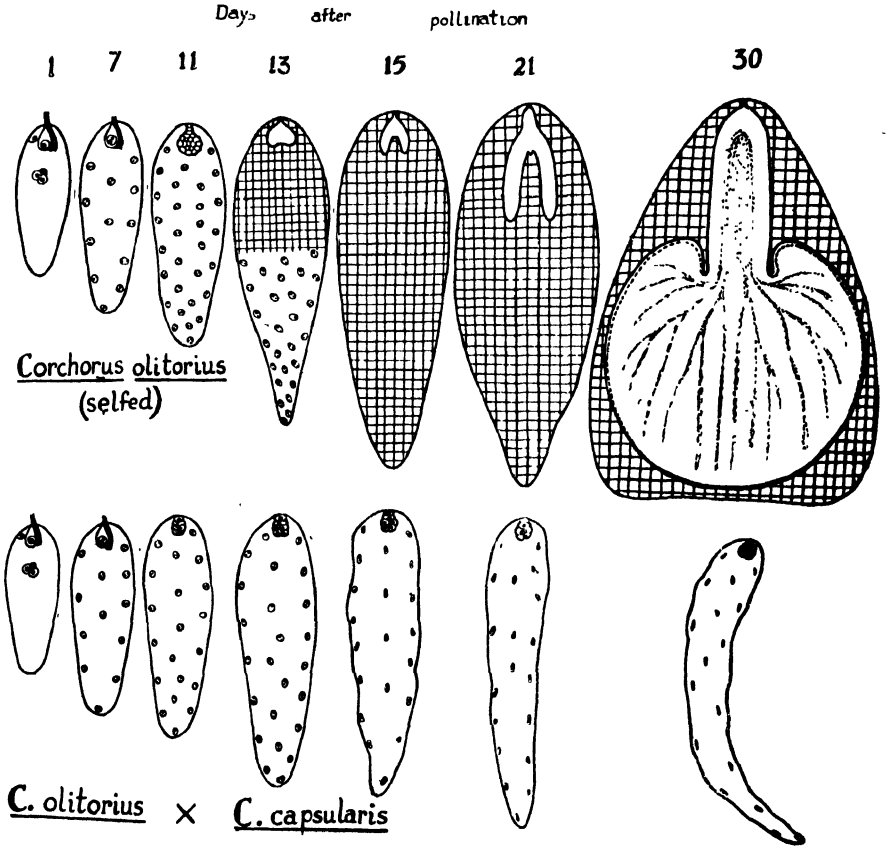

FIG. 1 — A SEMI-DIAGRAMMATIC COMPARISON OF THE EMBRYOLOGICAL SEQUENCES FOLLOWING SELF-POLLINATION IN *Corchorus olitorius* (UPPER SERIES) AND AFTER CROSSING IT WITH *C. capsularis* (LOWER SERIES)

in 1958 in this combination a single F_1 hybrid was isolated in 1959 and its F_2 progeny shows an interesting spectrum of segregation for characters of the two parents (Swaminathan & Iyer, 1961).

The use of *C. capsularis* pollen after irradiation with 600r to 1200r of X-rays did not prove effective. At higher dosages (2000r and 2500r), however, there was a marked improvement in the growth of hybrid embryo and endosperm. One more hybrid has been obtained from crosses made in 1959 between a grafted *C. olitorius* (♀) and *C. capsularis* whose pollen was irradiated with 2000r of X-rays.

As regards the other techniques tried to effect the cross, the smearing of cross-pollinated ovaries of *C. olitorius* with 10 ppm of IAA, IBA, NOA, and 5 ppm casein hydrolysate, resulted only in the swelling of the ovary wall without any effect on embryo or endosperm development.

DISCUSSION

One great handicap for all the previous workers interested in hybridizing the two jute species, was the lack of pertinent information on the nature of seed failure. Except for the work of Banerji (1932) on the development of the female gametophyte and fertilization in *C. olitorius*, no data were available on the embryological processes following self- and cross-pollinations between *C. olitorius* and *C. capsularis*. Srinath & Kundu (1952) observed the course of the pollen tube in these crosses and found it to be normal in either direction. Attempts have also been made at the JARI, Barrackpore, to achieve this cross using techniques like stigma cutting, style grafting, application of stigmatic paste, hormones and other chemicals, but without success (*see* Kundu, 1959).

Nothing further was known until the work of Ganesan *et al.* (1957) who showed for the first time that in the cross between *C. olitorius* (♀) and *C. capsularis* (♂) a hybrid embryo and nuclear endosperm are formed after normal process of fertilization, but that the embryo aborted at an early globular stage. They suggested that somatoplastic sterility is probably involved and embryo culture of the occasional heart-shaped stages noted by them, might prove useful in overcoming the incompatibility barrier. Following up this information, Islam & Rashid (1960) have been able to obtain hybrids from this cross by applying 300 ppm IAA to the pedicel of flowers after cross-pollination. Simultaneously, a similar hybrid has been obtained at IARI by Swaminathan *et al.* (1961) using reciprocally grafted parents in the cross. The isolation of one more F_1 hybrid from crosses made with irradiated pollen and the cytogenetic analysis of the F_2 progeny (Swaminathan & Iyer, 1961) enables us to postulate the probable nature of the incompatibility barrier that serves to isolate the two species of *Corchorus*.

From a histological study of maternal tissues of hybrid ovules, the possibility of somatoplastic sterility as the cause for seed failure in this cross is ruled out. The next hypothesis of the operation of complementary genetic lethals at the zygotic as well as endosperm levels, suggested by Sulbha &

Swaminathan (1959), is hard to reconcile with the fact that the mere application of IAA (300 ppm) or the use of reciprocally grafted parents in the cross could inactivate the incompatibility reaction. Studies on chromosome association during meiosis in microsporocytes of the hybrids and the pattern of recombination in F_2 plants indicate that cryptic structural differences between the chromosome complements may be involved in the divergence of the two species of jute.

In spite of these positive evidences, it is surprising to note that Patel & Datta (1960) do not find even the occurrence of a globular embryo in the cross, and believe that the two species are not crossable because they are phylogenetically wide apart. However, they have suggested the use of embryo-transplantation and embryo-culture techniques as the future possibilities for achieving success with this cross. Attempts so far made in this laboratory (Iyer *et al.*, 1959) indicate that both the above possibilities hold remote chances of success. It will be clear from the above survey, that only a systematic study of the embryological sequences in the parents and hybrid combinations can give us an insight into the nature and cause of seed failure in distant crosses.

SUMMARY

From a study of the embryology of the parents and reciprocal matings of the two cultivated species of jute, it has been shown that the premature abortion of the hybrid embryo and endosperm leading to seed failure in this cross, is not due to somatoplastic sterility. Of the various techniques tried to overcome the incompatibility barrier, such as hormone application, pollen irradiation and grafting of parents prior to crossing, the last two methods proved very effective, and viable hybrids have been obtained in this way. The efficacy of these techniques and the probable nature of incompatibility between these two species, have been discussed. It is concluded that embryological studies in interspecific crosses yield a wealth of information which the plant breeder can make use of in devising suitable techniques to overcome the barriers to crossability in plants.

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Since writing this paper, more detailed studies have been made on fertilization and seed development in this cross, and the results appear elsewhere (*see* Iyer, Sulbha & Swaminathan, 1961 cited above).

Apomixis in Some Species of *Pennisetum* and in *Panicum antidotale*

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According to Englebert (1941), Müntzing who postulated agamospermy in *Poa* in 1933 is usually credited with the discovery of apomixis in *Gramineae*. However, the possibility of apomictic seed formation in *Poa* had been suggested earlier by Zollikofer in 1930 (cited by Myers 1947) and also by Stenar (1932) in his paper on Parthenogenesis in *Calamagrostis*. Müntzing's (1933) careful work establishing this phenomenon in *Poa*, greatly stimulated further researches in this field resulting not only in an exhaustive work on this genus, but also in showing that apomixis exists in about 72 species of grasses.

The basis on which apomixis was postulated in *Poa* was the presence of unbalanced or aneuploid chromosome numbers in plants producing maternal progeny with the same chromosome number as the parent.

The present findings concern with the occurrence of apomixis in *Pennisetum* and in *Panicum antidotale*.

OBSERVATIONS

Megasporogenesis and embryo sac formation in *Pennisetum villosum* (2n = 45), *P. setaceum* (2n=27), *P. orientale* (2n=27), *P. pedicellatum* (2n=36 and 54 two races) show practically the same features as in other apomicts, but with some characteristics peculiar to this group. The archesporium consists of a single hypodermal cell which functions directly as the megaspore mother cell. It undergoes the usual reduction divisions, resulting in a linear tetrad of megaspores. In a small percentage of ovules the three micropylar megaspores degenerate, and the chalazal develops into a mature embryo sac while in a large number of ovules either the megaspore mother cell or its meiotic products undergo degeneration, either autonomously or by the aggressive growth of the aposporic initials in the nucellus. Ovules containing as many as nine such aposporous initials have been commonly observed. The differentiation of nucellar cells into aposporous embryo sacs

follows a set pattern. They may arise either in the vicinity of the megaspore mother cell or of its products, or in the chalazal region, integuments, funiculus and, in extreme cases, even from the pericarpic regions. The growth of these cells is marked by considerable elongation and vacuolation. The nuclei in the enlarging cells usually lie towards one of the poles but sometimes they may occupy a central position. The nucleus normally undergoes two successive divisions and the resulting four nuclei lie at the same pole. These nuclei organize themselves into an egg apparatus consisting of two synergid cells, one egg cell and a single polar nucleus, lying either close to the egg cell or in the centre of the embryo sac. The synergids are ephemeral and hence the mature embryo sac shows only an egg cell and a polar nucleus. In a majority of apomicts such four-nucleate embryo sacs are commonly found. In some species, however, the third division also occurs and the embryo sac becomes eight-nucleate.

The simultaneous origin of a large number of aposporous cells perhaps contributes to such irregularities as inverted polarity, and rarely, lack of organization, leading to the grouping of nuclei in any part of the embryo sac. The present material appears to be an extreme one, not only on account of a large number of such embryo sacs, but also due to their disposition in the nucellus and in their origin. Frequently, ovules full of embryo sacs, thus rendering them almost hollow, were met with.

The next group of plants comprises three diploids, namely, *P. ramosum* (2n=10), *P. hohenackeri* (2n=18) and *Panicum antidotale* (2n=18 and 36). There are two varieties of *P. ramosum*, one exhibiting complete male sterility and hence poor seed setting, and the other, being fertile, showing good seed setting. Both these varieties show normal megasporogenesis and the embryo sac development conforms to the Polygonum type. There are certain interesting features which deserve special mention. The fertile variety occasionally exhibited apospory. In one ovule a conspicuous nucellar cell resembling an aposporous initial was observed adjacent to the degenerated tetrad. In another ovule, two embryo sacs were observed one below the other. Each of these showed an egg apparatus and two polar nuclei, but there were no antipodal cells. A significant difference in the size of the nuclei in these two embryo sacs suggests that the upper embryo sac with larger nuclei is aposporous, while the lower with the smaller nuclei is the product of a megaspore. In the sterile variety of *P. ramosum* no such aposporous embryo sacs or aposporous initials were observed. A careful examination of the spikelets of this variety, however, showed that there was an occasional formation of seeds. The progeny raised from these seeds showed a remarkable resemblance to the mother plants in that they were also male sterile, thus indicating that the sterile variety occasionally formed seeds, presumably by apomixis.

A very striking feature of both the varieties of *P. ramosum* is the presence of conspicuous nucellar epidermal cells at the micropylar region. The number of these cells varies from two to nine. At the time when the chalazal megaspore is enlarging, these cells become clearly recognizable by their

conspicuous size. They become prominently vacuolated and the single nucleus undergoes one or even two divisions, leading to a two- or four-nucleate condition. Such conspicuous nucellar epidermal cells are also reported in other grasses like *P. latifolium* (Narayan, 1955b), but they appear to be unique in the present case as they become multinucleate.

P. hohenackeri (2n=18) (Saraswathi, unpublished), resembles *P. ramosum* in essential features. Megasporogenesis and embryo sac development conform to the Polygonum type and a normal eight-nucleate embryo sac is usually formed. However, two instances of occasional formation of two-embryo sacs in the same ovule were also observed. In another ovule, an aposporous cell was seen encroaching upon what looked like a haploid embryo sac. The above instances were the only three out of 600 ovules which presumably showed aposporous embryo sacs.

Panicum antidotale Retz. (Shamakumari, 1960) comprises two races, a diploid with 2n=18 and a tetraploid with 2n = 36. Microsporogenesis in the diploid form is fairly normal except for certain irregularities such as laggards and random distribution of chromosomes over the spindle. The pollen fertility is 70 per cent.

Microsporogenesis in the tetraploid race was marked by more frequent occurrence of aberrations such as irregular distribution of chromosomes, formation of micronuclei, eventually leading to the variations in size and shape of the pollen grains. Eighteen bivalents are usually observed at diakinesis, but the irregularities set in at later stages of development and pollen fertility is only 25 per cent.

Megasporogenesis and embryo sac development showed that both the races are apomictic, although the incidence of apomixis differs. In the diploid race, out of 700 ovules only three were suggestive of an aposporous development of the embryo sac while in the remaining the haploid embryo sac conforms to the Polygonum type. In a few ovules there was a degeneration of the megaspore mother cell or its meiotic products.

In the tetraploid race, on the other hand, there was a predominant development of the aposporous embryo sacs. In some ovules the megaspore mother cell or its meiotic products had degenerated. A large number of ovules showed the presence of many aposporous initials developing in the vicinity of the functional megaspore. Thus there appears to be the development of both aposporous and haploid embryo sacs. A striking feature of this material is the presence of what appears to be diplosporous embryo sacs. The position, size and shape of these cells which sometimes had reached the four-nucleate condition, and the fact that there are no degenerating masses representing either the megaspore mother cell or its meiotic products, together with the position and shape of the nuclei in these embryo sacs, suggest a diplosporous origin. Thus, in this material all the three types of embryo sac, namely aposporous, diplosporous and the haploid ones, are seen.

In the polyploid apomicts viz. *P. setaceum* (2n=27), *P. villosum* (2n=45), *P. orientale* (2n=27), the mature unreduced embryo sacs are usually four-nucleate, and the single polar nucleus contributes to the forma-

tion of endosperm whenever it is formed. Embryo sacs at later stages of development reveal well developed endosperm, while the zygote is still undivided, or a well developed multicellular embryo with the endosperm nucleus still undivided, thus suggesting that the endosperm and embryo develop independently of each other. However, well developed embryos without there being any trace of endosperm are also more commonly met with. In the same ovule embryo sacs with both embryos and endosperm, and others with only embryos and no endosperm are sometimes seen. The occurrence of two embryos facing opposite directions suggests that they probably arose from the disturbed polarity of one of the embryo sacs. More than one well developed embryo in different embryo sacs of the same ovule without any trace of endosperm was also observed.

DISCUSSION

Outside the Onagraceae the four-nucleate embryo sacs have been reported only in apomictic forms. Their presence was first recorded by Narayan (1951) in apomicts like *P. villosum*, *P. setaceum* and *P. orientale*. Recent work has shown that such functional four-nucleate embryo sacs are present in most of the apomicts of Panicoideae (Brown & Emery, 1958). The occurrence of four-nucleate embryo sacs in apomictic species prompted Warmke (1954) to speculate on their evolutionery significance. According to him the formation of an eight-nucleate unreduced embryo sac in apomicts leads to considerable unbalance of the tissues of the embryo, endosperm and the maternal plant when endosperm is formed by triple fusion. Such a situation would lead to a 2n embryo, 5n endosperm and 2n maternal tissue. On the other hand in apomicts that give rise to a four-nucleate unreduced embryo sac, the normal ratio of 2n embryo and 3n endosperm tissue is believed to be important for the proper development of the embryo. It is possible that such embryo sacs leading to the establishment of the ratio 2n: 3n: 2n would be favoured over those with an embryo-endosperm-maternal tissue ratio of 2n: 5n: 2n. Brown & Emery (1958) believe that a large number of apomictic Panicoideae produce typically unreduced four-nucleate embryo sacs with one or two exceptions such as *Paspalum secans* (Snyder, 1957), where eight-nucleate unreduced embryo sacs are formed. Examples of Panicoideae producing unreduced eigth-nucleate embryo sacs seem to be on the increase. Indications of the formation of such eight-nucleate embryo sacs have been seen not only in *Paspalum secans* (Snyder, 1957), but also in *Pennisetum clandestinum* (Narayan, 1955a), *Panicum maximum* (Warmke, 1954) and *Cenchrus ciliaris* (Sharatchandra, unpublished). In view of the very plausible speculation of Warmke that the four-nucleate embryo sacs have an advantage over the eight-nucleate embryo sacs, it would be very interesting to ascertain not only the incidence of the formation of the unreduced eight-nucleate embryo sacs, but also whether endosperm formation in such forms is autonomous or requires fertilization.

The endosperm is primarily a nutritive tissue. The embryo depends

upon this tissue for its nutrition, at least in the earlier stages of its development in the normal sexual forms. But the dependence of the embryo on the endosperm in the species which are predominantly aposporous is not obvious. This is indicated by the frequent occurrence of embryo sacs with embryos but without any endosperm. Such instances prompted some authors to suggest that the dependence of the embryos on endosperm has been relaxed among the apomicts. According to Brink & Cooper (1944) the absence of endosperm in the earlier stages of development indicates that either the embryos in such plants do not need the nutritive environment of the endosperm, or that some other mechanism exists which has taken over the role of endosperm. This suggests that the embryo-endosperm relationship in the apomicts may be different from that observed in the sexual forms. Presence of well developed endosperm with an undivided egg cell indicates that, the development of the one does not influence the development of the other and that no functional relationship between the embryo and the endosperm exists.

The presence of normal healthy embryos without any endosperm raises also the question as to how such embryos were nourished in the earlier stages of their development. Cooper & Brink (1949) observed one ovule possessing an embryo without endosperm in *Taraxacum officinale*, an autonomous apomict, and attributed the nutritive role to the food materials available in the ovules. Whether such a nutritive role can be attributed to the ovules in the species under present study is doubtful because of the extensive development of several aposporous embryo sacs in the same ovule, thus depleting the ovular tissue and rendering the ovule nutritionally inefficient. The presence of as many as seven well developed embryo sacs in one ovule, in three of which there were well developed embryos without any trace of endosperm, supports such a view.

Brown & Emery (1958) in their survey of 164 species of 64 genera belonging to 16 tribes of the Gramineae, from the point of ascertaining the method of reproduction, have come to the conclusion that a predominantly large number of Panicoideae, viz. 54 species, are probably apomictic. The basis on which they have arrived at this conclusion is that the four-nucleate embryo sacs are unreduced and that species producing these four-nucleate embryo sacs are apomictic. While it might be true that all four-nucleate embryo sacs are unreduced it cannot be held that all eight-nucleate embryo sacs are reduced, specially in view of the increasing number of species wherein there are indications of the development of eight-nucleate unreduced embryo sacs. Furthermore, species showing occasional apospory cannot be detected by examining a few ovules, especially in view of the rare occurrence of apospory as seen in *P. ramosum*, *P. hohenackeri* and *P. antidotale* which appear to be the only diploid grasses where apospory has been observed. This rare occurrence of apospory may be due to incomplete penetrance of factors responsible for apomixis. The presence of occasional apospory in diploid *Panicum antidotale* (2n = 18) and its predominant occurrence in a tetraploid race (2n = 36) of the species affords an example in support of the views expressed by Güstafsson (1947) that " the action of many of these apomixis influencing genes is stronger on the polyploid level than it is on the diploid level " (cited by Stebbins, 1950).

SUMMARY

Megasporogenesis and the development of the embryo sacs have been studied in *Pennisetum villosum* (2n=45), *P. setaceum* (2n=27), *P. orientale* (2n=27), *P. pedicellatum* (2n=36, 54, two races), *P. ramosum* (2n=10, two varieties), *P. hohenackeri* (2n=18) and in *Panicum antidotale* (2n=18 and 36, two races). The first four species are found to be predominantly aposporous. The megaspore mother cell and its products if any, either undergo degeneration or get crushed by the aggressively growing aposporous cells which give rise to diploid embryo sacs. The nucleus in these embryo sacs divides only twice to give rise to four nucleate embryo sacs. The aposporous initials arise in the chalaza, funiculus, integuments or even in the pericarpic regions. They are also more numerous ranging from three to nine in most of the ovules. The development of the embryo appears to be autonomous while the formation of the endosperm appears to depend upon the fertilization of the single polar nucleus. Thus the endosperm and the embryo develop independently of each other. Polyembryony occurs.

In *P. ramosum* and *P. hohenackeri*, the development of the embryo sac conforms to the Polygonum type. However, these species show the occasional development of aposporous embryo sacs. Progeny tests in *P. ramosum* reveal the occurrence of maternal plants indicating apomictic reproduction. In *Panicum antidotale* also, both the diploid (2n=18) and the tetraploid (2n=36) races show not only the Polygonum type of embyro sac development, but also the occurrence of apospory. The diploid race shows occasional apospory while the tetraploid shows frequent apospory. In addition the tetraploid race appears to show signs of diplospory also.

P. ramosum, *P. hohenackeri* and *Panicum antidotale* are diploid grasses in which apomixis is reported for the first time.

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The Ruminate Endosperm: Development and Types of Rumination

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The occurrence of a ruminate endosperm is one of the peculiar features met with in the mature seeds of higher vascular plants. Available data show (Periasamy, 1955) that ruminate endosperm occurs in representatives of at least thirty families of dicotyledons, one of monocotyledons and one of gymnosperms. The only study that has been made to investigate this phenomenon in detail is that of Voigt (1888). Since then there has been no attempt to study this phenomenon in detail, except for some casual observations that have been made by authors (*see* Periasamy, 1959) while studying the other embryological aspects of plants possessing a ruminate endosperm. Therefore, a study of the post-fertilization development of thirty plants with ruminate endosperm, representing the families Apocynaceae, Araliaceae, Aristolochiaceae, Caprifoliaceae, Ebenaceae, Polygonaceae, Rubiaceae and Vitaceae was made with a view to enhance our knowledge of this phenomenon. In this paper, certain general conclusions that have been arrived at as a result of the study in regard to the development and types of rumination are presented.

DEFINITION OF RUMINATE ENDOSPERM

The word ' ruminate ' is derived from the latin term ' ruminatus ' and means ' chewed '. Evidently the term ' ruminate endosperm ' must have been originally coined with the idea that the tissue of the endosperm becomes eaten away at the concerned places by ingrowth from the periphery of the seed. This is indicated in the meaning given by Asa Gray (1879) : " Ruminated — as if chewed; said of the albumen of a nutmeg ".

The studies of Hegelmaier (1886) and Voigt (1888) were probably the first to show that there is no ' chewing away ' of any bit of endosperm tissue but that the final appearance is brought about by peculiar types of growth activities of the seed coat and the endosperm. In the light of this information the term " ruminate endosperm " must have appeared as a rather

inappropriate one for describing this feature. Nevertheless, by virtue of its long usage the term has established itself in botanical literature as a descriptive one without any association of the idea with which it was originally employed.

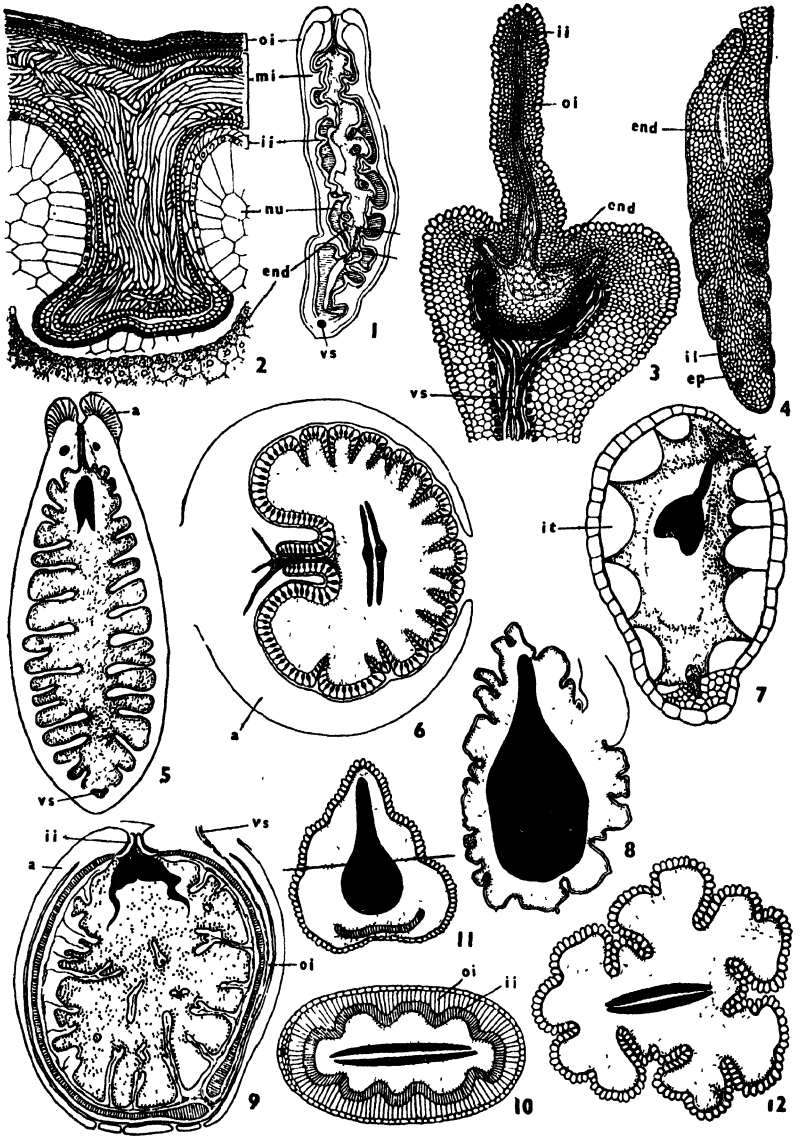
From the information available at present it may be stated that the only difference between a ruminate and a non-ruminate endosperm pertains to the surface which, in turn, is delimited by the inner surface of the seed coat. The surface of ruminate endosperm is irregular and uneven in contrast to the regular and even surface of the non-ruminate endosperm. The irregularity and unevenness of the surface of the ruminate endosperm is, however, of varying degrees and there is no possibility of fixing any exact limit to this variability. Thus, not only a deep furrowing of the surface as seen in *Myristica* (Fig. 9), *Areca* and others but also the less deep undulation of the surface as seen in *Passiflora calcarata* (Fig. 10) and *Apama siliquosa* could rightly be included under the category of ruminate endosperm. The ruminate endosperm may hence be defined as that endosperm which exhibits any degree of irregularity and unevenness in its surface contour within the mature seed, such contour being conditioned by the irregular inner surface of the seed coat.

BEHAVIOUR OF THE SEED COAT

Irregularities in the inner surface of the seed coat arise in two ways : (i) by an unequal radial elongation of the cells of any one layer or the only layer of the seed coat, and (ii) by a definite ingrowth or infolding of the seed coat.

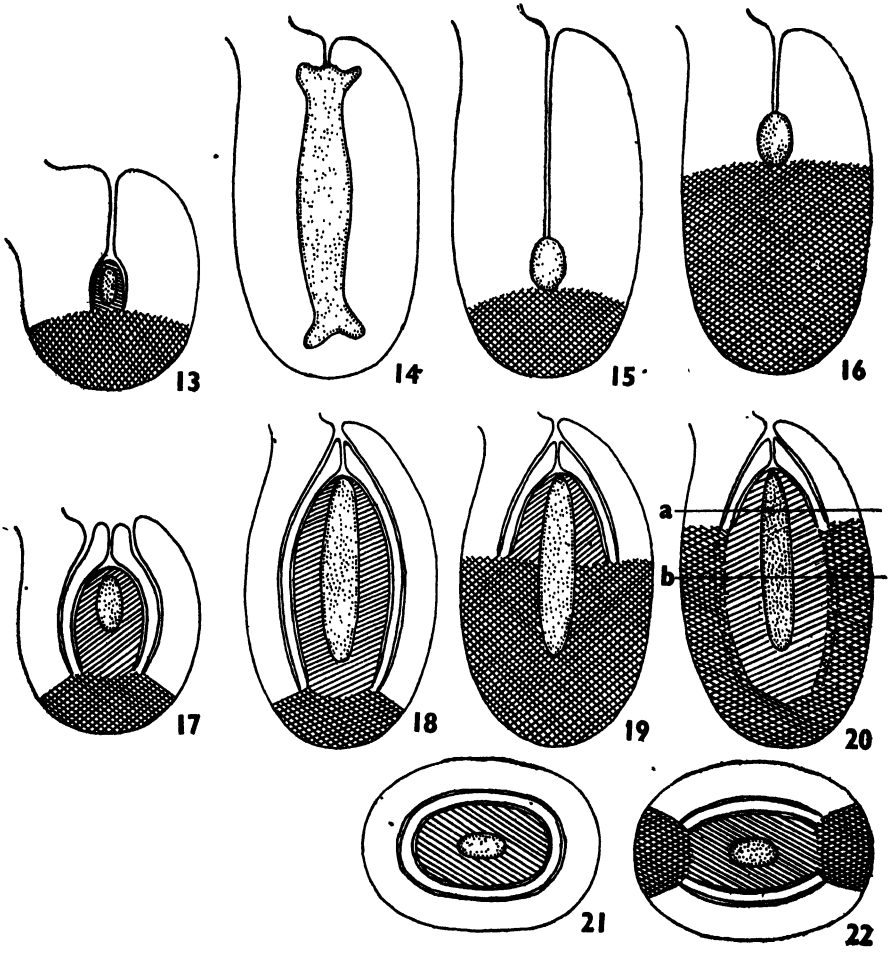
Only a few plants thus far investigated belong to the first category. In *Passiflora calcarata* (Raju, 1956) the innermost layer of the outer integument shows unequal radial elongation of the cells (Fig. 10). In certain plants of the *Scrophulariaceae* (Schmid, 1906; Krishna Iyengar, 1942) the cells of the integumentary tapetum which persists in the mature seed undergo unequal radial elongation and give rise to rumination of the endosperm (Fig. 7).

The majority of seeds having ruminate endosperm belong to the second category. In these, the ingrowths or infoldings are brought about in two ways : (1) by excessive elongation or enlargement of cells of the seed coat and (2) by localized meristematic activity, whereby each ingrowth is the result of a separate meristem in the seed coat. The former type of development is found in the members of the Annonaceae and Aristolochiaceae. In the Annonaceae either the inner or the outer integument or the peculiar middle integument which occurs in certain genera (Fig. 1) may give rise to the ingrowths of the seed coat (Corner, 1949a; Periasamy & Swamy, 1961). Whatever be the mode of origin, there is an excessive elongation of the cells in the integumentary portion of the seed coat while multiplication of cells remains confined to the vicinity of the perichalaza (Periasamy & Swamy, 1961). The cells elongate in a fibre-like manner in various directions and



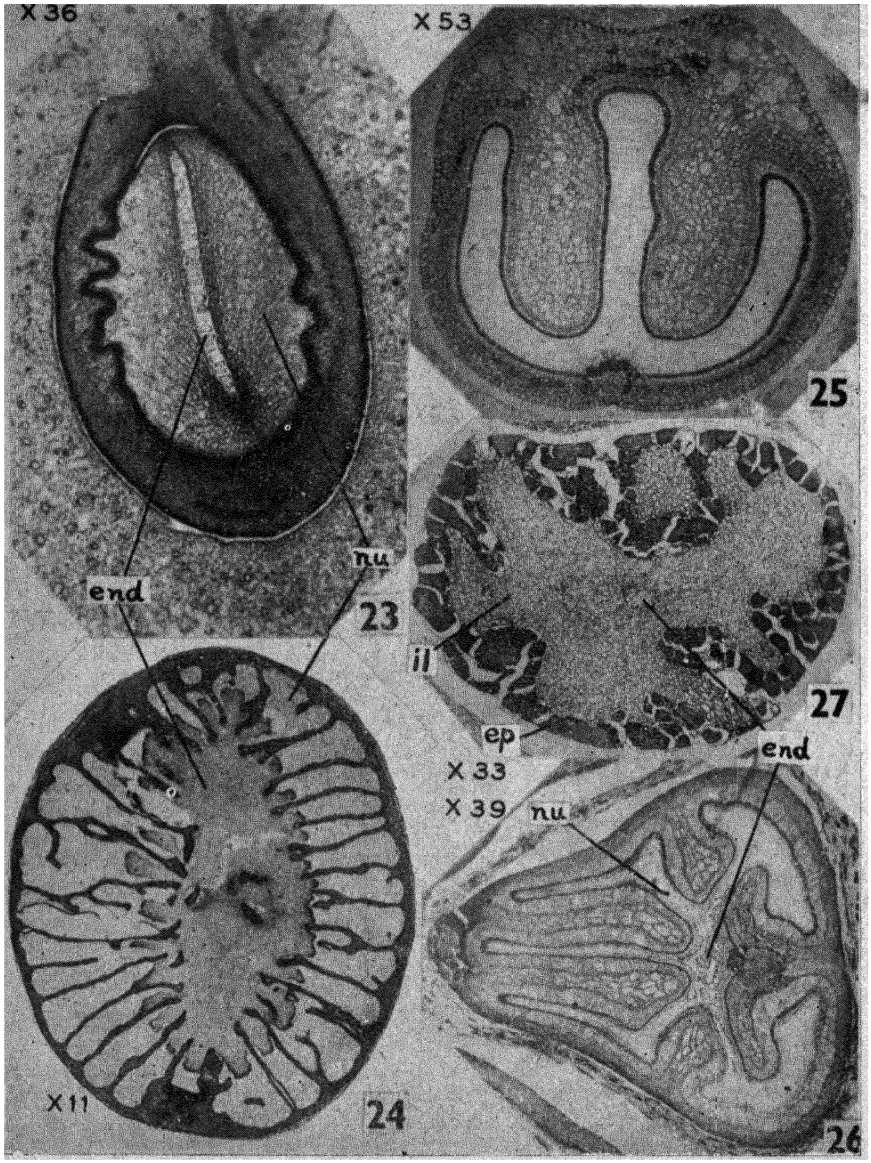
FIGS. 1-12 — (*a*, aril; *end*, endosperm; *ep*, epidermis; *ii*, inner integument; *il*, inner layers; *it*, integumentary tapetum; *mi*, middle integument; *nu*, nucellus; *oi*, outer integument; *vs*, vascular strand. Endosperm stippled, embryo black): Figs. 1, 2. *Cananga odorata*. Fig. 1. Trans-median longitudinal section of immature seed. Fig. 2. Enlargement of portion marked in Fig. 1. Fig. 3. L. s. developing seed of *Coccoloba uvifera*. Fig. 4. L. s. developing seed of *Psychotria congesta*. Fig. 5. L. s. mature seed of *Annona squamosa* (after Corner, 1949a). Fig. 6. T. s. mature seed of *Ervatamia heyneana*. Fig. 7. L. s. mature seed of *Tetranema mexicana* (after Krishna Iyengar, 1942). Fig. 8. L. s. mature seed of *Andrographis serpyllifolia* (after Mohan Ram, 1960). Fig. 9. L. s. mature seed of *Myristica fragrans* (after Voigt, 1888). Fig. 10. T. s. mature seed *Passiflora calcarata* (after Raju, 1956). Figs. 11, 12. *Coccoloba uvifera*. Fig. 11. L. s. mature seed. Fig. 12. T. s. seed along line shown in Fig. 11

form criss-cross arrangement or irregularly tangled arrangement in the ingrowths as well as in the other portions of the seed coat (Fig. 2). The ingrowths themselves are formed without any regular pattern of arrangement or shape in most members of this family. However, in *Alphonsea ceramensis*, *Artabotrys blumei*, *Desmos dasymaschala*, *Uvaria lowii* (Voigt, 1888; Corner, 1949 a) and *Miliusa wightiana* (Periasamy & Swamy, 1961) the ingrowths are all plate-like and arranged one over the other in four longitudinal rows. In



FIGS. 13-22 — DIAGRAMMATIC REPRESENTATION OF VARIATIONS IN THE BEHAVIOUR OF THE CHALAZA. ALL OVULES ANATROPOUS (Chalaza cross-hatched, endosperm and embryo sac stippled, nucellus striped): Figs. 13-16. Unitegmic ovules. Fig. 13. Median L. s. ovule at the time of fertilization. Fig. 14. Median L. s. developing seed with early endosperm development. Figs. 15, 16. Median L. s. developing seeds with normal-chalaza and massive-chalaza respectively. Figs. 17-22. Bitegmic ovules. Fig. 17. Median L. s. ovule at the time of fertilization. Figs. 18-20. Median L. s. developing seeds with normal-chalaza, massive-chalaza and perichalaza respectively. Fig. 21. T. s. along line *a* in Fig. 20. Fig. 22. T. s. along line *b* in Fig. 20

Apama siliquosa of the Aristolochiaceae (Periasamy, 1959). Excessive growth of the outermost layer of the inner integument produces an undulate configuration in the inner as well as the outer surface of the seed coat. The second type of development takes place in *Degeneria vitiensis* (Swamy,



FIGS. 23-27 — (*end*, endosperm; *ep*, epidermis; *il*, inner layers; *nu*, nucellus): Figs. 23, 24. *Miliusa wightiana*. Fig. 23. L. s. young developing seed. Fig. 24. T. s. developing seed at a later stage. Figs. 25-27. T. s. developing seeds of *Cissus pallida*, *Leea asmbucina* and *Tarenna asiatica* respectively

1949), *Myristica fragrans* (Voigt, 1888), *Shorea talura* (Nagaraja Rao, 1953), *Tiliacora racemosa* (Sastri, 1954), in the Vitaceae (Figs. 25, 26), in *Diospyros chloroxylon, D. tomentosa* (Periasamy, 1959) and the ruminate seeds of the Palmae (Voigt, 1888; Venkata Rao, 1959). Except in the Vitaceae, the distribution of the localized meristems and consequently the ingrowths do not have a definite pattern nor are the ingrowths formed in exactly similar shapes. In the Vitaceae, however, the meristematic activity becomes localized in the seed coat to vary definite places in each genus and the shape of the in-growths also appears to be constant for a species (Figs. 25, 26; Periasamy, 1959).

In most seeds that develop from unitegmic ovules, viz. *Spigelia splendens, S. anthelmia* (Dahlgren, 1922), *Voacanga grandifolia, Ervatamia heyneana* (Fig. 6), *Psychotria* spp. *Randia malabarica* and *Tarenna asiatica* and bitegmic *Coccoloba uvifera* (Fig. 12; Periasamy, 1959) the mature seed coat consists of only one layer of cells which develops from the outermost layer (epidermis) of the integumentary and chalazal tissues of the ovule. Definite infoldings of this layer make the endosperm ruminate. During seed development the epidermis becomes morphologically different from the inner layers and produces infoldings or irregular undulations chiefly by cell enlargement. Concurrent with the infoldings or undulations of the epidermis, there is a corresponding outgrowth and undulation of the inner layers of the integument and the chalaza, chiefly by meristematic activity (Fig. 27). Thus, in these instances, both excessive enlargement of cells and meristematic activity seem to be involved in the production of irregularities in the seed coat.

Elytraria acaulis (Johri & Singh, 1959), *Andrographis serpyllifolia* and *A. echioides* (Mohan Ram, 1960; Mohan Ram & Pushpa Masand, 1962) of the Acanthaceae show a striking difference from all the above described plants. In these plants the seed coat does not form infoldings of its own accord but becomes consumed and pushed in an irregular manner by the unequal peripheral activity of the enlarging endosperm.

BEHAVIOUR OF THE ENDOSPERM

The endosperm begins to increase in volume simultaneously with the increase in size of the seed in *Shorea talura* (Nagaraja Rao, 1953), *Diospyros chloroxylon, D. tomentosa, Myristica fragrans, Coccoloba uvifera* (Fig. 3), *Viburnum foetens* (Periasamy, 1959), the Scrophulariaceae (Schmid, 1906; Krishna Iyengar, 1942) and the Palmae (Voigt, 1888, Venkata Rao, 1959). In these plants the nucellus shows little or no increase following fertilization and becomes quickly replaced by the endosperm. The irregularities which arise in the inner surface of the seed coat affect the surface of the endosperm directly as they develop and make it ruminate even at the early stages of development. In *Elytraria acaulis* (Johri & Singh, 1959), *Andrographis serpyllifolia* and *A. echioides* (Mohan Ram, 1960; Mohan Ram & Pushpa Masand, 1962) the endosperm exhibits unequal peripheral activity during late stages of development and causes the seed coat to attain an irregular configuration. In a relative sense all the above mentioned plants may be considered as having an early endosperm development.

In contrast to the above mentioned plants with early endosperm development, in the seeds of the *Annonaceae* (Fig. 23; Periasamy & Swamy, 1961), *Degeneria vitiensis* (Swamy, 1949), *Tiliacora racemosa* (Sastri, 1954), *Passiflora calcarata* (Raju, 1956), those of the Vitaceae (Fig. 26), *Ervatamia heyneana*, *Voacanga grandifolia*, *Psychotria* spp. (Fig. 4), *Randia malabarica*, *Tarenna asiatica* (Fig. 27), *Apama siliquosa* (Periasamy, 1959), *Spigelia splendens* and *S. anthelmia* (Dahlgren, 1922), the endosperm does not increase in volume as rapidly as the other tissues of the seed and remains small in a rather quiescent state until the seed enlarges almost to its maximum size. The bitegmic ovules of this category show a marked increase of the nucellus after fertilization (Figs. 23, 26) and the unitegmic ovules show a similar increase of the inner layers of the chalazal and integumentary portions of the ovule (Fig. 27). Since the endosperm remains quiescent, the irregularities in the seed coat do not affect the surface of the endosperm directly as they develop, but give rise only to a ruminate nucellus (Figs. 23, 26) or an irregular seed surface (Fig. 27) as the case may be. Thus, rumination is performed in the tissues that surround the endosperm. Later on, however, the endosperm becomes actively meristematic, increases rapidly in volume and consumes all the surrounding tissues except those that serve as the mature seed coat. Since the seed coat has a preformed ruminate configuration, the enlarging endosperm exhibits during later stages an unequal peripheral activity to develop in the form of lobes in between the preformed rumination ingrowths or infoldings of the seed coat (Fig. 24). In these the endosperm may be considered as having a late development and the cause of rumination appears to rest finally with the endosperm.

BEHAVIOUR OF THE CHALAZA

Netolitzky (1926) considers the behaviour of the chalaza as an important feature of post-fertilization development. In regard to seeds with ruminate endosperm the chalaza shows three types of post-fertilization activity.

1. The chalaza may continue to remain as a comparatively smaller portion of the seed so that there is no appreciable alteration of its size (Figs. 15, 18) than that existed before fertilization (Figs. 13, 17).
2. The chalaza may show an overall increase in size so that a wider part of the seed becomes occupied by the chalaza (Figs. 16,19) than was the condition before fertilization (Figs. 13, 17).
3. The chalaza may show a unidirectional extension so that it loses its initial radial symmetry in the ovule (Fig. 17) and attains a bilateral symmetry (Figs. 20 – 22).

The last mentioned type of chalaza has already been termed as ' perichalaza ' and the seeds derived from this as ' perichalazal ' by Corner (1949a). In conformity with this term the first type of chalazal activity stated above may be termed 'normal-chalazal' and the second type as 'massive-chalazal'.

It is not always easy to find what particular type of behaviour the chalaza exhibits during post-fertilization development, because of the difficulty in

demarking the exact boundary of the chalazal tissue. According to Netolitzky (1926) the chalaza " is that region of the seed or its primordium which is the place of origin of the integuments and which reaches up to the base of the nucellus without being sharply demarkated from it..." Since chalaza is the place of origin of the integuments it necessarily follows that there would be no differentiation of individual integuments in the chalazal region. Therefore, it may be stated that any region in the seed coat of a bitegmic seed where there is absence of distinction between the individual integuments and other tissues, belongs to the chalaza (*see* Figs. 17 – 22). In order to apply this criterion it is necessary to study the developmental stages because the picture may become confusing in the mature seed due to complete disorganization of one of the integuments.

In the unitegmic ovlues, however, we have to rely upon the nucellus in order to demark the boundary of the chalazal tissue. All the tissues that lie below the level at which the nucellus joins the integument is to be considered as belonging to the chalaza (Fig. 13). However, when the unitegmic ovules are tenuinucellate the nucellus often disappears even before fertilization or in any case immediately after fertilization (Maheshwari, 1950). When the nucellus disappears, the only aid to demark the boundary of the chalaza is the chalazal end of the endosperm tissue. But if the endosperm develops early and destroys most of the surrounding tissues soon after fertilization, or if the endosperm elongates rapidly towards the chalaza and the micropyle as in haustorial formations (Maheshwari, 1950) it would be impossible to determine the relative contribution of the chalazal and integumentary parts to the tissues surrounding the endosperm (Fig. 14).

In seeds showing late endosperm development, however, the position of the quiescent endosperm tissue within the enlarging seed would give a sure indication of the relative growth of the chalazal and the integumentary parts of the ovule after fertilization. If the chalazal part contributes more to the tissues of the seed, the quiescent endosperm would appear to be pushed more and more towards the micropylar part of the seed, without any marked alteration of the distance between the endosperm and the micropylar end (Fig. 16). On the other hand, if the integumentary part takes the leading role, the quiescent endosperm would appear to be pushed more and more towards the chalazal part, without appreciable change in the distance between the endosperm and the chalazal end (Fig. 15). In the first instance there is a relatively greater growth of the chalaza, whereas in the second there is a relatively greater growth of the integument. The former would represent a massive-chalaza and the latter a normal-chalaza.

Apama siliquosa (Periasamy, 1959), *Passiflora calcarata* (Raju, 1956), members of the Scrophulariaceae (Schmid, 1906; Krishna Iyengar, 1942), *Elytraria acaulis* and *Andrographis serpyllifolia* show normal-chalazal development.

Instances of massive-chalazal development are seen in *Degeneria vitiensis* (Swamy, 1949), *Spigelia splendens*, *S. anthelmia* (Dahlgren, 1922), *Myristica fragrans* (Periasamy, 1961), *Coccoloba uvifera* (Fig. 3), *Diospyros chloroxylon*,

D. tomentosa, *Voacanga grandifolia*, *Ervatamia heyneana*, *Psychotria* spp. (Fig 4), *Randia malabarica*, *Tarenna asiatica* (Periasamy, 1959) and those of the Palmae (Venkata Rao, 1959). Perichalazal activity is seen in the Annonaceae (Voigt, 1888; Corner, 1949a; Periasamy & Swamy, 1961), Vitaceae and *Tiliacora racemosa* (Periasamy, 1959). In these, chalazal extension takes place along the median longitudinal plane of the ovule and the vascular strand traverses throughout the entire length of the perichalaza. In all seeds with perichalazal or massive-chalazal activity, rumination is due to a definite ingrowth or infolding of the seed coat (Periasamy, 1959).

According to Corner (1949a), the chalaza "seems to act as the organizational centre for the developing seed after fertilization, comparable with the neurophore, or the region of inflection, of the animal embryos". Further, he believes that rumination in the annonaceous seeds is due to overgrowth of the integuments. Indeed, in all cases of rumination, except perhaps in *Elytraria acaulis*, *Andrographis serpyllifolia* and *A. echioides*, there can be no doubt that some sort of overgrowth in the tissues that constitute the seed coat is involved in the production of irregularities which cause the rumination.

In the ruminate seeds that are associated with chalazal hypertrophy of the perichalazal or massive-chalazal type, it is seen that depending upon the degree and nature of chalazal growth, the distribution of the rumination ingrowths or infoldings and the activity of the nucellus become altered in a definite sequence. In the bitegmic perichalazal seeds which may be considered as having a special and limited chalazal growth, the perichalazal region is devoid of rumination ingrowths which therefore are confined only to the integumentary portion of the seed coat. The nucellus, in these instances, shows a marked increase after fertilization (Figs. 23, 24, 26). The bitegmic massive-chalazal seeds of *Coccoloba uvifera* and those of the Palmae show an overall expansion of the chalaza and also significant growth of the integumentary tissues. In these, the rumination becomes a product of not only the tissues of the integument but also that of the chalaza. The nucellus shows an increase after fertilization but never becomes as massive as in the case of perichalazal seeds since it is replaced by the endosperm at a comparatively early stage (Fig. 3). In *Myristica fragrans* (Fig. 9), the chalaza shows extensive overall growth after fertilization and the inner integument is completely arrested. The integuments do not take any part in producing the rumination which becomes wholly a function of the chalazal tissue (Periasamy, 1961). The nucellus proper is replaced by the endosperm at a very early stage.

During post-fertilization development of the unitegmic, tenuinucellate ovules of *Psychotria* spp. (Fig. 4), *Tarenna asiatica* (Fig. 27), *Randia malabarica*, *Voacanga grandifolia*, *Ervatamia heyneana* (Periasamy, 1959), *Spigelia splendens* and *S. anthelmia* (Dahlgren, 1922), the nucellus undergoes early degeneration and there is a massive chalazal growth. The outermost layer of the ovule becomes morphologically distinct from the inner layers, produces the infoldings which cause rumination and finally functions as the only layer of the seed coat. The inner layers increase by meristematic activity to surround the quiescent endosperm (Figs. 4, 27), remain thin-walled,

become ruminate by the infoldings of the outermost layer and finally get replaced by the endosperm (Fig. 6). In short the outermost layer behaves in the same manner as the seed coat of bitegmic ovules, whereas the inner layers undergo the same fate as the nucellus. Further, in *Psychotria* spp. which exhibit pronounced chalazal growth, the rumination infoldings of the epidermal layer are confined to the region of the chalaza alone (Fig. 4), whereas in others which do not show such marked chalazal growth, the infoldings of the epidermis develop from both the chalazal and integumentary regions.

Netolitzky (1926) has expressed the opinion that seeds which show chalazal growth after fertilization represent a derived form from those without chalazal growth. In conformity with this assumption and in accordance with the principle of "transference of function" enunciated by Corner (1949a, b, 1958), the above described chalazal behaviours indicate that in seeds with ruminate endosperm there is a transference of the functions of the integuments and those of the nucellus to the chalaza wherever it becomes hypertrophied after fertilization.

TYPES OF RUMINATION

Classification of rumination types can be done in various manners since the phenomenon involves a number of structural and developmental characteristics. Dahlgren (1922) has already classified rumination on the developmental basis and has distinguished four types : (1) The *Torreya* type, (2) The *Spigelia* type, (3) The *Calamus* type and (4) The *Coccoloba* type. The basis for his classification is the temporal relationship that obtains between the development of endosperm and the formation of irregularities in the seed coat. This, evidently, does not permit a clear-cut segregation of the types and Dahlgren (1922) himself says that rumination in the Annonaceae forms a transition between the *Spigelia* and the *Calamus* types. Further, in order to fix the type on such a basis it is necessary to study all the developmental stages, a procedure which will not be of much practical value. A classification based on morphological features alone would best serve for practical purposes and also permit a clear-cut segregation of the types. In this regard it is seen that variations which are exhibited in the behaviour of the mature seed coat and the bitegmic or unitegmic nature of the ovule are also indicative of the ontogenitic sequences that are involved in the phenomenon of rumination. Using these criteria seven types of rumination may be distinguished.

KEY TO THE IDENTIFICATION OF RUMINATION TYPES

1a. The mature seed with unequal radial elongation of any one layer or the only layer of the seed coat. Infolding of the seed coat absent

2a. Ovules bitegmic.....Passiflora type (Fig. 10)

2b. Ovules unitegmic.....Verbascum type (Fig. 7)

1b. The mature seed with definite ingrowths or infoldings of the seed coat

3a. The mature seed coat is more than one layered

4a. The ingrowths of the seed coat are supplied with vasculature..... .....Myristica type (Fig. 9)

4b. The ingrowths of the seed coat are not supplied with vasculature .....Annona type (Fig. 5)

3b. The mature seed coat is only one layered

5a. Ovules bitegmic.....Coccoloba type (Figs. 11, 12)

5b. Ovules unitegmic

6a. Rumination preformed in the tissues of the seed coat..... Spigelia type (Fig. 6)

6b. Rumination due to unequal peripheral activity of the endosperm during later stages.....Elytraria type (Fig. 8).

On the basis of development, the rumination types could be grouped as follows :

1a. Chalazal hypertrophy absent during post-fertilization development .....Passiflora type, Verbascum type, Elytraria type

1b. Chalazal hypertrophy present during post-fertilization development.

2a. Late endosperm development...Annona type (except *Shorea talura*), Spigelia type

2b. Early endosperm development...Myristica type, Coccoloba type

Plants with ruminate endosperm thus far investigated belong to the following types :

1. Passiflora type : *Passiflora calcarata* (Passifloraceae)
2. Verbascum type : *Verbascum montanum*, *V. nigrum*, *Celsia coromandalina*, *Vandellia hirsuta*, *V. scabra* and *Tetranema mexicana* (Scrophulariaceae)
3. Myristica type : *Myristica fragrans* (Myristicaceae), *Actinophloeus ambiguus**, *Areca catechu*, *A. concinna*, *Archontophoenix alexandrae*, *Caryota furfurcata**, *Chamerops humilis*, *Nenga wandlandiana*, *Pinanga kuhlii*, *Ptychococcus paradoxus*, and *Ptychosperma elegans* (Palmae)
4. Annona type : All the investigated plants of the Annonaceae, *Apama siliquosa* (Aristolochiaceae), *Degeneria vitiensis* (Degeneriaceae), *Shorea talura* (Dipterocarpaceae), *Diospyros chloroxylon*, *D. tomentosa* (Ebenaceae), *Tiliacora racemosa* (Menispermaceae) *Ampelocissus latifolia*, *A. tomentosa*, *Cayratia carnosa*, *C. pedata*, *Cissus pallida*, *C. vitiginea*, *Leea aspera*, *L. sambucina*, *Tetrastigma lanceolarium*, *Vitis vinifera* (Vitaceae), *Actinorhytis calaparia*, *Calamus* sp., *Caryota mitis*, *C. urens*, *Licula* sp., *Livingstonia* sp., *Medemia* sp., *Phoenix* sp., *Raphia* sp. (Palmae)

* According to Voigt (1888), *Actinophloeus ambiguus* and *Caryota furfurcata* have *Myristica* type of rumination. But Venkata Rao (1959) reports non-ruminate endosperm in *Actinophloes macarthurii* (even though his own figures and account indicate presence of rumination), and absence of vascular supply to the ingrowths in *Caryota mitis* and *C. urens*.

5. Coccoloba type : *Coccoloba uvifera* (Polygonaceae)
6. Spigelia type : *Spigelia splendens*, *S. anthelmia* (Loganiaceae), *Ervatamia heyneana*, *Vacanga grandifolia* (Apocynaceae), *Arthrophyllum diversifolium*, *Hedera helix* (Araliaceae), *Viburnum foetens* (Caprifoliaceae), *Psychotria congesta*, *P. dalzelli*, *P. elongata*, *P. macrocarpa*, *P. reevesii*, *P. serpens*, *Randia malabarica*, *Tarenna asiatica* (Rubiaceae)
7. Elytraria type : *Elytraria acaulis*, *Andrographis serpyllifolia*, *A. echioides* (Acanthaceae)

SUMMARY

A ruminate endosperm is known to occur in 30 families of dicotyledons, one of monocotyledons and one of gymnosperms. An investigation of 30 species belonging to 8 families of dicotyledons was undertaken.

The chief difference between a ruminate and a non-ruminate endosperm pertains to their surface. In the mature seed the surface of the ruminate endosperm is irregular and uneven to varying degrees in contrast to the regular and even surface of the non-ruminate endosperm.

The cause of rumination appears to rest with the activity of either the seed coat or the endosperm. Irregularities on the inner surface of the seed coat are of two types: (i) a definite ingrowth or infolding of the seed coat; and (ii) an unequal radial elongation of the cells of any one layer or the only layer of the seed coat.

The endosperm may begin active growth during the early stages of seed development, or remain in a quiescent state and of a small size until the seed almost reaches its mature size. In the latter case a ruminate nucellus precedes the ruminate endosperm.

Seven types of rumination have been distinguished on a morphological basis. These are typically present in the following genera : *Passiflora*, *Verbascum*, *Annona*, *Myristica*, *Coccoloba*, *Spigelia* and *Elytraria*.

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Review of Recent Work on the Embryogeny of Some Families and Genera of Disputed Systematic Position

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It is widely accepted at present that microscopic study of plants including anatomy, embryology and cytology is of considerable value in the proper assessment of the systematic status of a taxon; external morphological study alone is not adequate and has led to the anamolous placing of various families and genera of flowering plants. In recent years detailed work on the embryogenesis of a large number of species has been carried out by Souèges and his associates in France. The rigorous methods employed by them for a complete developmental history of the early embryo has revealed the existence of certain definite patterns of embryonal development and this has enabled them to formulate a system of embryogenic classification. While a number of families like Alismaceae, Juncaceae, Liliaceae, Chenopodiaceae, Caryophyllaceae, Ranunculaceae, Cruciferae, Malvaceae, Oenotheraceae, Lythraceae, Solanaceae, Scrophulariaceae, Labiatae, Campanulaceae and Compositae are remarkably uniform in their mode of embryonal development, other families, particularly the large families, Boraginaceae and Leguminosae show considerable heterogeneity. Considering the vast number of species of flowering plants in existence at present, the number of investigated members is, as yet, very small and further work on an extensive scale is necessary before a clear picture can emerge in respect of these heterogeneous families. However, the work that has already been done has helped in the elucidation of the evolutionary tendencies within the families and genera. In addition, several small families and individual genera have been subjected to rigorous studies in recent years.

It is proposed to discuss in this paper some of these recent studies of Souèges and his co-workers which have helped to clarify the relationships of certain families and genera of disputed systematic position.

CNEORACEAE

This small family includes the only genus *Cneorum* with about a dozen

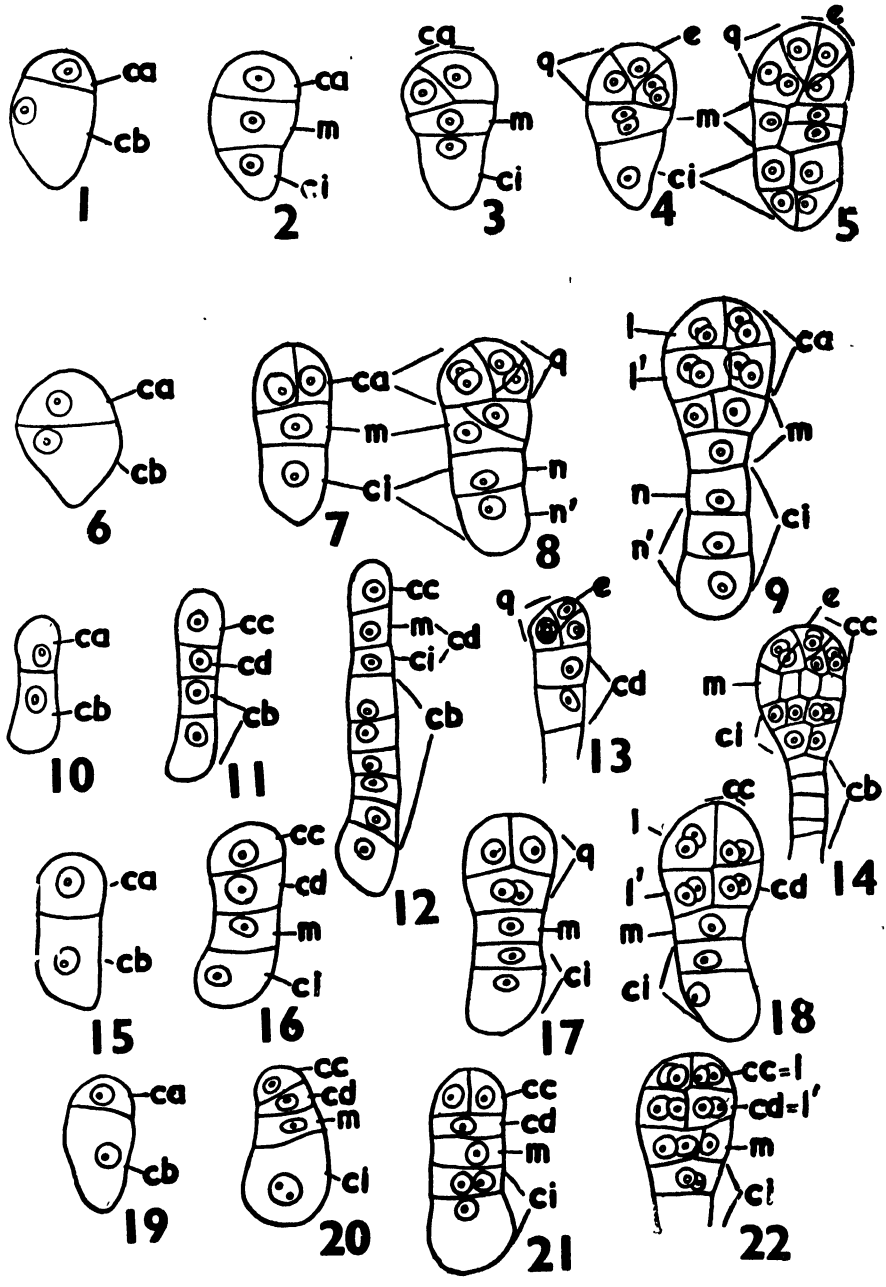
species which are restricted to the Mediterranean and Canaries. The genus is included in the Simarubaceae by Bentham & Hooker (1867) but is placed as a separate family in the Geraniales by Engler & Prantl (1897) with close affinities to the Zygophyllaceae. Earlier, Endlicher (1836-40) had placed it in the Rutales near the Burseraceae. It has also been placed near the Celastraceae (Hutchinson, 1926) and in Rhamnales near the Rhamnaceae (Van Tieghem, 1906). The recent work of Souèges (1955) has indicated that the relationship of the family should be looked for in Rhamnales near the Rhamnaceae. *Cneorum tricoccon* (Figs. 1-5) belongs to the first period in the second group and II megarchetype in the embryogenic classification. This megarchetype is typified by *Geum urbanum* and two members of the Rhamnaceae, *Ceanothus azureus* and *Rhamnus frangula* belong here. A tetrad of the B_2 category (Fig. 3) is formed which in the next stage gives rise to a group of eight cells disposed in three tiers. An epiphysis (Fig. 4) is differentiated from the quadrants and ultimately the cotyledons and the stem tip are derived from the quadrants. The hypocotyl in its entirety is derived from the intermediate cell, *m*, of the four-celled embryo. A suspensor is also differentiated. It may be stated here that the investigated members of the Zygophyllaceae show an entirely different mode of embryo development assignable to the second period in the 9th group.

CALLITRICHACEAE

Maheshwari (1950) has already discussed the systematic position of this small family and has referred to the work of Jφrgensen (1923, 1925) which indicates a close relationship of this family with Labiatae and Verbenaceae, rather than with any other family like Haloragidaceae, Caryophyllaceae, Euphorbiaceae with which this family had been associated in the past. Jφrgensen, however, to quote Maheshwari (1950) "wisely refrained from committing himself further in the absence of adequate anatomical and cytological data". The recent detailed work of Souèges (1952) on the endosperm and embryo of *Callitriche verna* lends further support to the view that the real affinities of this family are with the Verbenaceae. Jφrgensen did not give a detailed account of the development of the embryo and merely referred to it in general terms as of the dicotyledonous type. The precise study of Souèges reveals that *Callitriche verna* is ranged in the first period, first group and IV megarchetype with a tetrad in A_2 category and the destiny of *ca* and *cb* being :

$$ca = pvt + pco + phy + icc$$
$$cb = iec + co + s$$

The embryonal development of *Callitriche* thus conforms to the type in *Mentha* and more so with *Verbena* in regard to details and hence supports further the view put forward by Jφrgensen that the relations of Callitrichaceae are with the Labiatae and Verbenaceae rather than with any other family. The mode of endosperm development in *Callitriche* and *Verbena* is identical and thus indicates close affinities.



FIGS. 1-22—(*ca*, apical cell of two-celled proembryo; *cb*, basal cell; *cc* & *cd*, daughter cells of the apical cell; *m* & *ci*, superior and inferior cells derived from *cb*; *q*, quadrants; *l* & *l'*, octants; *e*, epiphysis initial; *n* & *n'*, derivatives of *ci*, all figs., selected from and redrawn after Souèges, 1953, 1955, to which papers reference may be made for details): Figs. 1-5. *Cneorum tricoccon* L. Figs. 6-9. *Ruta graveolens* L. Figs. 10-14. *Zygophyllum fabago* L. Figs. 15-18. *Radiola linoides* Roth. and Figs. 19-22. *Peganum harmala* L.

DATISCACEAE AND LOASACEAE

These two families are included in the Passiflorales of Bentham & Hooker (1862-83) and in Parietales of Engler & Prantl (1897). While the relationship of Datiscaceae to Begoniaceae has been clearly demonstrated by Crété (1952), the position of Loasaceae appears to be different on embryological grounds. The endosperm of *Loasa lateritia* with its haustoria and the embryo which resembles the course of development seen in the Solanaceae would suggest that the real position of the Loasaceae would be in one of the orders of the Gamopetalae. Further work on the other members of the family is needed before the family could be taken out of the Parietales.

PEGANUM AND OTHER GENERA

In regard to the systematic position of individual genera a few examples may be considered. *Peganum.* This genus with four species has been in turn included in the Rutaceae (Bentham & Hooker, 1862-67) and in the Zygophyllaceae (Engler & Prantl, 1897). Souèges (1953) has recently discussed the position of *Peganum harmala* in relation to *Ruta graveolens, Tribulus terrestris, Zygophyllum fabago* and *Radiola linoides* (Linaceae). He is of the opinion that *Peganum* be placed in a separate family, Peganaceae in the Geraniales near the Linaceae. The course of embryo development in the above members may be briefly discussed here. *Ruta graveolens* (Figs. 6-9) belongs to the first period, first group and IV megarchetype with a first tetrad of category, A_2 (Fig. 7), the destiny of *cb* being *iec* + *co* + *s*. The two members of the Zygophyllaceae investigated, *Tribulus terrestris* and *Zygophyllum fabago* (Figs. 10-14) show an altogether different course of embryo development being placed in the second period with a B_2 type of second tetrad. The destiny of the relevant cells taking part in histogenesis refers these species to the II megarchetype.

Radiola linoides (Figs. 15-18) of the Linaceae belongs to the first period with a first tetrad of C_2 (Fig. 16) category and as such in the 3rd group and IV megarchetype. The destiny of *cb* being *iec* + *co* + *s*, is the same as that of *Ruta* but with this fundamental difference that the first tetrad is in the 'C' series.

Peganum harmala (Figs. 19-22) belongs to the same period, same group and same megarchetype as *Radiola*. There are, however, some differences in later stages of development beyond the octant stage but these are not of much significance. The detailed studies of Souèges have thus clearly indicated that the Peganaceae are connected very closely with the Linaceae and the earlier placing of *Peganum* either in Rutaceae or in Zygophyllaceae cannot be justified on embryological grounds.

We may also, incidentally, consider the case of two other genera which have been the subject of dispute in so far as their systematic position is concerned. The genus *Parnassia* has been considered somewhat anamolous in the family Saxifragaceae and it has been in turn referred to widely different

families. Lula Pace (1912) on the basis of the characters of the ovule and embryo sac found it more closely related to the Droseraceae than to the Saxifragaceae. Diels (1906), however, refused to accept this genus in his treatment of the Droseraceae in Pflanzenreich. Bentham & Hooker as well as Engler & Prantl have both included the genus in the Saxifragaceae. A recent detailed study of the embryogeny of the genus by Lebègue (1953), however, reveals an entirely different picture. *Parnassia palustris* belongs to the first period in the first group in III megarchetype. This megarchetype has the destiny of the apical and basal cells of the two-celled proembryo as follows :

$$ca = pco + pvt + \tfrac{1}{2}phy$$
$$cb = \tfrac{1}{2}phy + icc + iec + co + s$$

To this megarchetype and group also belong *Polygonum*, *Oxybaphus* and *Claytonia*. The course of embryo development in *Parnassia palustris* and *Claytonia perfoliata* (Portulacaceae) is very similar. *Drosera* is, however, ranged in the second period, eleventh group and IV megarchetype and *Saxifraga* in the second period, ninth group and IV megarchetype and are thus embryonomically far removed from *Parnassia*. While evidence from other sources is wanting to establish definitely that the affinities of the genus are to be looked elsewhere than the Saxifragaceae, the genus could be tentatively placed in a separate family, Parnassiaceae and only further investigations can determine its real position.

Another genus of the Saxifragaceae, the monotypic Californian *Peltiphyllum* is an exception to the course of embryo development generally met with in the Saxifragaceae. In a recent study of *Peltiphyllum peltatum* supported by more than 80 figures of different stages of embryo development, Lebègue (1952) has conclusively shown that the course of embryo development in this member is unique among the Saxifragaceae. *Peltiphyllum* is ranged in the second period, ninth group and II megarchetype along with *Campanula* and *Lobelia* with the destiny of *cc* and *cd* as follows :

$$cc = pvt + pco$$
$$cd = phy + icc + iec + co + s.$$

All other investigated members of the Saxifragaceae with the exception of *Parnassia* already referred to above, are found to belong to IV megarchetype in the ninth group of the second period. It is not, however, possible to decide the systematic position of this genus on the basis of evidence of embryogeny alone.

The above account is intended mainly to draw the attention of the workers in the field to the great need for rigorous studies of embryo development and also to emphasize the value of these studies in the elucidation of systematic position and relationships of various genera and families though it should not be taken that assignment to their respective systematic position is to be on this basis alone. The recent studies have also indicated the various evolutionary tendencies that are evident within certain large families and genera. This is particularly illustrative, as indicated earlier, in the large families like Leguminosae and Boraginaceae.

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Pollen Development in Some Members of the Cyperaceae*

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Elfving (1879) and Strasburger (1884) working independently on *Heleocharis palustris* were the pioneers to establish the pollen development in the Cyperaceae. Juel (1900), Stout (1912) and Heilborn (1918) investigated the structure of pollen in *Carex acuta*, *C. aquatilis* and *C. ericetorum* respectively. Juel described the formation of the generative cell by the process of 'free cell formation' while Stout considered that the generative cell is cut off by a cell plate towards the periphery of the microspore. Working with *Scirpus lacustris*, *S. paluster* and *S. uniglumis*, Piech (1924, 1928) confirmed Juel's findings but their observations that the male cells become devoid of cytoplasm was explained by Kostrioukoff (1930) as an artifact due to poor fixation.

Tanaka (1937–1941, 1950) in his extensive work covering eight genera believes that a plasma membrane separates the functioning microspore nucleus from the three effete nuclei of a tetrad; the latter also get partitioned by similar membranes. In *Cyperus rotundus*, Khanna (1956) observed a distinct furrow separating the three smaller nuclei. Gupta (1958), however, denies a septum separating the functional nucleus from the three abortive nuclei in *Fimbristylis tenera*. Dnyansagar & Tiwari (1956) reported that in *F. quinquangularis*, the functional nucleus moves to one side prior to division.

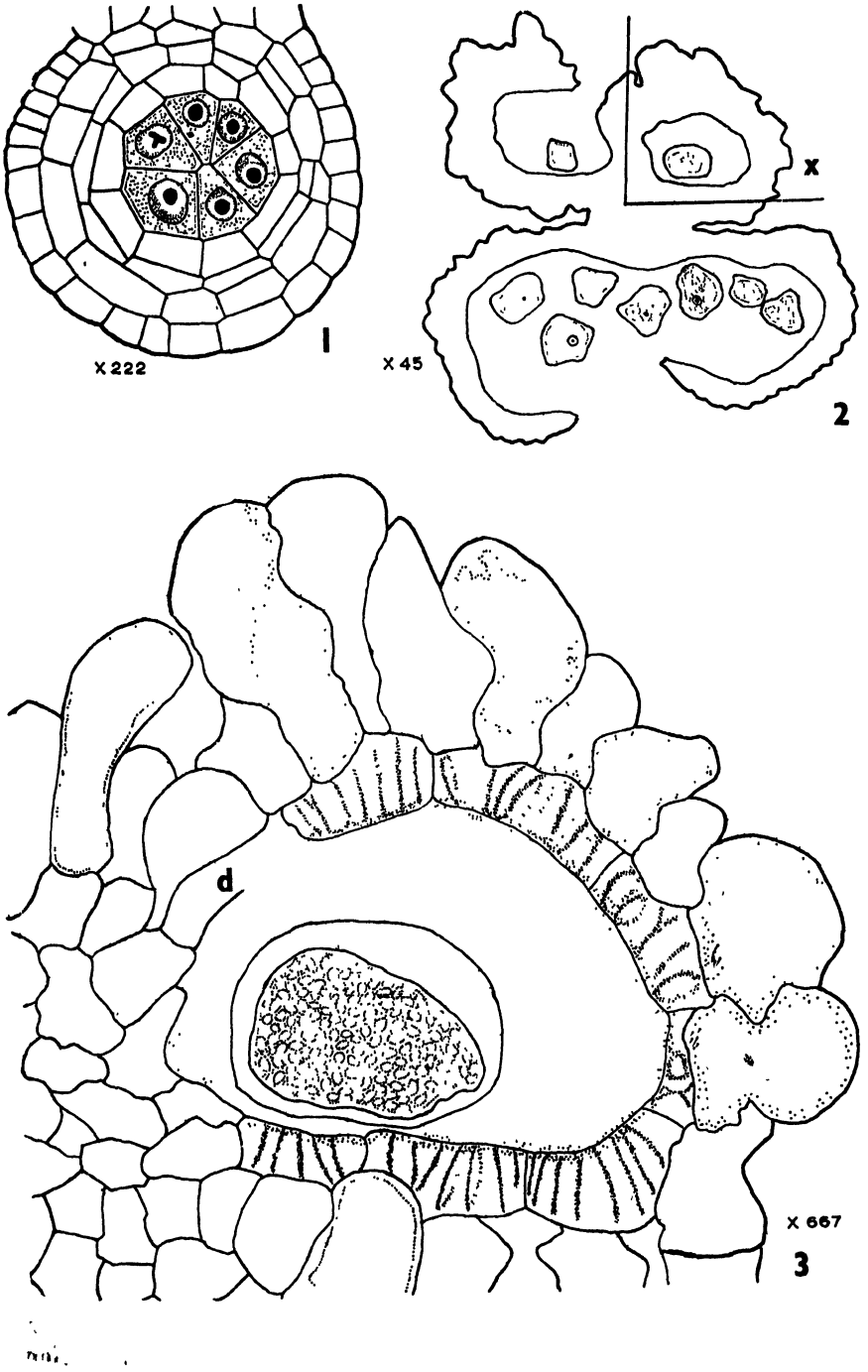
Selling (1947) suggested that the pollen of the Cyperaceae should be termed 'pseudomonads' and 'the wall of the mature grain is nothing but the wall of the pollen mother cell' (*see* Erdtman, 1943).

The present paper deals with the pollen development in five genera of the Cyperaceae.

OBSERVATIONS

The bisexual floret has a single stamen in *Bulbostylis barbata* Kunth., two in *Kyllinga triceps* Rottb. and three in *Cyperus articulatus* Linn.,

*This forms a part of the Ph.D. thesis submitted to the Department of Botany, University of Delhi in 1959.



FIGS. 1-3 — *Carex wahlenbergiana*. MICROSPORANGIUM (*d*, region of dehiscence): Fig. 1. T.s. of anther lobe showing parietal layers and sporogenous tissue. Fig. 2. T.s. mature anther (diagrammatic). Fig. 3. Magnified view of anther locule marked x in Fig. 2

C. niveus Retz., *Eleocharis palustris* R. Br. and *Scirpus maritimus* Linn. In *Carex wallichiana* Presc., the spikes are unisexual and the male floret has three stamens.

Each anther consists of four sporangia. Rarely, the two sporangia belonging to different thecae fuse in *Cyperus articulatus* resulting in a trilocular condition (Fig. 42).

In each loculus the hypodermal archesporium differentiates as a single file of cells running through its entire length (Figs. 17, 40). It undergoes a periclinal division giving rise to an outer primary parietal layer and an inner primary sporogenous layer. The former in turn divides to produce three layers of cells. Thus, at the microspore mother cell stage, the anther wall comprises an epidermis, the unthickened endothecium, one middle layer and tapetum (Figs. 1, 18, 41).

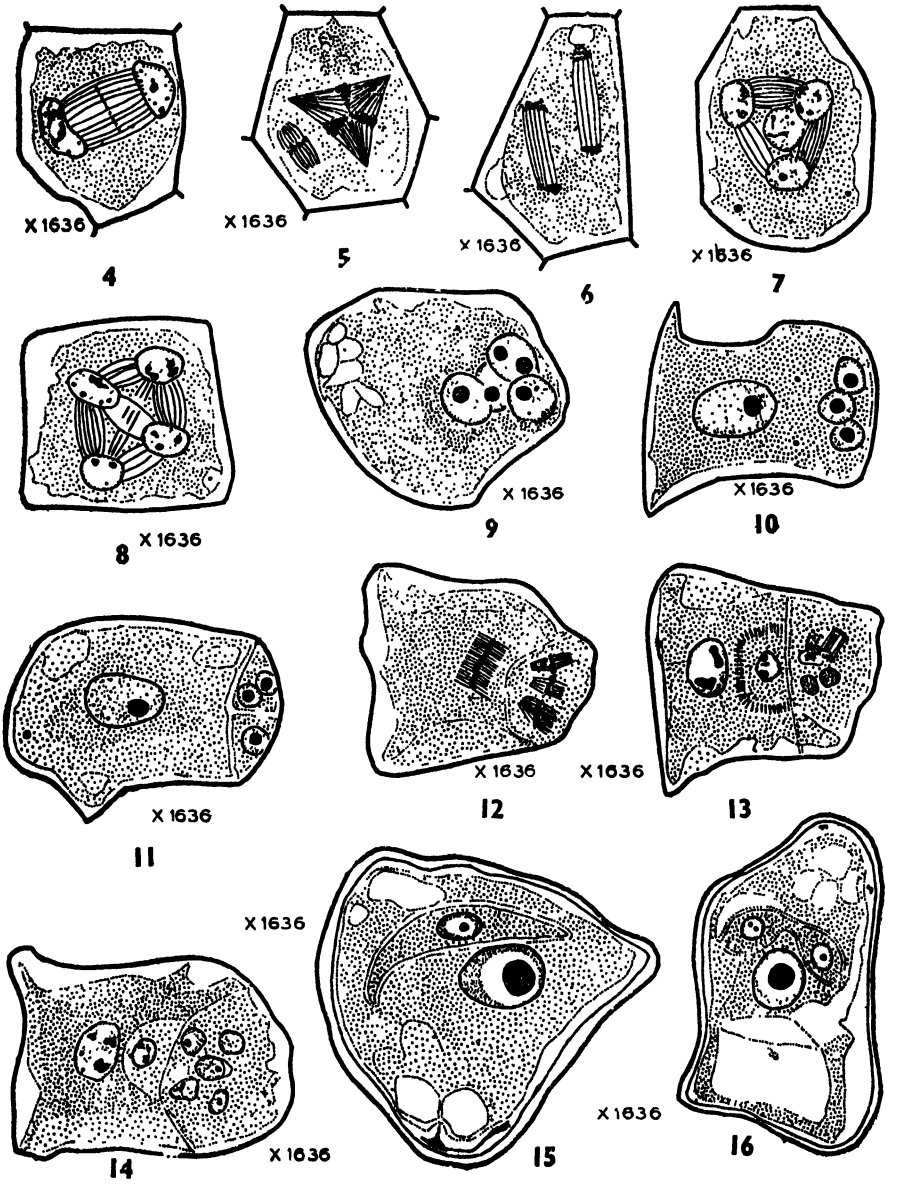
In *Carex* the papillate epidermal cells persist even at the time of shedding of the pollen (Figs. 2, 3). These show yellow granular contents and some of them may also contain tannin. In *Bulbostylis*, the epidermal cells shrink and form a flattened layer on the connective side, while the cells near the stomium remain turgid (Figs. 19, 20).

The middle layer degenerates at the tetrad stage. The cells of the endothecium become radially elongated and from their inner tangential walls, fibrous bands run upwards ending near the outer wall of each cell. In *Chamaegyne* the epidermis of the anther develops fibrous thickenings and functions as endothecium; hence the genus is regarded as a highly reduced form in the Cyperaceae (Süessenguth, 1954). In *Cyperus*, besides the endothecium cells lying close to the connective also show fibrous thickenings. The anther dehisces longitudinally by the breakdown of the thin-walled cells at the junction of the pollen sacs.

The tapetal cells are densely cytoplasmic and possess conspicuous nuclei. They become vacuolate at the time of the second meiotic division and remain uninucleate throughout. In *Carex aquatilis*, Stout (1912) sketched a tapetal cell at an early prophase but further stages are wanting to prove their multinucleate nature. In *Cyperus tegetum*, Padhye & Moharir (1958) have reported binucleate tapetal cells, but their figures are not at all convincing. The tapetum remains intact till the microspores enlarge and the prominent 'Ubisch' granules appear on its inner tangential walls. After the disappearance of the tapetum, the granules come to lie against the inner wall of endothecium (Fig. 43). Probably they contribute to the formation of exine (*see* Maheshwari, 1950).

The sporogenous cells divide in such a manner that the resulting microspore mother cells are wedge-shaped and disposed around a central axis. Their acute apices point towards the centre of the cylinder (Fig. 18).

While the microspore mother cell prepares for meiosis, a special mucilaginous wall is secreted between the protoplast and the cell wall. The meiotic divisions in the microspore mother cells proceed normally (Figs. 6-8, 21-24, 45, 47). After Meiosis I, an ephemeral cell plate is initiated but it disappears soon (Fig. 4). The haploid chromosome number in *Bulbostylis barbata* is



FIGS. 4-16 — *Carex wallichiana*. MICROSPOROGENESIS AND MALE GAMETOPHYTE: Figs. 4-8. Meiosis I and II; one of the dyad nucleus in Fig. 5 shows a tripolar spindle. Fig. 9. A tetrad; all the four microspore nuclei have migrated to one side. Fig. 10. Same, three of the microspore nuclei remain at one end of pollen grain while the functional nucleus migrates back to the centre. Fig. 11. Delimitation of pollen nucleus from the three micronuclei by a septum; one of the micronuclei is also separated from the other two. Fig. 12. Metaphase of the functional nucleus; the three micronuclei also form miniature metaphase plates. Figs. 13-15. Two-celled pollen grains. Fig. 16. Three-celled pollen grain

five as determined by metaphase counts during Meiosis I and II. The same number has also been reported by Tanaka (1941). In *Cyperus* numerous darkly stained bodies appear in the cytoplasm of pollen mother cells during meiosis (Fig. 44). The spindles may be parallel or at right angles to each other resulting in tetrahedral or isobilateral arrangement of the nuclei but cell plate is not formed. Linear arrangement of nuclei are not uncommon in *Cyperus* (Fig. 46). Finally, all the four nuclei move towards the inner wall of the mother cell.

Several cases of tripolar spindles have been observed in *Carex* during Meiosis II (Fig. 5). The spindle fibres are directed towards the three acute poles and the chromosomes are arranged on a triradiate plate in the centre with the fibres attached to them. The metaphase separation seems improbable; if the nuclei are at all formed, they may not have the complete haploid constitution. Such spindles have been induced in *Vicia faba* (Sakamura, 1920) by treating the anthers with chloral hydrate and in *Epilobium* (Michaelis, 1926) by intense cold treatment. Heitz (1925) and Hedayetullah (1933) have observed a similar feature in *Melandrium* and *Oenothera missouriensis* respectively. In *Hydrilla verticillata* (*see* Maheshwari, 1950), the multipolar spindles gradually revert to bipolar condition.

After the second division, the pollen grains assume queer forms. All the ' tetrads ' in an anther undergo ' contraction ' and appear to be degenerating. In sections, the grains show vivid forms. This is due to the uneven distribution of cytoplasm which is irregularly thin at the periphery and dense in the centre.

Wodehouse (1935) observed an interesting behaviour of microspores. He states: " When the grain dries and shrinks, it generally does so with the formation of three or four large concavities on its sides and a smaller one at the large end causing it to become polyhedral, sometimes more or less tetrahedral, though there is much variation in this. In the expanded condition, the grain as a whole assumes a symmetrically ovoid form. " Süessenguth (1920) and Tanaka (1939, 1940) also pleaded that the pollen grains give ' difficulties in observation ' at one or the other stage of development. The present findings support the view of the earlier workers. That this phenomenon is not due to plasmolysis has been checked by observations of fresh pollen grains mounted in plain water or acetocarmine.

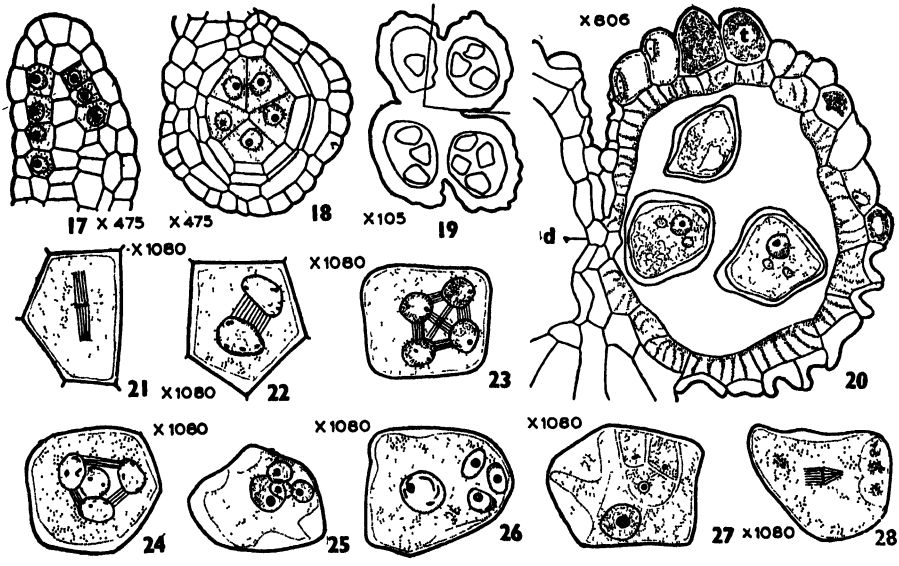
All the four microspore nuclei are of the same size in the beginning (Figs. 9, 47), but soon the one situated in the outermost position grows larger while the rest are pushed towards the inner corner (Figs. 10, 25, 26, 48) or rarely they migrate in various directions. The degenerating nuclei are delimited by a hyaline plasma membrane and may be further separated from each other by thin membranes (Figs. 11, 27, 49).

The three non-functional nuclei in *Carex* may undergo abortive divisions which are generally arrested soon after metaphase. Rarely, the division is completed and 5 or 6 small nuclei are formed (Figs. 12-14). These nuclei soon degenerate and their remnants lie closely adpressed to the intine and can be seen as dark streaks upto the shedding stage of the pollen. In *Cyperus*

also the non-functional nuclei show feeble capacity for division. Only in three cases, four degenerating chromatin masses were seen in the inner corner instead of the usual three (Fig. 53). It is inferred that of the three nuclei only one nucleus divided resulting into four masses. Excepting Juel (1900) and Tanaka (1939) no other investigator has reported the division of the three small nuclei.

The young microspore is richly cytoplasmic with a centrally situated nucleus and no vacuolation occurs in the cytoplasm prior to its division (Figs. 28, 50). The spindles in *Carex* are markedly symmetrical, broad and blunt (Fig. 12). The two daughter nuclei are always unequal in size. The generative cell rests upon the septum which separates the three effete nuclei (Figs.13, 29). It soon becomes spindle-shaped and appears round when cut across (Figs. 15, 30, 31, 52).

The pollen grains round up, begin to enlarge, and finally assume a symmetrically ovoid form. Meanwhile vacuoles also appear. The division of the generative cell results in two spherical male gametes (Figs. 16, 32, 33, 54, 55). The nature pollen grains accumulate numerous starch grains which mask the male nuclei.



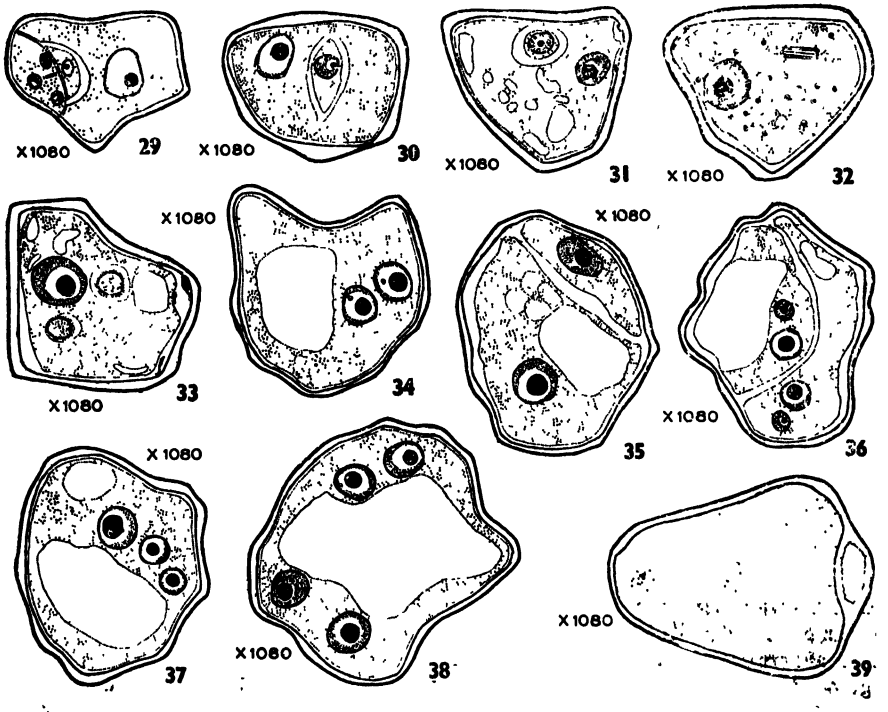
FIGS. 17-28 — *Bulbostylis barbata.* MICROSPORANGIUM, MICROSPOROGENESIS AND MALE GAMETOPHYTE (*d*, region of dehiscence; *t*, tannin) : Fig. 17. L. s. young anther showing hypodermal archesporium. Fig. 18. T. s. anther locule at microspore mother cells stage. Fig. 19. T. s. mature anther (diagrammatic). Fig. 20. Magnified view of anther locule marked *a* in Fig. 19. Figs. 21-24. Meiosis I and II. Figs. 25, 26. Tetrads; the functional nucleus in Fig. 26 has migrated to the centre. Fig. 27. Pollen grain with one functional and three non-functional nuclei. The nuclei are separated from each other by plasma membranes. Fig. 28. Division of the functional nucleus; note abortive divisions in non-functional ones

The pollen grain has a smooth exine and is monocolpate with a single distal ulceroid aperture located on the side opposite to the three degenerating nuclei (Fig. 39).

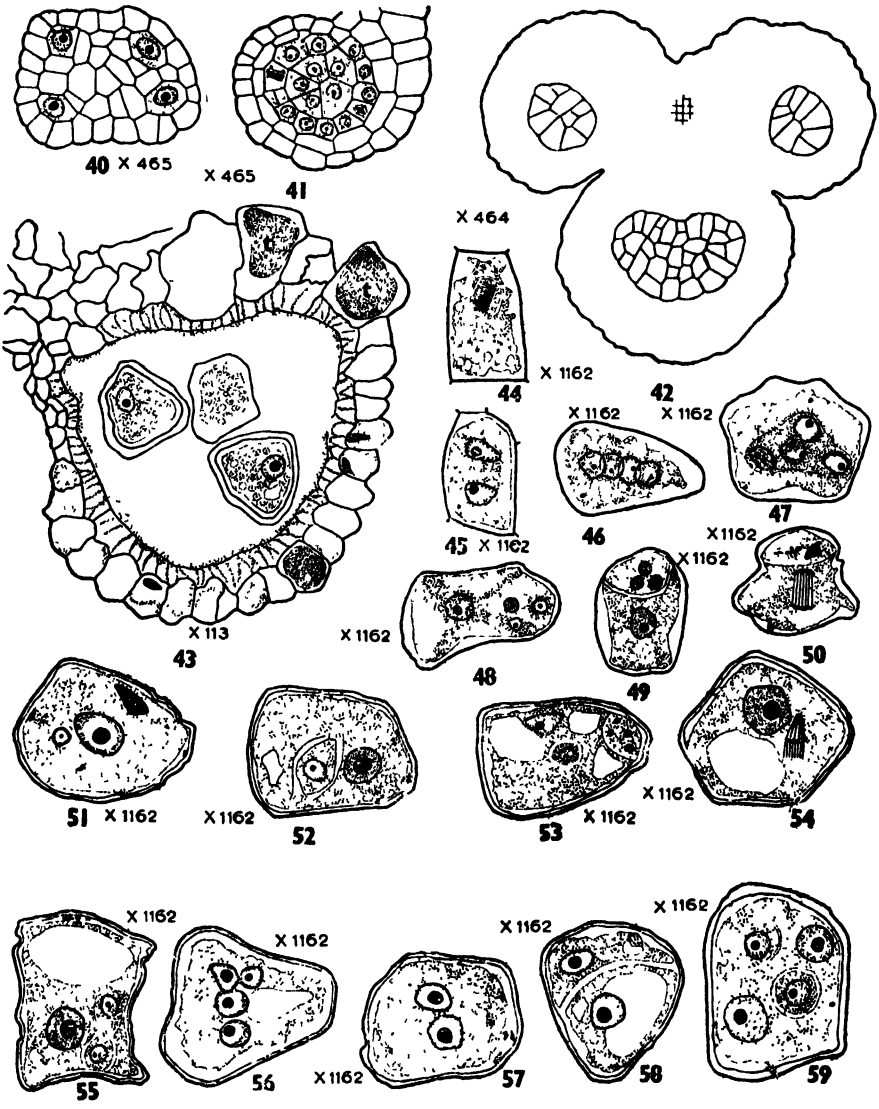
Deviations from the above course of development are also observed. Frequently, in *Bulbostylis* and *Cyperus* double pollen grains are formed in which the two cells are unequal in size having the usual vegetative and generative nuclei (Figs. 34-36, 57-59). In about 10 per cent of the abnormal pollen grains all the four nuclei remain healthy while the microspore wall differentiates into exine and intine (Figs. 37, 38, 56). It is of interest that the three abortive nuclei cannot be seen in either case. Sometimes the pollen grains in *Carex* show two nuclei of almost equal size, each of which may divide again to give rise to the vegetative and generative cells.

DISCUSSION

Cytokinesis. In *Carex acuta*, Juel (1900) described a transitory cell plate after Meiosis I. Based on this, Wulff (1939) considered it to be a reduced



FIGS. 29-39 — *Bulbostylis barbata*. MALE GAMETOPHYTE (CONTD.): Figs. 29-31. Two celled pollen grain; the three abortive nuclei are persistent although the membrane separating them have almost disappeared in Fig. 29. Fig. 32. Generative cell in telophase. Fig. 33. Three-celled pollen grain with the remnants of the three adpressed degenerated nuclei. Figs. 34-38. Abnormal pollen grains. Fig. 39. Psilate pollen grain showing one ulceroid aperture at the broad end (lateral view)



FIGS. 40-59 — *Cyperus articulatus*. MICROSPORANGIUM, MICROSPOROGENESIS AND MALE GAMETOPHYTE (*t*, tannin-filled cell) : Fig. 40. T. s. young anther showing an archesporial cell in each lobe. Fig. 41. T. s. anther lobe showing the development of the wall. Fig. 42. T. s. abnormal anther with three microsporangia. Fig. 43. T. s. mature anther lobe. Figs. 44, 45. Meiosis I. Note the presence of darkly stained bodies in the cytoplasm. Figs. 46, 47. Tetrads. Figs. 48, 49. 1:3 arrangement of microspore nuclei; in Fig. 49 the three nuclei are separated from the fourth one by a plasma membrane. Fig. 50. Functional nucleus at telophase. Figs. 51-53. Two-celled pollen grains; note the presence of four abortive nuclei in Figs. 51, 53. Fig. 54. The generative nucleus at metaphase. Fig. 55. Three-celled pollen grain. Fig. 56. Abnormal pollen grain where all the nuclei seem to be functional. Fig. 57. Abnormal pollen grain with two similar nuclei. Figs. 58, 59. Double pollen grains

form of successive type. In *Eleocharis palustris*, Håkansson (1954) states, " It seemed that the formation of a cell plate was initiated but it was never completed and was of an ephemeral nature. The changes in the cytoplasm observed in *Eleocharis* seem reminiscent of successive pollen formation. " Tanaka (1940) categorically writes, " It may be assumed that the 'reduzierter sukzendaner Type' assumed by Wulff (1939) may be accepted only partially. " Schnarf (1931) regarded the meiotic divisions to be of the simultaneous type. The present study on *Bulbostylis*, *Carex*, *Cyperus* and *Kyllinga* confirms his observation.

Septum Formation. Whether septa are formed amongst the quartet nuclei is a debated question. There are at least three different types of observations :

(i) Septum formation is entirely inhibited (Suessenguth, 1920; Piech 1924, 1928; Tanaka, 1939; Gupta, 1959).

(ii) Septa arise but are ephemeral (Elfving, 1879).

(iii) Septum formation occurs as a regular feature (Strasburger, 1884; Juel, 1900; Stout, 1912; Håkansson, 1928; Tanaka, 1939, 1940; Dnyansagar & Tiwari, 1956).

It is interesting to note that even for the same species different opinions have been expressed. In *Heleocharis palustris*, Elfving (1879) held the second view, Strasburger (1884) the third and Piech (1928) the first view. However, Piech has reported that the pollen nucleus and the effete nuclei in the corner were separated by the secondarily formed callose membrane. In *Scirpus lacustris*, Piech (1924) could recognize no cell plate or membrane but Tanaka (1940) reported clear septa among them. In *Fimbristylis*, Tanaka (1939) did not observe any septum, but Dnyansagar & Tiwari (1956) have noticed a distinct septum which Gupta (1959) refutes.

Maheshwari (1950) has stressed that the following points need clarification : (i) whether the functioning microspore nucleus is separated from the three non-functioning nuclei of the tetrad by a partition wall, (ii) whether the non-functioning nuclei are separated from one another by walls and (iii) what is the possible fate of the non-functioning nuclei ?

The author finds that during earlier divisions a septum is formed between the pollen nucleus and the three small nuclei and at the same time or somewhat later, septa are also formed in between the three small nuclei. These septa gradually disappear.

Thus, the septum formation is normal but the statement of previous authors (Piech, 1928; Tanaka, 1939, 1940) that there are extreme difficulties in observation is instructive. The phase of contraction in pollen mother cell probably explains their difficulty. They did observe contracted grains but concluded that those are abortive pollen grains having various shapes. The author has never observed the entire degeneration of the grains in any anther loculus.

Dnyansagar & Tiwari (1956) in *Fimbristylis quinquangularis* found that the septum persists even when the non-functional nuclei had degenerated. On the contrary, the author finds, the septa are formed in all the five genera

studied and are destroyed prior to the disintegration of the three nuclei. A septum between the functional and the three non-functional nuclei, additional to all the above mentioned septa, has been reported in *Cyperus tegetum* by Padhye & Moharir (1958) which in the author's opinion is very doubtful.

Finally, the three nuclei degenerate and appear as darkly stained rod-like bodies which are closely adpressed to the intine. Divergent views exist regarding the course of degeneration of the three abortive nuclei. Some authors regard that they are absorbed in the cytoplasm (Elfving, 1879; Strasburger, 1884). Wille (1882, 1886) reports their fusion with the pollen nucleus. Still others conjecture that degeneration is owing to lack of space for proper functioning (Juel, 1900; Häkansson, 1928; Piech, 1928; Tanaka, 1939; 1940).

Microspore Nucleus. In the majority of angiosperms the microspore nucleus comes to lie at one end of the cell due to the formation of a large vacuole. In the Cyperaceae, no vacuolation is seen in the microspores. Both asymmetrical and symmetrical spindles have been observed during the division of the functional nucleus. A cell plate laid between the two nuclei rests upon the septum separating the vegetative from the three effete nuclei.

According to Piech (1928) the division of the functional nucleus which gives rise to the generative and vegetative nuclei occurs in the centre of the pollen grain. Consequently the cell plate extends round the generative cell forming a complete sphere. Juel (1900) also reported a similar feature which he termed as " free cell formation ", but Stout (1912) did not support it.

Generative Cell. As mentioned earlier, the generative cell is not formed free in the cytoplasm but initially rests upon the plasma membrane separating the functional nucleus from the remaining three effete nuclei. Although some of Piech's figures (his text Fig. 8, Plate 2) shows a generative cell lying free in the cytoplasm, his other figure (his text Fig. 7, Plate 2) clearly indicates that the generative cell rests on the plasma membrane. *Rhyncospora* (Tanaka, 1940) is the only exception in which the generative cell is formed as a free cell due to the migration of the three effete nuclei on the reverse side to that of the generative cell. Further studies are necessary to decide this issue. Dnyansagar & Tiwari (1956) report that the young pollen grains of *Fimbristylis quinquangularis* have vacuolated cytoplasm and it is claimed that the functional nucleus which lies at first in the centre migrates to one side prior to its division. However, they do not give any figure in support.

Maheshwari (1949) writes that the pollen grains of the Cyperaceae are shed at 3-celled stage almost without exception. In the plants studied by the author the ' pseudomonads ' are always shed at the three-celled stage. However, Dnyansagar & Tiwari (1956) in *Fimbristylis quinquangularis* and Gupta (1959) in *Kyllingia triceps* report a 2-celled condition. Their observations are probably based on the study of immature pollen. It is surprizing that Tanaka (1950) reported the 3-celled condition only in *Carex capricornis.*

SUMMARY

Microsporogenesis and male gametogenesis have been studied in *Bulbostylis barbata*, *Kyllinga triceps*, *Cyperus articulatus*, *C. niveus*, *Eleocharis palustris*, *Scirpus maritimus* and *Carex wallichiana*.

Due to failure of cytokinesis after Meiosis II a tetranucleate mother cell is formed. Only one of the nuclei is functional and is delimited from the other three by an ephemeral plasma membrane. The abortive nuclei get further separated from each other by delicate membranes, and seem to have a feeble capacity for further divisions but eventually all the daughter nuclei coalesce together forming an irregular darkly stained black mass.

The pollen grain assumes queer forms after the second division. That this phenomenon of contraction is not due to plasmolysis has been checked by observations of fresh pollen grains. Piech (1924; 1928) and Tanaka (1937; 1950) did observe contracted grains but considered them to be abortive. The author has not observed the entire degeneration of the grains in any anther loculus.

The young microspores are richly cytoplasmic with a centrally situated nucleus. In the pollen grain cell plate separating the generative nucleus is not situated in the centre but along the wall separating the functional nucleus from the three effete nuclei. The 'pseudomonad' is shed at the 3-celled stage but the tube nucleus shows a tendency towards degeneration. Occasionally, double pollen grains or the four nuclei of a tetrad were observed in a healthy state.

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Embryology in Relation to Systematic Botany with Particular Reference to the Crassulaceae

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Bailey (1949) has rightly pointed out that if a natural system of classification is to be attained, it must be based upon " the analysis and harmonization of evidence from all organs, tissues and parts ". The recent researches from various disciplines of botany have clearly shown that contributions to systematics can come from any of these branches. Some of the branches which have played a significant role in plant taxonomy are embryology, wood and floral anatomy, palynology, cytology and cytogenetics and ecology.

Eminent botanists like Hofmeister (cited from Maheshwari, 1950) and Strasburger (1902), many years ago indicated the possibility of utilizing embryological characters in taxonomy. Unfortunately there was great delay in the recognition of its value because the preparation of the material for embryological study involves time and requires a great deal of patience and skill. If such studies are carried out carefully in a comparative manner it is possible to arrive at some conclusions of systematic significance. Embryological studies in the past fifty years have made rapid strides and the data accrued, considered along with information from other sources, can help to arrive at a natural system of classification. In this paper the importance of embryology in relation to systematic botany is presented with particular reference to the Crassulaceae.

In several important systems of classification (Bentham & Hooker, 1862-83; Bessey, 1915; Wettstein, 1935; Engler & Diels, 1936 and Rendle, 1952) the family Crassulaceae is placed in the order Rosales, closely adjacent to Saxifragaceae. Hutchinson (1926) includes the families Crassulaceae and Saxifragaceae in his order 14, Saxifragales under the division Archichlamydeae. Hallier (1912) treats Crassulaceae under Caryophyllineae. A comprehensive comparative investigation of six taxa of Sedum *(Crassulaceae)

**Sedum palmeri* S. Watson, *S. aizoon* L., *S. ternatum* Michx., *S. stenopetalum* Pursh. and *S. chrysanthum* (Boissier) Raymond-Hamlet

supplemented by available literature on the embryology of Crassulaceae was undertaken (Subramanyam, 1955) to assess how far embryological characters can be used in determining the relationship of this family with such closely allied families as the Saxifragaceae, Podostemaceae and Hydrostachyaceae. Bessey (1915) in his phylogenetic arrangement of flowering plants places the Rosales close to the Ranales. Hutchinson (1926) regards the Saxifragales as herbaceous groups closely connected with the Ranales but slightly more advanced. So an attempt has also been made to compare the embryological characters of Ranales and Saxifragales.

TABLE 1—RESEMBLANCES BETWEEN CRASSULACEAE AND SAXIFRAGACEAE

Embryological characters	Crassulaceae	Saxifragaceae
	Crété (1946a, b); Dahlgren (1927, 1939); Erdtman (1952); d'Hubert (1896); Johansen (1950); Martin (1946); Mauritzon (1930, 1933a); Souèges (1925, 1927); Present study	Dahlgren (1927, 1930, 1939); Erdtman (1952); Herr (1954); Johansen (1950); Martin (1946); Mauritzon (1933a); Raghavan & Srinivasan (1942); Souèges (1936b, c); Wiggins (1959)
Anther wall	Multilayered	Multilayered
Division of pollen mother cells	Simultaneous	Simultaneous
Number of nuclei in mature pollen grain	2	2
Number of germ pores in pollen grain	3-colporate	3-colporate to poly-colporate
Number of ovules	Many	Numerous
Ovule	Crassinucellate, bitegmic	Crassinucellate, bitegmic usually
Archesporial cells	One or more	One or more
Megaspore quartet	Linear, T-shaped, oblique T-shaped and isobilateral	Linear, T-shaped, oblique T-shaped
Embryo sac development	Polygonum type	Polygonum type
Fusion of polar nuclei	Before fertilization	Before fertilization
Antipodals	Usually 3 small cells	3 small cells
Starch grains	Present in embryo sac and sometimes even in egg cell	Sometimes present in embryo sac and occasionally in egg cell
Endosperm	Cellular	Cellular or Helobial or Nuclear
Endosperm haustorium	Present	Sometimes present
Embryo development	Sedum variation, Caryophyllad type	Sedum variation, Caryophyllad type

TABLE 2—DIFFERENCES IN EMBRYOLOGICAL CHARACTERS BETWEEN CRASSULACEAE AND SAXIFRAGACEAE

Embryological characters	Crassulaceae	Saxifragaceae
	Mauritzon (1930, 1933a); Present study	Dahlgren (1928, 1930, 1938); Herr (1954); Mauritzon (1933a); Raghavan & Srinivasan (1942); Souèges (1936a); Wiggins (1959)
Anther tapetum	Secretory type	Secretory or amoeboid type
Ovary	Apocarpous, superior	Syncarpous 1-3, sometimes even inferior
Ovule	Usually crassinucellate, bitegmic	Sometimes tenuinucellate, bitegmic or unitegmic
Integumentary tapetum or Endothelium	Absent	Sometimes present
Synergids	Not hooked	Usually hooked
Endosperm	Cellular always	Cellular, Helobial or Nuclear
Endosperm haustorium	Chalazal	Absent but when present may be chalazal or micropylar
Basal cell of two-celled proembryo	Does not divide but becomes large and vesicular in shape; encloses a prominent hypertrophied nucleus and develops haustorial processes	Slightly enlarges and divides into a group of cells; does not develop haustorial processes

Table 1 shows how the Crassulaceae resembles Saxifragaceae in a number of embryological characters. These evidences indicate that the families, Crassulaceae and Saxifragaceae are closely interrelated. Erdtman (1952) states : " Pollen grains ± similar to those in Crassulaceae occur in Rosaceae and Saxifragaceae, etc." The differences in the embryological characters of these two families are presented in Table 2. Furthermore, a survey of embryological characters shows that from an evolutionary point of view, the Saxifragaceae are more advanced than the Crassulaceae. Some of the more important of these characters noticed in the Saxifragaceae are : Syncarpy and epigyny; tenuinucellate ovule, which is sometimes unitegmic; and the development of an endothelium. It may also be recalled that these features are characteristic of the various families of the Sympetalae which are regarded as definitely advanced in the scale of evolution.

Podostemaceae and Hydrostachyaceae are two other families in the Rosales which are usually placed very close to the Crassulaceae and so a comparison of their embryological characters was made (Table 3) . It is interesting to find that such embryological characters as, presence of a large

number of tiny ovules, a narrow elongated nucellus with a small embryo sac situated at the apex and a characteristic behaviour of the basal cell which becomes vesicular and forms haustorial processes, are common to all these families. Further, the Podostemaceae resembles the Crassulaceae in having a secretory type of anther tapetum, binucleate pollen grain, presence of starch grains in both pollen and embryo sac and finally Dwarf type of internal morphology of the seed. It is also significant that the endosperm in both Crassulaceae and Hydrostachyaceae is cellular. In addition to these features *Crassula aquatica*, a member of the Crassulaceae, has a mode of life somewhat similar to that of Podostemaceae. It has the most reduced endosperm in the Crassulaceae and this may well form a transitional stage leading to the complete suppression of this tissue in the Podostemaceae. It may also be pointed out

TABLE 3—COMPARISON OF EMBRYOLOGICAL CHARACTERS OF CRASSULACEAE, PODOSTEMACEAE AND HYDROSTACHYACEAE

Embryological characters	Crassulaceae	Podostemaceae	Hydrostachyaceae
	Martin (1946); Mauritzon (1930, 1933a); Rombach (1911); Souèges (1927); Present study	Dahlgren (1927); Hammond (1937); Magnus (1913); Martin (1946); Mauritzon (1933b, 1939); Razi (1949); Went (1909, 1910, 1912, 1926, 1929)	Dahlgren (1927); Mauritzon (1933b, 1939); Palm (1915); Schnarf (1931); Warming (1882)
Anther tapetum	Secretory	Secretory	
Number of nuclei in mature pollen grain	2	2	
Size and number of ovules	Small and many	Small and many	
Shape of nucellus	Narrow and elongated	Narrow and elongated	
Position of embryo sac	Upper part of nucellus	Upper part of nucellus	
Starch grains	Present in mature pollen grain as well as embryo sac	Present in mature pollen grain only	Present in mature embryo sac only
Endosperm	Cellular	No endosperm	Cellular
Division of fertilized egg	Results in two unequal cells	Results in two unequal cells	
Basal cell of suspensor	Enlarges, becomes vesicular and forms a haustorium	Enlarges, becomes vesicular and forms a haustorium	
Seed (internal morphology)	Dwarf type	Dwarf type	

TABLE 4—DIFFERENCES IN THE EMBRYOLOGICAL CHARACTERS OF CRASSULACEAE, PODOSTEMACEAE AND HYDROSTACHYACEAE

Embryological characters	Crassulaceae	Podostemaceae	Hydrostachyaceae
	Erdtman (1952); Mauritzon (1930, 1933a); Souèges (1927); Present study	Erdtman (1945, 1952); Hammond (1937); Magnus (1913); Razi (1949); Went (1909, 1910, 1912, 1926, 1929)	Erdtman (1945, 1952); Palm (1915); Warming (1882)
Pollen grains	Single, 3-colporate	Single or united in dyads	United in tetrads, non-aperturate
Ovule	Crassinucellate, bitegmic	Tenuinucellate, bitegmic	Tenuinucellate, unitegmic
Pseudo embryo sac	Absent	Present	Absent
Development of embryo sac	Polygonum type	Reduced Allium type	Polygonum type
Number of polar nuclei	2	Usually a single polar nucleus or cell	2
Antipodals	3 small cells	Usually absent; rarely a single antipodal cell or nucleus	Cells (?)
Endosperm and haustorium	*ab inito* cellular; chalazal endosperm haustorium present	Absent right from the beginning	Cellular; endosperm haustorium absent
Basal cell of suspensor	Remains undivided; encloses a large hypertrophied nucleus and is not partitioned by a wall	Usually the nucleus divides and becomes coenocytic	At a later stage the coenocytic basal cell becomes cellular by the laying down of irregular walls

here that on the basis of embryological features Maheshwari (1945) has concluded that it is " almost certain that the Podostemaceae are much reduced apetalous derivatives of the Crassulaceae. "

At the same time it must be indicated that the Crassulaceae, Podostemaceae and Hydrostachyaceae show some differences in the embryological characters (Table 4).

The Podostemaceae differs strikingly from the Crassulaceae in having a highly reduced Allium type of embryo sac without antipodals (when present reduced to one), a new structure — the pseudo embryo sac and a complete lack of endosperm right from the beginning. These are probably derivative characters due to the special mode of life of these plants. Certain peculiarities in embryology are also found in other specialized families like the parasitic Loranthaceae (Correa, 1958; Dixit, 1958a, b; Maheshwari, Johri & Dixit, 1957; Narayana, 1958a, b; Rutihauser, 1937), Santalaceae (Paliwal, 1956; Ram, 1957, 1959a, b) and Balanophoraceae (Fagerlind, 1945), the

insectivorous Lentibulariaceae (Kaûsik, 1938; Khan, 1954), and in some of the mangroves (Treub, 1883; Mauritzon, 1939).

The present study has yielded some interesting data in respect of the origin of Saxifragales. A comparison of the important embryological characters of Crassulaceae, Saxifragaceae, Podostemaceae, Hydrostachyaceae and the various families of Ranales has been made (Tables 5, 5A, 5B, 5C and 5D). It is significant to find that the Crassulaceae and Saxifragaceae resemble the different families of the Ranales in having multi-layered anther wall; simultaneous division of pollen mother cells (except Lauraceae, Myristicaceae and Ceratophyllaceae, and some Annonaceae where it is of the successive type); two nucleate condition in mature pollen grain; apocarpous pistil; crassinucellate, bitegmic ovules; linear arrangement of megaspores; Polygonum type of embryo sac; fusion of polar nuclei before fertilization; three small antipodal cells (except Ranunculaceae and Berberidaceae, where they are large); and a cellular type of endosperm (except some Magnoliaceae *sensu stricto*, Lauraceae, Ranunculaceae and Berberidaceae, where it is nuclear). Further, multiple archesporial cells which are sometimes present in Crassulaceae and Saxifragaceae are also noticed in some families of the Ranales like Lauraceae, Ranunculaceae, Nymphaeaceae and Calycanthaceae. Starch grains in mature embryo sacs are reported to occur in some members of Crassulaceae, Saxifragaceae, Annonaceae, Ceratophyllaceae, Nymphaeaceae and Berberidaceae. Even the development of peculiar megaspore tubes noticed in two species of Crassulaceae is found in one member of Lauraceae (Bambacioni—Mezzetti, 1935). In Crassulaceae, after the first transverse division of the zygote, the basal cell enlarges and never divides further; this is also observed in Cercidiphyllaceae. These similarities in embryological features point towards a possible origin of Rosales (which includes the Saxifragales of Hutchinson) from the Ranales, thus supporting Bessey (1915) and Hutchinson (1926). The affinities of the Saxifragales from embryological grounds are brought out in the following diagram.

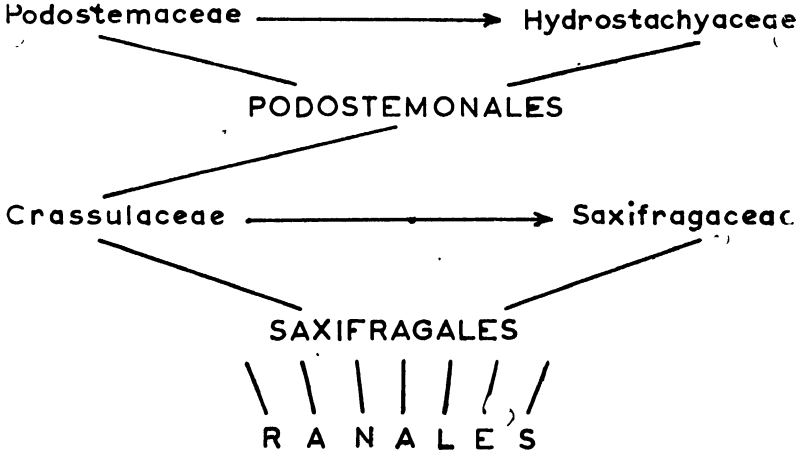

TABLE 5—COMPARISON OF EMBRYOLOGICAL CHARACTERS OF CRASSULACEAE, SAXIFRAGACEAE, PODOSTEMACEAE AND HYDROSTACHYACEAE OF ROSALES

ENGLER & PRANTL, 1930	ROSALES			
HUTCHINSON, 1926	SAXIFRAGALES		PODOSTEMONALES	
	Crassulaceae	Saxifragaceae	Podostemaceae	Hydrostachyaceae
	Crété (1946a, b)	Dahlgren (1927,	Dahlgren (1927)	Dahlgren (1927)
	Dahlgren (1927, 1939)	1930, 1939)	Erdtman (1945, 1952)	Erdtman (1945, 1952)
	Erdtman (1952)	Erdtman (1952)	Hammond (1937)	Mauritzon (1933b,
	Johansen (1950)	Herr (1954)	Magnus (1913)	1939)
	Martin (1946)	Johansen (1950)	Martin (1946)	Palm (1915)
	Mauritzon	Martin (1946)	Mauritzon	Schnarf (1931)
	(1930, 1933a)	Mauritzon	(1933b, 1939)	Warming (1882)
	Souèges (1925,	(1933a)	Razi (1949)	
	1927)	Raghavan &	Went (1909, 1910	
	Present study	Srinivasan (1942)	1912, 1926, 1929)	
		Souèges (1936b, c)		
		Wiggins (1959)		
Anther wall	5-layered	4-layered	4-layered	
Anther tapetum	Secretory type	Secretory or amoeboid type	Secretory type	
Division of pollen mother cells	Simultaneous	Simultaneous	Successive	
Number of nuclei in mature pollen grain	2	2	2	
Pollen grains single or united	Single	Single or rarely united in tetrads	Single or united in dyads	United in tetrads
Number of germ pores in pollen grain	3-colporate	Usually 3-colporate	3-colpate	Non-aperturate
Ovary	Apocarpous	1-3, syncarpous	1-3, syncarpous	1
Number of ovules	Many	Numerous	Numerous	Numerous
Ovule	Crassinucellate, bitegmic	Usually crassinucellate, bitegmic; sometimes tenuinucellate, bi- or unitegmic	Tenuinucellate, bitegmic	Tenuinucellate, unitegmic
Number of archesporial cells	One or more	One or more	One or two	One

TABLE 5 (*Contd.*)

ENGLER & PRANTL, 1931	ROSALES			
HUTCHINSON, 1926	SAXIFRAGALES		PODOSTEMONALES	
	Crassulaceae	Saxifragaceae	Podostemaceae	Hydrostachyaceae
Nature of megaspore quartet	Linear, T-shaped, oblique T-shaped; sometimes isobilateral	Linear, T-shaped, oblique T-shaped	No linear quartet	Linear
Development of embryo sac	Polygonum type	Polygonum type	Reduced Allium type	Polygonum type
Starch grains in mature embryo sac	Present	Present	Present	Present
Number and fusion of polars	2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization	Usually a single polar nucleus or rarely a polar cell	2 polar nuclei fuse before fertilization
Antipodals	3 cells, small, rarely large	3 cells, small	Usually absent, rarely a single antipodal cell or nucleus, small	Cells (?)
Nature of endosperm	Cellular	Cellular, Helobial or Nuclear	No endosperm	Cellular
Endosperm haustoria	Present, usually chalazal	Present, micropylar or chalazal	Absent	Absent
Development of embryo	Sedum variation, Caryophyllad type	Usually of the Sedum variation, Caryophyllad type		
Basal cell of embryo	Enlarges, does not divide; develops haustorial processes	Enlarges, but divides into a group of cells; does not develop haustorial processes	Enlarges and the nucleus divides, thus becoming coenocytic; develops haustorial processes	Enlarges, becomes coenocytic and later septate; develops into a haustorium
Nature of fruit	Follicle	Capsule	Septicidal capsule	Small capsule
Seed (internal morphology)	Dwarf type	Dwarf type, sometimes of the linear or spatulate type	Dwarf type	

Vacant columns mean no information available so far.

TABLE 5A—COMPARISON OF THE EMBRYOLOGICAL CHARACTERS OF MAGNOLIACEAE, TROCHODENDRACEAE, CERCIDIPHYLLACEAE AND ANNONACEAE OF RANALES

Engler & Prantl, 1930	Ranales			
Hutchinson, 1926	Magnoliales			Annonales
	Magnoliaceae* (*Sensu lato*)	Trochodendraceae	Cercidiphyllaceae	Annonaceae
	Bailey & Nast (1943a, b, 1945) Bailey & Smith (1942)	Erdtman (1952) Martin (1946) Nast & Bailey (1945)	Erdtman (1952) Swamy & Bailey (1949)	Asana & Adatia (1947) Corner (1949) Dahlgren (1927, 1939)
	Canright (1952, 1953, 1955, 1959)			Erdtman (1945, 1952)
	Earle (1938)			Martin (1946)
	Erdtman (1945, 1952) Johansen (1950)			Periasamy & Swamy (1956, 1959)
	Manewal (1914) Martin (1946)			Sastry (1955a, 1957a, b)
	Schnarf (1931)			Schnarf (1931)
	Swamy (1949, 1952)			
Anther wall	5-6 to many layered			5-6 layered
Anther tapetum	Secretory type		Secretory type	Secretory type
Division of pollen mother cells	Simultaneous		Simultaneous	Simultaneous or successive
Number of nuclei in mature pollen grain	2		2	2
Pollen grains single or united	Single, sometimes adhere in tetrads	Single	Single	Single or united in tetrads
Number of germ pores in pollen grain	1-sulcate	3(-4)-colporoidate	3-colpate	Non-aperturate or 1-sulcate
Ovary	Apocarpous or syncarpous or solitary	Almost apocarpous	Apocarpous	Apocarpous

TABLE 5A (*Contd.*)

ENGLER & PRANTL, 1930	RANALES			
HUTCHINSON, 1926		MAGNOLIALES		ANNONALES
	Magnoliaceae*	Trochodendraceae	Cercidiphyllaceae	Annonaceae
Number of ovules	1,2 or more	Several to 1	Many	Many to one
Ovule	Crassinucellate, bitegmic	Crassinucellate, bitegmic	Crassinucellate, bitegmic	Crassinucellate, bitegmic
Number of archesporial cells	One		One	One
Nature of megaspore quartet	Linear		Linear	Linear
Development of embryo sac	Polygonum type		Polygonum type	Polygonum type
Starch grains in mature embryo sac	Absent		Absent	Present
Number and fusion of polars	2 polar nuclei fuse before fertilization		2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization
Antipodals	3 cells, small		3 cells, small	3 cells, small
Nature of endosperm	Cellular, sometimes nuclear	Cellular	Cellular	Cellular
Endosperm haustoria	Absent	Absent	Absent	Absent
Development of embryo	Onagrad type			Onagrad type
Basal cell of embryo	Divides into a group of cells		Enlarges but does not divide	Divides into a group of cells
Nature of fruit	Follicle, capsule or berry	Follicle which is dehiscent or samaroid	Cluster of 2-6 follicles	Etaerio
Seed (internal morphology)	Rudimentary type; sometimes of the linear type	Spatulate type		Linear type

*Includes available information on Winteraceae and Degeneriaceae. Vacant columns mean no information available so far.

TABLE 5B—COMPARISON OF EMBRYOLOGICAL CHARACTERS OF MONIMIACEAE, LAURACEAE AND MYRISTICACEAE OF RANALES

ENGLER & PRANTL, 1930	RANALES		
HUTCHINSON, 1926	LAURALES		
	Monimiaceae* (*Sensu lato*)	Lauraceae	Myristicaceae
	Bailey & Swamy (1948, 1949)	Erdtman (1952)	Erdtman (1952)
	Erdtman (1945, 1952)	Johansen (1950)	Joshi (1946)
		Schnarf (1931)	Schnarf (1931)
		Schroeder (1952)	Voigt (1888)
		Stern (1954)	Sastry (1955b)
	Money, Bailey & Swamy (1950)	Sastry (1956, 1958)	
Anther wall	5-6 layered	4-5 layered	5-layered
Anther tapetum	Secretory type	Secretory or amoeboid	Secretory type
Division of pollen mother cells	Simultaneous	Successive	Successive
Number of nuclei in mature pollen grain	2	2	2
Pollen grains single or united	Single, rarely united in tetrads	Single	Single
Number of germ pores in pollen grain	Non-aperturate or 2 (-3) sulcate or monocolpate	Non-aperturate	1-sulcate
Ovary	Usually apocarpous	One	One
Number of ovules	One	One	One
Ovule	Crassinucellate, bitegmic	Crassinucellate, bitegmic	Crassinucellate, bitegmic
Number of archesporial cells		One or several	
Nature of megaspore quartet		Linear	
Development of embryo sac		Polygonum type	
Starch grains in mature embryo sac		Absent	
Number and fusion of polars		2 polar nuclei fuse before fertilization	
Antipodals		3 nuclei or cells, inconspicuous	
Nature of endosperm	Cellular	Nuclear	Nuclear
Endosperm haustoria		Absent	Absent
Development of embryo		Piperad or Asterad or Onagrad type	
Basal cell of embryo		Divides into a group of cells	
Nature of fruit	Drupaceous	Baccate or Drupaceous	Fleshy, dividing by two valves
Seed (internal morphology)			

* Includes available information on Amborellaceae. Vacant columns mean no information available so far.

TABLE 5C — COMPARISON OF EMBRYOLOGICAL CHARACTERS OF RANUNCULACEAE, CERATOPHYLLACEAE AND NYMPHAEACEAE OF RANALES

ENGLER & PRANTL, 1930	RANALES		
HUTCHINSON, 1926	Ranunculaceae	Ceratophyllaceae	Nymphaeaceae
	Coulter (1898)	Dahlgren (1927)	Cook (1902, 1906, 1909)
	Earle (1938)	Erdtman (1952)	
	Erdtman (1952)	Johansen (1950)	Dahlgren (1927)
	Hätfliger (1943)	Schnarf (1931)	Erdtman (1945, 1952)
	Johansen (1950)	Strasburgher(1902)	Johansen (1950)
	Martin (1946)		Martin (1946)
	Mottier (1895)		Schnarf (1931)
	Schnarf (1931)		
	Singh (1936)		
	Souèges (1934)		
Anther wall	4-layered	4-layered	
Anther tapetum	Secretory type	Amoeboid type	Secretory type
Division of pollen mother cells	Simultaneous	Successive	Simultaneous
Number of nuclei in mature pollen grain	2, 3	2	3
Pollen grains single or united	Single	Single	Single, rarely united in tetrads
Number of germ pores in pollen grain	(2-)3-colpate	Non-aperturate	Usually 3-colpate
Ovary	Apocarpous	One	Apocarpous or syncarpous
Number of ovules	Numerous	One	Many to one
Ovule	Crassinucellate, rarely tenuinucellate, uni- or bitegmic	Crassinucellate, unitegmic	Crassinucellate, bitegmic
Number of archesporial cells	Several or 1	One	1 or several
Nature of megaspore quartet	Linear, T-shaped, oblique T-shaped	Linear, T-shaped, oblique T-shaped	Linear
Development of embryo sac	Polygonum type	Polygonum type	Polygonum type
Starch grains in mature embryo sac	Absent	Present	Present
Number and fusion of polars	2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization
Antipodals	3 cells, large	3 cells, small	3 cells, small
Nature of endosperm	Nuclear	Cellular	Cellular, Helobial (?)
Endosperm haustoria	Absent	Absent	Chalazal haustorium present when cellular
Development of embryo	Onagrad type	Asterad type	Asterad type
Basal cell of embryo	Divides into a group of cells	Divides into a group of cells	Divides into a group of cells
Nature of fruit	Bunches of follicle or dry achenes or berry	Nut	Indehiscent pod or spongy berry
Seed (internal morphology)	Rudimentary type, sometimes of the linear type		Broad type

Vacant columns mean no information available so far.

TABLE 5D—COMPARISON OF EMBRYOLOGICAL CHARACTERS OF BERBERIDACEAE, LARDIZABALACEAE, MENISPERMACEAE AND CALYCANTHACEAE OF RANALES

ENGLER & PRANTL, 1930	RANALES			
HUTCHINSON, 1926	Berberidaceae	BERBERIDALES Lardizabalaceae	Menispermaceae	ROSALES Calycanthaceae
	Clark (1923)	Erdtman (1952)	Erdtman (1952)	Erdtman (1945,
	Dahlgren (1927)	Schnarf (1931)	Joshi (1937,	1952)
	Erdtman (1945, 1952)	Swamy (1953)	1939) Joshi & Raman	Schnarf (1931)
	Johansen (1950)		Rao (1935)	
	Johri (1935)		Martin (1946)	
	Martin (1946)		Sastri (1954a, b)	
	Mauritzon (1936)			
	Schnarf (1931)			
Anther wall	5-6 layered	5-layered	5-layered	
Anther tapetum	Secretory type	Secretory type	Secretory type	Secretory type
Division of pollen mother cells	Simultaneous	Simultaneous	Simultaneous	Simultaneous
Number of nuclei in mature pollen grain	2	2	2	
Pollen grains single or united	Single, sometimes united in tetrads	Single	Single	Single, sometimes united in tetrads
Number of germ pores in pollen grain	3-colpate	3-colpate	3-colpate or 3-colporate	2-3-sulcate
Ovary	One	Apocarpous	Apocarpous	Apocarpous
Number of ovules	Few to numerous	Numerous or solitary	Two to 1	Solitary or 2, superposed
Ovule	Crassinucellate, sometimes tenuinucellate, bitegmic	Crassinucellate, bitegmic	Crassinucellate, bitegmic	Crassinucellate, bitegmic
Number of archesporial cells	One	One	One	One to several
Nature of megaspore quartet	Linear	Linear	Linear	Linear
Development of embryo sac	Polygonum type	Polygonum type	Polygonum type	Polygonum type
Starch grains in mature embryo sac	Present	Absent	Absent	

TABLE 5D (*Contd.*)

ENGLER & PRANTL 1930	RANALES			
HUTCHINSON, 1926	Berberidaceae	BERBERIDALES Lardizabalaceae	Menispermaceae	ROSALES Calycanthaceae
Number and fusion of polars	2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization	2 polar nuclei fuse before fertilization	
Antipodals	3 cells, large	3 cells, small	3 cells, small	3 small
Nature of endosperm	Nuclear	Cellular	Nuclear	Cellular
Endosperm haustoria	Absent	Absent	Absent	
Development of embryo	Onagrad type		Onagrad type	
Basal cell of embryo	Divides into a group of cells		Divides into a group of cells	
Nature of fruit	Berry or capsule	Fleshy, indehiscent or dehiscent	Drupaceous	Achene
Seed (internal morphology)	Usually linear type; sometimes spatulate or rudimentary type		Usually linear type; sometimes spatulate type	

Vacant columns mean no information available so far.

SUMMARY

In several important systems of classification the Crassulaceae is placed in Rosales, closely adjacent to Saxifragaceae. Hutchinson includes Crassulaceae and Saxifragaceae in Saxifragales under Archichlamydeae. A comprehensive investigation on six taxa of *Sedum* (Crassulaceae) supplemented by available literature on the embryology of Crassulaceae was made with a view to find out how embryological characters can be used in determining the relationship of this family to such closely allied families as the Saxifragaceae, Podostemaceae and Hydrostachyaceae. Bessey places the Rosales close to the Ranales. Hutchinson regards the Saxifragales as herbaceous groups closely connected with the Ranales but slightly more advanced. The embryological characters of Ranales and Saxifragales have also been compared.

It is seen that the Crassulaceae resembles Saxifragaceae in a number of embryological characters which indicate that the Crassulaceae and Saxifragaceae are closely interrelated. Furthermore, a survey of embryological characters shows that from an evolutionary point, the Saxifragaceae are more advanced than the Crassulaceae.

Podostemaceae and Hydrostachyaceae which are usually placed very close to the Crassulaceae resemble the latter in a number of embryological characters. *Crassula aquatica* has a mode of life somewhat similar to Podostemaceae.

A comparison of the important embryological characters of Crassulaceae, Saxifragaceae, Podostemaceae, Hydrostachyaceae and the various families of Ranales has been made. It is significant to find that the Crassulaceae and Saxifragaceae have a number of embryological characters in common with different families of Ranales, thus supporting the views of Bessey and Hutchinson.

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The Embryo of Monocotyledons: A Working Hypothesis from a New Approach

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" In the early stages of development, the proembryo stages, the embryos of dicotyledons and monocotyledons follow similar sequences of cell division, and both become cylindrical or club-shaped bodies. The difference in development becomes evident when the formation of the cotyledon begins. In the absence of a second cotyledon the monocotyledon embryo does not become two-lobed at the distal end..." This quotation from Esau (1960) accurately sums up our contemporary understanding of the relationships of the monocotyledonous and dicotyledonous embryos.

If the early stages of embryonal development in the two groups of angiospermous taxa are alike, then at what precise step and in what manner does the divergence manifest itself ? Adequate answers to such queries have not yet been put forward.

Leaving aside those plants where the first division of the zygote is either by a vertical or oblique wall (such instances being rare) the partition wall is laid down in a transverse plane in the large majority of angiosperms, thus giving rise to a superposed arrangement of the daughter cells. Of these, the cell that is nearest the micropyle is the basal cell (conventionally designated *cb*) and the one that is away from the micropyle is the terminal cell (conventionally designated *ca*). During the division of these cells the wall is laid down generally in transverse or in longitudinal plane. The subsequent mode of segmentation and behaviour of the daughter cells of the basal cell (*cb*) are variable; on the other hand, the ontogenetic sequences in the daughter cells of the terminal cell (*ca*) are more stabilized. In the four-celled proembryo the cells are generally arranged either in a linear row (Fig. 1 A) or in a T-shaped manner in which the daughter cells of the terminal tier are adjacently placed in a single tier (Fig. 1 B).

In the linear kind of four-celled proembryo, the superposed daughter cells of *ca* first divide by a vertical wall, and the resulting cells again by another set of walls in the same plane but oriented at right angles to the previous

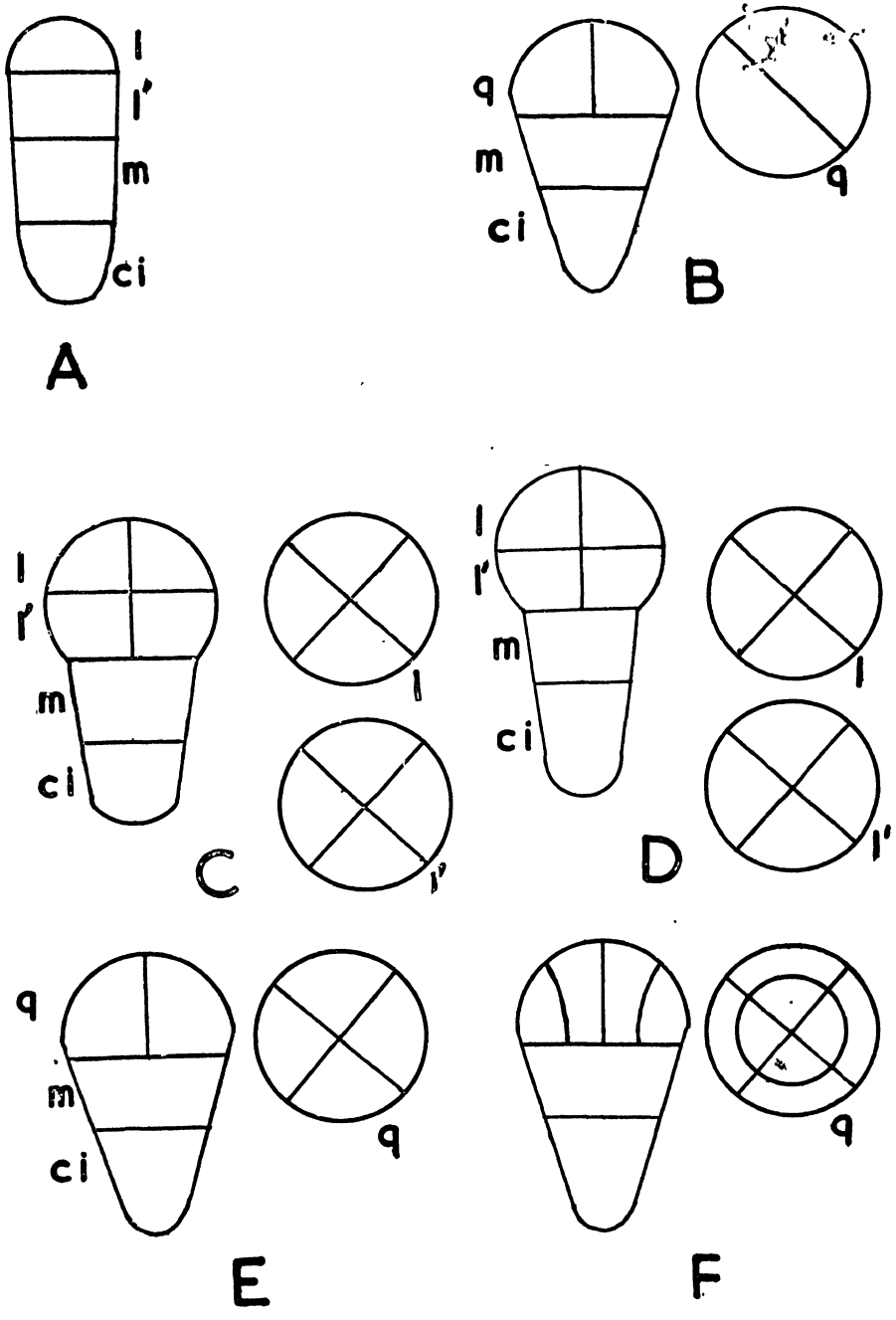

FIG. 1 — FORMATION OF OCTANTS

partition. Thus, a body of eight cells (octant) is formed in which the cells are arranged in two superposed tiers (*l* and *l'*) of four cells each; the component cells of each tier conform to the isobilateral plane of construction (Fig. 1 C). The formation of the octant stage in a T-shaped proembryo follows either of the two courses as follows: (i) The adjacently placed cells divide by a vertical wall that is at right angles to the previous partition, thus giving rise to a four-celled tier. These cells undergo the next division by transverse walls, thereby producing the octant stage. The component cells thus become disposed in two superposed tiers (*l* and *l'*) of four cells each. The end product resembles the octant derived from the linear type of four-celled proembryo (Fig. 1 D). (ii) The first division of the adjacently placed cells results in a four-celled tier as described for the previous course (Fig. 1 E). The next division in each of the cells is accompanied by the deposition of a wall in the periclinal plane. Thus an octant is produced in which an axial group of four cells becomes confronted peripherally by their sister cells (Fig. 1 F).

Thus, in the angiosperms in general, two types of octant configurations are seen irrespective of the category of the four-celled proembryo from which they are derived : (i) the component cells are disposed in two superposed tiers of equal number of cells in each tier, and (ii) all the eight cells are disposed in a single tier. The diverse methods through which the octant stage is reached is also common to both monocotyledons and dicotyledons. In this respect the embryos of these two groups of flowering plants bear obvious similarity until the attainment of the octant stage. At this stage of embryogenesis the destinations of the component cells become determined, thereby laying the foundation for future histogenesis and morphogenesis.

The share contributed by the derivatives of the basal and terminal cells of the two-celled proembryo toward the construction of the mature embryo is highly variable in angiosperms. In fact, the relative quantum of cells and tissues produced by these two cells has formed one of the major criteria for the delimitation of the presumed types of embryogenesis (*see* Johansen, 1950). At one end of the gamut of variability are the embryos wherein both the basal and the terminal cells contribute a somewhat equal share, while at the other end stand those embryos wherein the whole of their body is built solely by the derivatives of the terminal cell, the basal cell persisting as a suspensor. In all cases, however, the ultimate tier of the octant (if the component cells are disposed in two tiers) or the entire plate of octant cells functions as the seat of origin and differentiation of the cotyledons as well as of the shoot apex (epicotyl); frequently the function is shared by the terminal and subterminal tiers, the former engendering the shoot apex and the latter engendering the cotyledons. Therefore, in the dicotyledons as a whole, the ultimate tier of the octant or the octant itself may be looked upon as the telescoped shoot system of the plant.

Two methods of initiation of the shoot apex are generally encountered amongst the dicotyledons irrespective of the disposition of the octant cells: (i) directly from a group of cells of the terminal tier (Fig. 2 A), and (ii) through the formation of an epiphysis which in turn functions as the initial (Fig. 2 B).

The cotyledons also arise generally from the same tier that gives rise to the shoot apex. Frequently, however, as in the Papaveraceae, Gymnospermae (*pro parte*), Gesneriaceae and others the cotyledons originate from the subterminal tier (Fig. 2 C).

The recognized similarity in the early proembryonic development in monocotyledons and dicotyledons has led the embryologists to draw certain conclusions regarding the identity of octants in the two taxa. They visualize

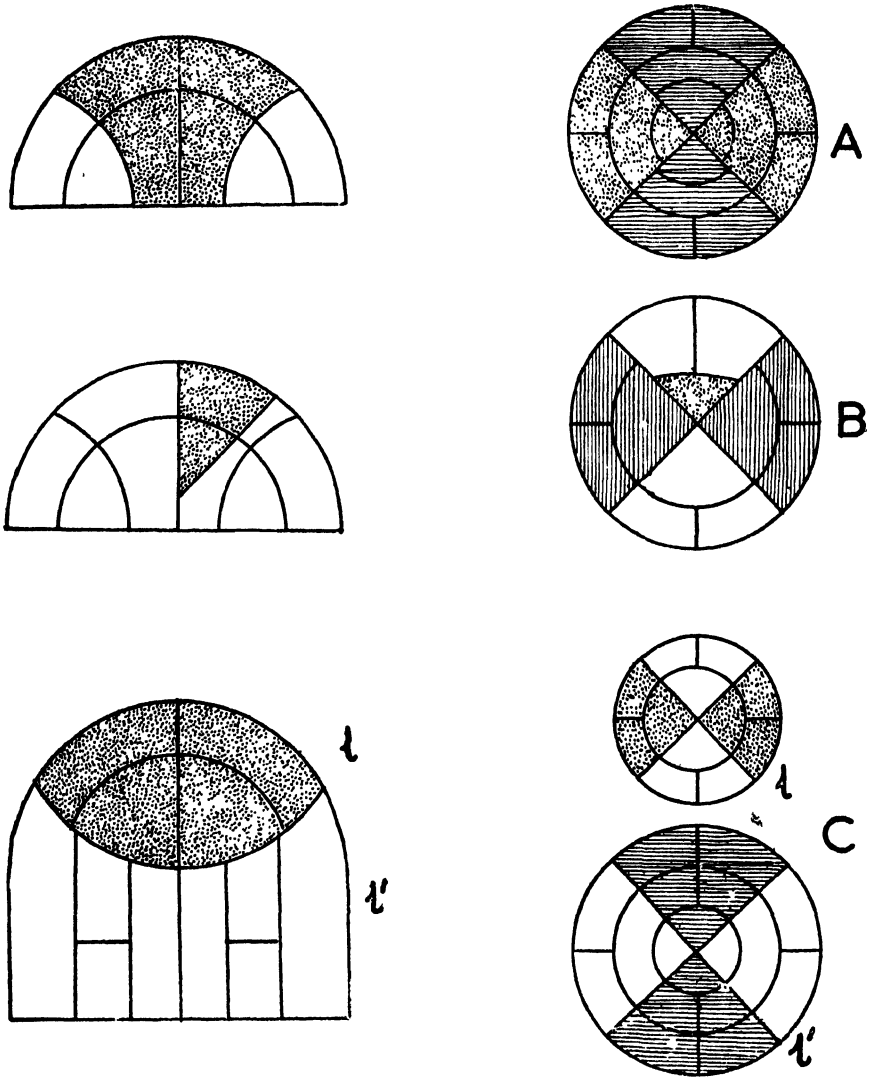

FIG. 2 — FORMATION OF SHOOT APEX (STIPPLED) AND COTYLEDONS (HATCHED IN DICOTYLEDONS). LEFT HAND COLUMN — MEDIAN LONGISECTIONS; RIGHT HAND COLUMN — CORRESPONDING TRANSECTIONS OF TIERS

two very different ontogenetic courses of the octant, the courses being specific to the taxon. Thus, what would have engendered a pair of lateral seed leaves (i.e. cotyledons) and terminal shoot apex in the dicotyledons develops into a single terminal cotyledon in the monocotyledons. The shoot apex in the latter group of plants, however, has been consistently described as arising from a lateral locus in the subterminal tiers, *m* or *l'* (Fig. 4). It is rather disappointing that in spite of the carefully documented studies on embryogenesis of angiosperms that have become available, very little attention has been devoted to the origin and early ontogeny of the cotyledons and of the future epicotyledonary part. Most contributors have contented themselves by giving a rough idea of the regions of the proembryo engendering the cotyledons. There are, however, a few investigators who have painstakingly and meticulously followed the detailed development of the cotyledons (Noll, 1935; Steffen, 1952; some contributions of Souèges and of his pupils).

In the dicotyledons, the initials of the stem apex and of the cotyledons are delimited at the octant stage itself. After the formation of dermatogen (protoderm) in the terminal, somewhat hemispherical tier (Fig. 3 A), the inner derivatives (the cotyledonary initials) divide by periclinal walls, thereby delimiting the outer *mc* and the inner *mv* (Fig. 3 B). Anticlinal divisions follow in the outermost and in the middle layers; divisions in the cell *mv* are essentially in the periclinal plane. As a result, the derivatives of the dermatogen, *mc* and *mv* give rise respectively to the epidermis, periblem and plerome of the cotyledons (Fig. 3 C, D). Due to this mode of growth, the cotyledonary primordia become raised above the level of the shoot apex initials (Fig. 3 D, E). The latter group of cells remains relatively quiescent, or if the cells divide, the divisions are few and the walls laid down are essentially in the anticlinal plane. In either case, the cotyledonary primordia grow in diverging directions and the terminal part of the embryo becomes notched while the embryo as a whole assumes a heart-shape (Fig. 3 E).

A review of available literature on the behaviour of the terminal tier in the monocotyledons leads to the recognition of two extreme patterns as follows :

A. After the delimitation of cells corresponding to those of the initials of the stem apex and cotyledons (Fig. 3 A) the divisions in the latter group of cells follow the same pattern as in the dicotyledons. The initials of the 'shoot apex', however, do not remain quiescent but divide simultaneously with the 'cotyledonary' initials (Fig. 3 F); the planes of cell division also simulate the pattern occurring in the cotyledonary initials of the dicotyledons. As a cumulative result, the hemispherical terminal tier as a whole expands *pari passu*; the general cell alignment in median longisections appears somewhat fan-shaped (Fig. 3 G). The subsequent growth, however, takes place in the direction of the vertical axis (Fig. 3 H).

B. The early planes of division in the cells corresponding to the initials of the shoot apex and cotyledons are predominantly transverse, divisions in other planes occurring sporadically (Fig. 3 I, J). Thus, from the beginning, the growth of the terminal tier is caused essentially in the direction of the proembryonal axis.

It should be noted that in both the extreme patterns of variability described above as well as in the intergrading kinds the cells corresponding to the initials of the dicotyledonous shoot apex do not remain quiescent, but keep pace in meristematic activity with the cells that correspond to the cotyledonary initials; in some cases, the bulk of tissue produced by the 'shoot apex initials' exceeds that engendered by the 'cotyledonary initials'. How-

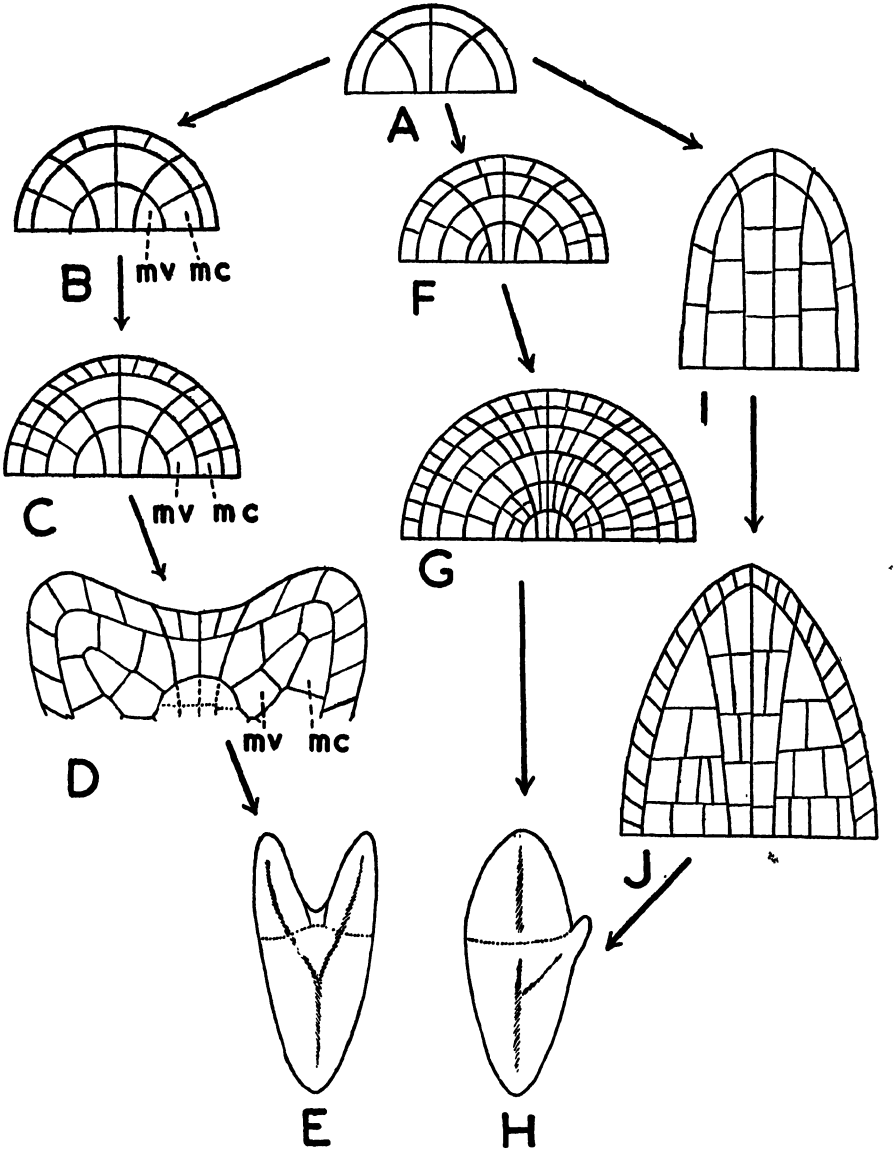

FIG. 3 — DEVELOPMENT OF THE TERMINAL TIER IN DICOTYLEDONS (B–E) AND MONOCOTYLEDONS (F–H)

ever, it is only in the initial stages of growth that the derivatives of the two types of initials are likely to be recognized distinctly, because, the borders merge with one another at later stages. The *en bloc* derivatives assume the form of a stub, and the embryo as a whole becomes somewhat cylindric or spindle-shaped (Fig. 3 H).

On the basis of available literature, the significant difference in the embryogeny of dicotyledons and monocotyledons thus appears to concern the behaviour of the hemispherical terminal tier, which is the potential locus of initiation of the cotyledons as well as of the shoot apex. In the dicotyledons, this tier engenders the corresponding structures while in the monocotyledons it is supposed to develop into a single cotyledon.

Considerations such as these raise a basic question. Are we justified in accepting that all the derivatives of the terminal tier of the monocotyledonous octant as representing a single cotyledon and in asserting that it occupies a terminal position in the embryo ? Judging from the consensus of opinion in regard to the homology of octants, it is to be assumed that both the cotyledonary and stem apical initials become involved in the morphological expression of the so-called " single terminal cotyledon ". Thus, it follows that, as compared with dicotyledons, the conventional cotyledon of the monocotyledons is identical and homologous with the complex of cells that builds the two cotyledons as well as the shoot system in the former taxon of angiosperms. The 'cotyledon' of the monocotyledons attains considerable morphological prominence in that it becomes exomorphologically differentiated into the so-called sheath, lamina, etc. In non-endospermous seeds it functions as a storage organ and in endospermous seeds, presumably as an absorptive organ, the feeder, foot, or sucker.

A strict adherence to the current opinion necessitates the following argument : The initials of the shoot apex and of the cotyledons having failed to produce normal structures in normal topography a new functional shoot meristem has become substituted in the monocotyledonous embryo. The locus of initiation of this meristem has invariably been assumed to lie in a lateral position either in the tier *m* or *l'*, both tiers being posterior to the so-called cotyledon (Fig. 4).

There are, however, stray records of the monocotyledons, wherein the shoot apex is said to arise from a terminal locus as in the dicotyledons, thereby contending that both the cotyledons and the stem apex are engendered by a common group of initials. Such a condition has been recorded by Solms-Laubach (1878) in *Tinnantia* and *Heterachtia* (Commelinaceae), by Campbell (1897) in *Zannichellia* (Zannichelliaceae), by Süessenguth (1921) and Goebel (1933) in *Tradescantia* (Commelinaceae), by Haccius (1952) in *Ottelia* (Hydrocharitaceae) and by Baude (1956) in *Stratiotes* (Hydrocharitaceae). The conclusions reached by Solms-Laubach and by Campbell, probably by virtue of their belonging to a period when the technique of preparation of material was inadequate and the methodology of the study of embryogenesis had not become standardized, have failed to appeal to modern embryologists. Because of the restricted scope of the studies of Süessenguth and Goebel,

their reports have also been viewed with suspicion; recent detailed studies on *Commelina forskalaei* (Maheshwari & Baldev, 1958), *Commelina communis* and *Rhoeo discolor* (Souèges, 1958a, b) have asserted the terminal origin of the cotyledon and the lateral origin of the shoot apex. A re-assessment of Haccius' text and illustrations has led Souèges (1954) to categorically conclude that the origin of the stem apex in *Ottelia* is truly lateral; this author has also re-affirmed such a condition to be present in *Sagittaria* and *Potamogeton*.

The concept of the division of flowering plants into two major categories on the basis of the number of cotyledons was codified by John Ray in 1682. This idea soon gained momentum and for more than the past 275 years has been the established foundation for prolific growth of diversified plant sciences, —taxonomy, general and comparative morphology, embryology, anatomy and histology. In the flush of enthusiasm that accompanied the post-Darwinian period, the entire edifice of phylogeny and discussions on the interrelationships of angiosperms have been and are being elaborated with implicit faith in the premise. As a result, unwarranted and unlimited controversies have centred around the nature of the monocotyledonous embryo for over half a century. The crux of the problem has been to find a way of reconciling the disparity not only in the number of cotyledons in the two groups of angiosperms but also the terminal origin of the cotyledon in the monocotyledons. All explanations and arguments that have been put forward in this regard have furthermore stemmed from yet another premature assumption that living monocotyledons have been derived from some extant dicotyledonous ancestors. Thus, by an unquestioned acceptance of a long-established assumption together with a hypothetical surmise the embryologists have reached a *status quo*.

The proponents of one school maintain that the single cotyledon of the monocotyledons is a product of fusion of the two cotyledons of dicotyledons,

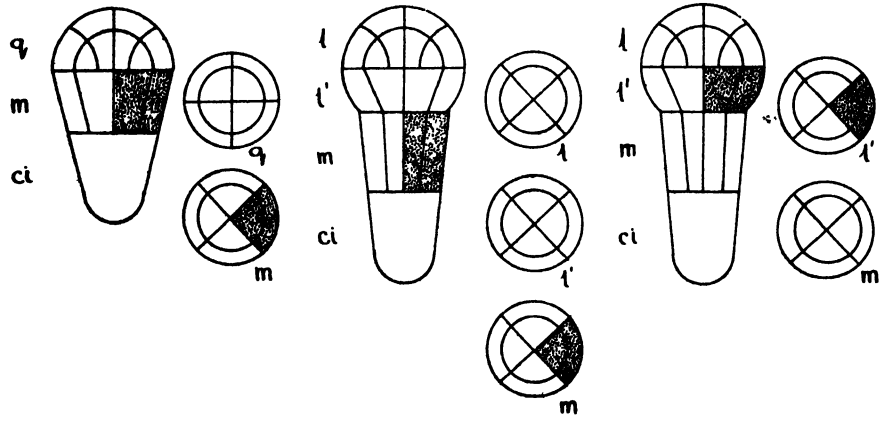

FIG. 4—ORIGIN OF SHOOT APEX (STIPPLED) IN MONOCOTYLEDONS. MEDIAN LONGISECTIONS AND TRANSECTIONS OF TIERS AT THE OCTANT STAGE OF THE PROEMBRYO

while those of the other school contend that the suppression or abortion of one of the cotyledons of dicotyledons has led to monocotyledonous condition. The existing ontogenetic evidence fails to support either view. Thus. if it is taken to be a fusion of cotyledons only, we are faced with the fact that it is not a fusion of these two structures alone, but it is an amalgamation of the two cotyledons *plus* the shoot apex enclosed between them. If it is taken to be a process of suppression of one of the cotyledons, we are faced with the problem of discovering the second cotyledon.

A word of comment is necessary in regard to the instances of ' monocotyledonous ' dicotyledons or of dicotyledons possessing one normally developed and the other somewhat ill-developed cotyledons that have often been adduced as representing transitional stages in the attainment of monocotyledony. That such endeavours are contemporaneous is instanced by the publication of Haccius (1954) on *Claytonia virginica*. Although there appears to be an apparent plausibility in this type of argument, more complete discussion is not possible for want of ontogenetic data on the monocotyledons. However, strictly following the current concepts, the so-called cotyledon of the monocotyledons cannot be considered to be the homologue of either one or both the cotyledons of the dicotyledons, as the so-called cotyledon of the monocotyledons represents the consolidated product of three distinct structures, the two cotyledons and the primary shoot apex. Thus, the latent cotyledonary number in the monocotyledons also should be two.

The salient points of the foregoing discussion may be briefly stated as follows :

1. In both dicotyledons and monocotyledons there is general agreement in regard to the obviousness of ontogenetic similarity until the attainment of the octant stage.

2. In both the groups of flowering plants, on the basis of contemporary understanding, the initials of the two cotyledons and of the terminally positioned shoot apex are recognizable.

3. In the dicotyledons the derivatives of the concerned initial cells engender the corresponding structures. In the monocotyledons, on the other hand, the derivatives should be considered as having become consolidated into a morphogenetically sterile structure which has been conventionally identified as the single terminal cotyledon.

4. In the dicotyledons the cotyledonary initials begin growth early while those of the shoot apex remain relatively quiescent at least until the cotyledonary growth is well ahead. In the monocotyledons, on the contrary, the growth of the ' shoot apex initials ' and of the ' cotyledonary ' initials begins simultaneously, the derivative cells soon merging into a single structure. Therefore, the monocotyledons also should be presumed to possess fundamentally two cotyledons.

5. Too much reliance on the concept of the monocotyledonous embryo as possessing a single terminal cotyledon and on the supposed dicotyledonous ancestry has obscured fundamental homologies and created wholly unwarranted problems.

Attention may be drawn at this stage to another feature of the monocotyledonous embryo, a feature that is well recognized and perhaps adds more flavour to the trend of argument presented in this paper. This concerns the radicle part of the embryo. It is this part that matures into the tap root in the dicotyledons. In the monocotyledons, in general, the radicle never develops into a permanently functional tap root; in some cases it may remain active for a certain length of time, in others it may not develop at all. In either case, a system of adventitious roots develops sooner or later and takes on the functions of anchorage and absorption. Thus, in the monocotyledons, just as the primary shoot pole of the embryo has been rendered morphogenetically sterile, the opposite pole also has suffered a similar fate although in a lesser and variable degree. Just as a new functional shoot meristem becomes substituted on a lateral side of the embryo, an adventitious system of functional roots becomes substituted at a relatively lateral position, generally on the opposite side of the functional shoot meristem.

Wardlaw (1955) has advanced the following working hypothesis : " A fertilized ovum may be regarded as a complex, gene-determined reaction system. According to the components of this system and the sustaining environmental conditions, characteristic chains of reactions will be set in motion, and the resulting biochemical pattern, or patternized distribution of metabolites, will constitute the basis for the visible morphological and histological developments. " A consideration of embryogenesis in dicotyledons and monocotyledons against this background leads to the following postulates :

The internal as well as external factors and the resulting metabolic gradient systems operate in such a way as to produce an octant proembryo through similar means in both dicotyledons and monocotyledons. Presumably as a consequence of a change in the gene-controlled biological system of gradient which becomes established at this stage, the consequent ontogeny follows wholly divergent courses in the two groups. In the dicotyledons, the change manifests itself in the establishment of an acropetal gradient of decreasing cell size and in the organization of a group of cells that further differentiate as the two cotyledons and the shoot apex. Working on the basis of contemporary concepts evolved by embryologists, in the monocotyledons, on the other hand, the establishment of the acropetal gradient should fail to involve the terminal tier of the octant. Therefore, the very poles that organize the root and shoot meristems of dicotyledons should be assumed as having been rendered morphogenetically sterile in the monocotyledons, the terminal pole more so than the basal pole. As a consequence, the principal seat of growth and morphogenesis should have become localized in the derivatives of the subterminal tier. The functional shoot meristem becomes organized at such a pole.

In conclusion, the essential differences between the dicotyledonous and monocotyledonous embryos will have to be looked for neither in the number of cotyledons nor in their topographical relationships, but in the morphogenetic potentialities of the primary terminal meristems, especially the one at the shoot pole. In the dicotyledons these tissues develop into the functional

root and shoot systems. In the monocotyledons, on the other hand, the primary shoot meristem should be assumed to have become sterile and reduced to a stub-like termination in which are consolidated the derivatives of the two cotyledons as well; the activity of the terminal root meristem is also similarly suppressed or is only transitory.

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Forms of Ovules in Euphorbiaceae

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The earlier classification of ovules into three types, viz. orthotropous, anatropous and campylotropous has been modified by Warming (1913) and Goebel (1933). Maheshwari (1950) stated that mature ovules are usually of five types. These types are based on the position of the micropyle in relation to the hilum and chalaza, and that various forms of ovules may sometimes intergrade into one another, or the same ovule may undergo various forms during the course of its development.

Bocquet (1959) has suggested that the classification of ovular forms should be based on the initial stages of development of ovules as well as the position of the vascular strand in the funiculus. According to him, the basic groups of ovule are orthotropous and anatropous. The curvatures which give rise to campylotropous or amphitropous conditions are modifications of these two basic types.

The earlier workers on the family Euphorbiaceae mention that ovules are mostly ana- or hemiana-tropous (*see* Schnarf, 1931; Banerji & Dutt, 1944; Banerji, 1949, 1951; Singh, 1954; Kapil, 1956). Thathachar (1953), however, considers that the ovules in *Breynia patens* are orthotropous. None of the above mentioned workers on the Euphorbiaceae have considered the ovular forms on the basis of their development, as well as on the nature of the vascular strand in the ovules. The present paper deals with the ovular forms in *Melanthesa rhamnoides*[1] Wt.; *Croton bonplandianum* Baill. and *Trewia polycarpa* Benth. belonging to the family Euphorbiaceae from the above mentioned points of view.

MATERIALS AND METHODS

Flowers and fruits of different stages of *Melanthesa rhamnoides* and *Croton bonplandianum* were collected locally while those of *Trewia polycarpa* were obtained from Dehra Dun. The materials were fixed in formalin-acetic-alcohol and stored in 70 per cent ethyl alcohol. These were dehydrated and cleared through ethyl alcohol-xylol series, as well as *ter.*-butyl alcohol-

[1] *Melanthesa rhamnoides* Wt. (syn. *Breynia rhamnoides* Muell. Arg.)

ethyl alcohol series, and imbedded in paraffin in the usual way. Sections were cut 9 to 20μ thick and stained in Heidenhain's iron haematoxylin, safranin fast green, and crystal violet-erythrosin combinations.

OBSERVATIONS

In *Croton bonplandianum* a single ovular primordium and in *Melanthesa rhamnoides* two ovular primordia arise on the axile placenta in each locule of the trilocular ovary. At their inception, these primordia are placed laterally at the base of loculus (Figs. 1, 2). They are composed of homogeneous mass of parenchymatous cells. Very soon the curvature starts so that their apices come to face the apical part of the ovary. The two integuments also make their appearance and the nucellar apex begins to grow into a beak-like structure. In the formation of the nucellar beak, the epidermal and the hypodermal cells divide predominently in anti- and peri-clinal planes. The two integuments grow further, the outer grows beyond the inner, but neither of them is ever able to enclose the nucellar beak completely; a micropyle is thus never formed in these two species.

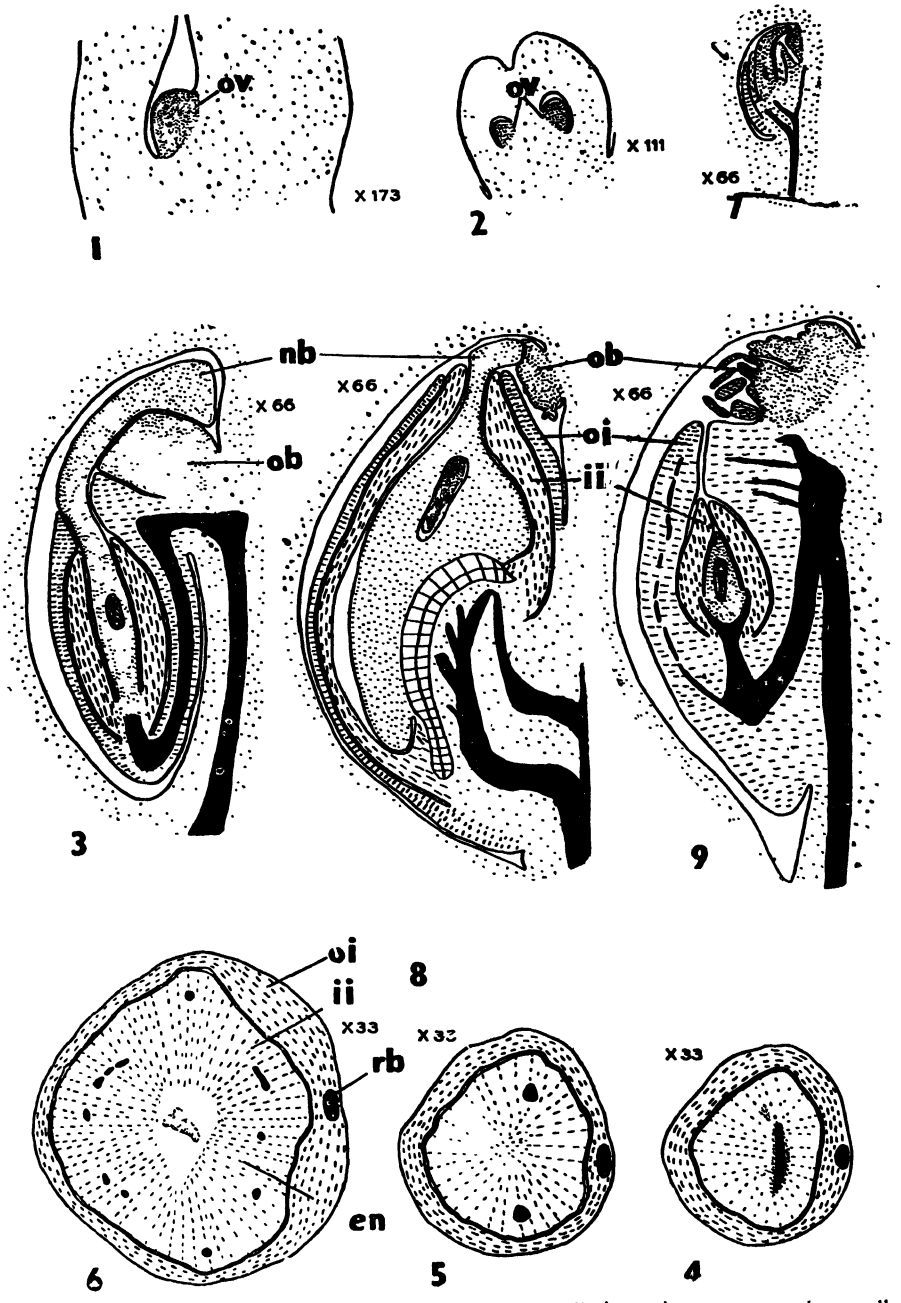
In *Croton bonplandianum* the ovular curvature is accompanied by the shifting of the position of its attachment region on the placenta. The ovule arises laterally on the placenta at the basal region of the loculus. The placenta below the region of attachment of the ovule grows and the latter is carried higher up so that the ovule finally becomes pendulous (Fig. 3). Landes (1946) also observes that in *Acalypha rhomboidea* the ovules arise from the placenta at the base of the loculi and on account of the more rapid growth of the basal part of the placenta the attachment region of the ovule is soon carried upward.

In *Melanthesa rhamnoides*, on the other hand, the attachment region of the ovule on the placenta always remains at a lower level in the locules as the growth of the placenta occurs mostly above this region. Thus, the ovule in this case is more or less erect and basal (Figs. 7, 8).

In *C. bonplandianum* the vascular supply to the ovule descends sufficiently down through the raphe to reach the chalaza. Thus the bundle in the raphe and the ventral carpellary bundles run more or less parallel (Fig. 3). After reaching the chalaza the bundle of the raphe divides into two or sometimes into three main strands which, while ascending through the inner integument undergo further divisions (Figs. 4-6).

The ovule in *M. rhamnoides*, on the other hand, has an appreciably broader attachment region. Besides this, the vascular supply to the ovule also originates in the basal region of the loculus from the ventral carpellary bundles and ascends more or less obliquely to reach the chalaza (Figs. 7, 8). There are no integumentary bundles in this plant.

The ovules in these two plants, in spite of having many features in common, such as two integuments, an elongated and curved nucellar beak, the micropylar end facing the apical part of the ovary, show that their form, as well



FIGS. 1-9—*Croton bonplandianum* (*en*, endosperm; *ii*, inner integument; *nb*, nucellar beak; *ob*, obturator; *oi*, outer integument; *ov*, ovular primordium; *rb*, raphe bundle): Fig. 1. L. s. part of ovary showing ovular primordium at the base of the locule. Fig. 2. *Melanthesa rhamnoides*. L. s. ovary showing one ovule primordium in each locule. Fig. 3. *C. bonplandianum*. L. s. ovule before fertilization. Figs. 4, 5, 6. *C. bonplandianum*. T. s. developing seed. Figs. 7, 8. *M. rhamnoides*. L. s. ovule at megaspore tetrad and zygote stages respectively. Fig. 9. *Trewia polycarpa*. L. s. ovule before fertilization. Vascular supply is partly reconstructed in Figs. 3, 7, 8 and 9

as organization and the course of the vascular supply are quite different before fertilization as well as in subsequent stages of seed development.

Trewia polycarpa, like *C. bonplandianum*, possesses a single ovule in each locule, but in contrast to the latter its ovules are fused with the placenta almost throughout their length with no indication of a definite raphe (Fig. 9). Due to non-availability of the material of earliest stages the development of ovule in this plant and its actual mode of fusion with the placenta could not be studied. At the mature embryo sac stage, the ovule in *T. polycarpa*, however, possesses a massive nucellus and two integuments and in contrast to *M. rhamnoides* and *C. bonplandianum* possesses a distinct micropyle. The beak-like nucellar apex in this plant is fully encircled by the two integuments and reaches only up to the base of the endostome which faces the stigmatic side of the ovary.

The vascular supply to the ovule in *T. polycarpa* is very interesting, for it presents marked difference over those commonly found in other members of the family (Singh, 1959). Fig. 9 shows that a number of vascular strands are given out from the ventral carpellary bundles to the ovule at different levels. A vascular strand, comparable to the raphe bundle of other members of the family, originates from the ventral carpellary bundles; it grows downward through the fused part of the ovule and the placenta and reaches the chalazal region. In the chalazal region this strand gives out branches which enter the outer integument. The residual vascular tissue grows upwards and organizes into a cup-shaped structure at the base of the nucellus. This is also confirmed from the transverse sections where the vascular strands are present in the form of a ring. No supply is given to the nucellus or the inner integument. Slightly above the origin of the raphe bundle, a number of vascular strands are given out from the ventral carpellary bundles. These strands enter the outer integument.

DISCUSSION AND CONCLUSION

From the above mentioned observations it is clear that in the ovules of *Croton bonplandianum* the shifting of the attachment region is brought about by the active growth in the region of the attachment, as well as in the region below it during the overall growth of the ovary. As a consequence of this, the vascular supply of the ovule has to descend considerably down to reach the chalaza. On the other hand, in *Melanthesa rhamnoides* the attachment region of the ovule remains confined in the lower region of the locule as more active growth takes place above this region. The vascular supply, therefore, only follows an oblique ascending course to the chalaza.

It may, therefore, be suggested that the pendulous and non-pendulous nature of the ovules in the family Euphorbiaceae can be achieved simply due to differential growth in varying regions of the placenta. The ovules which are pendulous approach the anatropous form while those which are attached only in the lower region extending up to the base of the chamber approach the orthotropous type.

Trewia polycarpa, because of the fusion of ovule with the placenta throughout the major part of its length, poses a fresh problem in the classification of ovular types. The course of the so-called raphe bundle, however, suggests that the ovule in this plant is a modification of the anatropous type where the ovule has fused with the placenta on the ventral side.

It is a pleasure to offer the sincere thanks of the author to Professor Bahadur Singh under whose guidance this work has been accomplished, and to Dr R. K. Singh, Principal B. R. College, Agra for facilities and encouragement.

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In vitro Induction of Adventive Buds from Embryos of *Cuscuta reflexa* Roxb.

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Attempts have been made to induce adventive embryony so as to obtain more than one plant from a single seed. Haberlandt (1921, 1922) claimed to have obtained two embryos in *Oenothera* by merely pricking the ovules. He thought that a so-called 'necrohormone' is liberated by the wounded cells which causes other cells to divide and form embryos. However, this work remains unconfirmed. Van Overbeek *et al.* (1941) injected various chemicals into the ovaries of *Datura stramonium*. In a few ovules integumentary proliferations resembling embryos were observed but these remained undifferentiated and are now considered to be tumorous growths. Fagerlind's (1946) experiments on *Hosta* indicate that though it is possible to induce the ovules of this plant to produce adventive embryos by the application of suitable chemicals, endosperm development fails without normal fertilization. In the absence of the endosperm the artificially induced adventive embryos failed to develop further and such ovules ultimately shrivelled and died. All the experiments mentioned above were carried out on flowers which were attached to the parent plant and it was naturally difficult to regulate the supply of nutrients to the embryo sac.

With the advent of the *in vitro* culture technique this problem has received a fresh impetus. By this method individual tissues or organs can be studied under controlled nutritional as well as environmental conditions. Such studies were undertaken on the embryos of *Cuscuta reflexa*, whose life-history has been studied earlier by Johri & Tiagi (1952).

MATERIAL AND METHODS

Cuscuta reflexa, a total parasite on many angiosperms, flowers and fruits at Delhi during the months of October–February. Cultures of embryos were started in January 1960. Fruits of various sizes were surface-sterilized by dipping in 95 per cent ethanol and then flaming. Seeds were dissected out of the fruits and the embryos excised with the help of needles under aseptic conditions.

Two different stages were chosen : (a) undifferentiated ovoidal embryos, 0·5–1·0 mm. long; and (b) older ones, 1·5–2·5 mm. long, with a well marked shoot apex and a large radicular end. They were inoculated on a basic medium containing minerals * + vitamins ‡ + glycine (7.5 mgl./l.) + sucrose (5%) + casein hydrolysate (400 mgl./l.) + indole-acetic acid (1 mgl./l.), unless mentioned otherwise.

Following the customary methods of dehydration and imbedding, the cultured embryos were cut at a thickness of 10μ and stained with safranin-fast green. Some whole mounts were also made after dehydrating in the alcohol-xylol series.

OBSERVATIONS

When the young and ovoidal embryos were cultured on the basic medium, they failed to show any appreciable response during the first two weeks. Later they became greatly swollen and rounded. A month after inoculation, outgrowths appeared on the surfaces of the embryos (Fig. 1R). In six weeks these overgrowths had coalesced to form a hypertrophied mass and small whitish or greenish protuberances simulating young embryos were observed all over the surface of this mass (Fig. 2). These have been designated here as adventive embryos.

Older embryos grown on the same medium responded differently. During the first week they showed very little growth (Fig. 3). In the second week the radicular end became swollen and produced many hair-like processes § while the plumule elongated into a slender stem (Fig. 4). Gradually the radicular end became still more massive; a month after inoculation it callused either at a few points or even over the entire surface (Fig. 5). As with the younger ovoidal embryos, adventitious growths emerged all over this callus mass or developed as separate entities from other regions of the radicle (Fig. 6).

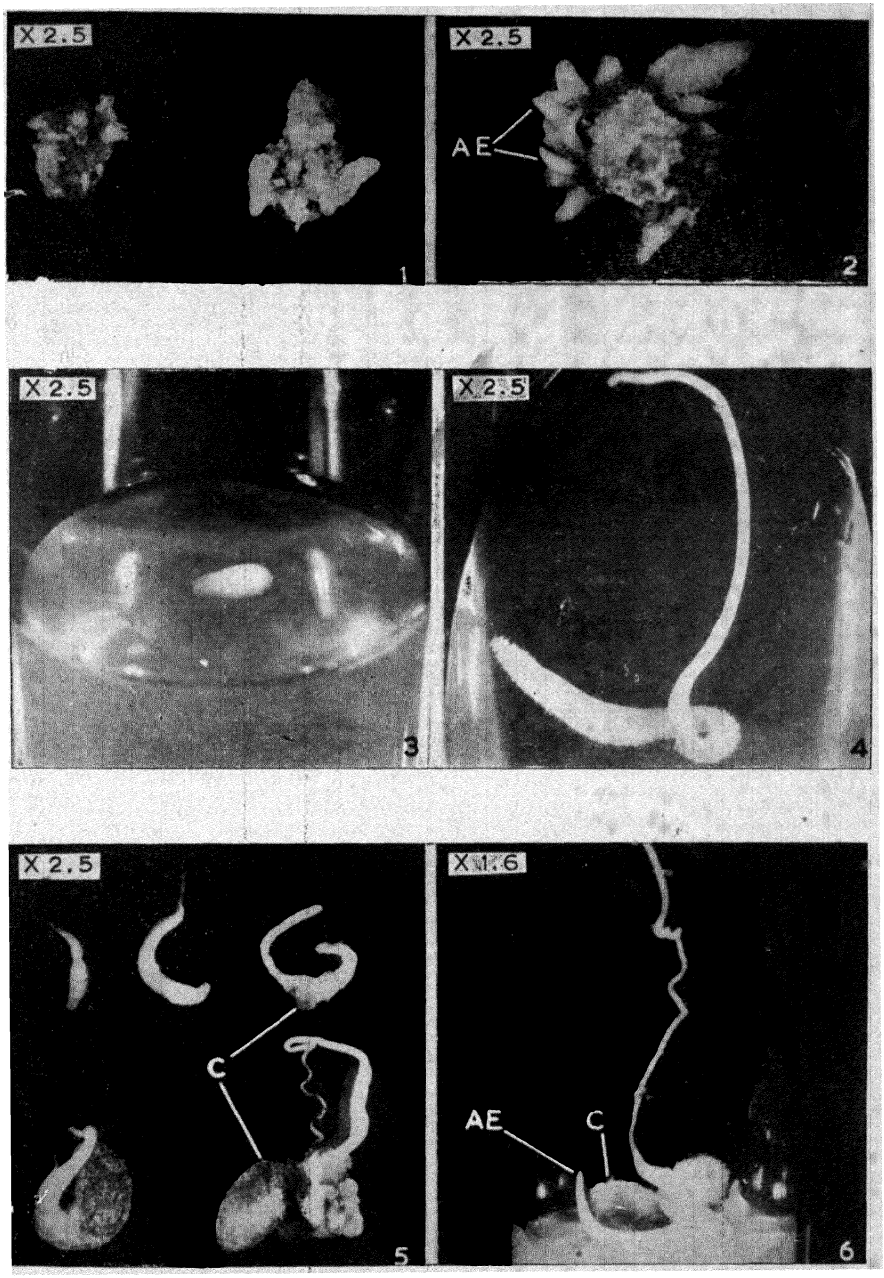
Most of the adventive growths closely resembled the embryo arising from a zygote (Fig. 14). They also had a dome-shaped shoot apex surrounded by two scale leaf primordia (Fig. 13). However, the ' plumular leaves ' often showed many abnormalities in their development. Some embryos had only one leaf, still others had none.

In Nature the zygote undergoes a transverse division resulting in a 2-celled proembryo. Further divisions produce a filamentous structure from whose apical end differentiates a globular embryo. The subsequent deve-

* $MgSO_4 \cdot 7H_2O$ (360 mg./l.), $Ca(NO_3)_2 \cdot 4H_2O$ (260 mg./l.), Na_2SO_4 (200 mg./l.), NaH_2PO_4 (165 mg./l.), KNO_3 (80 mg./l.), KCl (65 mg./l.), $MnSO_4 \cdot 4H_2O$ (3mg./l.), $ZnSO_4 \cdot 7H_2O$ (0·5 mg./l.), H_3BO_3 (0·025 mg./l.), $CuSO_4 \cdot 5H_2O$ (0·025 mg./l.), $NaMoO_4$ (0·025 mg./l.), CoCl (0·025 mg./l.), $FeC_6O_5H_7 \cdot 5H_2O$ (10 mg./l.).

‡ Niacin (1·25 mg./l.), thiamine hydrochloride (0·25 mg./l.), calcium pantothenate (·025 mg./l.) and pyridoxine hydrochloride (0·025 mg./l.).

§ Haccius & Troll (1961) point out that there is no primary root in *Cuscuta* and hence the hairs cannot be called root-hairs.



FIGS. 1-6 — CULTURES OF EMBRYOS GROWN ON BASIC MEDIUM (*AE*, adventive embryos; *C*, callus): Fig. 1. Thirty day-old cultures of undifferentiated embryos showing callus formation. Fig. 2. Forty day-old culture; adventive embryos arising from the mother embryo. Figs. 3-6. Stages in the germination of older and differentiated embryos. Adventive embryos differentiate from their radicular ends

lopment is unique in that the embryo does not attain the heart-shaped stage and cotyledons are not formed even at maturity. Instead, it elongates and a stem tip flanged by two plumular leaves differentiates at the apical end. Further growth results in the coiling of the embryo within the seed (*see* Johri & Tiagi, 1952).

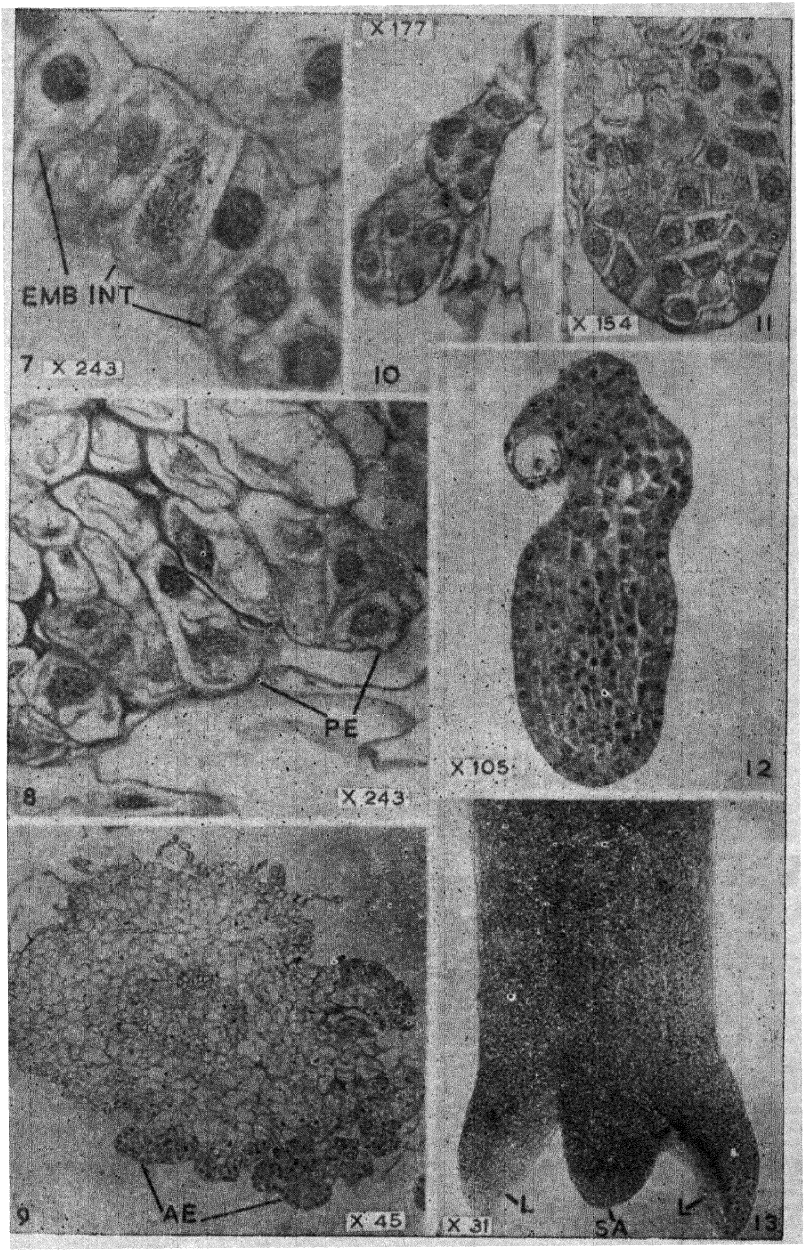
Microscopic observations showed that in the excised embryos, grown in the culture medium, some of the epidermal cells enlarged and became densely cytoplasmic and their nuclei became very prominent (Fig. 7). Such cells divided to produce 2-celled structures looking like proembryos (Fig. 8). Sometimes both of these cells again divided transversely but this was not always so. By further irregular divisions, they developed into globular embryos (Figs. 9-11) which elongated (Fig. 12) and ultimately their distal end differentiated into the characteristic stem tip surrounded by two 'plumular leaves' (Fig. 13). Since there was no problem of space, the embryos inside the culture tubes did not coil, but directly gave rise to normal vegetative shoots (Fig. 14).

When the callus obtained from zygotic embryos was subcultured on the basic medium, it showed a capacity for unlimited proliferation. In the first week of the transfer it usually turned brown and appeared like a dead tissue. Growth was, however, resumed; the callus grew profusely and after another eight weeks it produced a fresh crop of embryos (Figs. 15-17). On subculturing, the adventive embryos too callused on their 'radicular' end giving rise to a fresh crop of embryos (Fig. 19). When such embryos were transferred to the basic medium without indole-acetic acid many of them produced normal shoots (Figs. 14, 18).

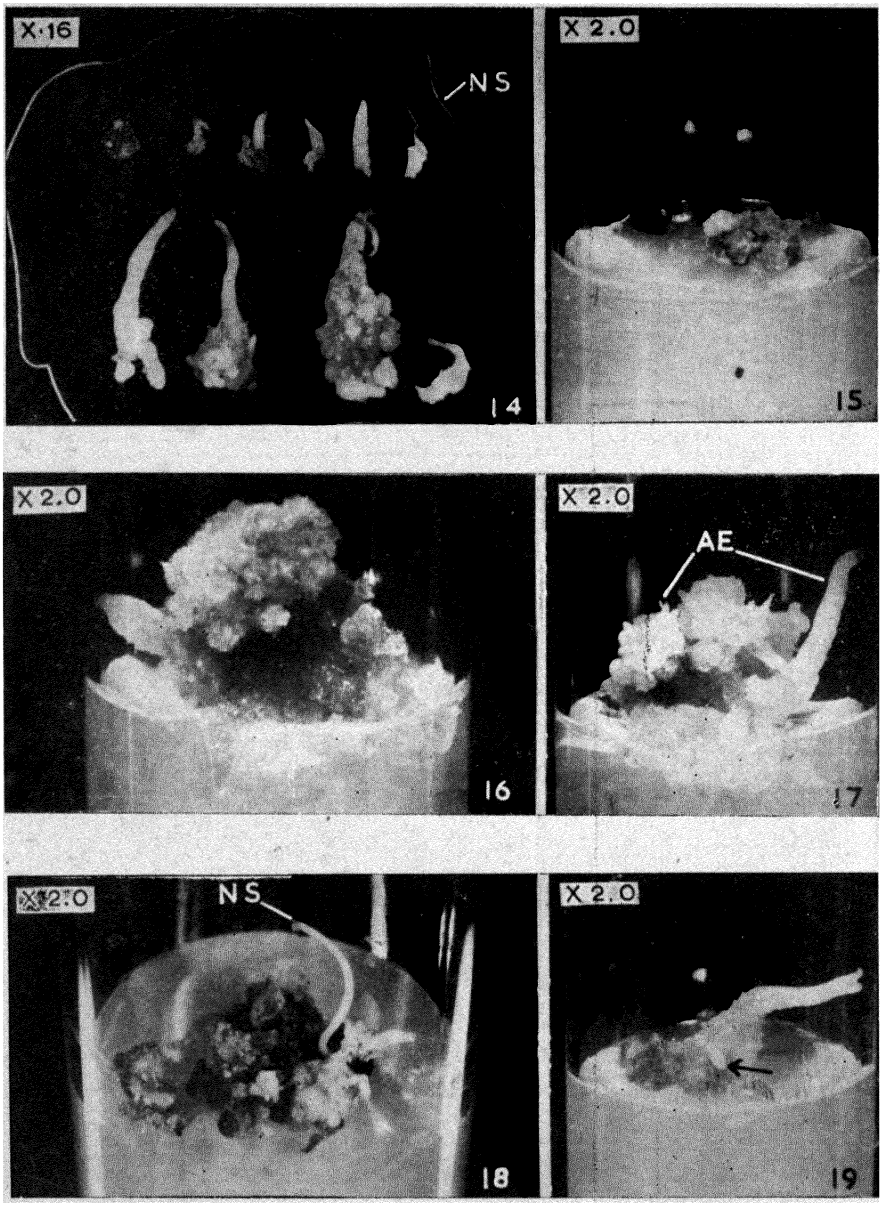
DISCUSSION

Attempts to induce the formation of additional embryos from the same ovule have generally proved unsuccessful. However, Skoog (1944) obtained root and shoot differentiation in cultures of the pith-tissue of tobacco. Similarly, Levine (1947) obtained differentiation in carrot root tissues grown *in vitro* in media containing α-NAA. From further work on tobacco callus tissue, Skoog & Tsui (1948), Miller & Skoog (1953) and Skoog & Miller (1957) postulated a synergistic effect of auxins and purine derivatives (adenine and kinetin) as a factor controlling organogenesis.

Steward *et al.* (1958a, b) went a step further and obtained carrot plants from free cells isolated from the root-phloem callus. The first few stages in the development of these free cells to differentiated plants resemble some events of the early embryogeny of carrot as described by Borthwick (1931). Of special interest are stages recalling the formation of a dicotyledonous embryo. This indicates that even plantlets produced from vegetative tissue have the tendency to pass through stages which strongly resemble those of a normal embryo. Coconut milk was found to be essential for differentiation (*see* Steward & Pollard, 1958; Mitra *et al.*, 1960) and shoots were obtained only when the rooting callus mass was transferred to an agar medium.



FIGS. 7-13 — PORTIONS FROM TRANSECTIONS OF ZYGOTIC EMBRYOS CULTURED ON BASIC MEDIUM SHOWING STAGES IN THE DEVELOPMENT OF ADVENTIVE EMBRYOS. (*EMB INT*, embryo initials; *L*, leaf primordium; *PE*, 2-celled proembryo; *SA*, shoot apex) : Figs. 7, 8. Some of the epidermal cells of the zygotic embryos have become richly cytoplasmic with a large nucleus. They divide transversely (Fig. 7) to produce 2-celled proembryo-like structures. Figs. 9-11. Stages in the formation of globular embryos Figs. 12, 13. The globular embryos undergo vertical elongation (Fig. 12) and ultimately the distal end differentiates into the shoot apex flanged by leaf primordia. Fig. 13 represents a whole mount



FIGS. 14-19 — (*AE*, adventive embryo; *NS*, normal shoot): Fig. 14. Stages in the development of adventive embryos leading to the formation of a normal shoot (in basic medium without IAA); note the proximal end which is always broader than the distal end which differentiates into the shoot apex. Figs. 15-17. Subcultures of callus on basic medium. Fig. 15 shows the initial size of the inoculum which proliferated in ten weeks into a mass of partly differentiated callus containing some adventive embryos. Fig. 18. Ten week-old culture grown on basic medium without IAA; note the formation of a normal shoot. Fig. 19. An adventive embryo isolated from the culture shown in Fig. 6; the arrow indicates the differentiation of a fresh embryo

The induction of 'adventive embryos' has also been reported by Reinert (1959) in cultures of carrot root-tissue. In tobacco callus also, Bergmann (1959, 1960) has illustrated the stages in the development of isolated cells to clusters which again recall the events of early embryogeny. Although the sequence of differentiation and development of vegetative buds from the callus has not been studied by many authors, it is probable that in all cases the plantlets have a similar beginning.

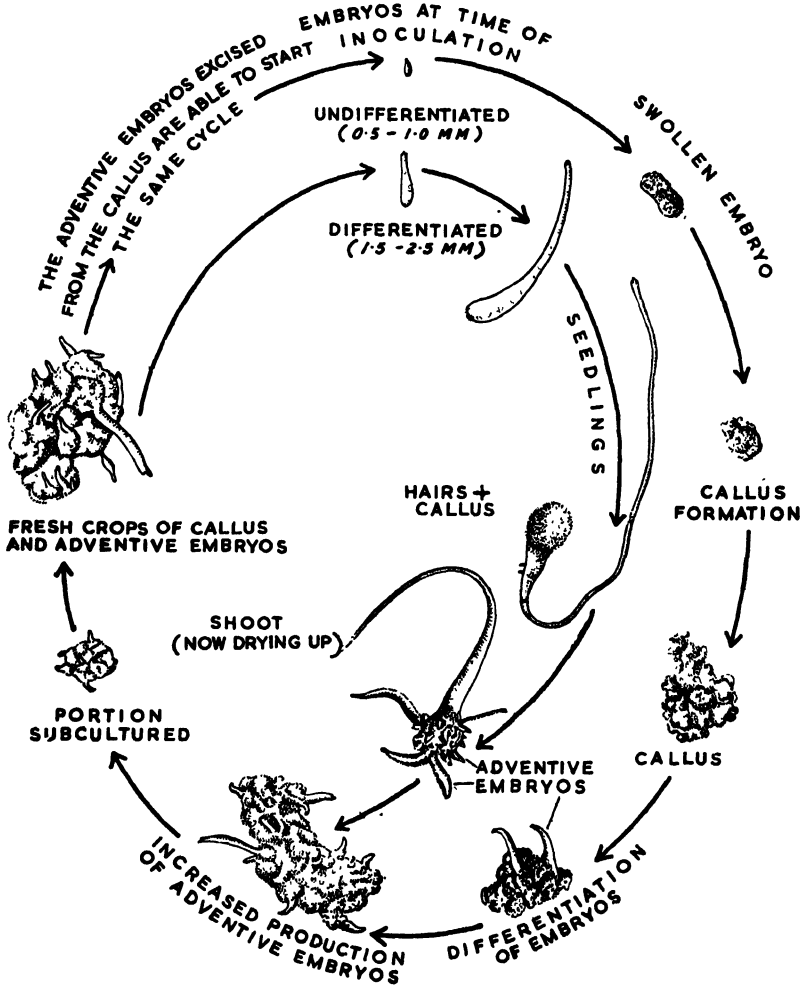


FIG. 20 — Diagram showing result of culturing young undifferentiated embryos as well as older embryos. The former produces callus all over, while the older differentiated embryos callus at their radicular end only. Several adventive embryos develop from these calli which on subculturing proliferate to produce a fresh crop of embryos. Some of the adventive embryos produce normal seedlings while the rest continue to callus and repeat the cycle

The above observations raise hopes that if appropriate measures are taken, almost any living cell grown in a culture medium could perhaps be induced to become meristematic and organize into a whole plant. This would naturally be even more applicable to a tissue which is a normal source of adventive embryos. In *Citrus microcarpa* Ranga Swamy (1958) has reported the proliferation of nucellar tissue and the formation of 'pseudobulbils'. Parts of this callus mass, on sub-culture, differentiated into embryos which ultimately gave rise to plantlets. Curtis & Nichol (1948) obtained proliferation from zygotic embryos of certain orchids. These calloid masses were capable of unlimited growth and produced numerous shoots. Another work, in this laboratory, on the embryos of *Dendrophthoe falcata* (Johri & Bajaj, unpublished), indicates similar possibilities. After passing through some stages of germination the embryo of this plant proliferates at the ' root ' region giving rise to vegetative buds.

In *Cuscuta* the young and undifferentiated embryos only callused, while differentitated embryos produced normal seedlings and also formed a callus at the ' radicular end '. The latter produced adventive embryos which on subculturing produced a callus and additional embryos, thus continuing the cycle (Fig. 20). Some of the newly differentiated embryos produced normal seedlings when IAA was excluded from the medium, while others merely grew into unorganized masses. The development of these artificially induced embryos has been traced and some important stages, otherwise typical of the zygotic embryo, have been illustrated.

The term ' adventive embryo ' is commonly used for the embryos arising from the nucellar or integumentary cells of a number of plants. They do not pass through all the conventional stages of embryo development but the mature form is similar to that of the zygotic embryo (*see* Maheshwari, 1950). The artificially induced embryos in *Cuscuta*, arising from the zygotic embryo itself, pass through stages of development which are not very different from those of nucellar embryos. Thus the extension of the term 'adventive embryos' to these structures seems justified. The plants arising from such adventive embryos should be genetically similar to those from the main embryo itself.

SUMMARY

Cultured embryos of *Cuscuta reflexa* proliferated and gave rise to adventive embryos. After passing through some stages of development resembling those of true embryos they produced normal shoots. The proliferations obtained on subculturing showed the capacity of the adventive embryos for unlimited growth and differentiation.

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Some Observations on the Embryology of *Dicraea stylosa* Wight

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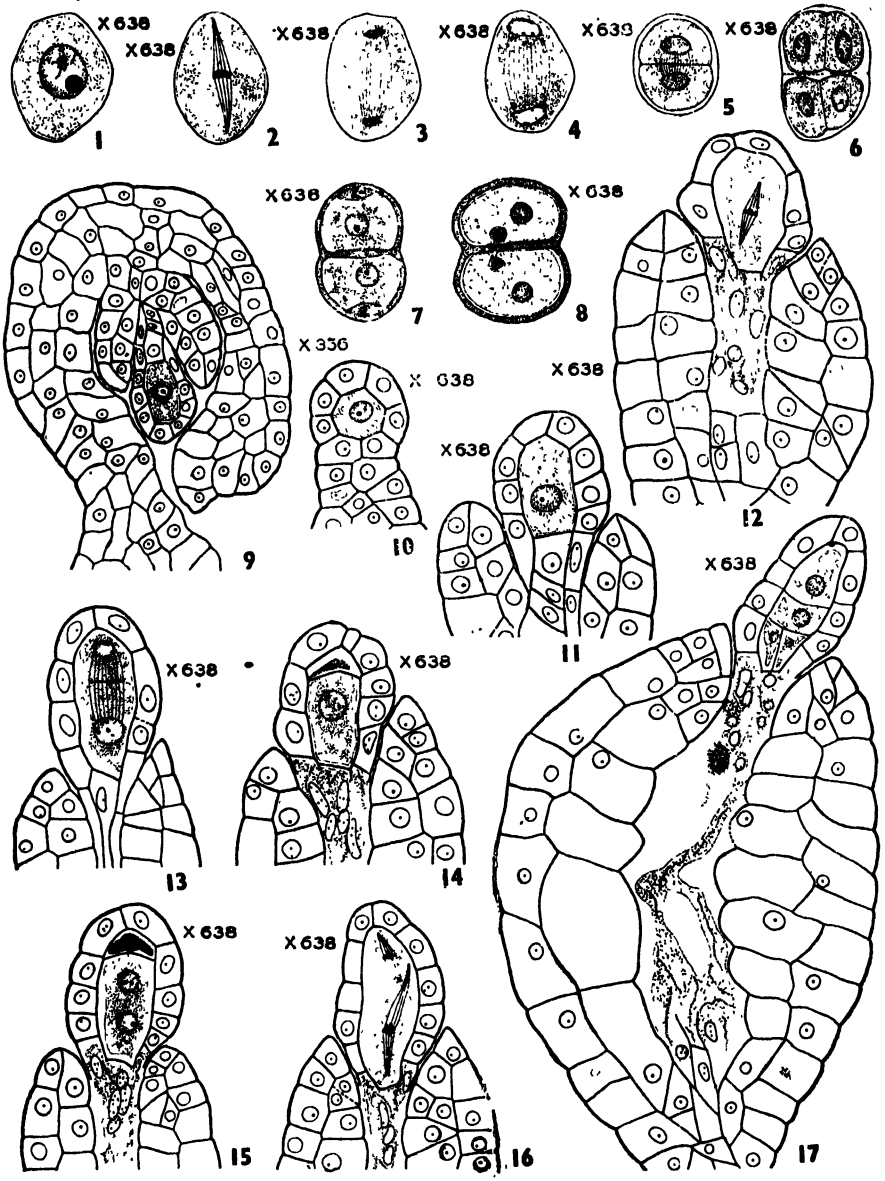
The Podostemaceae, as a group of aquatic dicotyledons, have posed some baffling morphological and embryological problems, among which may be mentioned the nature of the plant body, the mode of development of the female gametophyte, the occurrence and function of the pseudo embryo sac, and the nutrition and differentiation of the embryo in the absence of an endosperm. Embryological studies in the family were initiated by Went (1908, 1910) who observed a reduced Allium type of embryo sac in several members. He noticed that the nucleus of the functional dyad cell divides to form two nuclei of which the chalazal degenerates very early while the micropylar divides twice to give rise to a four-nucleate embryo sac which comprises an egg apparatus and a single polar nucleus. He also drew attention to the absence of triple fusion, the lack of endosperm, and the presence of a pseudo embryo sac. In his account of the embryology of *Podostemon subulatus*, *Hydrobryum olivaceum* and *Dicraea elongata*, Magnus (1913) reported a greatly reduced embryo sac—the so-called 'Podostemon' type—where the nucleus of the chalazal dyad cell divides only twice resulting in a four-nucleate embryo sac. According to him the first division of the nucleus of the functional dyad cell in *Dicraea* is followed by a wall resulting in two cells which by another division form a four-celled mature embryo sac comprising a single synergid, an egg and two antipodal cells.

The account of Magnus has often been criticized by other writers. With a view to verify his findings, material of a species, *Dicraea stylosa* Wight, was collected from the Punalur region of Kallada river, Kerala State, and fixed in formalin-acetic acid-alcohol. The flowers and fruits were prepared for microtomy by the usual methods. Sections cut at 7μ gave the best results. Heidenhain's haematoxylin counterstained with erythrosin proved most suitable.

OBSERVATIONS

Microsporogenesis and Male Gametophyte. The youngest anther



FIGS. 1-17 — Fig. 1. Microspore mother cell. Figs. 2-4. Same, meiosis I. Fig. 5. Dyad cells. Fig. 6. Microspore tetrad. Fig. 7. A double pollen grain with one lenticular generative cell cut off at each pole. Fig. 8. Same, with the generative cells freed from the wall. Fig. 9. L. s. ovule at megaspore mother cell stage. Figs. 10, 11. L.s. ovules at archesporial and megaspore mother cell stages respectively. Figs. 12, 13. Divisions of megaspore mother cell. The pseudo embryo sac has been drawn in full in Fig. 12. Fig. 14. Degeneration of the upper dyad cell and the enlargement of the functional dyad. Fig. 15. Two-nucleate embryo sac. Fig. 16. Same, with simultaneous division of the two nuclei. Fig. 17. Organized embryo sac; note also pseudo embryo sac below

showed the microspore mother cells (Fig. 1) which undergo normal meiotic divisions (Figs. 2-6). After tetrad formation the microspores separate in pairs, resulting in the double pollen grains characteristic of several members of the family. The exine is thick and echinulate. An ellipsoidal generative cell is cut off from each cell of the double pollen grain (Fig. 7). The separating wall later dissolves and the generative nucleus comes to lie in the general cytoplasm although still surrounded by a definite sheath of dense cytoplasm (Fig. 8).

Megasporogenesis and Female Gametophyte. The ovule is anatropous, tenuinucellate and bitegminal with the micropyle formed by the outer integument alone (Fig. 9). The inner integument is usually two-layered, but may be thicker towards the tip. The outer integument is usually more than two-layered.

The hypodermal archesporial cell functions directly as the megaspore mother cell (Figs. 10, 11). It divides to form two unequal dyad cells of which the micropylar degenerates promptly. The nucleus of the chalazal dyad cell divides to form a two-nucleate embryo sac (Figs. 12-15). According to Magnus (1913) in *Dicraea elongata* a wall is laid down at this stage separating the two nuclei. In the author's preparations of *Dicraea stylosa* no evidence of this has been found. Even in *D. elongata* the observations of the author failed to reveal a cell wall. Both the nuclei undergo a simultaneous division (Fig. 16) and cell formation takes place at the four nucleate stage. The mature embryo sac comprises a single synergid, an egg and two juxtaposed antipodal cells (Fig. 17).

Meanwhile, the nucellar cells just below the megaspore mother cell break down leaving a large cavity—the pseudo embryo sac—which is bounded by the inner integument (Figs. 12, 17). This sac contains several free nuclei and dense cytoplasm. It serves to enclose and nourish the embryo, thus making up for the absence of endosperm tissue. The cells of the inner integument undergo considerable enlargement.

Embryogeny. The first division of the zygote is transverse (Figs. 18, 19). This is followed by three further transverse divisions resulting in a five-celled linear proembryo (Figs. 20-22). The terminal cell then undergoes two vertical divisions at right angles to each other thus forming a quadrant (Figs. 23, 24). The subterminal cell also divides by a longitudinal wall (Fig. 25). The embryo then passes through the octant stage (Fig. 26) and proceeds to the globular stage (Fig. 27). By this time the embryo grows down into the pseudo embryo sac. Gradually the cotyledons develop (Figs. 28, 29). The embryogeny conforms to the Solanad type.

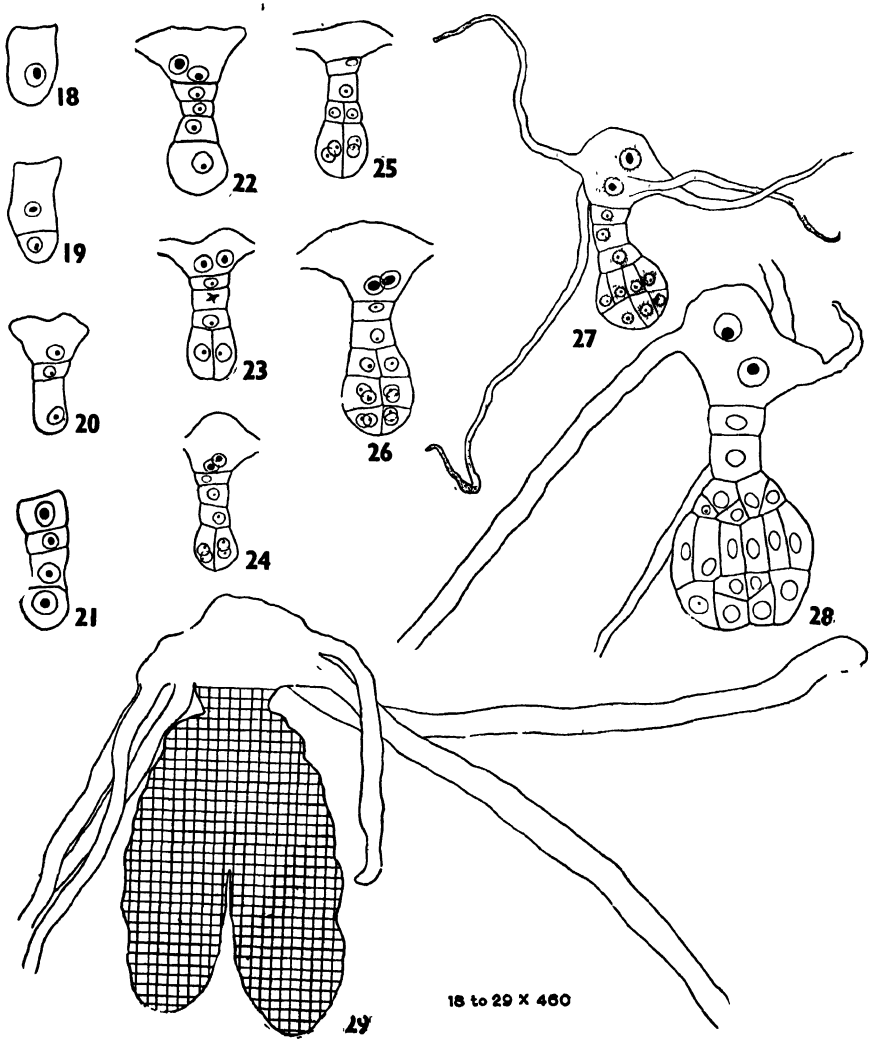
It is interesting to note that the basal cell of the proembryo enlarges considerably (Figs. 20, 22) and generally has two hypertrophied nuclei. It sends out a variable number of protuberances which later develop into conspicuous tubular haustorial branches (Fig. 27).

Since the inner layers of the outer integument break down, it has not been possible to trace the precise course of the haustoria in sections except

ascertaining that they grow in between the two integuments. However, the whole mounts (Figs. 27-29) reveal that the haustoria are invariably much longer than the embryos themselves. They are thin-walled and full of dense cytoplasm but the nuclei do not move into the haustorial branches.

DISCUSSION

As mentioned earlier, according to Magnus (1913) in *Dicraea elongata* the nucleus of the functional dyad cell undergoes only two divisions resulting in a four-nucleate embryo sac (*see* Fig. 30). In most other members of the



FIGS. 18-29 — EMBRYOGENY : Figs. 18-26. Early stages of embryogeny up to the octant stage. Fig. 27. Whole mount of globular embryo showing long, hypha-like haustoria branches. Figs. 28, 29. Whole mounts of older embryos

family, however, (*see* Went, 1908, 1910; Hammond, 1937; Razi, 1949, 1955) the functional dyad nucleus divides thrice but at the two-nucleate stage the primary chalazal nucleus degenerates so that the micropylar alone divides twice to form four nuclei (Fig. 30). It was suggested by Maheshwari (1950) that owing to the ephemeral nature of the chalazal nucleus Magnus might have missed it in *Dicraea*. Magnus also stated that the first division of the functional dyad is followed by wall formation resulting in a two-celled embryo sac. Of the two cells the upper divides transversely to give rise to a synergid and an egg while the lower divides longitudinally to form two antipodal cells. Thus, there are no free nuclear divisions preceding the organization of the embryo sac, a situation unique among angiosperms.

The present investigation seems to confirm the account of Magnus regarding the presence of a bisporic four-nucleate embryo sac, but wall formation after the first division of the dyad cell nucleus has not been found. In this the developmental details are extremely telescoped and it probably marks the culmination of reduction. In other members of the family, like *Terniola*, *Indotristicha* and *Zeylanidium*, the author was able to confirm the degeneration of the chalazal nucleus of the two nucleate embryo sac but this is not so in *Dicraea*.

Unlike other angiosperms where the synergids are sister cells *Dicraea* is also peculiar in that the synergid and the egg are sister cells. The egg apparatus with an egg and one superposed synergid is also unique. There

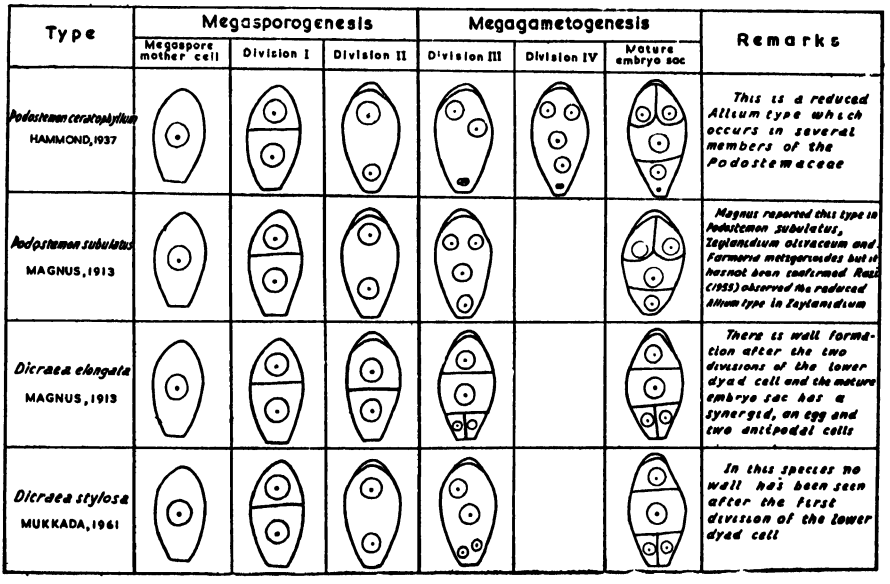


FIG. 30 — DIAGRAMMATIC REPRESENTATION OF VARIOUS TYPES OF EMBRYO SACS IN THE PODOSTEMACEAE

is no polar nucleus. The adjacent position of the two chalazal cells, their early degeneration, and the complete absence of the endosperm indicate their antipodal nature. Thus in *Dicraea* the polar nuclei and the accompanying triple fusion are completely eliminated. In other members of the family the embryo sac comprises two synergids, an egg and a chalazal nucleus which is referred to as the polar. Razi (1949) calls it a polar cell in *Griffithella hookeriana*. Razi's (1949, 1955) report that in *Lawia* and *Griffithella* the chalazal end of the mature embryo sac is continuous with the pseudo embryo sac is not true of *Dicraea*.

In the absence of an endosperm, the development of the suspensor haustorium may be an alternative device to draw nutriments from the external tissues. The pseudo embryo sac is likely to conserve food materials in the fluid that is reported to fill it in the early stages of embryogeny. The complete differentiation of the embryo even in the total absence of an endosperm, which besides nourishing the embryo is believed to supply certain morphogenetic substances, offers problems whose critical evaluation is bound to add fresh information to our concept of the role of endosperm in higher plants.

SUMMARY

Microsporogenesis is of the successive type and double pollen grains are formed. The ovule is bitegminal with the micropyle formed by the outer integument alone. A pseudo embryo sac develops just below the normal embryo sac. The organized embryo sac has a single synergid, an egg and two antipodal cells, but no polar nuclei. The development of the embryo conforms to the Solanad type.

To Professor P. Maheshwari and Dr B. M. Johri the author expresses his gratitude for suggesting the problem and encouragement. He is also grateful to Dr R. N. Chopra and Dr S. C. Maheshwari under whose guidance this work has been carried out.

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Intraovarian Pollination in *Eschscholzia californica* Cham. and *Papaver rhoeas* L.

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Plant breeders are usually confronted with many problems, such as (i) failure of pollen grains to germinate on a foreign stigma, (ii) dying or bursting of the pollen tubes in the style, and (iii) slow growth of the tubes so that the ovary shrivels before they are able to reach the ovules. Several mechanical devices have been suggested for overcoming these difficulties (*see* Maheshwari, 1950; Gardella, 1950; and Hecht, 1960). Intraovarian pollination seems to be one of the most promising of them as it aims to bring the pollen grains directly in the vicinity of the ovules. In the present study *Eschscholzia californica* and *Papaver rhoeas* were used for trying this technique.

MATERIAL AND METHODS

The method involves the following steps : (i) determination of the time of anthesis, dehiscence of anthers and pollination, (ii) emasculation and bagging of flower buds, (iii) collection of pollen grains, (iv) determination of the proper cultural conditions for optimum germination of the pollen grains, and (v) injection of pollen into ovary.

After emasculation the flower buds were enclosed in a cellophane bag. In some experiments the stigmatic lobes were smeared with collodion so as to render them incapable of supporting the germination of the pollen.

Pollen suspensions were made in 2 ml. of sterile double distilled water, or solutions of boric acid (100 mg./l. and 200 mg./l.). Each drop contained 100 to 300 pollen grains.

The ovary was surface-sterilized with rectified spirit. Two punctures were made, one at the base of the ovary and another on the opposite side near the top. The pollen suspension was injected through the former and the ovarian cavity was considered full when the fluid started oozing out from the second hole. For injections, an all-glass 'insuline' syringe was used. Care was taken to shake the syringe before each injection to prevent the pollen

grains from settling down. After the operation the holes were sealed with petroleum jelly.

Four sets of experiments were made. In the first set the ovaries of emasculated and bagged flower buds were pricked with the needle and left as such. The second and third sets of ovaries were injected with suspensions of pollen in double distilled water with 100 mg./l. and 200 mg./l. of boric acid. In the fourth dry pollen was introduced as such into the ovary through a slit. For control, one set of the flowers was allowed to become pollinated naturally.

Both freehand and microtome sections were prepared and stained by the customary methods. Dissections and whole mounts were stained with acetocarmine or cotton-blue in lactophenol. Chromosome counts were made by squash methods using seedling root-tips pre-treated with a solution of 8-hydroxyquinoline (0·002 *M*).

OBSERVATIONS

Eschscholzia californica. In Delhi gardens the plants flower in February. The flowers are self-compatible. The majority of them open in the morning and the anthers dehisce before noon. Pollination occurs soon after and on the following day many pollen grains may be seen germinating on the stigma.

The stigmatic papillae are present all over the radiating arms of the stigma and similar hairs are also present along the placentae. Each papilla is an elongated, uninucleate cell. After the pollen grains have germinated (36 hr after anthesis) the papillae begin to collapse. The stigma starts drying three days after anthesis as indicated by its curled condition (Fig. 1B).

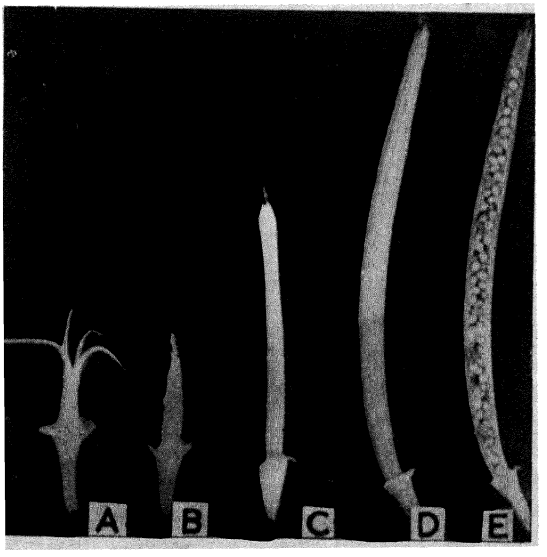

FIG. 1 — *Eschscholzia californica*, NATURALLY POLLINATED OVARIES (NAT. SIZE) : A. On the day of anthesis. B–D. Three, six and eighteen days after anthesis. E. Same as D, but vertically to show the developing ovules

In sterile double distilled water the pollen grains invariably burst. Germination occurs in 0·25 to 0·5 *M* sucrose solutions but the size of the pollen tubes remains small, and only 2-4 per cent of them attain a length of 200μ. In a boric acid solution (200 mg./l.) the pollen tubes attained a length of 594μ within 6 hr. The pollen grains are heteromorphic, the largest having a diameter of 48μ showed 50 per cent germination. The smaller (20-28μ diameter) invariably shrink or burst.

The ovary is about 0·9 cm. long on the day of anthesis (Fig. 1A). In the first week it grows to almost four times its original size (Fig. 1B, C). The maximum length is attained in nature in two weeks (Fig. 1D, E) after which it starts shrinking so that the mature fruit is much smaller. The ovules are bitegminal. Both the integuments form the micropyle. The mature seeds are opaque and have prominent ridges on their surface. The endosperm is free nuclear during the first week but by the second week the endosperm becomes cellular. The growth of the embryo is at first slow, but early in the second week a filamentous proembryo is formed and the globular stage is attained by the end of the second week; 27-day-old embryos show the differentiation of cotyledons. Pluricotyly is frequent.

Unpollinated ovaries remain healthy for about a week and even show some elongation, but later turn brown and dry up. A few ovaries (about 1 per cent) develop parthenocarpically but the seeds abort.

Pricking of unpollinated ovaries with the hypodermic needle does not hinder their early growth in any way, but like the emasculated and bagged ovaries they dry up after a week.

Ovaries injected with a pollen suspension made in boric acid (100 mg./l. or 200 mg./l.) show slower growth than naturally pollinated ovaries (Fig. 2A-F). The maximum size is attained by the third week. The wound generally remains although sometimes it heals up (Fig. 2E) due to a hypertrophy of the adjacent cells. Seventy-two hours after injection pollen grains with long pollen tubes are observed on the placentae and the surfaces of the ovules (Fig. 3 A, B). Many pollen tubes are also seen entering the micropyles (Fig. 3C, D).

The growth of the ovules is slow in the beginning but sometimes it surpasses even that of the ovules in naturally pollinated ovaries (Fig. 2 G-H).

Although stages in double fertilization were not seen by us, this may be assumed to take place normally, for the development of the embryo and endosperm follows the normal course reported by Sachar & Mohan Ram (1958) (Fig. 4 A-C). The embryo becomes 4-celled five days after injection and a long filamentous 8-celled proembryo is seen within 11 days. The embryo attains the heart-shape within 18 days and in a 27-day-old seed a well differentiated embryo showing pluricotyly is observed (Fig. 4 A–C). In seeds obtained by intraovarian pollination the mature embryos are as large or longer than those in naturally pollinated ovaries. The endosperm remains free nuclear for ten days, after which wall formation starts from the periphery. In mature seeds the endosperm is completely cellular and rich in food reserves.

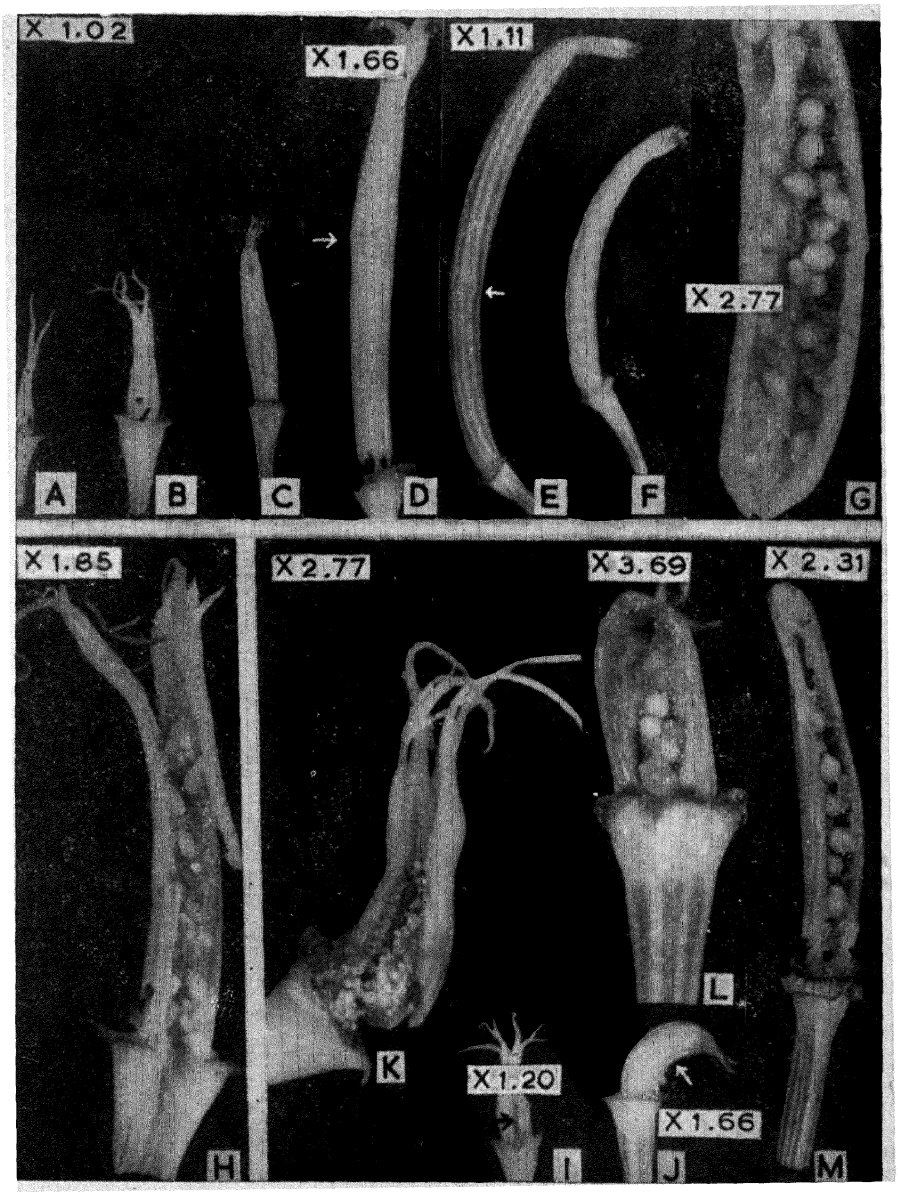

FIG. 2 — OVARIES OF *Eschscholzia californica* INTO WHICH POLLEN WAS INJECTED ARTIFICIALLY (ARROW SHOWS THE PLACE OF INJECTION) : A–H. Ovaries injected with pollen + 200 mg./l. boric acid. A. Ovary at the time of injection. B–F. Ovaries 3, 6, 11, 17 and 25 days after injection. G. Same as F, cut vertically to show developing ovules. H. Same as D, split vertically. I-M. Ovaries into which pollen was inserted as such through a slit. Two and nine days after insertion of pollen; slit through which pollen grains were introduced is clearly seen in I; in J it is obscured by a curvature of the ovary. K. An ovary 4 days after insertion of pollen, split to show the ovules, some of which are developing while others are abortive. L. Front view of the slit in J, enlarged to show the developing ovules. M. Fruit 20 days after insertion of pollen

The fruits require the same time for maturation as those from naturally pollinated ovaries (25-30 days). The number of seeds per fruit varies from 2 to 30. The size of the mature fruit is directly proportional to the number of seeds that develop within it. The seeds germinate after about five months and give rise to normal healthy seedlings (Fig. 4 D).

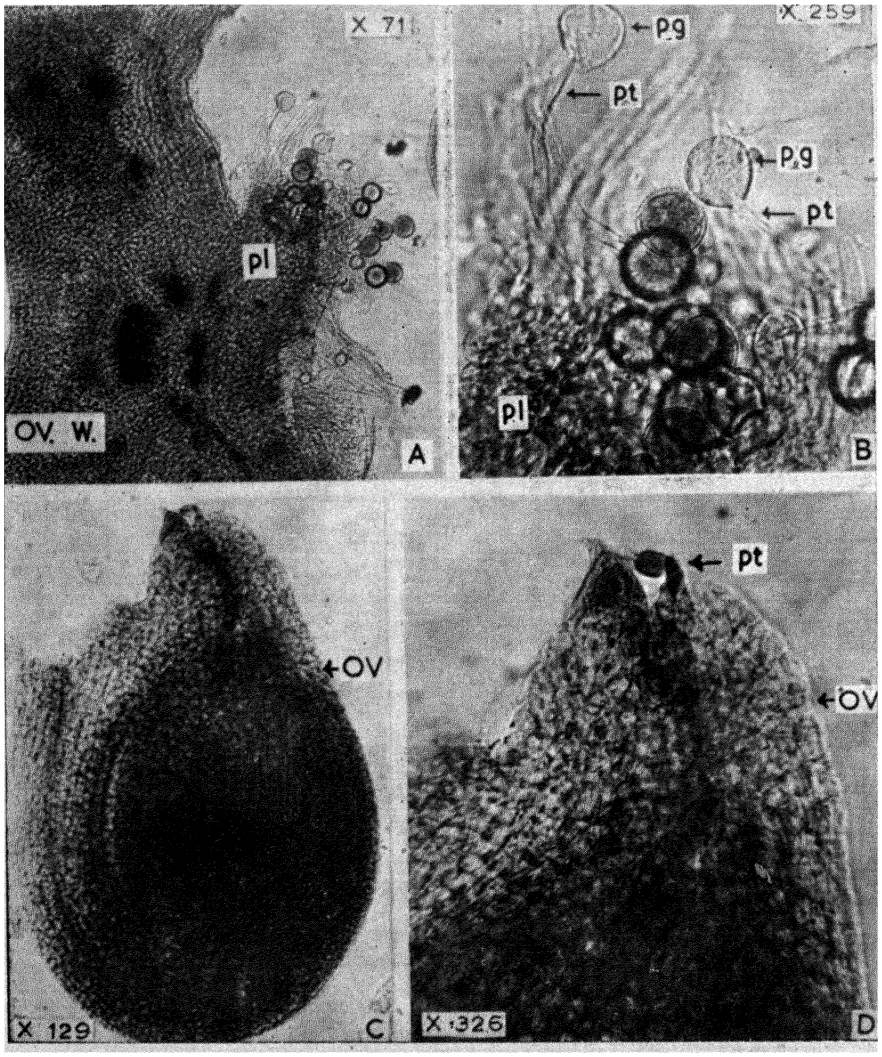

FIG. 3 — *Eschscholzia californica*, OVARIES INJECTED WITH A SUSPENSION OF POLLEN + 200 MG./L. BORIC ACID (*ov*, ovule; *ov. w.*, ovary wall; *pg*, pollen grain; *pl*, placenta; *pt*, pollen tube): A. T.s. of part of an ovary 72 hours after injection of pollen suspension. A few of the pollen grains have germinated. B. Portion of placenta enlarged from an ovary 72 hours after injection; some of the pollen grains have germinated. C. Whole mount of an ovule from an ovary, injected 4 days earlier with a pollen suspension; note pollen tubes in micropyle. D. Same, micropylar end enlarged to show germinating pollen grain

In some ovaries pollen is introduced directly through a slit in the ovary wall (Fig. 2 I-M). Due to some arrest in growth on the cutside the ovary often curves as shown in Fig. 2 J-K. The fruits are smaller than those from naturally pollinated ovaries or from ovaries injected with pollen suspensions. The ovules near the slit grow better than those away from it (Fig. 2 K). This is no doubt due to their more favourable position from the point of view of pollination. The number of seeds per fruit is smaller than that in ovaries injected with suspensions of pollen (Fig. 2 M), but the endosperm and embryo show the normal course of development.

Papaver rhoeas. At Delhi the flowers start opening in January in the morning and continue to do so until about 10 a.m. Dehiscence of the anthers begins nearly 12 hr before anthesis. In the peak blooming season (February) it continues till next day, although later (March) it is completed by 10 a.m. on the same day as anthesis.

The nodding flower buds straighten and become completely erect on the day of anthesis. In the bud the ovary is pale yellow, but it turns green by the day of anthesis when it measures 1·6 cm. in length (inclusive of the stigma) and 1·5 cm. in diameter. The stigmatic papillae are unicellular, elongated and blunt. The nucleus is situated in the centre and on each side of it there is a large vacuole. Rarely a papilla shows two nuclei.

The flowers are generally self-pollinated. In a flower, which has just opened, the stigmatic lobes already show germinating pollen grains As in *Eschscholzia* the pollen grains are dimorphic. The large pollen grains (36μ across) are full of starch and oil globules. Along with these occur many smaller ones (diameter, 28μ) in the same anther. Their percentages, however, vary from anther to anther. The smaller grains stain lightly and are mostly nonviable.

In sterile double distilled water only 2 per cent of the pollen grains germinate, but the tubes burst soon. The addition of sucrose in low concentrations (0·1*M* to 0·3*M*) enables better germination but a boric acid solution (100 mg./l.) in distilled water proves to be the best. In this 73 per cent of the pollen grains germinate and form fairly long tubes (990μ). Combinations of boric acid and sucrose solutions of different concentrations do not improve the percentage of germination. Solutions of ascorbic acid promote the length of the tubes (1050μ) but the percentage of germination (20%) is much lower than that in boric acid. The best results are obtained in boric acid within a *p*H range of 5·5 to 6·8.

During the first three days, the naturally pollinated ovary grows rapidly. It is twice its original size (3·2 cm. in length and 2·9 cm. in diameter) by the end of the first week. Nine days after pollination the size of the ovaries ranges from 3·0 to 4·6 cm. in length, and 3·1 to 3·6 cm. in diameter. The maximum length (4·4 cm.) is attained in two weeks (Fig. 5 A-C), but the ovaries continue to increase in diameter until the third week. After this the ovary starts drying and shrinking. The capsules ripen and dehisce in about 30 days.

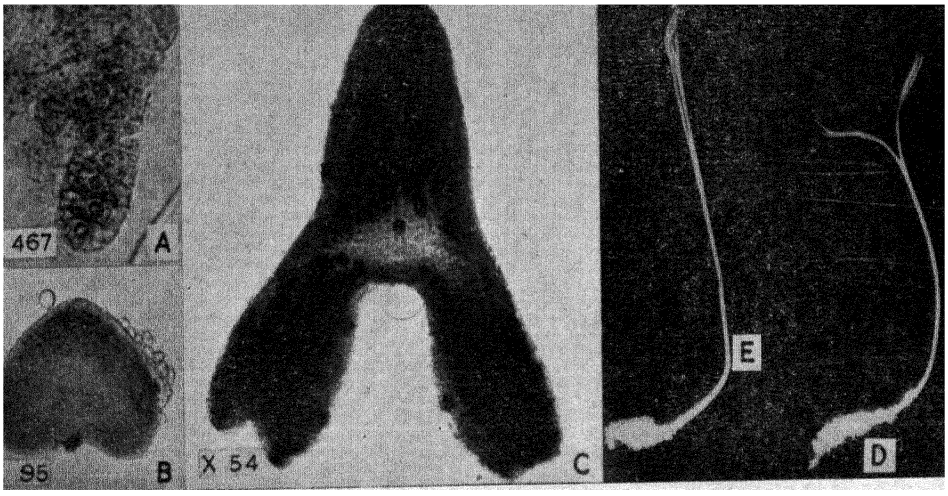

FIG. 4—*Eschscholzia californica*: A-C. 11, 18 and 27 day-old embryos from ovaries injected with pollen suspension. D. Ten day-old seedling resulting from seed obtained from injected ovary. E. Same, from naturally pollinated ovary

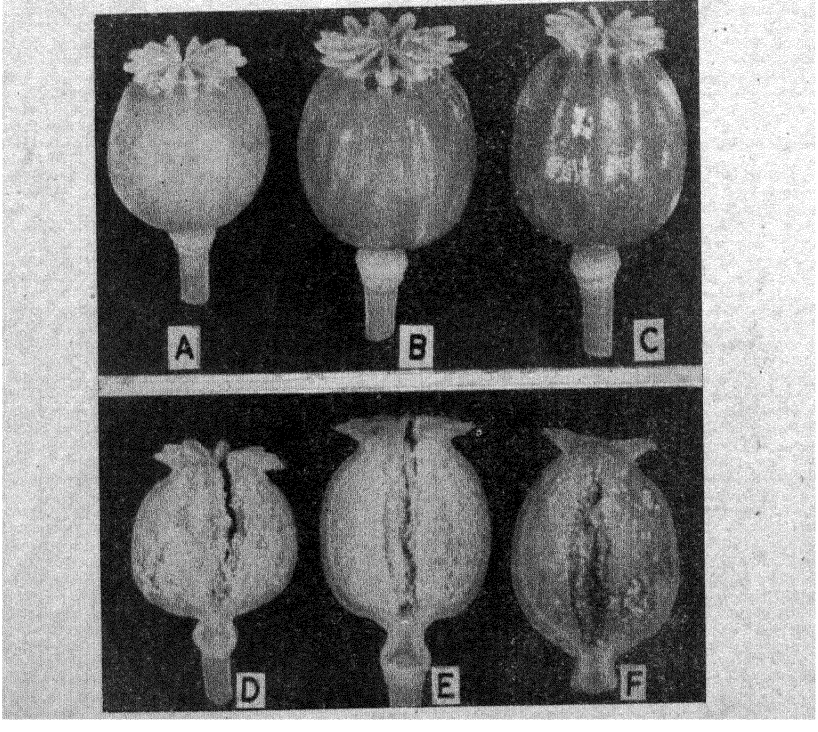

FIG. 5—*Papaver rhoeas*, NATURALLY POLLINATED OVARIES (NAT. SIZE): A–C. 9, 15 and 21 days after anthesis. D–F. Same as in A–C, vertically cut to show the developing ovules. In F some of the ovules became detached during fixation

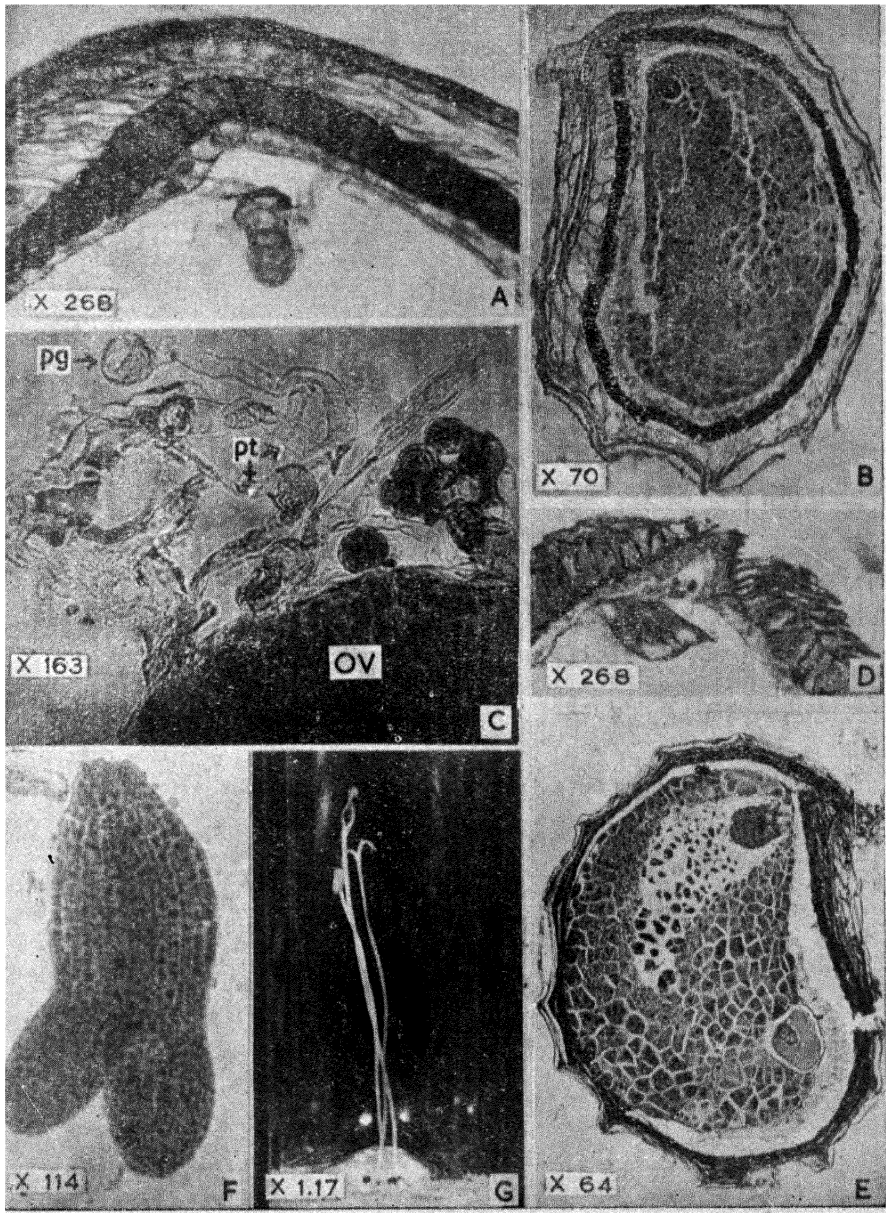

FIG. 6 — *Papaver rhoeas* (*ov*, ovule; *pg*, pollen grain; *pt*, pollen tube) : A-B. L. s. of ovules from naturally pollinated ovaries after 6 and 9 days respectively. C. Germinating pollen grains on the surface of an ovule; 3 days after insertion of pollen grains through a slit. D. Micropylar end of an ovule from an ovary injected with pollen + 100 mg./1. boric acid, 6 days after injection. E. L.s. of an ovule 21 days after pollination from an ovary injected with pollen suspension made in 100 mg./1. boric acid; note a persisting antipodal cell. F. 21 day-old embryo from ovary injected with pollen suspension in double distilled water. G. 26 day-old seedling reared in White's basic medium from seed obtained by intraovarian pollination

The ovules grow at a more rapid rate than the ovary. On the day of anthesis they generally measure 1·2 mm. × 0·9 mm. and show mature embryo sacs but attain a size of 2 7 mm. × 2·3 mm. within a week. After this the increase in size is negligible, and 21 day-old ovules measure 2·9 mm. × 2·5 mm. Six days after pollination the ovules show many free endosperm nuclei distributed along the periphery of the embryo sac. After nine days the ovules show endosperm cells full of oil globules (Fig. 6 B).

The zygote remains undivided for 4 or 5 days from the day of anthesis. Six days after pollination the ovule shows a 2- to 4-celled filamentous proembryo (Fig. 6 A) and after nine days a globular proembryo (Fig. 6 B) is observed. In another week the embryo becomes heart-shaped, and is about 200μ long and 130μ broad. By the third week it measures 764μ × 191μ, and the seeds are ready to be shed.

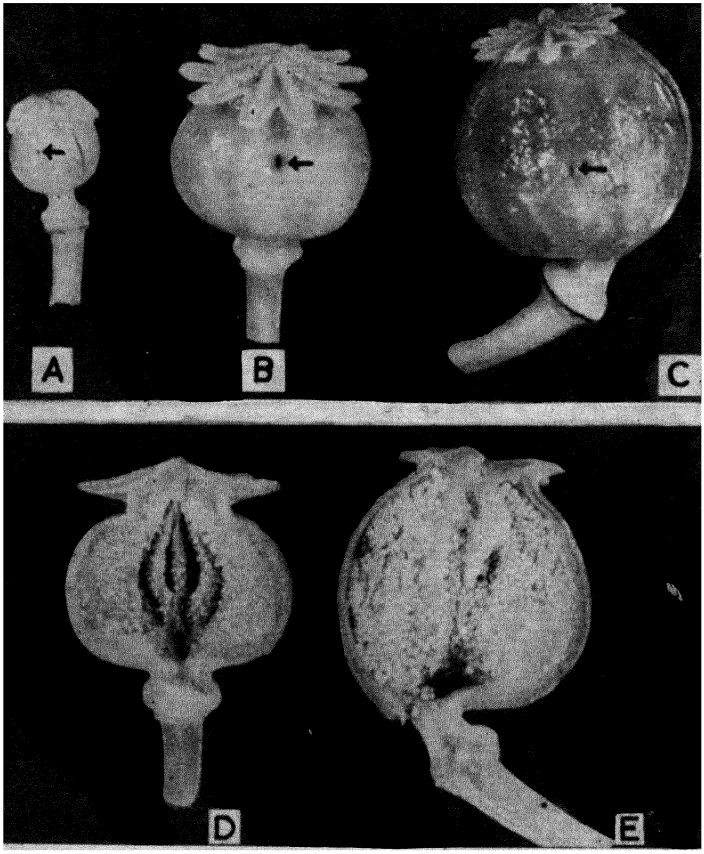

FIG. 7 — *Papaver rhoeas*, OVARIES IN WHICH A POLLEN SUSPENSION WAS INJECTED (ARROW SHOWS THE PRICK) (NAT. SIZE): A–C. 1, 6 and 9 days after injection. D–F. Same as B and C, cut vertically to show the ovules

In early stages the growth of ovaries of even emasculated and bagged flowers matched well with that of the naturally pollinated ovaries, but it gradually declined. The ovules in these parthenocarpically developed ovaries were transparent and devoid of contents. The growth of the unpollinated ovaries pricked with a hypodermic needle did not differ much from that of the unpricked ovaries.

When unpollinated ovaries were injected with a suspension of pollen grains in a solution of boric acid (100 mg./l.), their growth matched that of naturally pollinated ovaries (Fig. 7 A-C). However, the seeds thus obtained were slightly smaller (2·8 mm. × 2·5 mm.) than naturally formed seeds and the seed set was only about 40-75 per cent of the naturally formed seeds (Fig. 7 D-E).

Ovules from ovaries fixed six days after injection showed zygotes or two-celled proembryos (Fig. 6 D). During the second week the embryo grew faster than in nature. The plumule and root tip became differentiated in the third week. However, in some ovules the egg remained undivided even up to fifteen days after injection and these were abortive.

Very often the antipodals persist even in mature seeds (Fig. 6 E).

When ovaries were injected with a pollen suspension made in double distilled water only, they grew normally but the seed setting was only about 20 per cent. Although a longer time was needed, the maximum size attained by such ovaries sometimes surpassed that of the controls and the mature embryo was also slightly larger (Fig. 6 F) than in nature.

Even dry pollen grains when introduced into the ovary through a vertical cut, germinated readily in the ovarian cavity (Fig. 6 C). However, only about 10 per cent of the ovules grew to maturity.

Seeds obtained from intraovarian pollinations are fully viable and their behaviour was identical with that of normal seeds. Both sets of seeds remained dormant for nearly three months. After this, soaking in tap water for 24 hr and then culturing on a modified White's medium results in 100 per cent germination (Fig. 6 G). The root tips of the seedlings show 24 chromosomes.

SUMMARY AND CONCLUSIONS

From time to time several techniques have been tried to overcome the various barriers in fertilization. Yasuda (1931) grafted the style of one flower on the ovary of another in *Petunia violacea*. In a cross between *Zea* (♀) and *Tripsacum* (♂) Mangelsdorf & Reeves (1931) shortened the style of *Zea* to suit the pollen tubes of *Tripsacum* and obtained an intergeneric hybrid. Buchholz *et al.* (1932) removed the middle portion of the style in *Datura* and joined the upper and lower parts together. Hecht (1960) successfully grafted the upper part of the style of an incompatible strain of *Oenothera organensis* on the lower half by means of a "splint" made of lactose gelatin. The pollen tubes grew into the scion overcoming stylar incompatibility. This method, however, is impracticable in plants having thin styles.

A more effective method may be to introduce the pollen grains directly inside the ovarian cavity. But for a few cursory reports such intraovarian

pollinations have not been tried extensively. Dahlgren (1926) was able to bring about fertilization in the ovules of *Codonopsis ovata* by removing the style and placing the pollen grains on the cut end of the top of the ovary. The experiments of Cappelletti (1937) on *Digitalis purpurea*, and of Bosio (1940) on *Helleborus foetidis*, *H. viridis*, *Paeonia anomala*, *P. officinalis* and *P. peregrina* also gave some promising results. The most recent report is that of Kanta (1960) on *Papaver*.

Intraovarian pollinations may prove useful to geneticists when failure to secure desired hybrids is due to incompatibilities resulting in lack of proper growth of pollen tubes and fertilization. By the direct introduction of pollen into the ovary it may be feasible to cross species or varieties and obtain plant types which might otherwise be impossible.

We are indebted to Dr N. S. Ranga Swamy for his help and keen interest during the course of this study.

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Embryological Studies on the Loasaceae with Special Reference to the Endosperm Haustoria

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Embryological data on the family Loasaceae are rather meagre. After some fragmentary observations of Hofmeister (1859), the first investigation was by Kratzer (1918) comprising nine species belonging to the genera *Loasa, Cajophora, Blumenbachia, Mentzelia* and *Gronovia.* Although he pointed out the presence of chalazal and micropylar endosperm haustoria, many of his descriptions are either not illustrated at all or are accompanied by only a few stages of development. Later Crété (1946a, b) gave a more detailed account of the endosperm and embryo development in *Loasa lateritia* Gill.

MATERIALS AND METHODS

The three species studied by the author are : *Cajophora silvestris* (Poepp.) U b et Gilg, *Loasa bergii* Hier. and *Mentzelia laevicaulis* (Doupl.) Gray. The first two were collected in Junin de los Andes, Neuquén, R. Argentina. Material of the last was obtained by Prof. P. Maheshwari through the courtesy of Prof. H. Savitsky from Salt Lake City, Utah, U. S. A. The flowers were fixed in formalin-acetic acid-alcohol and the customary methods followed for preparing the material for microtomy. Sections were cut 8-20 microns thick and stained with Heidenhain's haematoxylin counterstained with fast green or erythrosin. Whole mounts of dissections stained with acetocarmine proved more useful than sections for a study of the fully developed endosperm haustoria.

OBSERVATIONS

An interesting feature of this family is the presence of bristles showing

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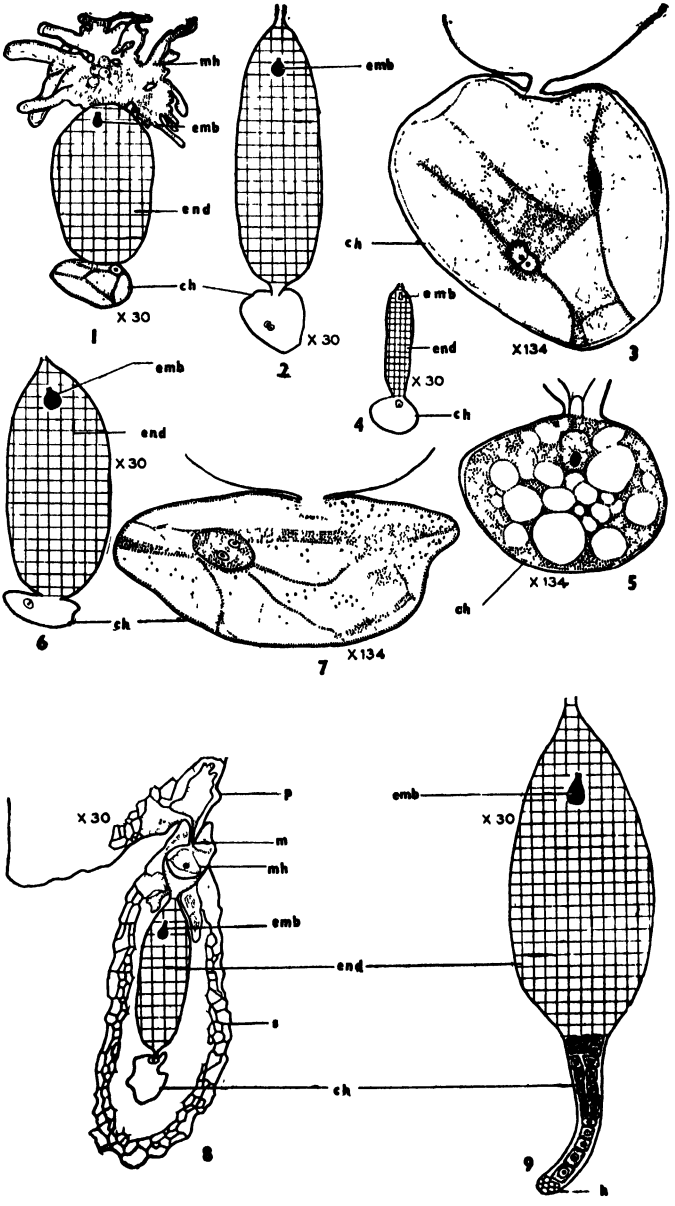
slight variations in the different genera and sometimes accompanied by stinging hairs. The flowers are hermaphrodite, actinomorphic and pentamerous. In some genera the petals are induplicately valvate. There are numerous stamens with their filaments free or joined into bundles. Sometimes the external stamens are petaloid and in *Cajophora* and *Loasa* they function as nectaries. The inferior ovary is unilocular and the calyx tube is adnate to the ovary wall. There are numerous ovules borne on parietal placentae. The ovary wall is ribbed; in *Cajophora* it is spirally twisted and develops into a multiseeded capsule.

In most members of this family the ovules are anatropous, tenuinucellate and unitegmic. There is a long micropyle. According to Kratzer (1918) the megaspore mother cell differentiates from the single cell of the archesporium without cutting off the parietal cells. This, he says, forms a tetrad of which the uppermost cell develops into the embryo sac. However, in *Loasa bergii* it is found that it is the chalazal megaspore that functions. Occasionally the third megaspore may also remain healthy for some time. Like other tenuinucellate ovules, the innermost layer of the integument develops into a well differentiated endothelium. The functional megaspore develops in the normal way giving rise to an 8-nucleate embryo sac of the Polygonum type. The mature embryo sac consists of enlarged micropylar and chalazal portions separated by a narrow constriction. From these enlarged portions the micropylar and chalazal endosperm haustoria originate.

Although the first division of the primary endosperm nucleus is not observed, many ovules show a linear row of about a dozen endosperm cells which divide longitudinally. In all the three species well-developed micropylar and chalazal haustoria are present. In *Loasa bergii* the chalazal haustorium (Figs. 1-8) is balloon-shaped and shows conspicuous vacuoles connected by cytoplasmic strands. Usually a single hypertrophied nucleus is present, but haustoria with more than one nucleus, possibly originated by fragmentation, have also been observed. The chalazal haustorium can be easily detached from the neighbouring tissue consisting of partially digested cells and was studied in sections as well as in dissections.

The micropylar haustorium is coenocytic and possesses hypha-like projections, which sometimes attain an enormous size (Fig. 1). These branches ramify inside the ovule and the funiculus, sometimes reaching even the placentae (Fig. 8). It cannot be easily separated from the neighbouring tissues. The presence of branches in this haustorium is not a constant feature for it is found in some fruits but not in others. Kratzer (1918) suggested that the development of the branches is associated on the one hand with the vigorous development of the embryo and on the other with the differential resistance of the surrounding tissues. The chalazal haustorium remains healthy up to the time of initiation of the cotyledons. Even in later stages the remains can be seen between the main portion of the endosperm and the seed coat. Kratzer reported the branching of the chalazal haustorium in all the three species of *Loasa* studied by him. However, the author has never observed any branching of the chalazal haustorium in *L. bergii*.

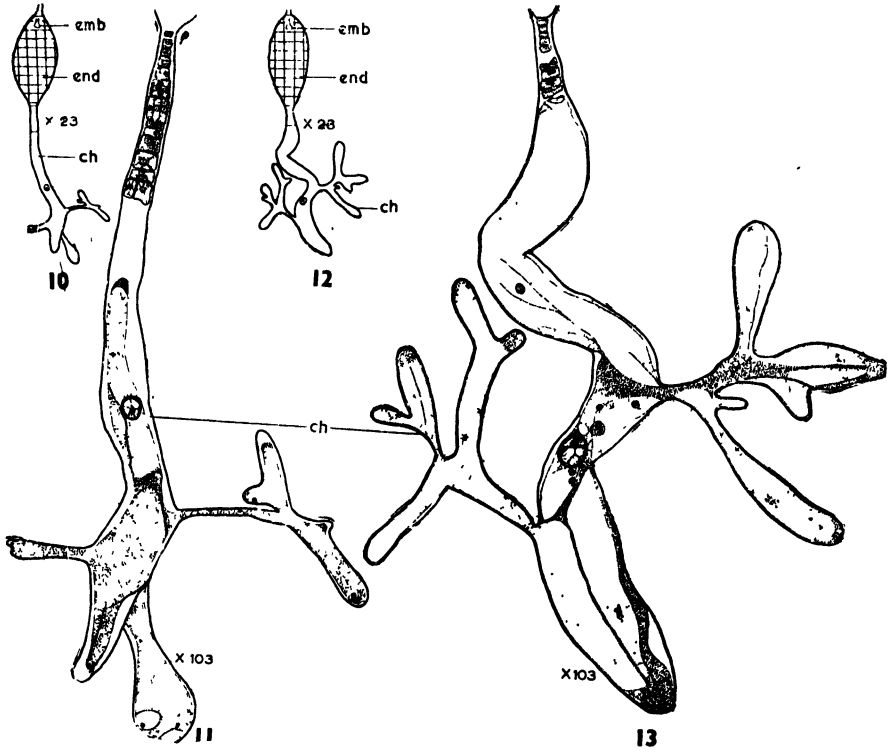


FIGS. 1-9 — Figs. 1-8. *Loasa bergii*; Fig. 9. *Mentzelia laevicaulis* (*ch*, chalazal haustorium; *emb*, embryo; *end*, endosperm; *h*, hypostase; *m*, micropyle; *mh*, micropylar haustorium; *p*, placenta; *s*, seed coat) (All figures except 8 have been drawn from dissected whole mounts) : Fig. 1. Endosperm showing chalazal and much branched micropylar haustorium. Figs. 2, 4, 6. Endosperm with chalazal haustorium. Figs. 3, 5, 7. Magnified views of the chalazal haustorium shown in Figs. 2, 4 and 6. Fig. 8. L. s. seed showing the micropylar haustorium ramifying into the placental tissue. Fig. 9. Endosperm with chalazal haustorium and well developed hypostase

In *Cajophora silvestris* the elongated chalazal haustorium has a multicellular narrow base and a much branched terminal portion with delicate walls (Figs. 10-13). The latter contains a single hypertrophied nucleus. In *C. lateritia*, Kratzer (1918) has reported that the chalazal haustorium remains unbranched. It would be interesting to investigate this and other species of the genus with the help of dissections. The micropylar haustorium is similar but shorter and broader; it has numerous nuclei and occasional apparent wall formation.

In *Mentzelia laevicaulis* the chalazal haustorium is elongated and unbranched, and comprises many enlarged cells with conspicuous nuclei (Fig. 9). Here the ovule also shows a well-developed hypostase which is absent in the other two genera. The growth of the endosperm compresses the chalazal haustorium against the seed coat but its remains can be seen at the chalazal end for a long time. The micropylar haustorium is shorter and broader.

During the development of the embryo, a part of the endosperm, the endothelium and most of the remaining integumentary cells are absorbed.



FIGS. 10-13 — *Cajophora silvestris*. (All figures have been drawn from whole mounts of dissections) (*ch*, chalazal haustorium; *emb*, embryo; *end*, endosperm) : Figs. 10, 12. Endosperm showing chalazal haustorium. Figs. 11, 13. Magnified views of the chalazal haustoria shown in Figs. 10 and 12

Out of the ten or more layers originally present in the integument only the thickened epidermis and two layers of compressed hypodermal cells remain.

In conclusion, it may be said that a comparative study of the embryology of the different genera and species of the Loasaceae may throw some light on the systematic position of the family about which there is so far no agreement. Bentham & Hooker (1862-83) include it in the Passiflorales along with Samydaceae, Turneraceae, Passifloraceae, Cucurbitaceae, Begoniaceae and Datiscaceae. Engler & Diels (1936) include it in the Parietales, an order of doubtful phylogenetic significance and the same has been done by Wettstein (1935). Hutchinson (1959) assigns it to the order Loasales along with the Turneraceae. Recently Takhtajan (1959) has kept it with some reserve, in the Polemoniales on the basis of some affinities with the Boraginaceae and Hydrophyllaceae.

The embryological data so far available on this family indicate a close resemblance to the gamopetalous families. The development of the embryo is comparable to that found in Solanaceae, some forms being related to *Hyoscyamus* and others to *Solanum*. A similar embryonal development associated with endosperm haustoria is also found in some members of Hydrophyllaceae (*see* Crété, 1951). Further work is in progress.

The author is indebted to Prof. P. Maheshwari for critical suggestions and advice. Thanks are due to Dr R. C. Sachar for his help during this investigation and to Ing. A. Burkart of the Darwinion Botanical Institute, Buenos Aires, for the identification of the species of *Loasa* and *Cajophora*.

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Embryology of *Quinchamalium chilense* Lam.

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The embryology of *Quinchamalium chilense* (Santalaceae), a native of South America, has not been studied so far.

The material was processed through *tert.*-butyl alcohol series and prepared in the usual way for microtomy. The study of tortuous embryo sacs and endosperms was supplemented with dissections. For this purpose the ovaries were pretreated with 4 per cent potassium hydroxide solution at 40° C. for 2 to 4 hr. The whole mounts were stained with acetocarmine, mounted in glycerine jelly tinged with acetocarmine and sealed with gold size.

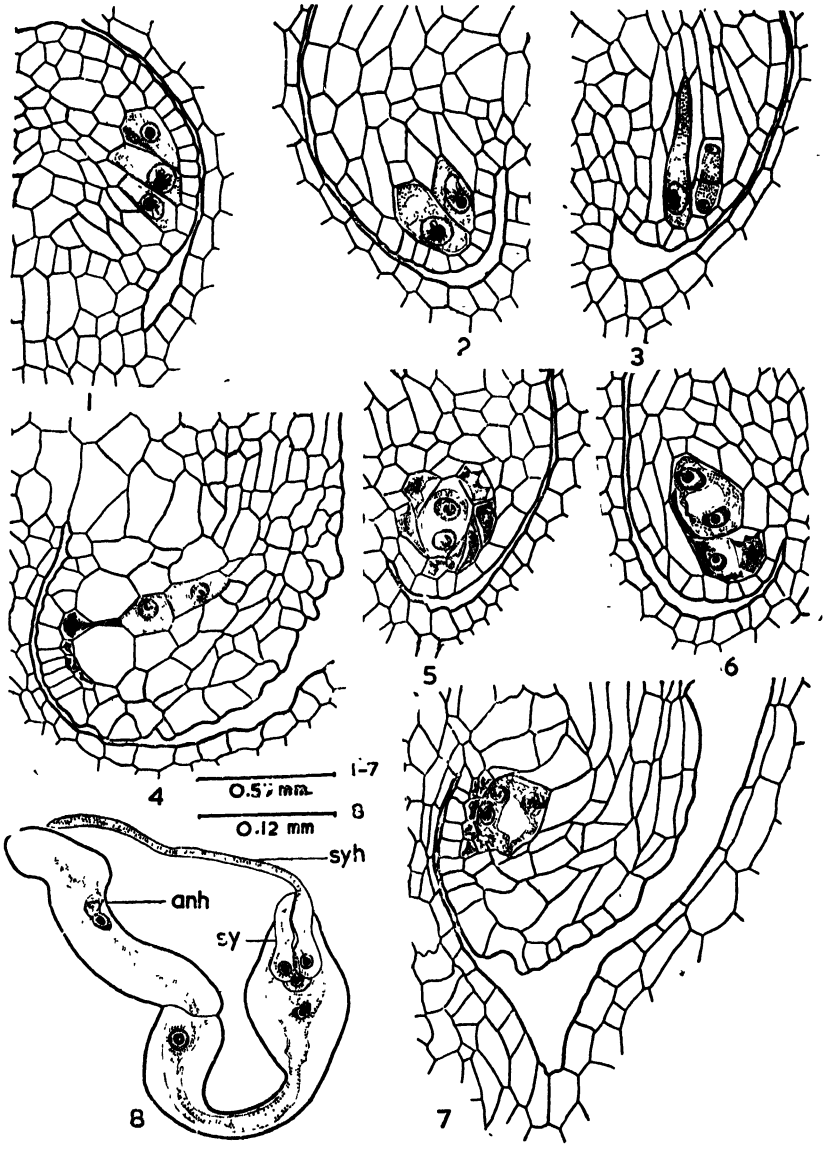
OBSERVATIONS

The flowers are spirally arranged in compressed spikes. They are sessile, bisexual, pentamerous and monochlamydeous, and are borne in a cupular outgrowth of the peduncle. The morphological nature of the cupule is controversial and while Miers (1878) designated it as a calycle, Pilger (1935) considered it to be formed by the coalescence of bract and bracteoles. The perianth is preceded by a whorl of four appendages which surrounds the ovary, the anterior member being much larger than the others. Each of these shows a distinct vascular supply (Figs. 10, 26). These appendages were regarded as calyx by Miers (1878) and as 'Becherkelch' (calyx cupule) by Pilger (1935). Smith & Smith (1942) interpret them as bracts because* (i) in other genera of the Santalaceae, below the ovary, there are one or more appendages which are obviously bracts, (ii) in *Buckleya*, where a whorl of similar appendages is fused with the ovary, the vascular tissue in upper part of the ovary is derived only from the inner whorl of appendages, and (iii) it seems illogical to have the calyx tube free from the ovary and the corolla-staminal tube fused with it.

The perianth forms a long tube and in the bud condition its lobes remain united due to interlocking of the marginal epidermal cells (Fig. 10). There

* Personal communication to Dr B. M. Johri

are five epiphyllous stamens with long filaments and dorsifixed anthers. In most of the santalaceous plants, hairs are present at the base of the stamens but they are absent in *Quinchamalium*. There is a 5-lobed hypogynous disc and its lobes alternate with those of the perianth. The ovary is inferior



FIGS. 1-8 — MEGASPOROGENESIS AND FEMALE GAMETOPHYTE (*anh*, antipodal haustorium ; *sy*, synergid; *syh*, synergid haustorium) : Figs. 1, 2. L.s. ovules showing megaspore mother cells. Figs. [illegible]. Same, showing dyad and tetrad respectively. Figs. 5-7. Two and 4-nucleate embryo sacs. Fig. 8. Whole mount of a mature embryo sac; note the prolongation of the tips of the synergids and the antipodal chamber containing three nuclei

with a long style which grows beyond the level of the anthers making self-pollination rather difficult.

Ovule. The conical placenta bears three hemianatropous ovules which show a massive integument and a reduced nucellus.

Megasporogenesis and Female Gametophyte. The archesporium comprises a single cell or a group of cells which function directly as megaspore mother cells (Figs. 1-3). They undergo reduction divisions forming linear tetrads (Fig. 4) and 2- and 4-nucleate embryo sacs are formed as usual from the chalazal megaspore (Figs. 5-7). At the 4-nucleate stage, the tip of the gametophyte extends beyond the ovular epidermis and grows upwards in between two ovules or between the placenta and the inner wall of the ovary. Similar extension of the embryo sac is also known in *Santalum* (Paliwal, 1956; Bhatnagar, 1959), *Leptomeria* (Ram, 1959) and *Mida* (Bhatnagar, 1960). However, in *Leptomeria* the gametophyte becomes extra-ovular only after organization.

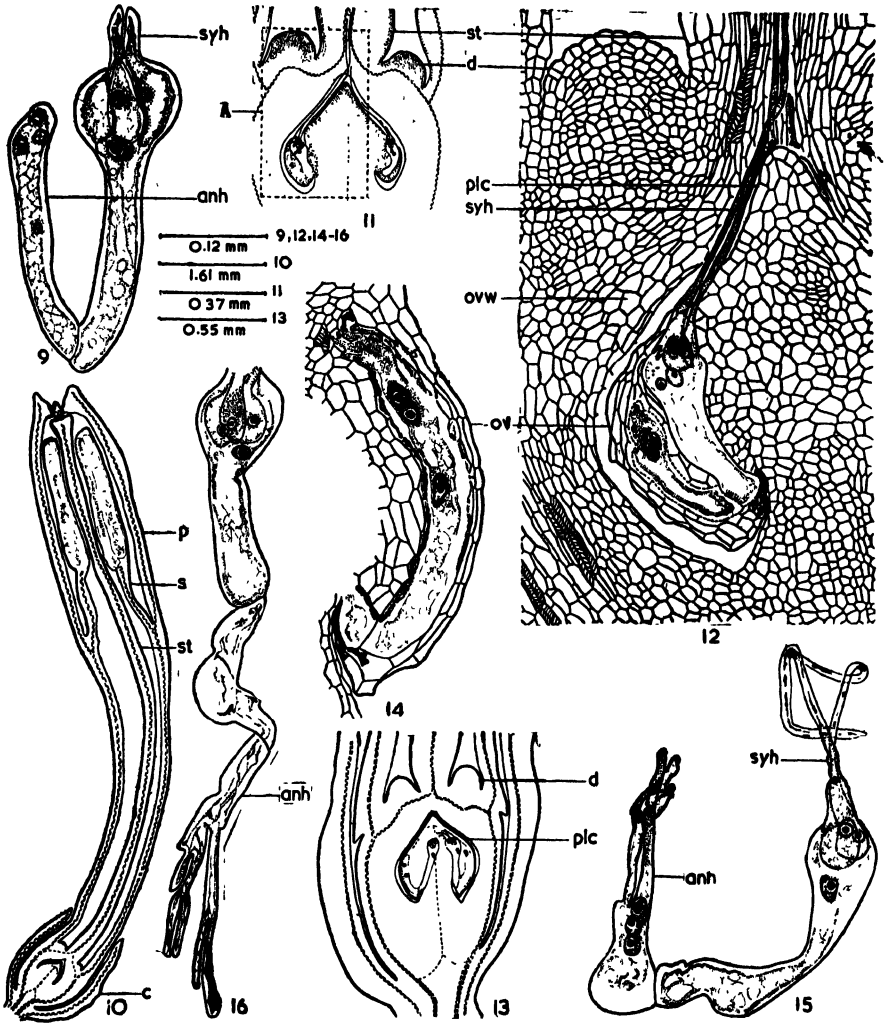
Another nuclear division produces eight nuclei which organize into the egg apparatus, two polars and three antipodal nuclei (Fig. 8). The mature embryo sac is either U- or J-shaped (Fig. 9). Sometimes as many as seven embryo sacs may develop within the same ovary.

The synergids are large with prominent nuclei. Even before fertilization, in closed buds, their tips elongate and protrude beyond the wall of the embryo sac (Fig. 9) forming tubular extensions (Figs. 11, 12, 15, 17-24). The latter grow through the stylar tissue along the vascular supply reaching up to one-third the length of the style (Figs. 11, 12). Occasionally the activity of one of the synergids may be checked (Figs. 8, 20, 23) and, therefore, sometimes only one synergid persists.

Similar but less extensive synergid haustoria are known in some members of the Compositae. Dahlgren (1924) observed that in *Calendula arvensis*, *Mutisia candolleana* and *Ursinia anthemoides* the synergids elongate so much that their tips project to a considerable extent into the micropyle and even beyond it, sometimes reaching as far as the funiculus.

Paliwal's (1956) report about the protrusion of tips of synergids of *Santalum album* and *S. yasi*, after fertilization, to form dichotomously branched haustoria has been contradicted by Bhatnagar (1959). According to Bhatnagar, in *Santalum album* and *S. freycinetianum*, the synergids degenerate soon after fertilization and what have been interpreted as synergid haustoria are really the pollen tubes which adhere to the embryo sac. In fact, as early as 1836 and 1843, Griffith had observed such persistent pollen tubes in *Santalum* and had pointed out that these tubular structures are formed only after the action of pollen on the stigmatic surface.

The antipodal nuclei are separated by a transverse wall from the rest of the embryo sac (Fig. 8) and they do not organize into individual cells. The tip of the antipodal chamber elongates, traverses through the funiculus reaching up to the apex of the placenta where it branches (Figs. 13-16) and invades the placental tissue (Figs. 13, 14). The nuclei become hypertrophied, multinucleolate (Fig. 15) and sometimes undergo divisions forming 5-7 nuclei (Fig. 9).



FIGS. 9-16 -- FEMALE GAMETOPHYTE (*anh*, antipodal haustorium; *c*, cupule; *d*, disc; *ov*, ovule; *ovw*, ovary wall; *p*, perianth; *plc*, placental column; *s*, stamen; *st*, style; *syh*, synergid haustorium) (Figs. 15, 16 from whole mounts, rest from microtome sections) : Fig. 9. Mature embryo sac with five nuclei in the antipodal chamber, secondary nucleus and egg apparatus; note the elongation of the tips of the synergids, the nucleus of one of the synergids could not be traced. Fig. 10. L.s. flower showing disposition of floral organs. Fig. 11. Enlarged view of the lower portion from Fig. 10. Fig. 12. Magnified view of portion marked A in Fig. 11; the tip of the embryo sac has become extra-ovular and has grown along the outer margin of the placenta, the tips of the synergid haustoria have reached up to the base of the style, and tip of the antipodal chamber has also extended into the funiculus. Fig. 13. L.s. ovary showing antipodal haustoria. Fig. 14. Enlarged view of antipodal haustoria from Fig. 13. Fig. 15. Mature embryo sac showing extensive synergid and antipodal haustoria. Fig. 16. Same, note the branched antipodal haustorium, the nuclei were not traceable; the synergid haustoria have been omitted

A pronounced antipodal haustorium has also been reported by Rao (1942) in *Scleropyrum wallichianum*. He says that the antipodal nuclei lie free in the cytoplasm and after fertilization migrate into the antipodal extension (haustorium) of the embryo sac. Subsequently the haustorium grows rapidly and the antipodal nuclei also enlarge. On reaching the base of the placenta, the haustorium branches and curves upwards growing along the endocarp reaching up to the base of the style. The antipodal nuclei also multiply, become hypertrophied and migrate into the branches. He interprets the antipodal haustorium and endosperm haustorium as separate entities. However, his illustrations (*see* his Figs. 44-47) show a haustorium only after the formation of the endosperm and it appears that he mistook the antipodal caecum for the antipodal haustorium which, after the division of the primary endosperm nucleus, acts as the chalazal endosperm haustorium. This is a common feature in the Santalaceae.

Endosperm. The division of the primary endosperm nucleus is accompanied by a transverse wall forming a micropylar and a chalazal chamber (Fig. 20). The micropylar chamber divides transversely and the two daughter cells (Fig. 21) divide vertically (Fig. 22). Further divisions are irregular and the derivatives contribute to the endosperm proper (Figs. 23-28). The chalazal chamber undergoes two successive divisions at right angles to each other forming four cells arranged in an isobilateral fashion (Figs. 21-24). These are the initials of the endosperm haustoria. They elongate and extend along the course of the antipodal haustorium of the embryo sac (Figs. 25, 26), reaching up to the apex of the placenta. Their remnants can be recognized till the late globular stage of the embryo (Fig. 26).

The mature embryo is dicotyledonous, and occupies almost the entire length of the endosperm (Fig. 28).

SUMMARY AND CONCLUSIONS

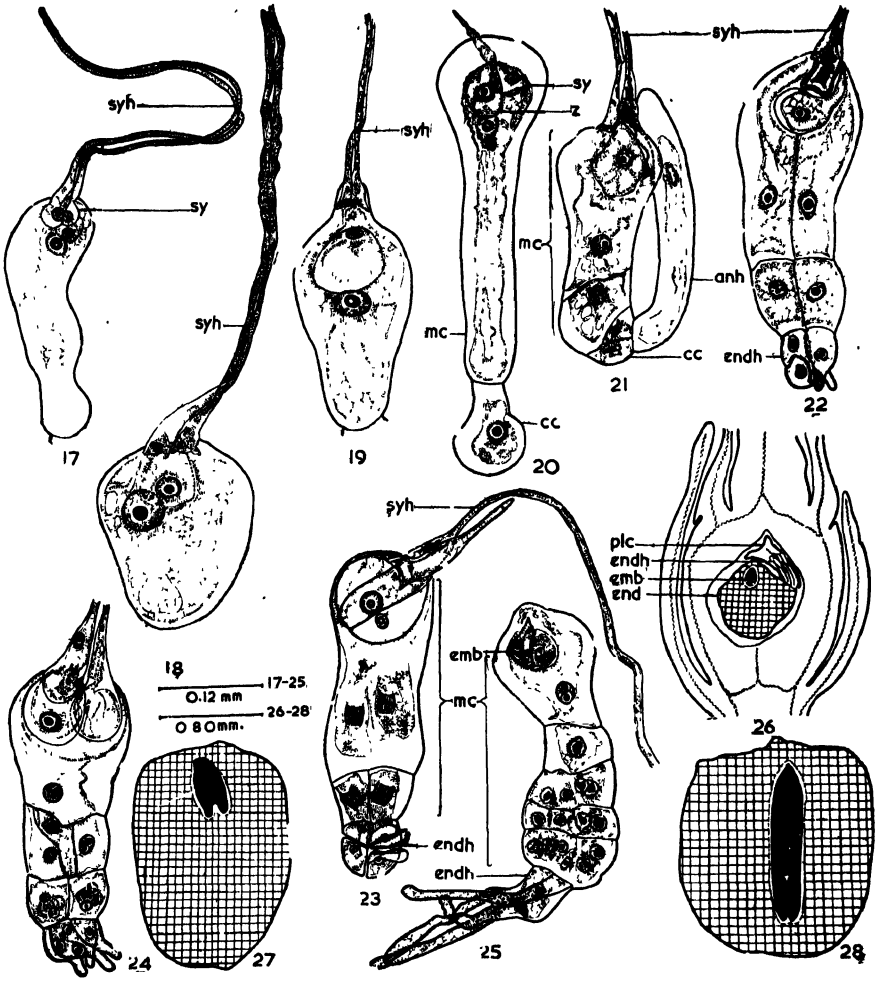
The flowers are sessile, bisexual and pentamerous and are enclosed in a cupular structure. The ovary is inferior with a short placenta which bears laterally three hemianatropous ovules. The latter show a massive integument and a reduced nucellus.

The embryo sac conforms to the Polygonum type. At the 4-nucleate stage the micropylar end becomes extra-ovular and grows towards the style. The mature embryo sac is U- or J-shaped. The antipodal nuclei are separated by a wall from the rest of the embryo sac and do not organize into individual cells. The tips of the synergids protrude beyond the wall of the embryo sac and grow in between the placenta and inner wall of the ovary reaching up to nearly one-third the length of the style. The tip of the antipodal chamber also extends through the funiculus forming a conspicuous haustorium which ramifies along the vascular tissue.

The endosperm is Cellular. The first division of the primary endosperm nucleus is transverse forming a micropylar and a chalazal chamber. The micropylar chamber alone forms the endosperm proper. The chalazal

chamber undergoes two divisions forming four cells which give rise to endosperm haustoria. The embryo is dicotyledonous.

On the basis of the presence of a perigonium, the genus *Quinchamalium* was first assigned to the family Santalaceae (De Candolle, 1856). The pre-



FIGS. 17-28 — ENDOSPERM (*anh*, antipodal haustorium; *cc*, chalazal chamber; *emb*, embryo; *end*, endosperm; *endh*, endosperm haustorium; *mc*, micropylar chamber; *plc*, placenta; *sy*, synergid; *syh*, synergid haustorium; *z*, zygote) (Figs. 21, 26-28 from microtome sections, rest from whole mounts. The remnants of antipodal haustoria have been omitted in Figs. 17-20): Figs. 17-19. Fertilized embryo sacs, note the synergid haustoria, the nuclei of the synergids were not seen in Fig. 19. Fig. 20. Two-celled endosperm. Fig. 21. Two-celled micropylar chamber, the chalazal chamber has divided into four cells; the antipodal haustorium is still persisting. Figs. 22-25. Progressive stages in the development of endosperm and chalazal endosperm haustoria. Fig. 26. L. s. ovary showing endosperm at the globular stage of proembryo, the endosperm haustoria have extended through the funiculus. Figs. 27, 28. Endosperms at early and late dicotyledonous stages of embryo

sence of a distinct calyx outside the corolla tube led Miers (1851) to remove this genus (along with *Arjona* and *Myoschilos*) to the family Olacaceae. In 1878 he placed them under a separate tribe Arjoneae, where each flower is supported by a calycle. Van Tieghem (1896) kept *Quinchamalium* and *Arjona* in a separate family Arionacées and justified it on the basis of (i) the epigynous disc instead of calycinal as is common in the Santalaceae, (ii) the hairs at the base of the stamens arising from the epidermal cells of the perianth in *Arjona* instead of from the hypodermis as in the Santalaceae, and (iii) the ovary being unilocular in the upper region and plurilocular with one ovule in each loculus in the lower region.

As far as the first point is concerned, Smith & Smith (1942) pointed out that the epigynous disc is not a distinctive character of the family. Secondly, even in the Santalaceae the hairs originate from the epidermis and not from the hypodermis and in this respect Van Tieghem's observation is incorrect. Lastly, the plurilocular condition of the ovary in the basal region is met with in several genera of the Santalaceae, e.g. *Choretrum*, *Leptomeria*, *Osyris*. Thus, in all the three characters, *Arjona* and *Quinchamalium* resemble other members of the Santalaceae. Besides, there are additional similarities like the nature of the placenta, the extra-ovular extension of the embryo sac at the 4-nucleate stage, Cellular endosperm, and formation of endosperm haustoria from the chalazal chamber.

Therefore, the taxonomic assignment of *Quinchamalium* should await studies of the two allied genera, *Arjona* and *Myoschilos*.

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Effect of Some Growth Substances and Calyx on Fruit and Seed Development of *Althaea rosea* Cav.

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The *in vitro* culture of ovaries was initiated by LaRue (1942), who apart from obtaining rooting of excised floral parts, achieved appreciable growth of ovaries of many plants. Since then this technique has been improved upon and is useful for investigating problems of fruit development. Ovaries of tomato (*Lycopersicum esculentum*), tobacco (*Nicotiana tabacum*), strawberry (*Fragaria chiloensis* × *F. virginiana*), gherkin (*Cucumis anguria*) and bean (*Phaseolus vulgaris*) were grown *in vitro* with varying degrees of success by Nitsch (1949, 1951, 1952). Jansen & Bonner (1949) duplicated the result with another species of tomato, *Lycopersicum pimpinellifolium*. From *in vivo* studies on a self-sterile strain of tomatoes (John Baer), Leopold & Scott (1952) concluded that fruit set is dependent upon the presence of mature leaves. When flowers were grown on different media, it was found that a large number of nutritive materials could be substituted for leaf requirement. Rédei & Rédei (1955) cultured pollinated ovaries of wheat and later obtained normal plants from the embryos excised out of these. de Capite (1955) grew the ovaries of *Fragaria* and *Pisum*, but the fruits produced *in vitro* were smaller than those *in vivo*. Anantaswamy Rau (1956) studied the influence of colchicine on the embryo and endosperm of *Phlox drummondii*, from the ovaries maintained on artificial medium.

Nitsch (1951) reported that the general pattern of ovary growth *in vitro* is the same as *in vivo*. This indicates that the growth responses of the ovaries maintained in artificial media are dependable, and allow for reasonable comparisons.

The present paper deals with the effects of some growth substances and calyx on fruit and seed development in *Althaea rosea*.

MATERIAL AND METHODS

Plants of *Althaea rosea* (hollyhock), grown in the Botanical Gardens of the Department of Botany, University of Delhi, were used for the present

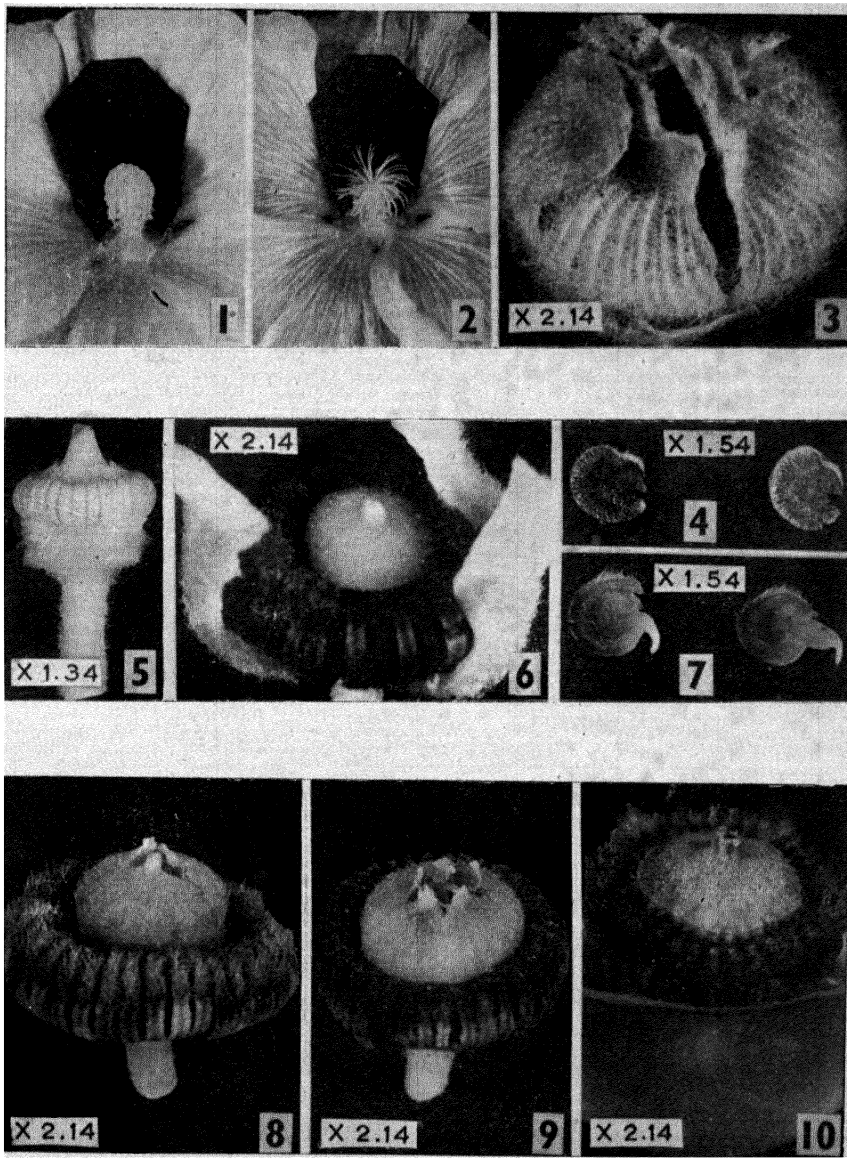


FIG. 1—Flower at anthesis; the stigmas are still within the staminal tube. Fig. 2. Same, showing the emergence of stigmas. Fig. 3. A mature fruit produced in nature 30 days after pollination. Fig. 4. Seeds of the above. Fig. 5. Ovary at the time of inoculation (3 days after pollination). Fig. 6. A 25-day-old mature fruit from an ovary planted with intact calyx on NBV. Fig. 7. Germinating seeds from another fruit (40-day-old) maintained on NBV. Fig. 8. A 20-day-old fruit cultured on NBV + GA (25 ppm). Fig. 9. Another fruit (30-day-old) grown on NBV + IBA (20 ppm). Fig. 10. A 25-day-old fruit cultured on NBV + IAA (5 ppm) + kinetin (0·5 ppm)

study. The flowers were tagged on the day of anthesis and were picked three days after pollination. The corolla and the staminal tube were removed. The calyx and the epicalyx were trimmed in most of the flowers, but were retained in some. Surface sterilization was done by keeping the ovaries in a 15 per cent decanted solution of calcium hypochlorite for 15 minutes. At the time of inoculation a short distal portion of the pedicel was trimmed.

The basic medium (abbreviated as NBV) comprised Nitsch's (1951) mineral salts and sucrose and a mixture of vitamins and glycine (modified from White, 1943). This medium was supplemented by indoleacetic acid (IAA, 5 ppm), indolebutyric acid (IBA, 1, 5, 10, 20 ppm), gibberellic acid (GA, 1, 5, 10, 25 ppm), kinetin (0·1, 0·2, 0·5, 1, 2 ppm) and colchicine (50, 100, 200 ppm) and was jelled with 0·8 per cent of Bacto agar. Ovaries were also maintained on plain agar to see how far the development proceeds without any nutrients.

Rimless 'pyrex' tubes, plugged with nonabsorbent cotton (wrapped in a piece of cheese-cloth), were used as containers. The *p*H of the medium was adjusted to 5·5. In each tube 10 ml. of the medium was dispensed and these were autoclaved at 15 lb. pressure for 20 minutes. The cultures were maintained at laboratory temperatures (17-21° C.), and while the majority of them received diffused daylight, some sets were kept in total darkness.

Twenty-four ovaries were planted in each medium, except the one with gibberellic acid in which only 12 were sown. An increase in diameter was taken as an index for growth, and a change in colour, from greenish yellow to black, as the criterion for maturity.

The development of endosperm and embryo was studied mainly by dissections. Microtome sections of the seeds were cut at the thickness of 12-14μ, and Heidenhain's haematoxylin was used for staining.

OBSERVATIONS

Studies In Vivo. The anthers start dehiscing a day or two after anthesis and the stigmas, which are at first enclosed in the staminal tube (Fig. 1), come out and spread in 3-4 days (Fig. 2). After pollination the corolla closes in a couple of days and is eventually shed along with the style and the staminal tube. The calyx and the epicalyx are persistent (Fig. 3). Depending upon the season, the fruits attain their maximum size of 17-20 mm. diameter in 10-18 days after pollination. Maturation occurs in about 30-35 days (in February) and this period gradually shortens with the advance of season; in April the fruits mature in 20-25 days. After maturation the mericarps separate and are dispersed by wind. Fruit set is about 95 per cent.

The removal of the calyx from the pollinated flowers did not affect the growth of the fruits (Fig. 11).

Development of Seed. At the time of inoculation (three days after pollination) the ovules showed free nuclear endosperm and a globular proembryo (Fig. 12). In some only the zygote and primary endosperm nucleus were observed. Eight days after pollination the ovules showed mostly heart-

shaped embryos and the endosperm had become cellular, with the conical chalazal part alone containing free nuclei. Within 25 days the normally developing seeds showed a mature embryo (Fig. 19). However, about 15-20

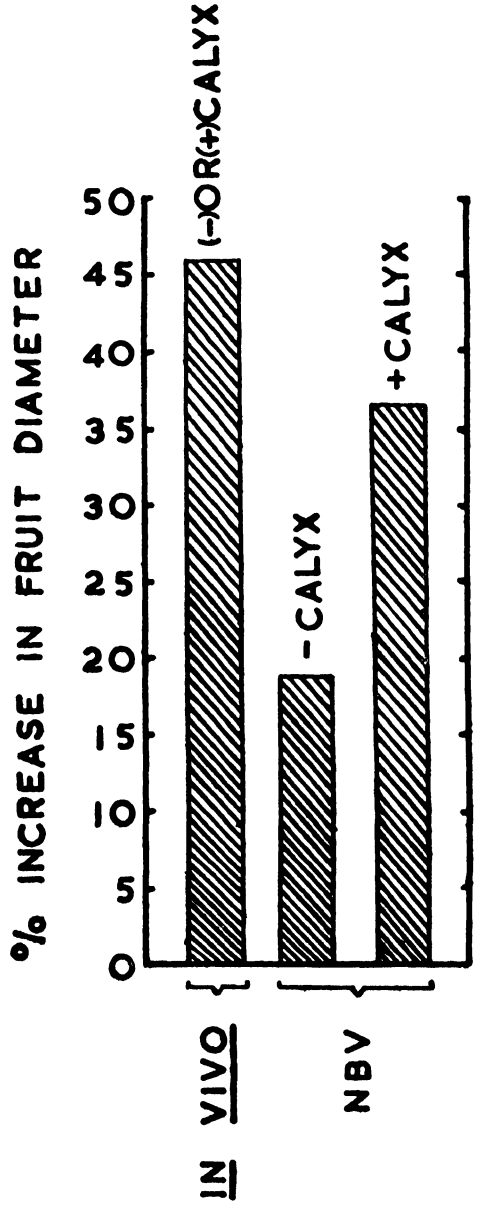


FIG. 11 — HISTOGRAMS GIVING THE INCREASE IN FRUIT DIAMETER IN TERMS OF THE PERCENTAGE OF THE TOTAL POSSIBLE GROWTH. THE EFFECT OF CALYX ON FRUIT GROWTH STANDS OUT PROMINENTLY ON NBV

per cent seeds did not develop properly and were either empty or contained globular or heart-shaped embryos.

Studies In Vitro. With the advance in season the diameter of the ovary at the time of inoculation also increased. In February the maximum diameter was 12 mm., in March 14 mm. and in April 15·8 mm. This resulted in a lesser and lesser capacity for growth after inoculation. Therefore, the comparisons between different media are based on the percentage of the total possible increase in diameter in each experiment (Table 1).

Fig. 5 shows an ovary three days after pollination in the month of February. Unless otherwise mentioned all the ovaries were planted without calyx and were maintained in diffused daylight. The behaviour of the ovaries is given separately under each of the media used.

Plain Agar. The increase in diameter of the ovary was only to the extent of 0·4 mm. and there was no maturation.

NBV. About 58 per cent of the ovaries showed growth and the average diameter obtained was 12 mm. No maturation was observed. In another set of 24 ovaries kept in dark, the average fruit diameter was 14·4 mm. and one of the fruits showed signs of maturation 40 days after inoculation.

The presence of the calyx was beneficial for the growth and maturation of the fruits (Fig. 11). Nearly 85 per cent fruits attained an average diameter of 19 mm. The seeds were also comparable to those in nature (Figs. 4, 7). Of the 12 ovaries planted late in season (April), eight started turning black after 15 days and four of these were fully mature by the 20-25th day (Fig. 6). In one fruit many of the embryos germinated *in situ* (Fig. 7).

NBV + Colchicine. In 50 ppm of colchicine only 20·8 per cent of the ovaries increased in diameter by 0·5 mm. while the rest did not grow at all. No signs of maturation were observed. With 100 ppm 66·6 per cent of the ovaries increased appreciably in diameter. Two fruits showed signs of maturation after 50 days. A still higher concentration (200 ppm) was less effective than 100 ppm (Table 1).

NBV + Gibberellic Acid. Only 16·6 per cent of the ovaries planted on NBV + GA (1 ppm) grew well. One of the fruits started maturing after 30 days. With 5 ppm, 33·3 per cent of the ovaries grew and one fruit turned slightly black. In 10 ppm, 40·2 per cent of the ovaries grew and the final diameter exceeded that in 5 ppm. Two fruits showed signs of maturation but only one turned fully black. The best results were obtained with 25 ppm GA (Table 1). In this 50 per cent of the ovaries increased in diameter, two fruits turned almost black by the 20th day (Fig. 8) and a third started maturing after 30 days.

NBV + IBA. Out of the 24 ovaries planted in each concentration, a set of 12 was kept in diffused daylight while the other set was kept in total darkness.

In the first set, (1 ppm) IBA did not prove any better than the basic medium. In 5 ppm about 50 per cent of the ovaries showed good growth and a couple of fruits matured. The next two concentrations (10, 20 ppm) did not improve

growth very much over the basic medium and only two fruits matured in 20 ppm (Fig. 9).

In darkness, with 1 ppm IBA, 25 per cent of the ovaries showed an average increase of 0·8 mm. in diameter and one fruit exhibited signs of maturation. In higher concentrations (5, 10 ppm) fruit growth increased considerably. The best results were obtained in 20 ppm IBA (Table 1). About 90 per cent

TABLE 1—COMPARISON OF DIFFERENT MEDIA BASED ON THE PERCENTAGE OF INCREASE IN DIAMETER

Treatment	Initial diam. mm.	Total possible increase (19-x)*	Actual increase mm.	Increase %
In vivo	14·5	4·5	4·5	100·0
NBV	10·1	8·9	1·9	21·4
(dark)	11·3	7·7	3·1	40·3
(+ calyx)	14·5	4·5	4·5	100·0
NBV + colchicine				
50 ppm	15·8	3·2	0·2	6·0
100 ppm	9·9	9·1	3·2	35·2
200 ppm	9·3	9·7	2·6	26·8
NBV + GA				
1 ppm	15·6	3·4	0·6	17·6
5 ppm	15·1	3·9	0·8	20·5
10 ppm	15·6	3·4	1·3	38·0
25 ppm	15·0	4·0	1·8	45·0
NBV + IBA (dark)				
1 ppm	15·1	3·9	0·8	20·5
5 ppm	15·3	3·7	1·1	29·7
10 ppm	11·1	7·9	2·7	34·2
20 ppm	15·1	3·9	3·4	87·0
NBV + IAA				
5 ppm	14·6	4·4	0·4	9·0
NBV + kinetin				
0·1 ppm	15·8	3·2	0·1	3·0
0·2 ppm	15·3	3·7	0·6	16·2
0·5 ppm	13·1	5·9	1·7	28·8
1 ppm	14·3	4·7	2·1	44·7
2 ppm	13·7	5·3	1·7	32·0

* 19 mm. is the average final fruit size, x is the initial fruit diameter.

of the ovaries showed good growth and the average fruit diameter was 18·5 mm. The maximum diameter was attained in 10-15 days after inoculation and 6 fruits out of 12 matured in 25-30 days.

NBV + IAA. Only 8·3 per cent of the ovaries increased in diameter from 14·6 to 15 mm. One of them started turning black after 30 days, but did not mature.

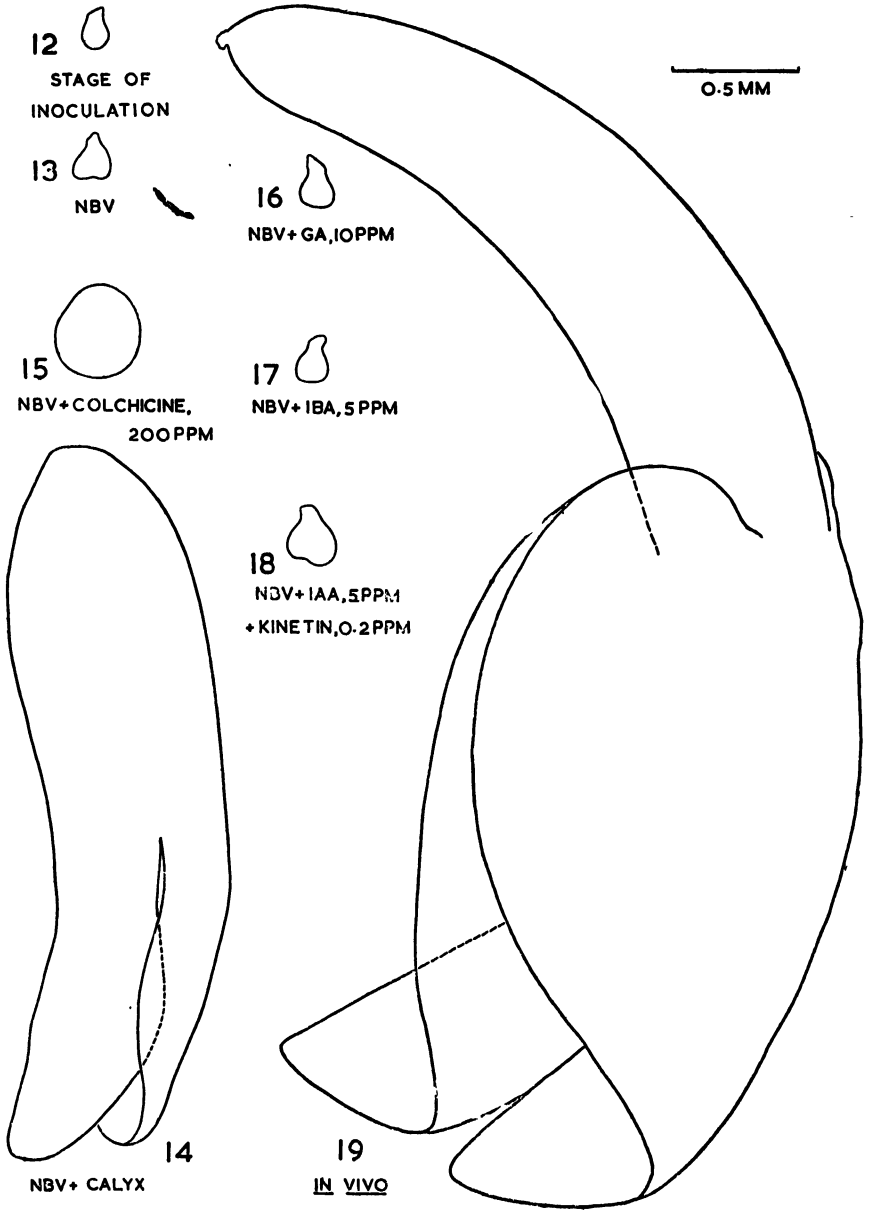
NBV + Kinetin. In 0·1 ppm kinetin the increase in fruit diameter was only 0·1 mm. and none of the fruits matured. The addition of 0·2 and 0·5 ppm kinetin improved growth. A couple of fruits started turning black, but full maturity was not attained. A slight increase in the girth of pedicels was noticed in some cultures. With 1 ppm kinetin the increase in fruit diameter was still greater. Four fruits started maturing but only one matured fully. In 2 ppm, although the percentage of ovaries which grew was greater, the increase in fruit diameter was less. A couple of fruits turned slightly black.

NBV + IAA + Kinetin. The concentration of IAA (5 ppm) was kept constant while that of kinetin was varied. With a combination of 0·1 ppm kinetin and IAA, fruit growth and maturation improved as compared to 0·1 ppm kinetin or 5 ppm IAA used individually. In this case 40·2 per cent of the ovaries grew and the average fruit diameter was 17 mm. With 0·2 ppm kinetin fruit growth also improved. The next concentration of kinetin (0·5 ppm) proved to be even better. About 70 per cent of the ovaries grew and increase in diameter was 55 per cent of the total possible increase. The maximum diameter was attained in 10-15 days, and maturation started in three fruits, of which two ripened in 20-30 days (Fig. 10). In a few cultures the pedicels showed a slight increase in girth. With 1 ppm kinetin, 50 per cent of the ovaries showed good growth, and one fruit turned slightly black. With 2 ppm kinetin, the percentage of fruit set as well as the increase in fruit diameter was lower, and maturation of fruits failed to occur.

Development of Seed. Dissections of thirty-day-old ovaries, maintained on plain agar, revealed only unhealthy heart-shaped embryos. No endosperm could be found. Development of the endosperm and embryo showed marked differences in fruits grown on NBV with and without calyx. When grown without calyx the endosperm remained free nuclear and the embryo progressed only up to the pre-heart or heart-shaped stage (Fig. 13). On the other hand, many of the embryos showed well developed cotyledons in fruits cultured with intact calyx (Fig. 14). In one of the fruits most of the embryos had reached their normal size and germinated *in situ*. In general, however, the size of the embryos was much smaller as compared to that in nature. Endosperm development seemed to be normal.

In NBV + colchicine (50 ppm) some of the embryos showed the initiation of cotyledons, and the endosperm became cellular in the micropylar region. In higher concentrations (100, 200 ppm), on the other hand, the embryo remained at the globular stage and there was no cell formation in the endosperm. The embryo at times became very large (Fig. 15), but its differentiation was checked.

With other supplements (e.g. GA, IBA, IAA, kinetin) the growth of the embryo and endosperm was inhibited to a greater extent than with colchicine. Most of the embryos remained at the globular stage, or rarely develop-



FIGS. 12-19 — DIAGRAMS OF EMBRYOS DISSECTED FROM 25–30-DAY-OLD FRUITS. THE RESPECTIVE MEDIA ARE GIVEN BELOW EACH FIGURE

ed to heart-shaped stage (Figs. 16-18). The endosperm showed just a few cells, if at all, at the extreme micropylar end. In all such cases the contents of the embryo sac gradually degenerated and the seeds were reduced to flat papery structures.

DISCUSSION

LaRue (1942) reported a fivefold increase in the size of tomato ovaries. The fruits of *Kalanchoë* developed practically to full size, though the seeds did not contain embryos. *Forsythia* fruits, whether rooted or not, remained alive in culture for a year and reached almost their maximum size. *Caltha* fruits grew to about half of their normal size and burst open, exposing immature seeds.

Nitsch (1951) observed that pollinated ovaries of tomato could be easily raised on a basic medium comprising mineral salts and sucrose only. With the addition of tomato juice to the medium, fully mature tomatoes were obtained, but their final size was smaller than that of fruits ripening on the plant. Ovaries of gherkins, strawberry, bean and tobacco also produced small fruits in the basic medium.

Sachar & Baldev (1958) report that in *Linaria maroccana* the most favourable medium for ovary culture was Nitsch's basic medium supplemented with vitamins, glycine and yeast extract (0·5 per cent), but the control ovaries in field grew to a larger size. Similarly in *Tropaeolum majus* (Sachar & Kanta, 1958) even the largest fruit, produced in Nitsch's basic medium with vitamins, glycine and thiamin (10 ppm), failed to come up to the size of natural fruits. In *Iberis amara* (Nirmala Maheshwari & Lal, 1958) the fruit growth *in vitro* paralleled to that *in vivo*. With the addition of IAA to the medium the fruits were sometimes even larger than those in nature.

In *Althaea rosea* the best results were obtained with IBA, when the cultures were maintained in darkness (*see* also Chopra, 1958). The average diameter of the fruits was 18·5 mm. as compared to 19 mm. in nature.

The above findings indicate that the requirements for best fruit growth vary with different plants and in some none of the tested chemicals give good results.

Effect of Calyx on Fruit Growth. The calyx has a beneficial effect on fruit growth. Sachar & Kanta (1958) observed that in *Tropaeolum* the presence of the calyx and corolla increased the diameter of the fruit from 2·5 to 5 mm. (in plain agar), while ovaries planted without calyx attained a diameter of only 4·2 mm. Nirmala Maheshwari & Lal (1958) also report that ovaries of *Iberis* with calyx and corolla always outgrew those in which these had been removed. In *Althaea* the ovaries raised without calyx in the basic medium grew to a diameter of 12 mm., while those with intact calyx produced the largest fruits measuring 19 mm. in diameter.

Seed Development. Nitsch (1951) stated that in the basic medium normal seeds failed to develop in the pollinated ovaries of tobacco and tomato. In gherkins also, most of the fruits showed only underdeveloped seeds with

soft seed coats, although when tested for germination a few produced "apparently normal seedlings." In *Phlox*, Anantaswamy Rau. (1956) observed multinucleate embryonal cells and a nodular organization of the endosperm under the influence of colchicine. Early embryogeny proceeded normally, but the ovaries which remained in the colchicine medium for more than 12-14 days showed considerably malformed seeds with degenerated endosperm and embryos. In *Tropaeolum* (Sachar & Kanta, 1958) the embryos from ovaries grown in the basic medium did show some growth and differentiation comparable to that in nature. However, these embryos were always smaller than the normal embryos of the same age and later showed signs of degeneration. The addition of various growth substances did not bring about any improvement (*see* also Sachar & Chopra, 1959).

Sachar & Baldev (1958) report that the development of endosperm and embryo in *Linaria* proceeded alike both in cultured and in natural fruits, except that there was a greater deposition of starch in the endosperm of the former. In *Iberis* as well (Nirmala Maheshwari & Lal, 1958) the ovaries maintained on Nitsch's basic medium with vitamins and glycine (NBV) and NBV with IAA (5 ppm) showed normal growth of the endosperm and embryo. In other cultures of *Iberis*, with 2, 4-D and kinetin, the growth of the endosperm and embryo was arrested.

In *Althaea* none of the chemicals promoted the growth of the endosperm and embryo. The endosperm mostly remained free nuclear, while the growth of the embryos was arrested at the heart-shaped stage. All such seeds were eventually reduced to thin papery structures.

Effect of Calyx and Other Maternal Parts on the Development of Endosperm and Embryo. Rédei & Rédei (1955) planted ovaries of *Triticum* in the culture medium 3-4 days after pollination. If the ovaries were deprived of the lemma and palea, the embryo seldom developed further. On the other hand, in ovaries enclosed by these parts the embryo displayed continued differentiation, although the endosperm showed only a jellied appearance. When the embryos were excised 8-12 days after inoculation and transferred to a fresh medium, they produced normal plants.

In *Althaea* normal endosperm and embryo development took place only in the ovaries planted with calyx on the basic medium. Although in some fruits germination started in the test tube, the size of embryos was usually much smaller than that in nature.

SUMMARY

Pollinated ovaries of *Althaea rosea* have been successfully cultured in artificial medium. The presence or absence of the calyx did not make any appreciable influence on fruit growth *in vivo*. *In vitro*, on the other hand, the calyx not only affected fruit growth and maturation, but also the development of the endosperm and embryo. The ovaries planted in the basic medium without calyx produced fruits which were only 12 mm. in diameter,

while those with intact calyx formed much larger fruits (19 mm.) which were comparable to those in nature. Further, normal endosperm and embryo development was observed only in ovaries cultured with calyx. The ovaries devoid of calyx revealed only free nuclear endosperm and heart-shaped embryos.

Of the supplements used, IBA proved to be the best for the growth and maturation of fruits. In 20 ppm IBA the average fruit diameter was 18·5 mm. (in dark) and 50 per cent of the fruits matured. Darkness increased fruit size and the percentage of maturation.

None of the growth substances promoted the growth of the endosperm and embryo. The former remained mostly in the free nuclear condition and the latter did not progress beyond the heart-shaped stage. In all such cases the embryo sac contents eventually degenerated and the seeds were nonviable.

The author takes this opportunity to thank Professor P. Maheshwari for his keen interest in this work.

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In Vitro Production of Onion Seeds

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In recent years extensive researches have been carried out on the culture of dicotyledonous tissues and organs, but the monocotyledons have received much less attention. Robb (1957) cultured explants from the bulb scales of *Lilium speciosum* which proliferated and differentiated into bulblets in 15-16 weeks. Clark & Heath (1959) studied the changes in the growth substance-content of onion plants during bulbing.

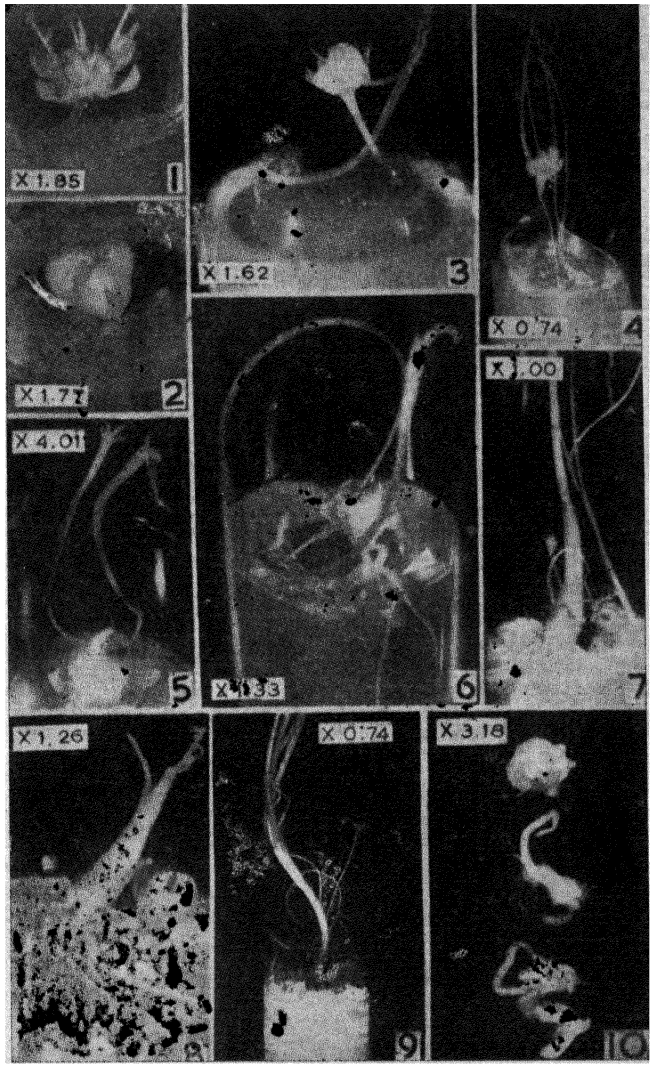
The present paper deals with the *in vitro* production of seeds of *Allium cepa* var. *Pusa red.*

EXPERIMENTAL PROCEDURE

Pollination of flowers occurs on the day of anthesis. The flowers were collected two days after pollination (Fig. 1), sterilized for 5 minutes in chlorine water, washed thoroughly with sterile water and inoculated aseptically in ' Pyrex ' culture tubes containing 15 ml. of sterile nutrient medium. The basal medium (BM) comprised half-strength Knop's major salt solution, minor salt solution (Nitsch, 1951), vitamin solution (White, 1943; modified by the addition of calcium pantothenate and increasing the original concentration by 2·5 times), 5 per cent sucrose, 0·7 per cent Difco Bacto agar and 10 mg./l. ferric citrate. To this medium were added several growth substances like casein hydrolysate (CH), yeast extract (YE), gibberellic acid (GA), indoleacetic acid (IAA), naphthalene acetic acid (NAA), kinetin (K) and tryptophane.

OBSERVATIONS

Growth In Vivo. Two days after pollination the ovary is 3 to 4 mm. in diameter, and in 21 days attains the optimal size (6-7 mm.). The ovary has three, rarely four loculi, each of which usually contains two ovules. The fruits mature and dehisce in six weeks. Seeds germinate immediately after shedding, first producing a coleorrhiza which grows into the soil and with the development of the cotyledonary leaf, the seed is carried upwards above the surface of the soil. The second leaf originates from the upper part of the



FIGS. 1-10 — GROWTH OF EXCISED FLOWERS : Fig. 1. Flower at the time of inoculation (2 days after pollination). Fig. 2. A six-week-old mature fruit on BM + IAA (1 ppm) + GA (4 ppm) + K (0·5 ppm). Fig. 3. Seedling formed on BM. Fig. 4. Fruit cultured on BM + GA (4 ppm) showing *in situ* germination of seed; the ovary is pushed up due to the elongation of the cotyledon (nat. size). Fig. 5. *In situ* germination of seeds on BM + IAA (1 ppm); instead of growing downwards the root-end extended upwards, note the initiation of root from the tip of the axis. Fig. 6. Seedling produced on BM+ NAA (2 ppm); arrow points to the root bearing root hairs outside the medium, the portion inside the medium lacks them. Fig. 7. Bulb produced on BM+IAA (0·5 ppm); the roots are short and thick. Fig. 8. Seedling showing branched root system produced on BM + IAA (1 ppm) + GA (2 ppm). Fig. 9. Bulbs produced on BM + IAA (1·0 ppm) + K 0·5 ppm. Fig. 10. Eight-week-old abnormal embryos on BM + NAA (2 ppm)

compressed stem. At the time of pollination the ovule contains the 8-nucleate organized embryo sac. Fertilization occurs about three days after pollination and in another seven days a 2-celled proembryo (Fig. 11), and after four weeks mature embryo (length 5 mm.) is produced.

Growth In Vitro. Flowers sown on BM remain fresh up to three weeks and the ovaries attain a diameter of 5-6 mm. In a 15-day-old culture only about 6 to 7 per cent seeds show the embryo (length 0·5 mm.) and normal endosperm. The mature embryos attain the natural size (5 mm.). The remaining seeds fail to advance beyond the stage of inoculation. Five per cent seeds germinate *in situ* 7 to 8 weeks after inoculation (Fig. 3).

Addition of 0·5 or 1 ppm IAA to BM did not stimulate growth and while the fruits measured 6 mm. in diameter after 6 weeks, the percentage of viable seeds increased to 20 per cent. There is also a slight increase in the size of the mature embryo (6 mm.) and seeds germinate *in situ* six weeks after inoculation.

At 3 and 5 ppm IAA the fruits grow to natural size but due to the degeneration of embryos the seeds do not germinate. On BM + 2 ppm IAA germination is rare but the addition of 0·5 ppm of kinetin induces frequent germination.

On BM+4 ppm GA, 20 per cent seeds show mature embryo (7 mm.) and well developed endosperm. The remaining seeds are abortive and shrunken. With GA 2 or 6 ppm the ovules abort at an early stage and viable seeds are not produced.

On BM+IAA 2 ppm+GA 2 ppm although mature embryos develop after 6 weeks, the rate of growth is much slower as compared to that in nature. Eleven days after inoculation the ovules show 3-celled proembryos (Fig. 13) and free nuclear endosperm. In this medium the synergid frequently enlarges so much that it appears much larger than the egg (Fig. 12).

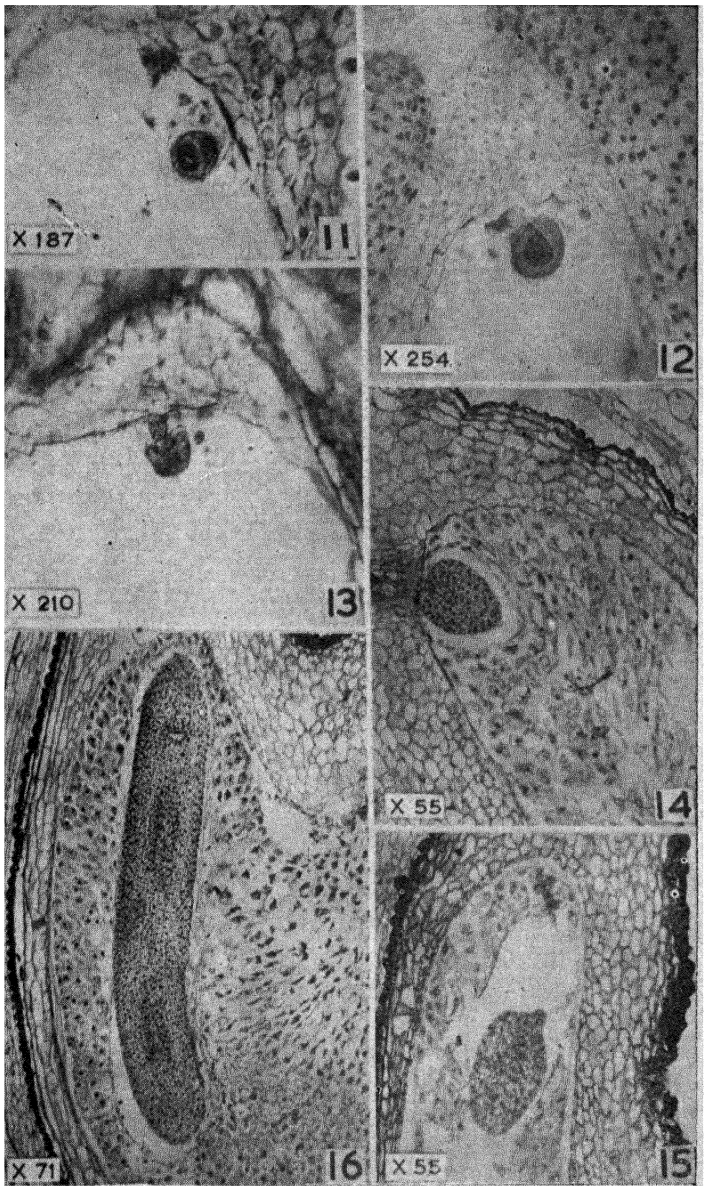
Fruits of optimal size, diameter 9 mm. (natural size 5 to 6 mm.), are produced (Fig. 2) on BM+IAA 1 and 2 ppm + GA 4 ppm + kinetin 0·5 and 1 ppm but viable seeds are rarely produced. Only one per cent of the seeds germinate nine weeks after inoculation while rest of the ovules fail to develop beyond the stage at excision. In four-week-old cultures the ovules often contain embryo sacs with healthy primary endosperm nucleus and degenerated egg.

On BM + NAA 2 ppm the ovary shows satisfactory growth and mature embryos are produced in six weeks. Besides normal embryos some unusually large embryos also develop (Fig. 10) but such seeds do not germinate.

BM + tryptophane 2 and 5 ppm produces best growth of the endosperm and embryo. About 40 per cent seeds show globular proembryo 11 days after inoculation (Fig. 14) and differentiated embryo after 16 days (Fig. 15). 30 per cent develop to maturity in the next three weeks (Fig. 16). Thus, the time required for the development of the embryo *in vitro* is almost the same as in nature.

The *in vitro* seedlings show some differences in different media. In those produced on BM + IAA the root end extends upwards rather than growing

downwards as on BM alone (Fig. 5). After it attains a length of about 3 cm. a number of scale leaves develop from the base of the small discoid stem.



FIGS. 11-15 — HISTOLOGICAL STRUCTURE OF OVULES : Fig. 11. L. s. upper half of ovule, seven days after pollination, showing two-celled proembryo. Fig. 12. Embryo sac from ovules reared for two weeks on BM + IAA (2 ppm) + GA (2 ppm) showing egg and a hypertrophied synergid. Fig. 13. A three-celled proembryo produced in 11 days on BM + IAA (2 ppm) + GA (2 ppm). Figs. 14, 15, 16. Embryo and cellular endosperm from 11, 16 and 35 day-old cultures on BM + tryptophane (2 ppm) respectively

These scales turn crimson and form a miniature bulb. The roots remain stunted and thick (Fig. 7). Green leaves rarely appear on BM + IAA even four weeks after germination. BM + IAA + K also fails to induce the formation of green leaves but bulb formation is much better than on BM + IAA (Fig. 9). BM + GA gave strikingly different results as compared to BM + IAA. The radicle entered into the medium and produced a long root. After two weeks many roots developed and attained a length of 12 to 15 cm. Many leaves also developed from the upper portion of the stem (Fig. 4).

On BM + IAA + GA, the relative concentrations of IAA and GA determine the type of seedling, e.g. with IAA 1 ppm and GA 2 ppm there is profuse leaf formation and a branched root system (Fig. 8) but bulb formation is rare.

On BM + NAA 2 ppm the seedlings have a much smaller bulb (Fig. 6) than on BM + IAA.

When tryptophane is substituted for IAA, bulb formation is completely inhibited, but a number of leaves and fibrous roots are produced.

SUMMARY AND CONCLUSIONS

At the time of inoculation of flowers of *Allium cepa*, the ovules contained the organized embryo sac and in nature the embryo develops in four weeks. In flowers reared on BM only 5 per cent seeds showed normal embryo and endosperm. On BM + IAA (0·5, 1 ppm) and BM + GA (4 ppm) 20 per cent fertile seeds were produced. The best growth of seeds was obtained on BM + tryptophane (2 and 5 ppm) where 30 per cent seeds were viable.

Usually, the fruits grown in artificial media remain smaller than the size of natural fruits, e.g. in *Cooperia* (Sachar & Kapoor, 1958), *Linaria* (Sachar & Baldev, 1958) and *Tropaeolum* (Sachar & Kanta, 1958). In *Iberis* (Maheshwari & Lal, 1958), however, natural-sized or even slightly bigger fruits are produced. This is also true of *Allium*. The natural size of fruits is reached on BM + IAA (0·5, 1 ppm) and BM + GA (4 ppm) but larger fruits are produced on BM + IAA + GA + K although in the latter case only 1 per cent seeds are viable. It may be presumed that the role of fertile seeds in fruit development has been replaced by the supplements added to the nutrient medium. Similar results were also obtained in *Ranunculus* (Sachar & Guha, 1960).

The development of seed coat is independent of the growth of endosperm and embryo since healthy-looking seeds were frequently empty. In almost all the media tried, the size of embryo was comparable to that produced in nature.

The author records with pleasure her gratitude to Dr B. M. Johri for his guidance and to Professor P. Maheshwari for comments and advice. Thanks are also due to the Council of Scientific & Industrial Research, New Delhi, for financial assistance.

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Development of Exembryonate Seeds in *Foeniculum vulgare* Mill.*

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Some of the healthy-looking seeds of fennel are often non-viable and this may be due to (i) failure of endosperm development, (ii) occurrence of rudimentary or otherwise defective embryos, or (iii) embryolessness. The third cause of non-viability has been studied.

OBSERVATIONS

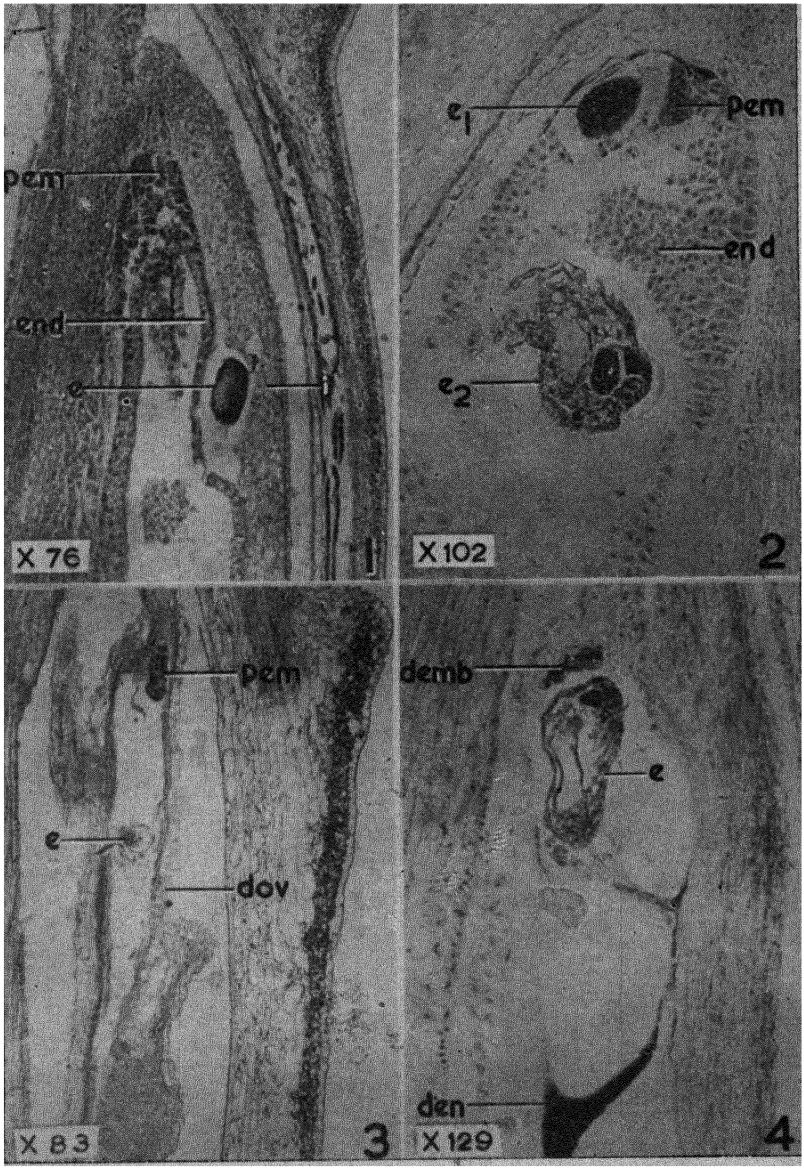
A cursory examination of the mericarps of *Foeniculum vulgare* showed a hole in the fruit-wall indicating the escape of an insect. The microtome sections of the young fruits containing globular embryo and cellular endosperm revealed an insect larva and the dissection of mature fruits yielded a phytophagous chalcid fly, *Systole albipennis* Walk. (family Eurytomidae) or one of the two hyperparasites, *Tetrastichus* sp. (family Eulophidae) or *Liodontomerus* sp. (family Torymidae).

Usually one (rarely two) egg of *Systole* is laid in the mericarp between the integument and the endosperm (Fig. 1). At this time the endosperm is partially or completely cellular, and the proembryo is filamentous or globular. Concomitantly with the growth of the fruit, the chalcid egg develops into the larva, pupa and adult fly (Fig. 6) at the cost of the endosperm and embryo (Fig. 2). After maturation, it gnats a hole in the pericarp and escapes (Fig. 5).

Owing to the insect attack the embryo, sometimes even the endosperm, fails to develop in approximately 40 per cent mericarps (Fig. 4). Occasionally the proembryo may continue to develop for some time although the growth of the endosperm is arrested (Fig. 3). Since the insects which escape from the fruits infect fresh mericarps, the percentage of exembryonate seeds increases during the late season. These seeds cannot be distinguished from the embryonate ones by their external appearance, size or weight.

*Part of the thesis entitled "Morphological and Embryological Studies of some Umbelliferous Spices" approved for the Ph.D. degree by the University of Delhi.

At times, the phytophagous chalcid larva may be overlaid by the egg of one of the two hyperparasites (Fig. 7), *Tetrastichus* sp. or *Liodontomerus*

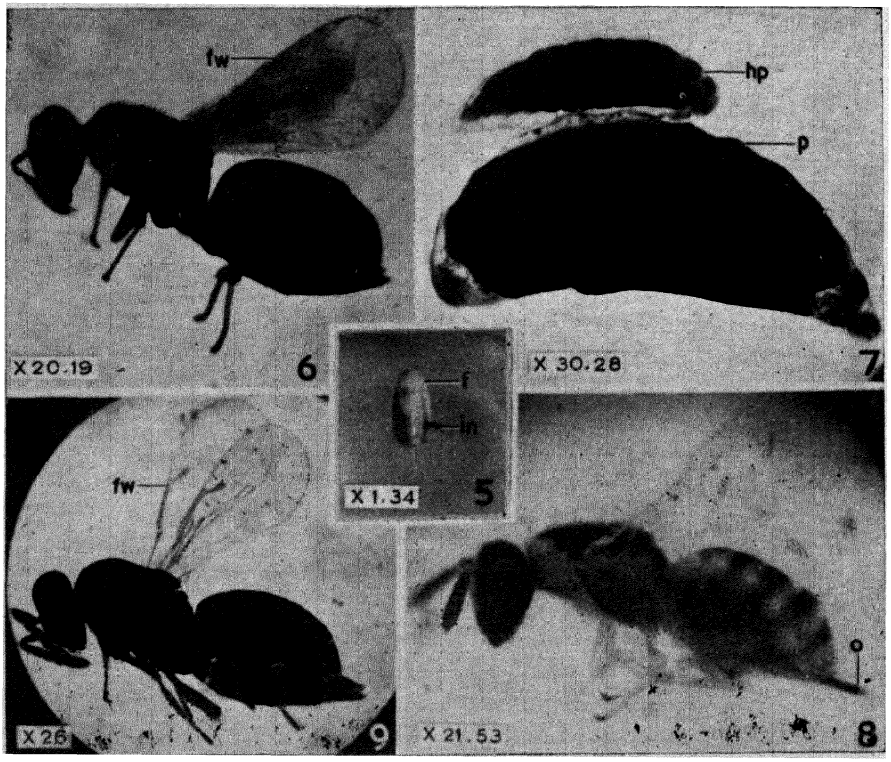


FIGS. 1-4—LONGISECTIONS OF DEVELOPING FRUITS OF *Foeniculum vulgare* (*demb*, degenerated embryo; *den*, degenerated endosperm; *dov*, degenerated ovule; e, e_1, e_2, developing insects; *end*, endosperm; *i*, integument; *pem*, proembryo) : Fig. 1. Shows insect egg between the endosperm and integument. Fig. 2. The proembryo and endosperm have been partially damaged by the two developing insects. Fig. 3. Arrested growth of endosperm. Fig. 4. Both the endosperm and embryo have degenerated

sp. The hyperparasites develop into adults at the cost of *Systole* larva but do not destroy either the embryo or the endosperm. However, as the hyperparasites grow in size, some of the surrounding cells may be crushed. Thus, in some fruits an adult *Tetrastichus* or *Liodontomerus* may be lying side by side with a normal embryo.

To trap the insects (*Systole*, *Tetrastichus* and *Liodontomerus*), the fertilized ovaries of *Foeniculum* were cultured on modified White's medium (*see* Ranga Swamy, 1961) jelled with 0·8 per cent Difco Bacto agar. Sucrose (5 per cent) was used as the carbon source. The *p*H of the medium was adjusted to 5·9. The cultures were kept at ± 25° C. and ±50 per cent relative humidity in diffuse sunlight.

At the time of inoculation the young mericarps contained globular proembryo and cellular endosperm with the larva of *Systole*, or it had been already hyper-parasitized by *Liodontomerus* (Fig. 8) or *Tetrastichus* (Fig. 9). In a few cultures, 10 days after inoculation, the adult insects escaped by boring a hole in the fruit-wall and survived for 11 to 17 days inside the culture tubes.



FIGS. 5-9 — PARASITE AND HYPERPARASITES (*f*, fruit; *fw*, forewing; *hp*, hyperparasite; *in*, insect; *o*, ovipositor; *p*, parasite) : Fig. 5. Insect emerging out of the fruit. Figs. 6-8. Whole mounts of insects dissected from the fruits of *Foeniculum*. Fig. 6. *Systole albipennis*. Fig. 7. Parasite and hyperparasite larvae. Fig. 8. *Liodontomerus* sp. Fig. 9. *Tetrastichus* sp. from a fruit of *Foeniculum* cultured *in vitro*

SUMMARY AND CONCLUSIONS

Flemion and her co-workers (1941; 1949; 1954; 1955) have reported that embryolessness in the umbelliferous seeds is due to the external feeding by *Lygus* bug which is said to deposit toxic oral secretions inside the fruits causing abortion of the embryo and endosperm (*see* Flemion, 1955). This view has also been supported by Kho & Braak (1956).

Neither the toxic nature of the oral secretions has so far been demonstrated, nor is there any direct evidence to support the view of Flemion *et al.* (1954) that *Lygus* transfers micro-organisms to the ovaries which cause embryolessness. According to Flemion & Henrickson (1949) the climatic conditions, types of soil, genetic influence, etc. have no effect on embryolessness. The author did not notice *Lygus* bug feeding on the fruits of *Foeniculum* and his observations indicate that the exembryonate seeds are produced due to damage caused to the embryo and endosperm by the growth of *Systole albipennis.*

It gives the author great pleasure to express his gratitude to Dr B. M. Johri, under whose guidance this work was carried out, and to Professor P. Maheshwari for his constant encouragement and advice. The insects were identified by Dr B. D. Burks (U. S. National Museum, Washington) and Dr J. F. Perkins (British Museum, Natural History, London) to whom the author is most grateful. Thanks are also due to the Indian Council of Agricultural Research, New Delhi, for financial assistance.

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Female Gametophyte of the Santalales

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The earliest embryological studies on the Santalaceae and Loranthaceae are those of William Griffith (1836a, b), Assistant Surgeon in the Madras Establishment of the East India Company and later attached to the Botanical Garden and the Medical College at Calcutta, who studied several species of these families. He not only used spirit for preserving the material to be examined later but also employed the technique of dissection. Without the latter method it would be almost impossible to study the tortuous course of embryo sacs in the Santalales and the author also used it with great advantage.

During the last ten years, many members of the Santalales have been investigated at the University of Delhi and the results summarized by Johri & Bhatnagar (1960). They agree that this order should comprise two suborders: Santalineae and Loranthineae, but point out that the Santalineae should include only four families—Olacaceae, Grubbiaceae, Santalaceae and Myzodendraceae, and the Loranthineae two families—Loranthaceae and Viscaceae. The Santalaceae may be subdivided into six tribes—Comandreae, Thesieae, Osyrideae, Santaleae, Opilieae and Anthoboleae.

OLACACEAE

In *Olax imbricata* the development of the embryo sac conforms to the Polygonum type (Fagerlind, 1947) whereas in *O. wightiana* (Shamanna, 1954) it is of the Allium type. Saroj Agarwal (University of Delhi) has recently confirmed bisporic development in *O. wightiana* and observed Polygonum type in another species—*O. scandens*. The tip of the 4-nucleate embryo sac grows between the ovule and the placental column or the inner wall of the ovary. All the nuclei of the embryo sac now divide producing the 8-nucleate gametophyte and the egg apparatus and polar nuclei are organized as usual. The antipodal nuclei do not organize into individual cells but remain limited to a common cell cut off at the base of the embryo sac. Sometimes two cells may organize and one of these is binucleate. At this stage a lateral caecum arises just above the level of the antipodal chamber,

branches repeatedly and traverses through the funiculus reaching up to the apex of the placental column.

The embryo sac of *Anacolosa frutescens* (Fagerlind, 1947) and *Strombosia ceylanica* (Agarwal, 1961) also conform to the Polygonum type and it does not extend beyond the ovule. In *Strombosia* starch appears at the 4-nucleate stage and sometimes it is so abundant that the nuclei may be altogether masked. The antipodal nuclei organize into individual cells.

GRUBBIACEAE

The development of the female gametophyte has not been studied in *Grubbia*.

SANTALACEAE

In this family all the members so far investigated show the Polygonum type of embryo sac. Some of the characteristic features of each tribe are dealt with below.

Comandreae. *Comandra umbellata* (Ram, 1957) has been studied in detail. Just below the level of the egg apparatus a lateral caecum arises on the funicular side, grows through the ovule, enters into the placenta and extends along the vascular strand. The polar nuclei lie at the mouth of the caecum. Sometimes only two antipodal cells (one binucleate) are formed. The synergids and antipodal cells degenerate even before fertilization. A single embryo sac develops in each ovule and by the time it reaches maturity most of the ovular tissue is consumed.

Thesieae. Bhatnagar & Agarwal (1961) have recently studied *Thesium alpinum* and *T. wightianum*. The embryo sac shows the normal organization and a caecum arises from the chalazal end leaving the antipodal cells *in situ*. The latter degenerate before fertilization. The tip of the embryo sac is somewhat swollen and it gradually consumes the integumentary tissue.

Agarwal (1962) has pointed out several new features in *Quinchamalium chilense*. In contrast to other members of the Santalaceae, neither the synergids nor the antipodal nuclei are ephemeral. Moreover, even before fertilization, the tips of synergids produce tubular haustorial processes which grow through the stylar tissue reaching up to one-third the length of the style. *Quinchamalium* has perhaps the longest synergid haustoria so far known in any angiosperm. There is also a much branched antipodal haustorium which invades the placental tissue.

Osylideae. Joshi (1960) reports that in *Osyris wightiana* (syn. *O. arborea*) the 4-nucleate embryo sac elongates considerably, destroys the ovular epidermis and its tip lies exposed in the ovarian cavity. A lateral caecum arises from the chalazal end leaving the antipodal cells *in situ*. The caecum elongates and invades the funiculus and the placental column.

In *Scleropyrum wallichianum* (Rao, 1942) the tip of the gametophyte does

not extend beyond the ovule and the antipodal nuclei are said to lie free in the embryo sac. An aggressive "antipodal haustorium" develops from the chalazal end and it enters the placental column before fertilization. Later on the haustorium grows up to the base of the placental column and undergoes branching. These processes curve upwards, undergo further branching, grow into the endocarp and some of them reach up to the base of the stylar canal. The antipodal nuclei enlarge and divide and the daughter nuclei migrate into the haustorium. It is likely that Rao mistook the endosperm haustorium for the antipodal haustorium and this point requires a careful reinvestigation.

Santaleae. We have considerable information on this tribe and the genus *Santalum* has been studied by several persons (*see* Paliwal, 1956; Bhatnagar, 1959). The tip of the 4-nucleate embryo sac extends beyond the ovule and grows towards the style between the placental column and the inner wall of the ovary. A chalazal caecum is also produced which grows downwards into the placental column leaving the antipodal cells *in situ*. The so-called synergid haustoria reported by Paliwal appear to be the remnants of the persistent pollen tubes (*see* Bhatnagar, 1959). The embryo sac of *Leptomeria cunninghamii*, *L. acida* (Ram, 1959b) and *Mida salicifolia* (Bhatnagar, 1960) is similar to that of *Santalum*, except that in *Leptomeria* the gametophyte becomes extra-ovular only after organization of the nuclei.

Opilieae. The tip of the embryo sac of *Opilia amentacea* (Shamanna, 1955) does not grow beyond the ovule but the chalazal end produces a large caecum leaving the antipodal cells *in situ*. The caecum invades the placenta. Swamy (1962) has pointed out that it extends into the solid part of the gynoecium in the direction of the pedicel so that the chalazal arm of the embryo sac becomes nearly three times longer than the micropylar arm.

Swamy (1960) has also studied *Cansjera rheedii*. The embryo sac resembles that of *Opilia*. With the initiation of the chalazal caecum the antipodal nuclei migrate into it but rapidly degenerate. The upper part of the embryo sac enlarges and crushes the adjoining integumentary tissue (except the outermost layer) so that the micropyle is demolished and the egg apparatus becomes exposed. Considerable amount of starch accumulates in the gametophyte.

Anthoboleae. Ram (1959a) investigated three species of *Exocarpus*—*E. cupressiformis*, *E. sparteus* and *E. strictus*, and *E. menziesii* has been worked out by Fagerlind (1959). Ovules, which normally develop on the placenta, are distinguishable into the integument and nucellus but in this genus the embryo sac develops in the placenta itself and the ovules are absent (*see also* Fagerlind, 1959). The tip of the embryo sac grows upwards beyond the placenta and the egg apparatus becomes exposed. Finger-like haustorial processes develop in *E. strictus* and these are much more extensive in *E. menziesii*.

In *Myzodendron punctulatum* and *M. quadriflorum* (Skottsberg, 1913) the embryo sac is of the Polygonum type and the antipodal end of the embryo sac extends into the placental column.

LORANTHACEAE

The embryo sac is of the Polygonum type and in some members, e.g. *Macrosolen* (Maheshwari & Singh, 1952), *Lepeostegeres* (Dixit, 1958b) and *Atkinsonia* (Prakash, 1961) the two lower nuclei of the 4-nucleate embryo sac divide earlier than the upper nuclei. Thus, a 6-nucleate stage precedes the 8-nucleate gametophyte. In the latter two plants, embryo sacs may sometimes show reversed polarity.

In most of the members the lower end of the embryo sac extends up to the base of the hypostase leaving the antipodal cells *in situ*. The upper end may remain limited to the ovary or may ascend into the style and stigma. This is the only family of angiosperms where such a behaviour has been observed. The ovarian, stylar and stigmatic tissues adjoining the gametophyte are usually rich in starch and sometimes starch accumulates in the embryo sac also.

Elytrantheae. The tip of the embryo sac extends only up to the base of the style in *Macrosolen* (Maheshwari & Singh, 1952) and *Peraxilla* (Prakash, 1960), slightly above the base of the style in *Lepeostegeres* (Dixit, 1958b), and up to almost half the length of the style in *Lysiana* (Narayana, 1958a).

Lorantheae. The upper end of the embryo sac reaches up to middle to two-thirds the height of the style in *Amyema* (Dixit, 1958a), *Dendrophthoe* (Singh, 1952), *Helicanthes* (Johri, Agrawal & Garg, 1957) and *Tolypanthus* (Dixit, 1961), and up to the stigma in *Barathranthus* (Garg, 1959), *Helixanthera hookeriana* (Schaeppi & Steindl, 1942), *Tupeia* (Smart, 1952) and *Tapinostemma* (Garg, 1959). In *Helixanthera ligustrina* (Maheshwari & Johri, 1950) it comes to lie just below the stigmatic epidermis.

Nuytsieae. The apical portion of the embryo sac does not extend beyond the base of the style in *Atkinsonia* (Prakash, 1961) but in *Nuytsia* (Narayana, 1958b) it ascends up to two-thirds the length of the style. In both the genera a caecum develops from the upper end of the embryo sac.

VISCACEAE

This is the only family in the Santalales where the development is bisporic and the problem of polarity of the embryo sac is of considerable interest (*see* Maheshwari, 1950).

Phoradendreae. The embryo sac is U-shaped in *Dendrophthora* (York, 1913), *Ginalloa* (Rutishauser, 1937), *Korthalsella* (Correa, 1958) and *Phoradendron* (Billings, 1933; Rizzini, 1950). The curvature takes place at the 4-nucleate stage so that the lower end of the embryo sac containing two nuclei grows out of the mamelon, curves and elongates in the carpellary tissue. Eight nuclei are formed as usual and the egg apparatus and the upper polar nucleus organize in the outer arm of the embryo sac which reaches a higher level than the other arm. The latter remains within the mamelon and contains the antipodal cells and the lower polar nucleus. Three synergids have been

reported in *Dendrophthora* and *Phoradendron*. Billings also reported four antipodals in the latter plant which appears to be unlikely since the synergids and antipodals are usually ephemeral. In fact, Billings and York's observations are far from satisfactory and tetrasporic origin of embryo sac in *Phoradendron* may be rejected. This genus as well as *Dendrophthora* require thorough reinvestigation.

Arceuthobieae. The embryo sac is straight and only seven nuclei have been observed in *Arceuthobium americanum* (Dowding, 1931), A. *oxycedri* (Johnson, 1888) and *A. minutissimum* (Correa, 1958). This is probably due to an early fusion of the polar nuclei.

Visceae. In *Viscum* (Steindl, 1935; Schaeppi & Steindl, 1945) also the embryo sac is straight and the synergids simulate the egg.

SUMMARY AND CONCLUSION

In the entire order Santalales the embryo sac is of the Polygonum type, except in the family Viscaceae where it is bisporic. The extension of the embryo sac at both ends and the formation of prominent haustoria are also common features. The extensive synergid haustoria of *Quinchamalium* is a unique feature and it would be of great interest to know if this is common to some other species in this order. In the family Loranthaceae the upper part of the gametophyte may ascend to various heights in the style until in some genera it comes to lie directly below the stigmatic epidermis.

We have practically no knowledge about the female gametophyte of several important genera, e.g. *Agonandra*, *Anthobolus*, *Arjona*, *Gaiadendron*, *Grubbia*, *Myoschilos*, *Octoknema*, *Okoubaka*, *Psittacanthus*, etc. An investigation of these and allied genera would be very helpful in correcting some of the earlier observations and tracing the inter-relationships of the Santalales.

The Santalales are widely distributed, sometimes in inaccessible regions, and the collection of the required material is not an easy task. We have been greatly helped in this project by numerous friends and co-workers and it would be appreciated if fellow botanists could send us preserved material of the plants mentioned above.

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In-Vitro Growth of Cotton Ovules

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During recent years artificial rearing of embryos, ovules and ovaries has received much attention (*see* Maheshwari, 1959). Controlled growth of ovules is helpful in understanding their growth requirements and interdependence of the various parts, namely, integument, nucellus, endosperm and embryo.

Withner (1943) advocated ovule culture as a quick method for obtaining seedlings of orchids. White (1932) obtained callus from the integumentary cells of *Antirrhinum* ovules and LaRue (1942) grew the ovules of *Erythronium americanum* which increased to four times their initial size. None of these authors followed the histological changes or the development of the endosperm and embryo *in vitro*. This credit goes to Nirmala Maheshwari (1958) who cultured the ovules of *Papaver somniferum* containing the 2-celled proembryo and a few endosperm nuclei. The ovules developed to maturity and germinated *in situ*. Ranga Swamy (1959) obtained normally developed embryos in the ovules of *Citrus microcarpa* which were inoculated at the time of initiation of nucellar embryos. Kapoor (1959) reared to maturity the ovules of *Zephyranthes* at the zygote and primary endosperm nucleus stage.

This paper deals with a preliminary account of the *in vitro* growth of ovules of *Gossypium hirsutum* var. Indore-2.

MATERIAL AND METHODS

The basal nutrient medium (BM) included modified White's minerals, vitamins, glycine (*see* Ranga Swamy, 1961), sucrose (4 per cent) and indoleacetic acid (IAA—1 ppm), and was jelled with 0·7 per cent Difco Bacto agar. The *p*H was adjusted to 5·5. Casein hydrolysate (CH — 250, 500, 750, 1000, 1500, 2000 ppm), gibberellic acid (GA — 2, 4, 6, 10 ppm), IAA (0·5, 1·5, 2 ppm), kinetin (K — 0·5, 1, 2, 4 ppm) and yeast extract (YE — 500 ppm) were used as supplements. In some experiments ovule extract was also added. This was prepared by crushing 100 g. of ovules (11 days after pollination) and diluting the extract to 1 litre.

The flowers of *G. hirsutum* were plucked six days after pollination and

after removing the epicalyx and calyx, the ovaries were surface sterilized by dipping in rectified spirit and flaming. The ovaries were then excised, ovules scooped out with a sterilized scalpel and inoculated. The cultures were maintained at 23-25° C., 40 to 50 per cent relative humidity, under diffuse sunlight.

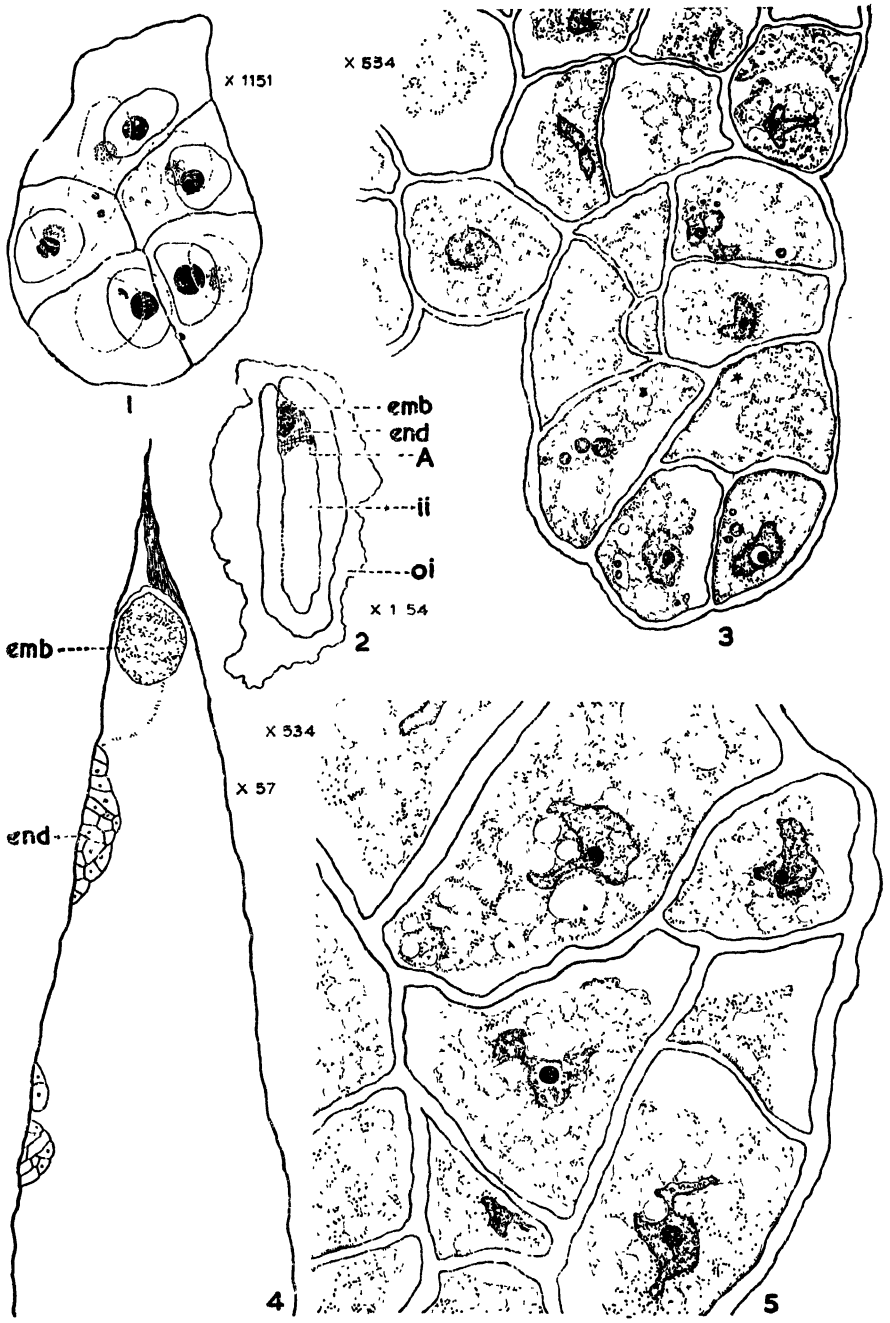
OBSERVATIONS

Ovule. At the time of inoculation the ovules mostly showed a 12-celled proembryo (Fig. 1) and about 500 endosperm nuclei. The inner integument was starchy and 13 or 14 cells thick while the outer was five or six cells thick with prominent unicellular epidermal hairs. The ovules developed satisfactorily on BM + 1000 ppm CH. The growth was much better if 5 ppm GA was also added. The ovules started turning brown in 30 days after inoculation and the maximum size (16 × 7 mm.) was recorded in 96 days after inoculation. In nature the maximum size of ovule (10·7 × 6·3 mm.) is attained in about 45 days after pollination (Figs. 6-8). Callusing often occurred from the outer integument (Fig. 9) and occasionally chlorophyll appeared in the epidermal cells. The epidermal hairs failed to grow and some darkly-staining contents appeared in them.

Embryo. Attempts to culture the young excised embryos of cotton have not been successful so far. Embryos planted 15-20 days after pollination only callused and finally succumbed (Lofland, 1950). Addition of casein hydrolysate, coconut milk, sodium nucleate and tomato juice extract proved of no value. When mature embryo (27 days after pollination) was cultured, it germinated into a normal seedling.

Dure & Jensen (1957) studied the influence of IAA and GA on the *in vitro* growth of embryos in which cotyledons had already developed. The embryo weighing 35 mg. did not show any division or elongation of cells while the axis of embryo weighing 64 mg. divided and elongated. Mauney (1958) has been able to rear them from heart-shaped stage to maturity on White's medium (salts raised to 5 times the original level) supplemented with coconut milk, adenine and CH. K, IAA and GA were found to be detrimental to normal growth. Weaver (1958) cultured hybrid embryos of *Gossypium arboreum* × *G. hirsutum* to maturity on White's medium, age ranging from 20 days after pollination. Varying degrees of growth were obtained and only one embryo produced a normal seedling.

In the author's experiments a mature embryo was produced by rearing the ovules containing a 12-celled proembryo but the rate of growth was invariably slower than that in nature. *In vivo* the embryo matures within 35 to 40 days after pollination. On BM + 500 ppm CH it reached the heart-shaped stage 30 days after inoculation and dicotyledonous stage after 81 days. However, the size (1·1 × 0·4 mm.) was less than one-fifth of the natural embryo (5·5 × 3·5 mm.). Addition of 750 and 1000 ppm CH produced a heart-shaped embryo in 75-80 days after inoculation but if the concentration was raised to 2000 ppm a young dicotyledonous embryo



FIGS. 1-5 — (*emb*, embryo; *end*, endosperm; *ii*, inner integument; *oi*, outer integument): Fig. 1. Embryo at the stage of inoculation. Fig. 2. Index figure for Fig 3. Fig. 3. Endosperm cells (marked A) magnified from Fig. 2 Fig. 4. Outline figure showing the embryo and groups of endosperm cells, 36 days after inoculation, on BM + 1500 ppm CH. Fig. 5. Magnified view of suspensor cells, 66 days after inoculation on BM + 1·5 ppm IAA

(0·3 × 0·2 mm.) developed during the same period. The addition of 5 ppm GA or 1 ppm K to BM + 1000 ppm CH did not improve the growth of the embryo. However, it was slightly better if GA and K were replaced with 500 ppm YE. The cotyledons differentiated 111 days after inoculation but one of them remained arrested.

Different concentrations of IAA did not prove stimulatory to the embryo, 0·5 ppm produced only a heart-shaped embryo in 66 days after inoculation and occasionally the growth was irregular. The embryo reached only up to late globular stage with 1·5 ppm while 2 and 2·5 ppm proved inhibitory.

The addition of 0·5 ppm K produced a dicotyledonous embryo (0·8 × 0·4 mm.) with enlarged, rounded cotyledons 15 days after inoculation, while in 1 ppm a much larger embryo (1·8 × 0·7 mm.) with folded cotyledons developed within 81 days after inoculation (Fig. 10). 2 and 4 ppm were inhibitory.

BM + 30 per cent ovule extract stimulated the growth of the embryo and it reached the heart-shaped stage 15 days after inoculation and dicotyledonous stage (0·6 × 0·3 mm.) after 36 days.

The following abnormalities were met with :

(i) The cells of the suspensor became hypertrophied and accumulated starch grains on BM + 250, 1000 ppm CH, 1 ppm K, 10 ppm GA and 1·5 ppm IAA (Figs. 5, 11). The nuclei became amoeboid and the cell walls became thickened.

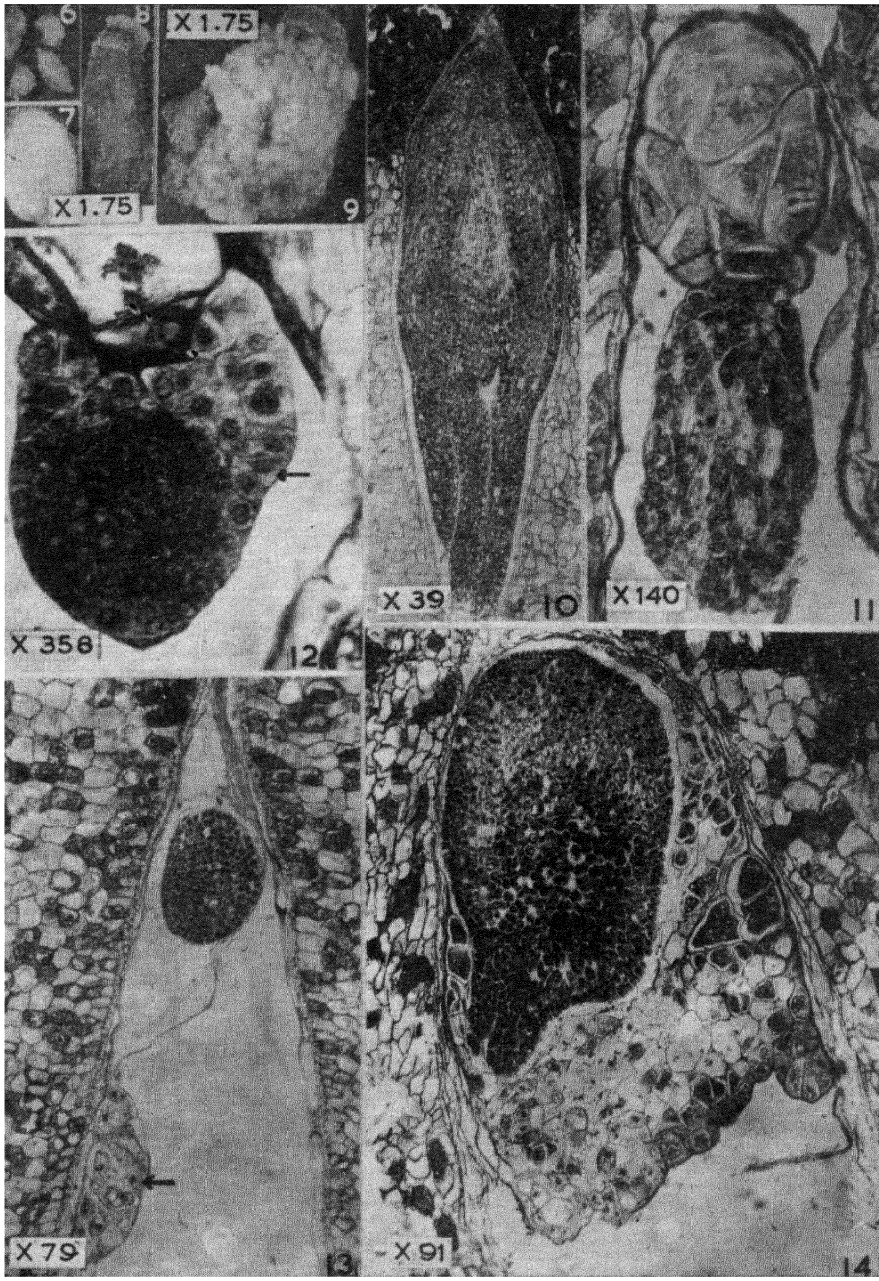
(ii) Budding of the embryonal mass, either at the apical or the basal end, was very common on BM + 500, 1000 ppm CH, 0·5 ppm K (Fig. 12) and 10 or 20 per cent ovule extract. One of the cotyledons usually developed much better with 0·5 ppm K.

Endosperm. In nature more than 1000 free nuclei are formed in seven to eight days after pollination when centripetal wall formation is initiated. The endosperm becomes completely cellular within the next eight to nine days. The sequence of endosperm development could not be followed *in vitro* but cellular condition was observed on (i) BM + 250, 750, 1500 and 2000 ppm CH, (ii) BM + 1000 ppm CH + 1 ppm K, (iii) * BM + 0·5 K (Fig. 14), (iv) * BM + 2 and 6 ppm GA, (v) BM + 0·5 ppm IAA and (vi) BM + 10 per cent ovule extract. 2 and 2·5 IAA; 1, 2 and 4 ppm K and 10 ppm GA proved inhibitory. The cells of the endosperm became much enlarged, accumulated starch grains, cell walls thickened and the nuclei became amoeboid (Figs. 2, 3). Occasionally, the endosperm cells developed in isolated groups along the periphery of the embryo sac (Figs. 4, 13). The walls of the endosperm cells showed pittings on BM + 250 ppm CH.

SUMMARY AND CONCLUSION

Maximum growth of ovules was observed on BM + 1000 ppm CH + 5 ppm GA. The addition of 0·5 and 1 ppm K produced a satisfactory develop-

*In these experiments the basal medium did not contain IAA.



FIGS. 6-14 — Fig. 6. Ovules at the stage of inoculation, note the epidermal hairs. Fig. 7. Mature ovule in nature, note the hairs. Fig. 8. Ovule, 96 days after inoculation reared on BM + 1000 ppm CH + 5 ppm GA. Fig. 9. Same, note the callusing of the outer integument. Fig. 10. 81-day-old dicotyledonous embryo with folded cotyledons in 1 ppm K. Fig. 11. 66-day-old embryo with hypertrophied suspensor cells on BM + 1·5 ppm IAA. Fig. 12. 15-day-old embryo on BM + 0·5 ppm K showing budding at the basal end marked with an arrow. Fig. 13. 36-day-old embryo and a group of endosperm cells (marked with an arrow) on BM + 1500 ppm CH. Fig. 14. 66-day-old embryo and endosperm on BM+0·5 ppm K

ment of the embryo but the rate of growth was slower than that in nature. The addition of 250, 750, 1500 and 2000 ppm CH, 1000 ppm CH + 1 ppm K, 0·5 and 1 ppm K, 2 and 6 ppm GA, 0·5 ppm IAA and 10 per cent ovule extract stimulated the growth of endosperm.

The author is grateful to Dr B. M. Johri and Professor P. Maheshwari for guidance. Thanks are also due to the Indian Central Cotton Committee for financial assistance.

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Studies in the Family Ranunculaceae : I. The Embryology of *Caltha palustris* L.

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The embryological literature on the genus *Caltha* is confined to the works of Mottier (1895), Thomas (1900), and Grafl (1941). Mottier gave an account of the development of embryo sac in nine species of the Ranunculaceae and noted a multicelled archesporium, absence of a parietal cell and the Polygonum type of embryo sac in *C. palustris*. Thomas observed that triple fusion precedes syngamy. According to Grafl the fusion of mitotic spindles in the antipodal cells results in irregular polyploid nuclei.

Kavina (1943) has made interesting teratological observations on this plant. The chromosome number in *Caltha* varies with the altitude, and tetra- and hexaploid forms are common in *C. palustris* (*see* Leoncini, 1950, 1951).

MATERIAL AND METHODS

The genus comprises nearly 40 species distributed in the cooler parts of the world, mostly in southern hemisphere and especially in New Zealand and the Andes of South Africa. In India, *C. palustris* forms extensive perennial tufts beside brooks and streams on the Himalayas (usually above 7,500 ft).

Preserved buds, flowers and fruits of different ages were obtained by Professor P. Maheshwari through the courtesy of Professors Th. Eckardt (Berlin), M. Ernst-Schwarzenbach (Zürich), O. Hagerup (Copenhagen), E. Söderberg (Stockholm), and W. van Heel (Netherlands); Drs F. A. L. Clowes (Oxford), Rhoda Garrison (Petersham), T. M. Harris (Reading), B. M. Johri (Swansea), Nirmal Kapil (Wisconsin), G. C. Mitra (Manchester), and J. L. Ramaut (Belgium); B. N. Kaul (Srinagar), and Miss Usha Bhagat (Ithaca). Our grateful thanks are due to all of them. Some material was also collected from Kedarnath (Western Himalayas, altitude 11,500 ft) during May 1959, by Dr Hardev Singh and one of us (S. Jalan).

The material was fixed in formalin-acetic-alcohol and later preserved

in 70 per cent ethyl alcohol. Customary methods of dehydration and imbedding were followed. Sections were cut at 7-14 microns and stained with safranin and fast green. Acetocarmine smears of pollen mother cells were also examined.

OBSERVATIONS

The flowers appear during April-May and are borne singly or in groups of two or three in the axils of cauline leaves. They are large (diam., 1-2 in.), yellow, pedicellate, actinomorphic and bisexual. The perianth consists of five or six deciduous tepals. The androecium shows numerous free, centripetal stamens. The gynoecium is apocarpous and comprises 6-14 superior pistils arranged in a whorl. Each carpel is laterally compressed and ends in a narrow, linear stigma. Numerous nectar secreting glands are present on the lateral sides of the ovary near the dorsal suture (Figs. 33-35).

Microsporogenesis and Male Gametophyte. The anther is dithecous and its wall consists of five or six layers—the epidermis, endothecium, two or three middle layers and a secretory tapetum. The epidermal cells are isodiametric and divide anticlinally in the young anther. Subsequently as the pollen sac enlarges, they elongate, their outer walls become wavy and a thick cuticle is deposited. The endothecial cells attain twice their original size and develop fibrous thickenings. The cells of the middle layers are narrow and become compressed due to the enlargement of the cells of the endothecium and the tapetum. The middle layer next to the tapetum is the first to disorganize, gradually the others also degenerate. The tapetal cells become prominent due to increase in their size and density of cytoplasm. Their nuclei undergo mitotic divisions to produce two or more nuclei which often fuse to form irregular masses. After the formation of microspore tetrads the tapetal cells begin to degenerate but their remnants may persist as granular bodies even after the dehiscence of the anther.

The meiotic divisions in the microspore mother cells are simultaneous. Tetrahedral and decussate tetrads are formed as a result of centripetal furrowing. The young microspore is oval but becomes more or less spherical at maturity. Its nucleus divides to form a large vegetative and a small generative cell. The mature pollen grain is tricolpate but sometimes pollen grains with four or five colpi were also observed. The pollen is shed at the 2-celled stage. The squash preparations of stigma also showed pollen tubes containing only two nuclei. Usually a single pollen tube emerged from a pollen grain but rarely polysiphonous condition was also noticed.

During maturation of the anther, the region between the adjacent sacs of each theca becomes slightly grooved and the cells of the septum start degenerating. To begin with a few cells disorganize below the groove but gradually the remaining layers also disintegrate and the adjacent sacs become confluent. The flaps of the anther wall at the grooved region may be held together for some time by a few deformed cells of the epidermis. As a re-

sult of shrinkage of the cells in the epidermis and the connective region, the flaps become disconnected and the pollen grains are liberated.

Ovule. Six to twelve ovules are borne in two rows on the marginal placenta of unilocular ovary. They are anatropous, bitegminal and crassinucellar. Each ovule appears as a papillate outgrowth and becomes anatropous during the development of the embryo sac. Rarely orthotropous ovules were also observed; Figure 7 shows one such ovule in which the outer integument has grown in reverse direction. Both the integuments arise almost at the same time but during the curvature of the ovule, the inner integument grows beyond the outer and forms the micropyle.

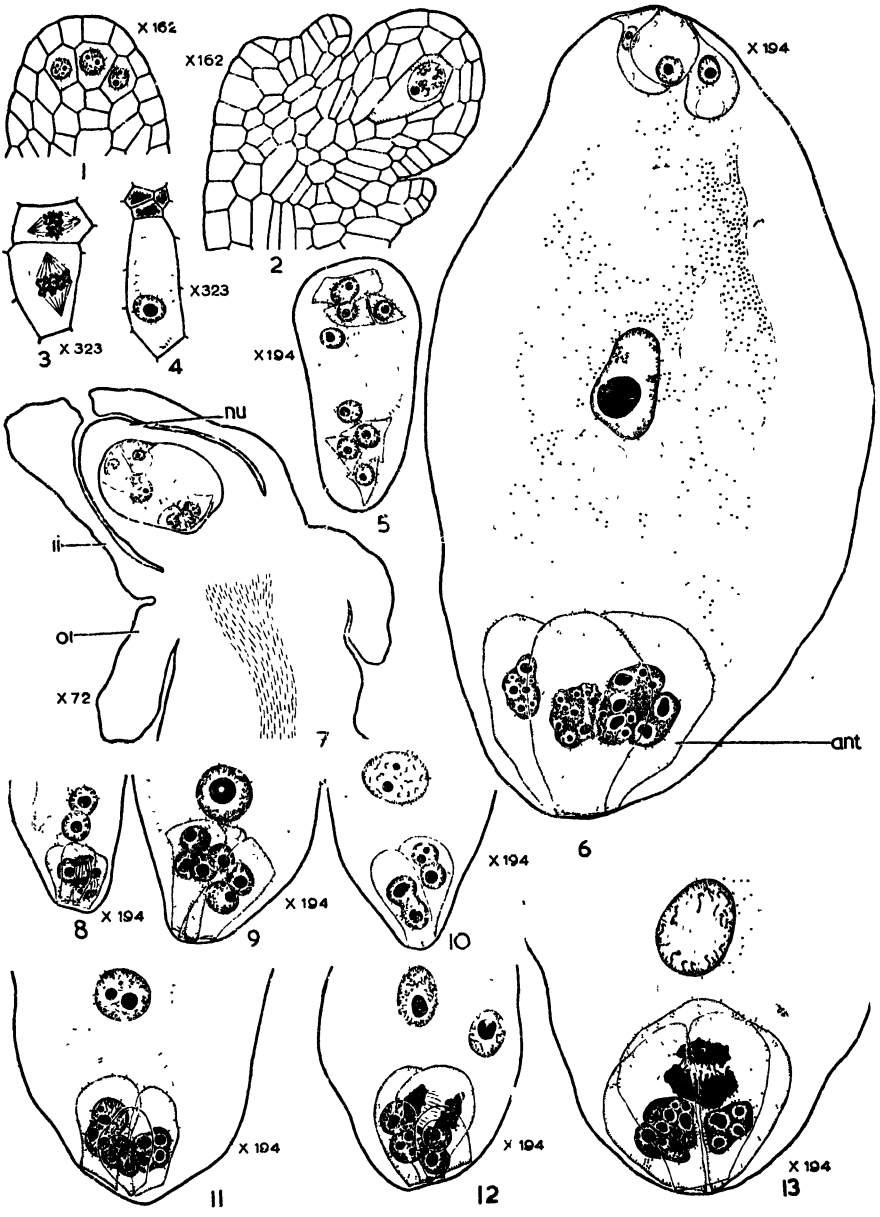
Megasporogenesis and Female Gametophyte. A multicelled archesporium differentiates in the young nucellus (Fig. 1). However, only one archesporial cell divides to form the primary parietal and the primary sporogenous cells. The epidermal cells of the nucellus often divide earlier than the archesporial cell and contribute a major share in the formation of the parietal tissue. Consequently the megaspore mother cell comes to lie below 2–5 layers of nucellar cells (Fig. 2). The first meiotic division of the megaspore mother cell results in two dyad cells of which the lower one is bigger. Rarely the spindles are obliquely oriented during Meiosis I. During Meiosis II the orientation of the spindle in the upper dyad cell may be in line with or at right angle to that in the lower dyad cell (Fig. 3) resulting in a linear or T-shaped tetrad (Fig. 4). Rarely, however, the upper dyad cell degenerates without undergoing division and leads to the formation of a triad. The chalazal megaspore functions and the upper three degenerate in an acropetal order. This is in contrast to Mottier's observation that the micropylar megaspore may also function. The nucleus of the functioning megaspore divides to form two nuclei which move to opposite poles of the young embryo sac. Subsequent divisions lead to 4- and 8-nucleate stages (Fig. 5). Thus, the development of the female gametophyte conforms to the Polygonum type.

The antipodal cells are large and persistent. In the beginning they are uninucleate but before the polar nuclei fuse, they undergo mitotic division and become binucleate (Figs. 8, 9). The two daughter nuclei either fuse (Fig. 10) or divide further and the resultant nuclei also fuse (Fig. 11). These nuclei repeat the processes of division and fusion and form irregular polyploid nuclei (Figs. 6, 12, 13). A fully developed antipodal cell is obovate and rich in cytoplasm; vacuoles sometimes appear towards its broader end (Fig. 6). In mature embryo sacs of the same ovary, the antipodal cells are frequently of different size and show various stages of development. They usually remain healthy up to the octant stage of the embryo and degenerate thereafter.

Pollination is effected by insects. Figure 14 shows a fertilized embryo sac. According to Thomas (1900) in *C. palustris* triple fusion precedes syngamy. Our observations confirm this statement.

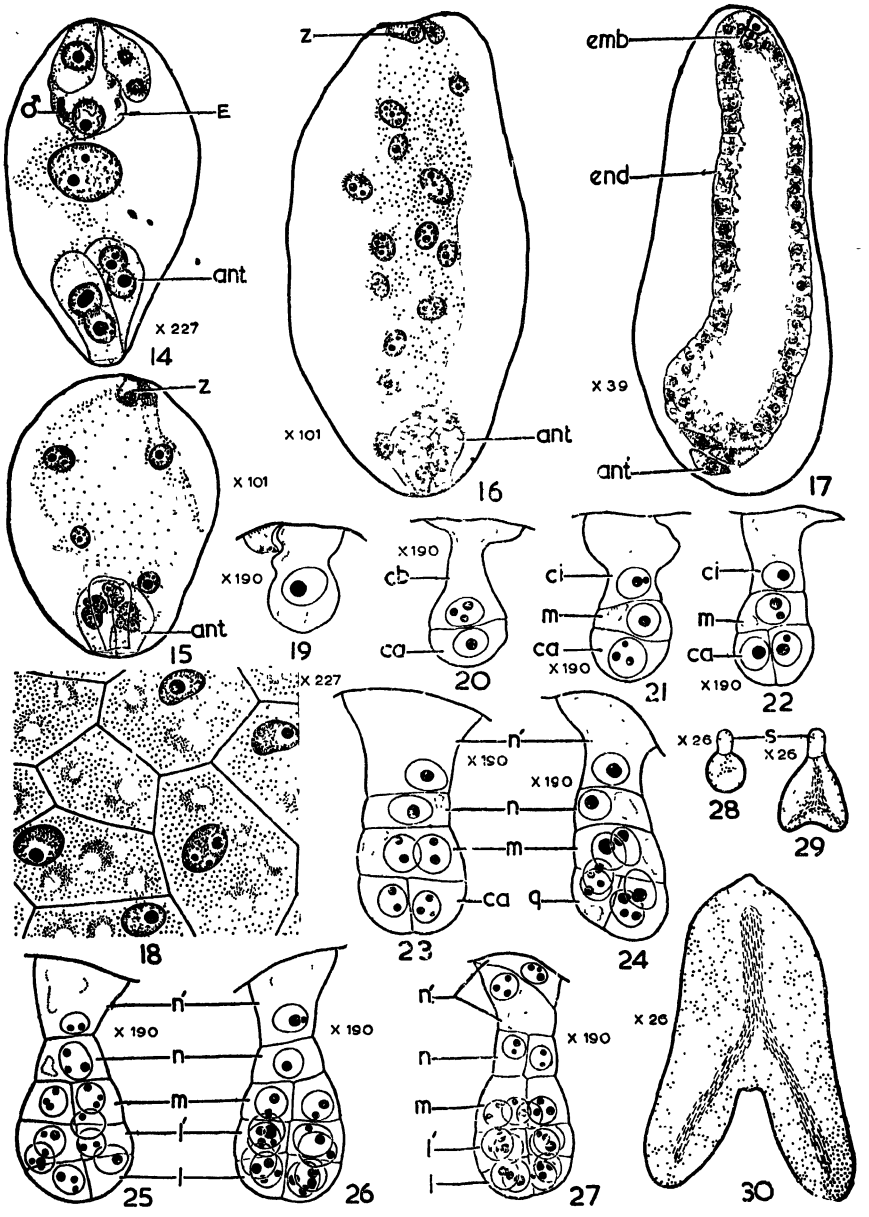
Endosperm. After fertilization the embryo sac enlarges considerably and the primary endosperm nucleus undergoes repeated divisions to form a number of nuclei (Figs. 15, 16) which occupy the periphery of the embryo

sac. Owing to centripetal wall formation (Fig. 17) the endosperm becomes two- or three-layered along the periphery and six- or seven-layered at the



FIGS. 1-13 — FEMALE GAMETOPHYTE (*ant*, antipodal cells; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument) : Fig. 1. L. s. nucellus showing multicelled archesporium. Fig. 2. Same, showing megaspore mother cell. Fig. 3. Dyad cells in Metaphase II. Fig. 4. T-shaped tetrad with functioning megaspore. Figs. 5, 6. Young and old embryo sacs. Fig. 7. Ovule showing orthotropous condition. Figs. 8-13. Chalazal portions of embryo sacs enlarged to show the division and fusion of antipodal nuclei

micropylar and the chalazal ends. Eventually it fills up the entire embryo sac (Fig. 31). The mature endosperm cells are rectangular or polygonal



FIGS. 14-30 — ENDOSPERM AND EMBRYO (*ant*, antipodal cells; *emb*, embryo; *end*, endosperm ; *s*, suspensor; *z*, zygote) : Fig. 14. Fertilized embryo sac. Figs. 15, 16. Four- and 16-nucleate endosperm. Fig. 17. Initiation of wall formation in the endosperm; note the persistent antipodal cells. Fig. 18. Endosperm cells from a mature seed. Figs. 19-30. Stages in the development of embryo

in shape. They are uninucleate, richly cytoplasmic and contain oil globules (Fig. 18).

Embryo. After the initiation of wall formation in the endosperm, the zygote (Fig. 19) divides transversely into a smaller apical cell and a larger basal cell (Fig. 20). Next, the cell *cb* divides transversely to form *m* and *ci*, and the cell *ca* divides vertically to give rise to a 4-celled proembryo (Figs. 21, 22).

The cell *ci* undergoes a transverse division to produce the tiers *n* and *n'* (Figs. 23-27) which by subsequent irregular divisions develop into a short suspensor of 6-8 poorly cytoplasmic, vacuolate cells. The tier *m* divides twice vertically to produce four cells (Fig. 27) which by tangential divisions form the hypophysis.

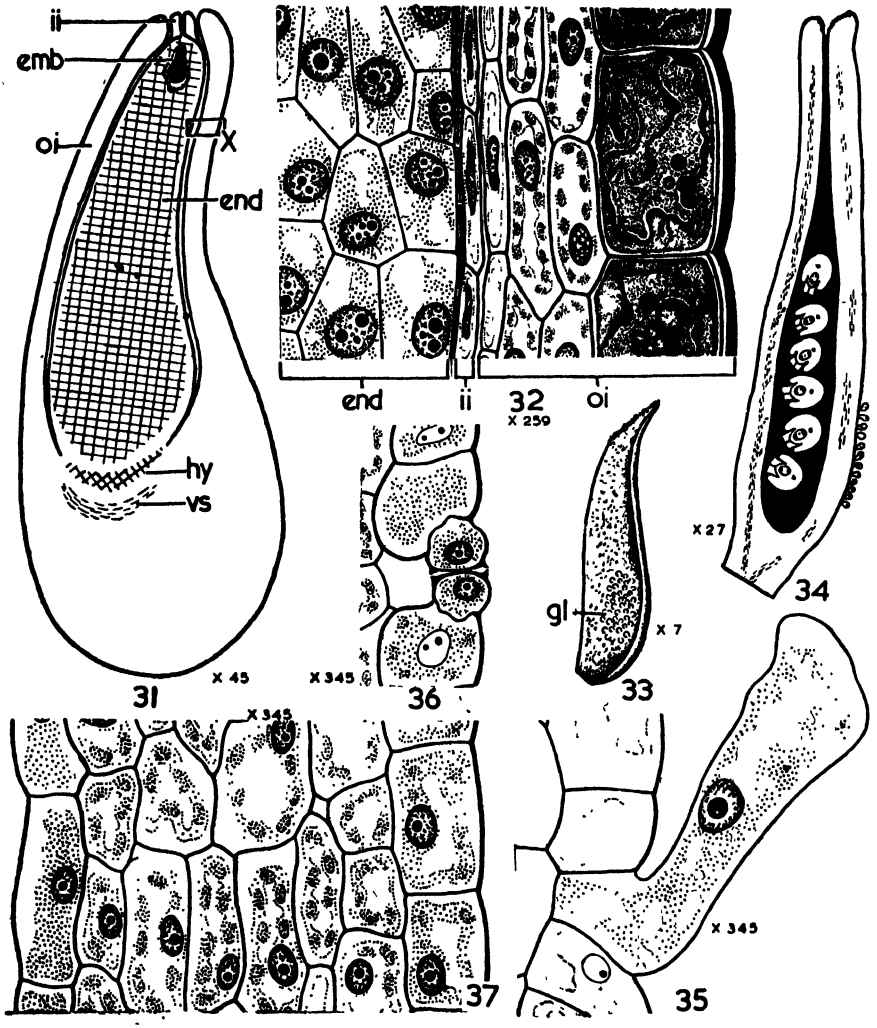
The apical cell undergoes longitudinal and transverse divisions to give rise to the quadrant and octant stages (Figs. 24-27). Further divisions result in globular and heart-shaped embryos (Figs. 28, 29). The derivatives of *l* form the cotyledons and the stem apex while those of *l'* give rise to the hypocotyl. The mature embryo is small and straight with two cotyledons (Fig. 30).

Seed and Testa. The mature seeds are small (0·2 – 0·4 mm. long) with a smooth, reddish brown testa. A ring-like groove across the seed separates the upper tough endospermous portion from the lower flaccid non-endospermous region.

In the young ovule the outer integument shows four or five layers of cells while the inner is always two-cell thick. The cells of the outer integument are isodiametric except those of the outer epidermal layer which are longer than broad. After fertilization the cells of the hypodermal layers including those of the inner epidermis enlarge considerably and their cytoplasm becomes more granulate. During subsequent growth of the endosperm and the enlargement of the outer epidermal cells, the inner epidermis is crushed while the cells of the middle two or three layers become compressed (Figs. 31, 32). In a nearly mature seed the cells of the epidermis develop a thick cuticle (Fig. 32) and are filled with tannin.

Concomitant with the changes in the outer integument the inner integument becomes flattened and crushed in the mature seed (Figs. 31, 32). However, its cells at the micropylar region become thick-walled and constitute the hard beak of the mature seed.

Fruit and Pericarp. The fruit comprises 6-14 follicles, each containing 6-12 seeds arranged in two rows along the ventral suture. Prior to fertilization the ovary wall consists of five or six layers of parenchymatous cells. Later, some of the cells divide periclinally and the wall becomes eight- or nine-layered (Fig. 37). A large number of unicellular glands arise from the epidermal cells (Figs. 34, 35). Stomata are also present on the pericarp (Fig. 36). During maturation of the fruit, the middle layers of the pericarp become flattened (Fig. 37) while the outer and inner epidermal layers elongate radially and a thick cuticle is deposited on the epidermis.



FIGS. 31-37 — SEED COAT AND PERICARP (*emb*, embryo; *end*, endosperm; *gl*, glands; *hy*, hypostase; *ii*, inner integument; *oi*, outer integument; *vs*, vascular supply): Fig. 31. L. s. young seed. Fig. 32. Enlargement of the portion marked 'X' in Fig. 31. Fig. 33. Carpel showing glands. Fig. 34. Same, in. 1. s. Fig. 35. One gland enlarged. Fig. 36. Stoma on the ovary wall. Fig. 37. Enlarged view of a portion of pericarp

SUMMARY AND CONCLUSIONS

The flowers of *Caltha palustris* arise singly or in groups of two or three in the axils of cauline leaves. They are incomplete, actinomorphic and bisexual. The androecium consists of numerous centripetal stamens and the gynoecium of 6-14 free, superior pistils. Rendle (1952) mentions that in *C. palustris* " there are no honey-leaves but a nectary is present on the carpel ".

According to Hutchinson (1955) "nectar is secreted in a shallow depression on each side near the base of the carpels". Our observations, however, indicate that abundant unicellular glands are present on the ovary wall rather than a nectary or a shallow depression.

The anther wall comprises the persistent epidermis, fibrous endothecium, two or three middle layers and a secretory tapetum. Coulter (1898) reported that the tapetum arises from the sporogenous tissue in some species of *Ranunculus*. A similar origin has been noted for *Myosurus minimus* by Swingle (1908). The validity of a sporogenous derivation of the tapetum has already been questioned by Singh (1936) who has shown that in *Ranunculus sceleratus* the tapetum has a parietal origin. Our preparations reveal that in *C. palustris* also the tapetum originates from the primary parietal layer. The reduction divisions in the microspore mother cells are simultaneous. Tetrahedral and decussate tetrads are formed by centripetal furrowing. The mature pollen grains are tricolpate and two-celled at the time of shedding.

The ovules are anatropous, bitegminal and crassinucellar. Rarely they remain orthotropous. The archesporium is multicelled but only one cell functions to cut off the primary parietal and the primary sporogenous cells. Mottier (1895) recorded that in the family Ranunculaceae, except in *Aquilegia canadensis* where a parietal cell is occasionally cut off, the functioning archesporial cell directly develops into the embryo sac. Dahlgren (1927) also remarked: "Innerhalb dieser Familie kommen indessen Teilungen von Epidermiszellen haufig vor. Deshalb erhielt man manchmal leicht den Eindruck, dass Deckzellen abgegeben werden, auch wenn das nicht der Fall ist". This statement obviously rules out any possibility of a parietal cell being cut off in the family Ranunculaceae. A perusal of the literature, however, reveals that the situation is somewhat different. Dahlgren himself has listed five species, namely *Clematis vitalba*, *Helleborus foetidus*, *Helleborus* sp., *Thalictrum purpurascens* and *Paeonia* sp. where a parietal cell has been reported. A parietal cell is also formed in *Ranunculus parviflorus* (Salisbury, 1931) and our preparations leave no doubt about its occurrence in *C. palustris*. The megaspore tetrads are linear or T-shaped. The female gametophyte follows the Polygonum type of development. Mottier (1895; his text Fig. 15) has noted that in one ovule the upper dyad cell failed to divide while the lower one gave rise to a two-nucleate embryo sac. This will lead to a bisporic development. Such a condition was, however, never observed by us.

The antipodal cells are large and persistent. Grafl (1941) concluded that in *C. palustris* the nuclei in the antipodal cells undergo synchronous mitotic divisions and become hexa- or octaploid due to the fusion of spindles. In contrast to this we find that polyploid nuclei are formed due to nuclear fusions rather than the fusion of spindles (*see* also Thomas, 1900). Mottier (1895) noted a similar phenomenon in *Anemonella thalictroides*, *Hepatica acutiloba* and some species of *Ranunculus*. However, in *Aconitum napellus*, *Thalictrum purpurascens*, and *Delphinium* spp. the process of polyploidization is different. In *Thalictrum* the antipodal nuclei undergo amitotic divisions (Overton,

1902) while in *Aconitum* and *Delphinium* they show endomitosis (Osterwalder, 1898; and Tschermark-Woess, 1956). In *Trautvetteria palmata* and some species of *Hepatica* not only the antipodal nuclei become polyploid but the antipodal cells also increase in number (*see* Schnarf, 1931).

Triple fusion precedes syngamy. The endosperm is Nuclear and the embryogeny conforms to the Onagrad type.

The seeds are small with a smooth reddish brown testa which is formed by the epidermis and the compactly arranged middle layers of the outer integument. The inner integument is crushed leaving a thin discontinuous strip of degenerated cells bordering the endosperm. The fruit is an etaerio of follicles. The pericarp comprises 6-8 layers of parenchymatous cells which become elongated and are closely placed at maturity.

We are indebted to Professor P. Maheshwari and Dr B. M. Johri for guidance, invaluable help and suggestions throughout the course of this investigation. One of us (S. Jalan) is grateful to the Council of Scientific & Industrial Research, New Delhi, for the award of a Junior Research Fellowship.

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Embryology of *Paeonia* Together with a Discussion on its Systematic Position

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In 1957, Yakovlev & Yoffe published their observations on the embryogeny of *Paeonia*. The embryo development in this plant has been described as unique in that the first division of the zygote is not accompanied by wall formation. The resulting two nuclei also undergo many free nuclear divisions so as to form a coenocyte in which the nuclei are arranged peripherally, the centre being occupied by a vacuole. Wall formation takes place at a later stage after which certain peripheral cells become meristematic and form localized groups of cells which serve as the embryonal primordia While there are several such meristematic centres, normally only one develops further and attains maturity. The close resemblance of the embryogeny of *Paeonia* to that of certain gymnosperms like *Ginkgo* has been considered by Yakovlev & Yoffe to be a feature of great importance.

This unique type of embryo development prompted the present investigation.

MATERIAL AND METHODS

Paeonia is a genus of temperate regions. In India it grows in the Western Himalayas at an altitude of 5,000 – 10,000 ft (*see* Collett, 1921). Material of several species was obtained by Professor P. Maheshwari from various countries and botanical gardens through the courtesy of Professors Th. Eckardt, O. Hagerup, E. Söderberg, P. Créte, H. Kihara, M. Kumazawa, M. Ernst-Schwarzenbach, N. Higinbotham, T. M. Harris and W. A. van Heel; and Drs Y. Nozu , B. M. Johri, Nirmal Kapil, A. N. Rao , B. Saha and H. Y. Mohan Ram.

The customary methods were followed for preparing the material for microtomy. Sections were cut 15-30μ thick and stained with safranin-fast green and crystal violet-erythrosin. The latter proved more satisfactory. The study of endosperm was also supplemented by dissections.

The seeds were treated with 5 per cent KOH solution for 12-16 hr to facilitate the dissection of the endosperm which was then stained with

Delafield's haematoxylin and mounted in a mixture of the stain and Zirkle's medium (*see* Johansen, 1940).

OBSERVATIONS

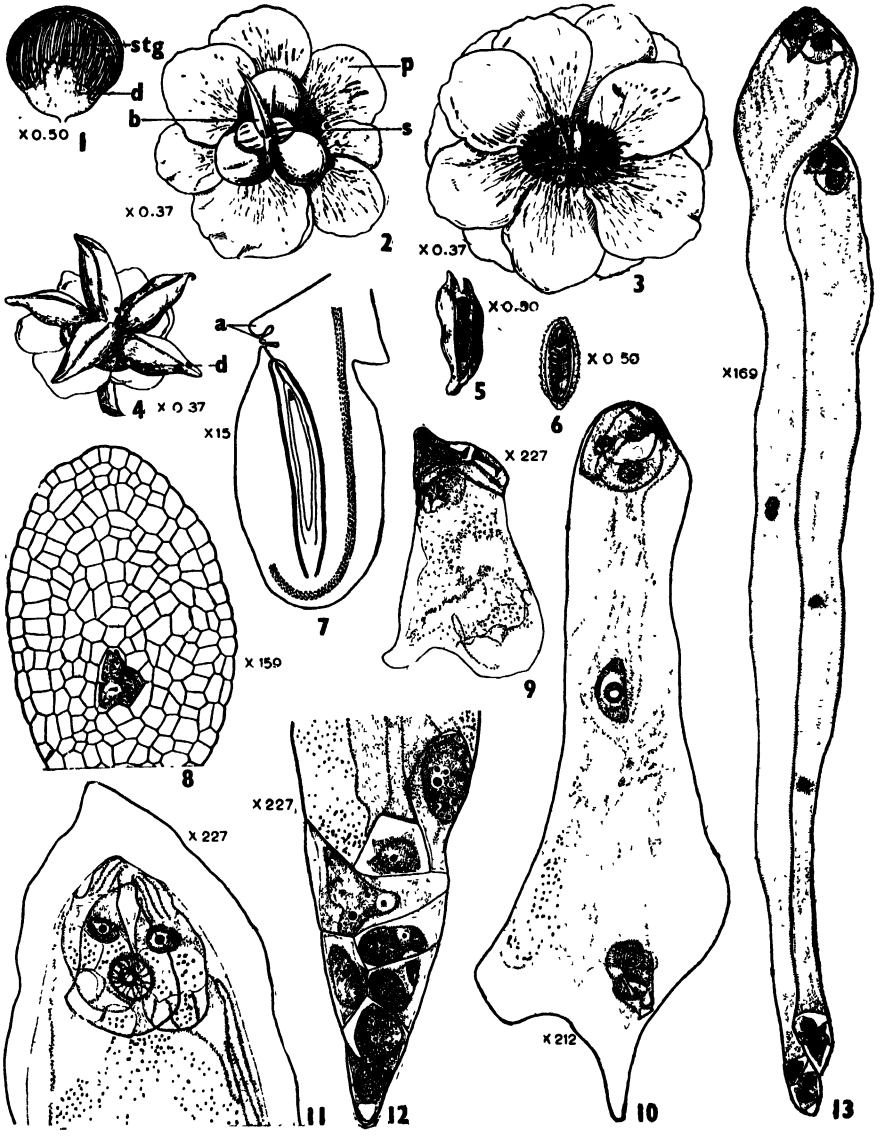
External Morphology. *Paeonia* is generally a herb with large, solitary bisexual flowers of various colors. The sepals vary from 5-8 in number. In Fig. 2, the three bigger sepals are more or less rounded while the smaller 2 have a pointed apex. The petals are numerous and spirally arranged (Fig. 3). There are many centrifugal stamens which are also spirally arranged (Figs. 1, 3). The pistil is apocarpous and comprises 2-5 carpels. The carpels as well as the stamens are borne on a hypogynous disc (Figs. 1, 4). Each carpel has a style that ends in a sessile bifid stigma (Fig. 1). The placentation is marginal (Fig. 6). The fruit is an etaerio of follicles (Figs. 4, 5).

The Ovule. The ovule is anatropous, crassinucellate, bitegmic and is borne on a placental projection (Fig. 7). Both the integuments form the micropyle. The outer integument is 25-30 layers thick at the sides and grows beyond the inner one which is only 4-6 layers thick. A hypostase is present at the chalazal end which becomes more pronounced during the post-fertilization stages.

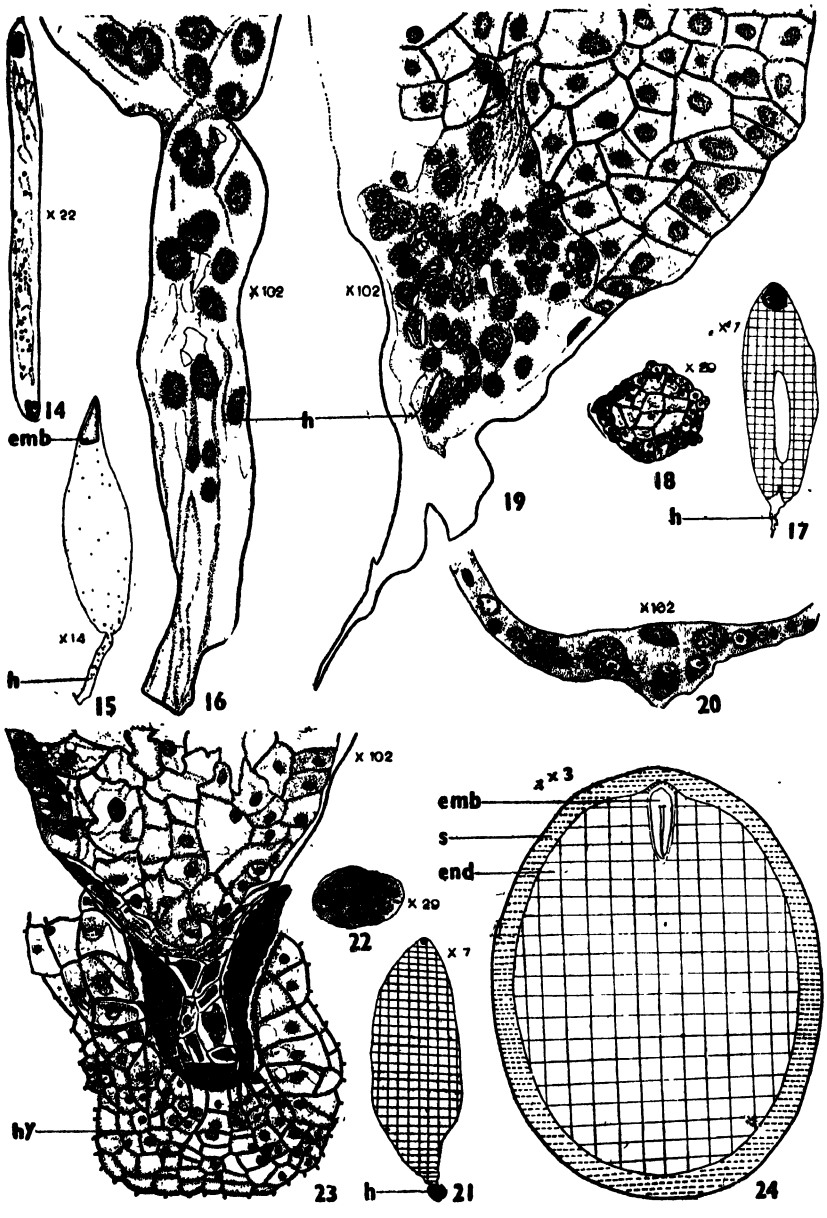
Megasporogenesis and Female Gametophyte. The archesporial cells vary from few to many in number. Figure 8 shows three megaspore mother cells which might have been formed from a corresponding number of archesporial cells. Generally a T-shaped tetrad is formed of which the chalazal megaspore functions (Fig. 9) to give rise to an 8-nucleate embryo sac. The development of the embryo sac thus conforms to the Polygonum type. An organized embryo sac has a well developed egg apparatus, two polar nuclei and three antipodal cells (Fig. 13). The polar nuclei fuse in the centre of the embryo sac soon after their formation (Fig. 10). The synergids are beaked and have a filiform apparatus (Fig. 11). The antipodal cells have large nuclei (Fig. 10) and persist during the early post-fertilization stages (Fig. 14). Occasionally accessory antipodal cells are also formed (Fig. 12). Formation of twin embryo sacs is a common feature (Fig. 13). They probably develop from two megaspore mother cells.

Endosperm. The endosperm is of the nuclear type. The division of the primary endosperm nucleus precedes that of the zygote and a large number of free nuclei are formed (Fig. 14). Nuclear fusions giving rise to irregular polyploid nuclei are very common especially at the chalazal end (Figs. 19, 20). The chalazal portion of the endosperm grows out into an haustorium (Fig. 16) at a stage when the embryo has a coenocytic suspensor (Fig. 15). The nuclei in the haustorium present varying shapes and contain many nucleoli (Figs. 16, 19). Wall formation is centripetal and is usually initiated at the micropylar end just when the coenocytic suspensor has become completely cellular (Figs. 17-19). In Fig. 17 wall formation has extended almost up to the base of the embryo sac; the haustorium is still free nuclear.

The haustorium attains its maximum activity and becomes completely cellular at the early globular stage of the embryo. At a somewhat advanced



FIGS. 1-13 — EXTERNAL MORPHOLOGY, MEGASPOROGENESIS AND FEMALE GAMETOPHYTE (*a*, placental projection; *b*, bract; *d*, disc; *p*, petal; *s*, sepal; *stg*, stigma) : Fig. 1. *Paeonia officinalis*, l. s. bud showing centrifugal development of stamens. Fig. 2. *P. bakeri*, back view of a flower. Fig. 3. *P. bakeri*, an open flower. Fig. 4. *P. delavayi*, an apocarpous gynoecium. Fig. 5. *P. lutea*, a dehisced follicle. Fig. 6. *P. officinalis*, l. s. ovary showing the ovule borne on a marginal placenta. Fig. 7. *P. albiflora*, l. s. ovule borne on a placental projection. Fig. 8. *Paeonia anomala*, l. s. nucellus with three megaspore mother cells. Fig. 9. *Paeonia anomala*, a megaspore tetrad. Fig. 10. *P. lutea*, an organized embryo sac. Fig. 11. *P. albiflora* micropylar end of the embryo sac showing the beak-shaped synergids. Fig. 12. *P. delavayi*, chalazal end of the embryo sac with accessory antipodal cells. Fig. 13. *P. tenuifolia*, twin embryo sacs



FIGS. 14-24 — ENDOSPERM (*emb*, embryo; *end*, endosperm; *h*, haustorium; *hy*, hypostase; *s*, seed coat) : Fig. 14. *P. delavayi*, l. s. embryo sac showing free nuclear endosperm. Fig. 15. *P. delavayi*, dissection of the embryo sac showing the free nuclear chalazal endosperm haustorium. Fig. 16. Haustorium from Fig. 15 enlarged. Fig. 17. *P. officinalis*, diagrammatic figure of the embryo sac showing the embryo and the chalazal endosperm haustorium. Fig. 18. Embryo from Fig. 17 enlarged. Fig. 19. Haustorium from Fig. 17 magnified. Fig. 20. *Paeonia veitchii*, fusion of endosperm nuclei at the chalazal end. Fig. 21. *P. officinalis*, diagrammatic representation of the embryo sac showing the endosperm haustorium. Fig. 22. Enlargement of embryo from Fig. 21. Fig. 23. Enlargement of haustorium from Fig. 21. Fig. 24. *P. delavayi*, l. s. mature seed

stage of the globular embryo, the haustorium degenerates (Figs. 21-23). The thick-walled cells of the hypostase (Fig. 23) give a famished appearance with the growth in the haustorium. The mature seed is filled with endosperm cells (Fig. 24). The seeds are thus albuminous.

Embryo. As already mentioned on page 215, Yakovlev & Yoffe (1957) reported a free nuclear embryo in *Paeonia*. They reported omission of wall formation during the first few divisions of the zygote and compared this unique type of embryo development to that of certain gymnosperms.

I studied the embryogeny in *P. actiflora*, *P. albiflora*, *P. suffruticosa*, *P. bakeri*, *P. delavayi* and an undetermined species. According to my observations (*see* also Murgai, 1959), in all the species the first division of the zygote nucleus is definitely followed by a wall resulting in an apical and a basal cell (Fig. 25). Free nuclear divisions now follow in the basal cell (Figs. 26-29) and give rise to a coenocyte (Figs. 30, 31) in which the nuclei occupy a peripheral position with a vacuole in the centre (Fig. 30). The apical cell divides only rarely, although in one ovule it was found to have undergone a vertical division (Fig. 27). The further fate of the apical cell could not be traced because of the paucity of good material.

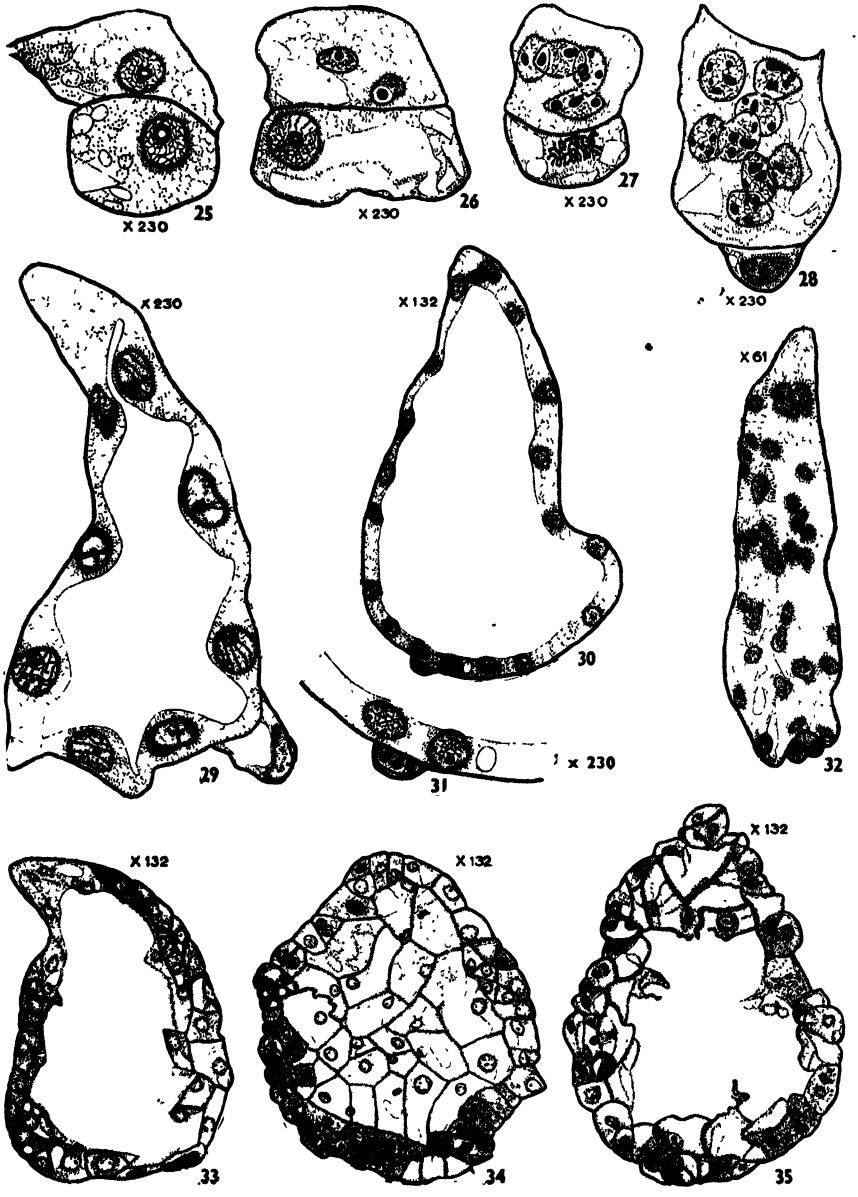
In 1961 Cave *et al.* published their observations on the embryogeny of *P. californica* and *P. brownii*. In both species they confirmed the results of the Russian workers in that the first and the subsequent divisions of the zygote nucleus were not followed by a wall. They interpreted the two cells of the proembryo obtained by me as the result of juxtaposition of the zygote and a persisting synergid. Encouraged by this Yakovlev & Yoffe (1961) once again emphasized the occurrence of the coenocytic phase in the embryogeny of *Paeonia* and its significance in the phylogeny of higher plants. They too have interpreted my two-celled proembryo as the zygote plus one of the persisting synergids.

My more recent investigations based on the material of *P. anomala* obtained from Srinagar have confirmed that a cell plate *is* laid down after the karyokinesis of the zygote. The next and the subsequent divisions of the basal cell are free nuclear. On the other hand, the apical cell seems to degenerate and its remnants are clearly visible at the 2-, 4- or 8-nucleate basal cell stage. In that case the embryo proper must be derived from the basal cell only.

After many free nuclear divisions, the coenocytic basal cell becomes cellular (Figs. 33-35). Some of the peripheral cells now divide more actively than others and form groups of cells. One such group develops into an embryo. The mature embryo is small, dicotyledonous and surrounded by copious endosperm (Fig. 24).

Systematic Position. The systematic position of the genus has been much disputed.

In 1908, Worsdell studied the vascular anatomy of different families of Ranales and found that *Paeonia* offers greater resemblance to the Magnoliaceae than to the Ranunculaceae. On the basis of vascular anatomy and



FIGS. 25-35 — EMBRYO : Fig. 25. *Paeonia* sp., 2-celled proembryo. Fig. 26. *P. actiflora* 2-celled proembryo; the basal cell shows two free nuclei. Fig. 27. *P. delavayi*, 4-nucleate basal cell and an apical cell at metaphase. Fig. 28. *P. delavayi*, 8-nucleate basal cell and an undivided apical cell. Fig. 29. *P. bakeri*, 8-nucleate basal cell and an undivided apical cell. The nuclei in the basal cell have attained a peripheral position. Fig. 30. *Paeonia bakeri*, a large coenocytic suspensor and an undivided apical cell. Fig. 31. Apical cell from Fig. 30 magnified. Fig. 32. *Paeonia* sp., large suspensor haustorium; the apical cell has divided vertically into two cells. Figs. 33-35. Cellular embryos (for explanation *see* text) : Figs. 33, 35. *Paeonia veitchii*; Fig. 34. *P. delavayi*

certain characters of the leaves and fruits, Worsdell came to the conclusion that "*Paeonia* constitutes a natural and independent order of plants, viz. the Paeoniaceae, and is much more closely allied to the Magnoliaceae rather than the Ranunculaceae."

On the basis of wood anatomy, Kumazawa (1935) separated *Paeonia* from the Ranunculaceae and assigned to it the status of a family with closer affinities to the Magnoliaceae than to the Ranunculaceae or the Berberidaceae.

The floral structure of *Paeonia* differs much from all other genera in the Ranunculaceae. Eames (1951) studied the floral anatomy of the Ranunculaceae and found that *Paeonia* is entirely different from other members of the family.

Wodehouse (1936) distinguishes *Paeonia* from the rest of the family on the basis of structure of exine of the pollen grain.

Gregory (1941) has found that the basic chromosome number in *Paeonia* is 5 in contrast to 7, 8 and 9 met with in other genera.

Embryologically also the genus deviates from most genera of the family in possessing ovules borne on placental projections, massive bitegmic ovules in which the outer integument is longer than the inner (in other genera the condition is just the reverse), long embryo sacs with beaked synergids (beaks are absent in the other members excepting *Caltha palustris*, Kapil & Jalan, 1960), occurrence of multiple embryo sacs, a chalazal endosperm haustorium, a large multinucleate suspensor cell and hypogeal germination.

Thus evidences from various fields of study strongly support the removal of *Paeonia* from the Ranunculaceae and establishment of a separate family, the Paeoniaceae. The next question that naturally arises is : What are the probable affinities of this new family Paeoniaceae ?

In the anatomy of the leaf and the stem, the Paeoniaceae resemble the Magnoliaceae while in a few morphological and embryological points it shows similarities with the Ranunculaceae.

Corner (1946) considers the order of development of the stamens to be of considerable phylogenetic significance and places *Paeonia* with its centrifugal stamens in a separate family Paeoniaceae close to the Dilleniaceae. Hutchinson (1959) is not at all convinced that this character alone is sufficient to class *Paeonia* near the Dilleniaceae and he therefore places the Paeoniaceae between the Ranunculaceae and the Helleboraceae in the order Ranales. According to him the most distinctive features of the Paeoniaceae are : the arillate seeds, the hypogynous disc and the centrifugal stamens. Further work is necessary before it is possible to make a choice between the view points of Corner and Hutchinson.

Both the Ranunculaceae and the Dilleniaceae have centrifugal stamens, an androecial disc and similar vascular supply to the stamens. Further, the Paeoniaceae resemble the Dilleniaceae in having scalariform vessels, abundant wood rays and a massive bitegmic ovule in which the outer integument is longer than the inner.

SUMMARY AND CONCLUSIONS

Paeonia is a herb growing in temperate climates. The flowers are bisexual, large and showy. There is a spiral arrangement of the floral parts. The sepals vary from 5 to 8 and the petals are indefinite. The centrifugal stamens as well as the 3-5 free carpels are borne on a hypogynous disc. The placentation is marginal and the fruit is a follicle.

The anatropous ovule is bitegmic and crassinucellate and is borne on a placental projection. The outer integument is massive and longer than the inner.

The presence of multiple archesporial cells is a common feature. A T-shaped tetrad of megaspores is formed and the chalazal megaspore functions. The development of the embryo sac is of the Polygonum type. The mature embryo sac possesses a well developed egg apparatus, a large central nucleus and three antipodal cells. The synergids are beaked and have a filiform apparatus. The antipodals persist during the early post-fertilization stages. Occasionally accessory antipodal cells are also formed. Formation of twin embryo sacs is very common in *Paeonia* and these are probably formed from two megaspore mother cells.

The endosperm is of the nuclear type. The chalazal portion of the endosperm forms a haustorium. Later the haustorium becomes cellular and degenerates at the advanced globular stage of the embryo. A hypostase is present at the chalazal end and is gradually consumed as the embryo advances in age. Almost the entire cavity of the mature seed is filled with endosperm thus rendering the seeds albuminous.

Yakovlev & Yoffe (1957, 1961) and Cave *et al.* (1961) reported a free nuclear embryo in *Paeonia*. Investigation on a number of species has clearly demonstrated that the embryo *is* cellular and not free nuclear. The coenocytic structure misinterpreted as the embryo by the Russian and American workers is in reality the massive suspensor haustorium. The mature embryo is small and surrounded by copious endosperm.

The systematic position of the genus has been discussed. Morphological, anatomical, cytological and embryological evidences point to the removal of *Paeonia* from the Ranunculaceae to a separate family, the Paeoniaceae. The affinities of the Paeoniaceae have been discussed.

The author is deeply indebted to Professor P. Maheshwari under whose valuable guidance this research was carried out. Thanks are also due to Dr R. C. Sachar for help and interest and to the many botanists abroad who supplied the material.

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Embryological Studies in Some Members of the Zingiberaceae

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The order Scitamineae has been generally accepted as well-knit assemblage of highly advanced monocotyledons, distributed in the tropics and sub-tropics. It forms a natural group of four allied families: Musaceae, Zingiberaceae, Cannaceae and Marantaceae. Among these perhaps, Zingiberaceae is the best represented in India, there being 21 genera including more than 200 species (Chakravorti, 1948). Several members are prized sources of spices and other food products.

The Zingiberaceae are of special interest from the taxanomic stand point. On the basis of morphological characters, Schumann (1904) divided them into two subfamilies: Zingiberoideae and Costoideae. This distinction is further supported by the available data from other fields such as geographical distribution (Loesner, 1930), vegetative anatomy (Tomlinson, 1956), cytology (Raghavan & Venkatasubban, 1943; Chakravorti, 1948), seedling morphology (Boyd, 1930, 1932) and palynology (Erdtman, 1952).

The work on the embryology of the family is very meagre and fragmentary. The earliest work is that of Humphrey (1896) on the development of the seed in Scitamineae, based on free-hand sections only. The investigations of subsequent workers namely Boehm (1931), Gregory (1936), Mauritzon (1936), Banerji (1940), Raghavan & Venkatasubban (1941) and Harling (1949) are confined mainly to sporogenesis and gametogenesis. Recently, Berger (1958) has studied the seed structure of certain species of *Elettaria*, *Amomum* and *Aframomum*.

It can be said that the embryogeny is diagnostic in deciding the disputed systematics. But it has not been studied by previous workers perhaps due to the difficulties in dealing with the material. Such a study was considered all the more important, since these plants that occur in inaccessible areas of the world, have remained practically unexplored. Therefore, with this point in view, the embryological study of some five genera namely *Costus speciosus* Smith, *Elettaria cardamomum* Maton, *Hitchenia caulina* Baker and *Zingiber macrostachyum* Dalz. was undertaken.

MATERIAL AND METHODS

The materials were collected from the districts of Belgaum, Karwar and Dharwar in Mysore State and fixed in formalin-acetic acid-alcohol. The ovary wall was trimmed to facilitate infiltration. Hard seeds were softened with 15 per cent hydrofluoric acid (in 70 per cent ethyl alcohol) or 5 per cent potassium hydroxide. Both ethyl alcohol-xylol and ethyl alcohol-*tert.*-butyl alcohol series were used for dehydration. Heidenhain's haematoxylin with erythrosin as counterstain proved quite satisfactory. Pollen grains were stained in basic fuchsin and mounted in glycerine jelly (*see* Wodehouse, 1935).

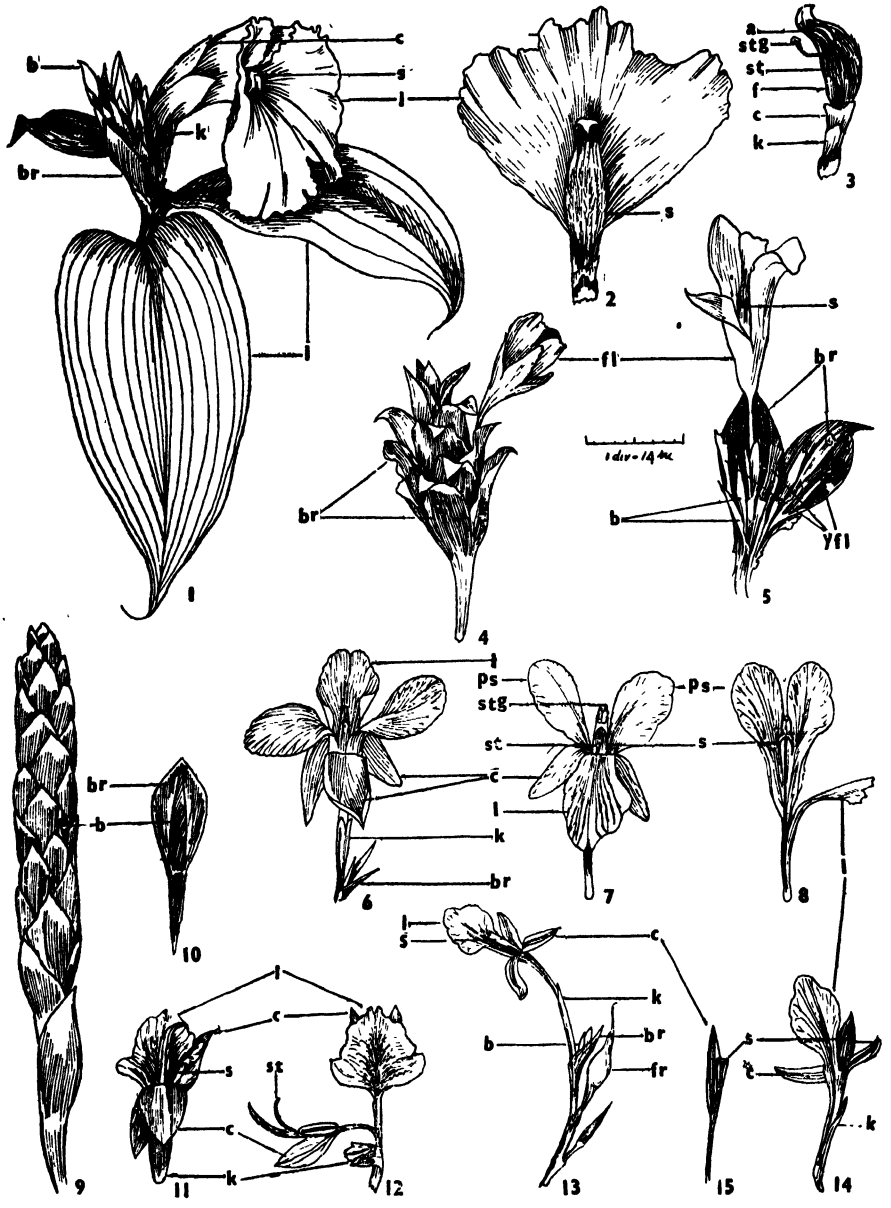
OBSERVATIONS

Flowers. These are arranged in spikes (Figs. 1, 4, 5, 9, 10, 13). They show a typical trimerous arrangement and are characterized by the presence of a large labellum and a single fertile stamen (Figs. 1-3, 6-8, 11-15). The staminodes (Figs. 6-8) and "epigynous glands" are present in *Elettaria* and *Hitchenia*. The ovary is inferior, tricarpellary and syncarpous. The seeds are numerous and arillate.

Anther and Male Gametophyte. The archesporial cells are hypodermal in origin which divide to form parietal wall layers and sporogenous cells (Figs. 16, 21). The anther wall consists of 6-8 layers of cells, innermost forming the secretory tapetum (Figs. 17, 22). The periplasmodial type has been reported only in *Nicolaia atropurpurea* (Boehm, 1913). The uninucleate cells of the tapetum commonly undergo periclinal divisions, intrude in between the sporogenous cells and degenerate when microspores are being formed. In *Costus speciosus* some of the tapetal cells also show binucleate condition (Fig. 17).

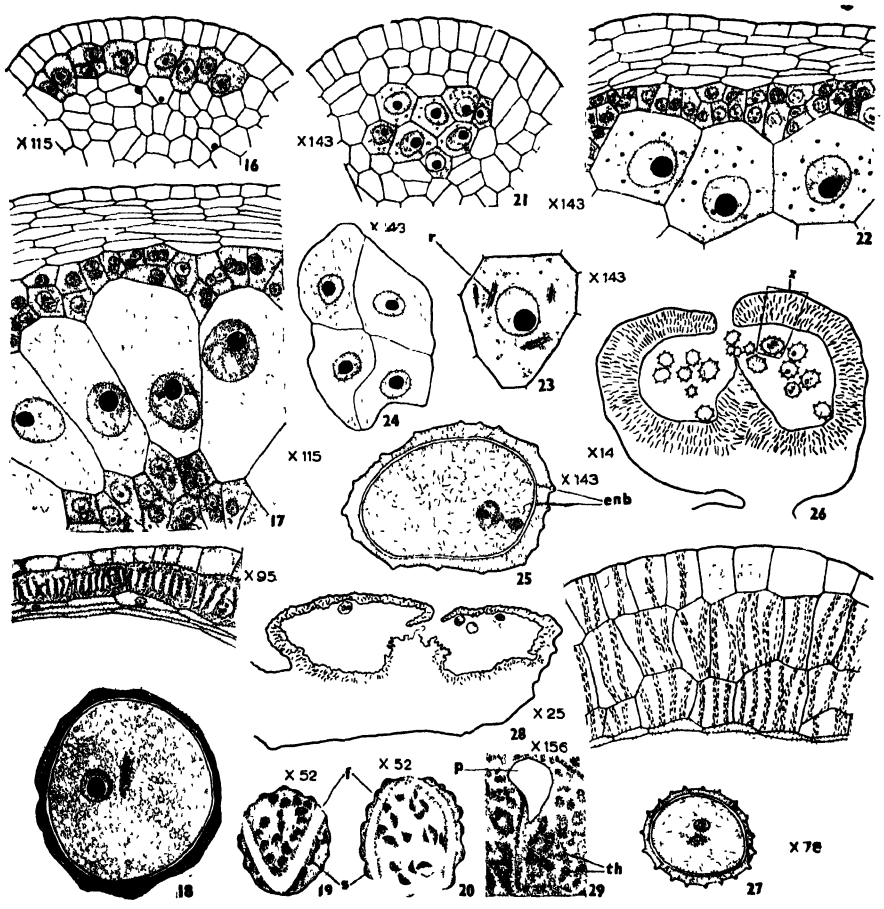
The sporogenous cells are one-layered in *Costus* (Fig. 17). The spore mother cells undergo successive meiotic divisions and give rise to isobilateral tetrads (Fig. 24). In *Nicolaia atropurpurea*, tetrahedral type has been reported by Boehm (1931). Extranuclear bodies of unknown constitution that take haematoxylin stain were observed in microspore mother cells, spore tetrads of *Costus* and *Elettaria* and even in the cells of the anther wall of the latter (Figs. 21-23). These seem to be feulgen negative and are abundant in *Elettaria* in which raphide bundles were also observed frequently in the microspore mother cells (Fig. 23).

The pollen grains contain large amount of starch (Fig. 18), but in *Elettaria cardamomum* some of them contain worm-shaped bodies (Figs. 25, 27). These are refractive and their chemical nature could not be determined. The exine is spinuliferous in *Costus* (Figs. 19, 20), reticulate with longitudinal ridges in *Zingiber macrostachyum* (Fig. 29) and smooth in other members. It shows a single furrow of various shapes in *Costus speciosus* (Figs. 19, 20). Occurrence of two furrows reported by Banerji (1940) is erroneous. In *Zingiber*, only a single pore could be observed (Fig. 29).

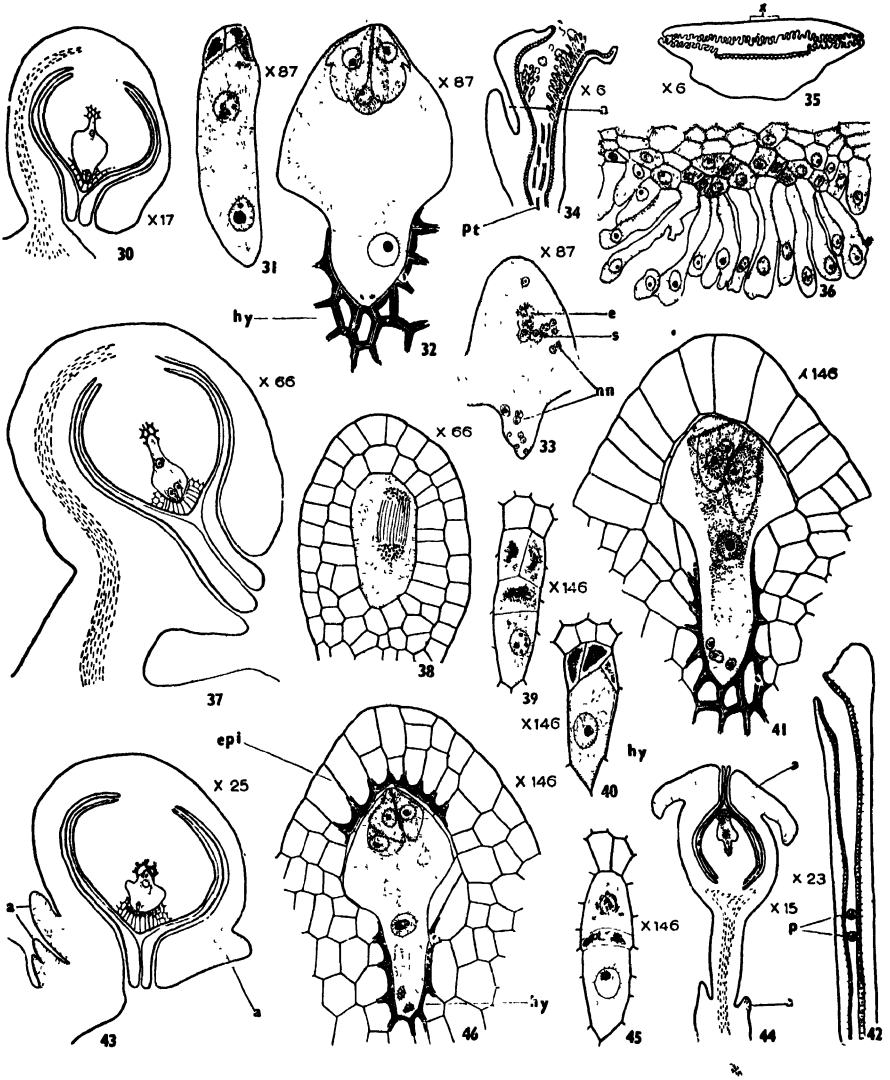


FIGS. 1-15 — (*a*, anther; *b*, bud; *br*, bract; *c*, corolla; *f*, filament; *fl*, flower; *fr*, fruit; *k*, calyx; *l*, labellum; *ps*, petaloid staminode; *s*, stamen; *st*, style; *stg*, stigma; *yfl*, young flower): Figs. 1-3. *Costus speciosus*. Fig. 1. Spike. Fig. 2. Labellum facing the stamen. Fig. 3. Front view of stamen. Figs. 4-8. *Hitchenia caulina*. Fig. 4. Compound spike. Fig. 5. Spikes in the axil of bracts, exposed. Figs. 6-8. Flowers and their parts. Figs. 9-12. *Zingiber macrostachyum*. Fig. 9. Long peduncled spike. Fig. 10. A bud at the axil of a bract. Figs. 11, 12. Flowers and their parts. Figs. 13-15. *Elettaria cardamomum*. Figs. 13. A portion of a panicle. Figs. 14, 15. Flower and its components

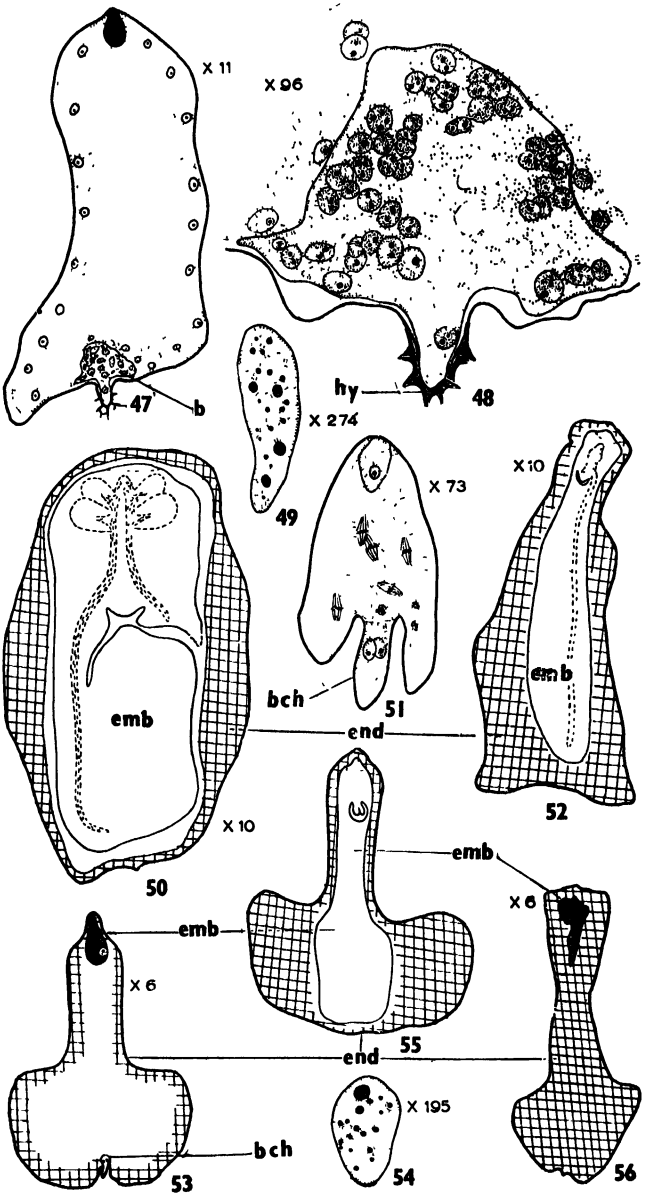
At the time of dehiscence, only a single fibrous layer is present in *Costus* (Fig. 18), whereas it extends to 2-4 layers in other members and even to the epidermis in *Elettaria* and *Zingiber* (Figs. 26-28). The dehiscence takes place longitudinally along the unthickened region of the locule and the pollen grains are shed at 2-celled stage (Figs. 18, 26-28).



FIGS. 16-29 — (*enb*, extranuclear bodies; *f*, furrow; *p*, pore; *r*, raphides; *s*, spinescent outgrowths; *th*, thickenings): Figs. 16-20. *Costus speciosus*. Fig. 16. Portion of the anther to show the archesporial cells. Fig. 17. Same at microspore mother cell stage. Fig. 18. Portion of the mature anther at the time of dehiscence showing endothecial thickenings and the starchy two-celled pollen grain. Figs. 19, 20. Surface view of mature pollen grains. Figs. 21-27. *Elettaria cardamomum*. Figs. 21, 22. Portions of the young anther locule at the stage of sporogenous cells and the spore mother cells respectively. Fig. 23. A spore mother cell enlarged to show raphides and the extranuclear bodies. Fig. 24. Spore tetrad. Fig. 25. Two-celled pollen grain showing refractive worm-like bodies in the cytoplasm. Fig. 26. T. s. dehisced anther lobe. Fig. 27. Portion marked *x* in Fig. 26, enlarged to show fibrous thickenings in the wall layers and the epidermis. Fig. 28. *Zingiber macrostachyum*. T. s. dehisced anther. Fig. 29. Surface view of a mature pollen grain showing the germ pore



FIGS. 30-46—(*a*, aril; *e*, egg; *epi*, epistase; *hy*, hypostase; *nn*, nucellar nuclei; *p*, pollen grains; *pt*, pollen tubes; *s*, synergid): Figs. 30-36. *Costus speciosus*. Fig. 30. L. s. ovule. Fig. 31. Two-nucleate embryo sac; note the remnants of degenerating megaspores at the top. Fig. 32. Mature embryo sac showing degenerated antipodals. Fig. 33. Abnormal embryo sac showing displaced egg apparatus and several nucellar nuclei. Fig. 34. L. s. glandular bilipped stigma with cut ends of pollen tubes in the stylar canal. Fig. 35. Transections of the style at the level *a* in Fig. 34. Fig. 36. Portion marked *x* in Fig. 35, magnified to show the glandular outgrowths lining the mouth of the stylar canal. Figs. 37-42. *Elettaria cardamomum*. Fig. 37. L. s. ovule. Fig. 38. Megaspore mother cell at anaphase. Fig. 39. T-shaped tetrad. Fig. 40. Enlargement of chalazal megaspore; Note the degenerating megaspores. Fig. 41. L. s. portion of the ovule at mature embryo sac stage with degenerating antipodal nuclei. Fig. 42. L. s. style and stigma. Figs. 43-46. *Hitchenia caulina*. Fig. 43. L. s. anatropous ovule with arillar outgrowths from the outer integument and funiculus. Fig. 44. L. s. orthotropous ovule, a rare occurrence. Fig. 45. Triad. Fig. 46. L. s. ovule showing mature embryo sac



FIGS. 47-56—(*b*, basal apparatus; *bch*, basal chamber; *emb*, embryo; *end*, endosperm; *hy*, hypostase): Figs. 47-50. *Costus speciosus*. Fig. 47. L. s. embryo sac showing Helobial endosperm. Fig. 48. Basal apparatus magnified from Fig. 47. Fig. 49. One of the nucleus of the basal apparatus enlarged to show the hypertrophy. Fig. 50. L. s. embryo sac showing cellular endosperm and the mature embryo. The basal apparatus has entirely degenerated. Figs. 51, 52. *Elettaria cardamomum*. Fig. 51. Embryo sac showing the Helobial endosperm. Fig. 52. Cellular endosperm enclosing the mature embryo. Figs. 53-55. *Hitchenia caulina*. Stages in the development of endosperm (cross-hatched). Note the two-nucleate basal chamber in Fig. 53. Fig. 54. One of the two nuclei of the chalazal chamber shown in Fig. 53, enlarged. Fig. 56. Cellular endosperm and the differentiated embryo

Ovule and Female Gametophyte. The ovules are numerous, anatropous, bitegmic, crassinucellate and the micropyle is formed by the inner integument only (Figs. 30, 37, 43). They are arranged on axile placenta. Gregory (1936) reported hemianatropous condition in *Elettaria*, but the ovule which he has illustrated are immature. A rare instance of orthotropous condition was noticed in *Hitchenia caulina* (Fig. 44). The cells of the nucellar epidermis at the micropylar end are rich in cytoplasm and become radially elongated forming the " nucellar pad " (Figs. 41, 46, 95, 96). These by periclinal divisions add to the persistent nucellar tissue in all the members except in *Costus*.

The hypodermal archesporial cell gives rise to a parietal cell and the megaspore mother cell. The development of embryo sac follows Polygonum type (Figs. 31,32, 38-41, 45, 46). However, " Lilium type " has been reported in an unidentified species of *Costus* (Humphrey, 1896) and *Costus igneus* (Mauritzon, 1936) which need reinvestigation. The synergids and the antipodals are ephemeral and the fusion nucleus lies in the narrow "ventricle" (Figs. 32, 41, 46).

Hypostase and epistase are formed during the development of the ovule (Figs. 32, 41, 46). These apparently inhibit the extension of the embryo sac, but the latter continues its downward growth around the hypostase which appears to project into the former forming the " postament " (Figs. 47, 48, 51, 53, 105).

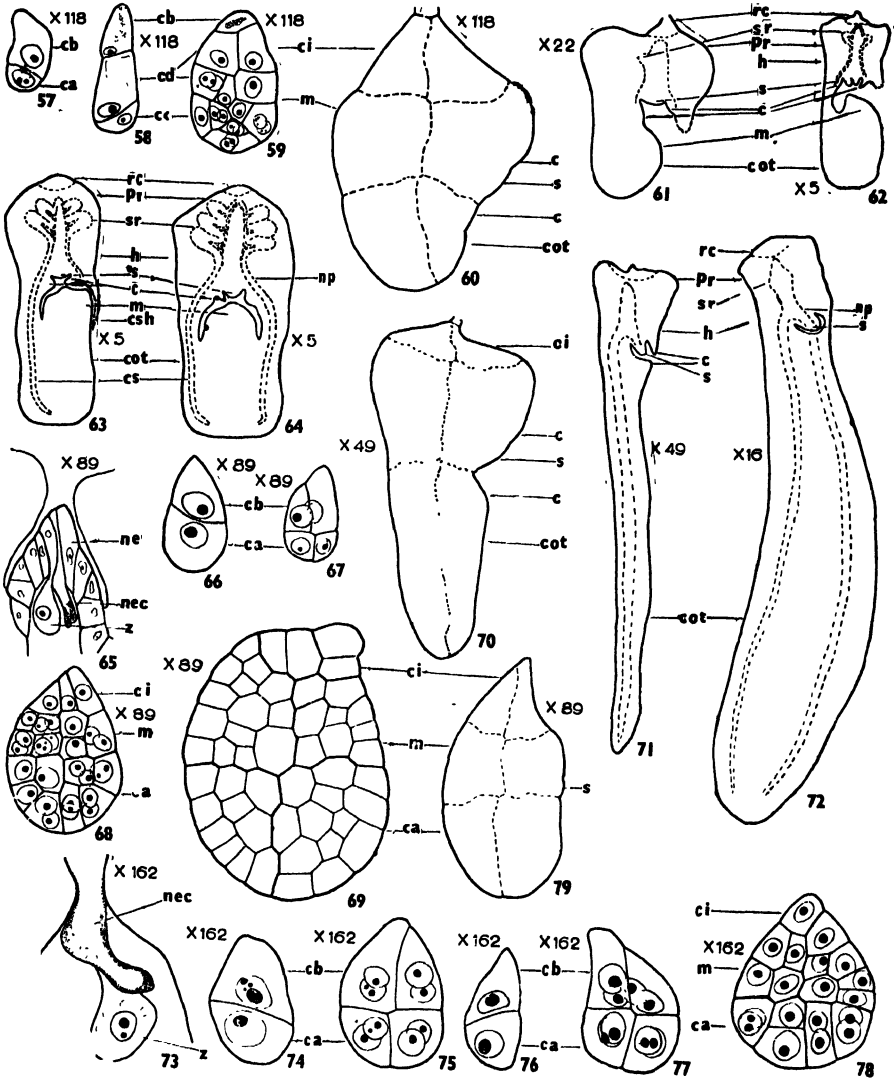
Abnormalities. In *Costus* some of the embryo sacs were observed to contain supernumerary nuclei (Fig. 33) termed as " wandering nuclei " by Madge (1934). These are perhaps nucellar in origin and get consumed by the enlarging embryo sac. Such instances have been reported by Boehm (1931), Madge (1934) and Harling (1949).

Pollination and Fertilization. The style is hollow. The stigma is simple, but the inner surface is lined with glandular cells in *Costus* (Figs. 34-36, 42). Pollen grains germinate on the surface of stigma excepting in *Elettaria* in which they enter the stylar canal (Fig. 42). The division of the generative cell takes place in the pollen tube, as observed in *Costus*. Fertilization is porogamous. Syngamy and double fertilization occur in close succession.

Endosperm. The endosperm is Helobial (Figs. 47, 51, 53). The wall layer, separating the two chambers, is conspicuous in *Costus speciosus* (Figs. 47, 48), whereas in others, it is merely a thin plasma membrane (Fig. 51). The micropylar chamber becomes cellular and stores plenty of aleurone grains and also starch in *Hitchenia caulina* and *Zingiber macrostachyum*, but only fat in *Costus*. At the micropylar end, a thin layer called " aleurone layer " persists (Figs. 50, 52, 55, 56).

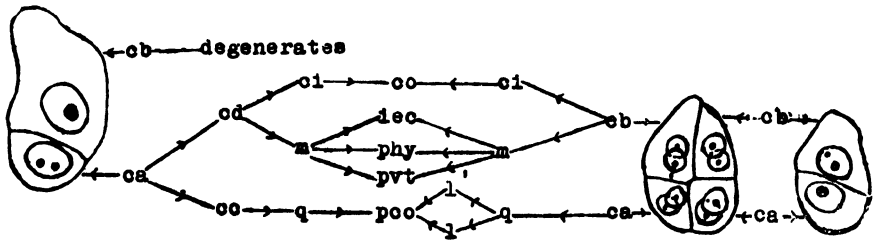
The chalazal chamber does not proceed beyond 2-nucleate stage and finally degenerates (Figs. 51, 53). However, *Costus* is exceptional in possessing a multinucleate basal apparatus containing dense cytoplasm and hypertrophied nuclei (Figs. 47-49). The latter phenomenon has also been observed in *Hitchenia caulina* (Fig. 54).

Embryo. The zygote divides transversely (Figs. 57, 66, 74, 76). The



•FIGS. 57-79 — (*c*, coleoptile; *cot*, cotyledon; *cs*, cotyledonary strand; *csh*, cotyledonary sheath; *h*, hypocotyl; *m*, mound; *ne*, nucellar epidermis; *nec*, nucellar epidermal cell; *np*, nodal plate; *pr*, primary root; *rc*, root cap; *s*, shoot apex; *sr*, secondary root; *z*, zygote): Figs. 57-64. *Costus speciosus*. Fig. 57-60. Proembryos. Fig. 61. L. s. differentiating embryo. Fig. 62. Median longitudinal section of nearly mature embryo. Figs. 63, 64. L. s. mature embryos at nearly median and tangential planes respectively. Figs. 65-72. *Elettaria cardamomum*. Fig. 65. Enlarged micropylar part of the nucellus showing the intrusion of a nucellar epidermal cell into the embryo sac. Figs. 66-68. Stages in the embryo development. Fig. 69. Globular embryo. Figs. 70, 71. Differentiating embryos. Fig. 72. Mature embryo. Figs. 73-75. *Zingiber macrostachyum*. Fig. 73. L. s. micropylar portion of embryo sac showing zygote and the intruding epidermal cell of the nucellus. Figs. 74, 75. Proembryos. Figs. 76-79. *Hitchenia caulina*. Figs. 76-78. Stages of embryo development. Fig. 79. Older embryo at the time of differentiation

embryo development conforms to Sagittaria variation of Caryophyllad type in *Costus speciosus* (Figs. 58-60), whereas Penaea variation of the Asterad type in other members (Figs. 65-69, 73-79). The difference in the development of both the types can be represented as follows :



The suspensor is absent, although a solitary cell derived from the basal tier, may be regarded as a rudimentary suspensor (Fig. 69).

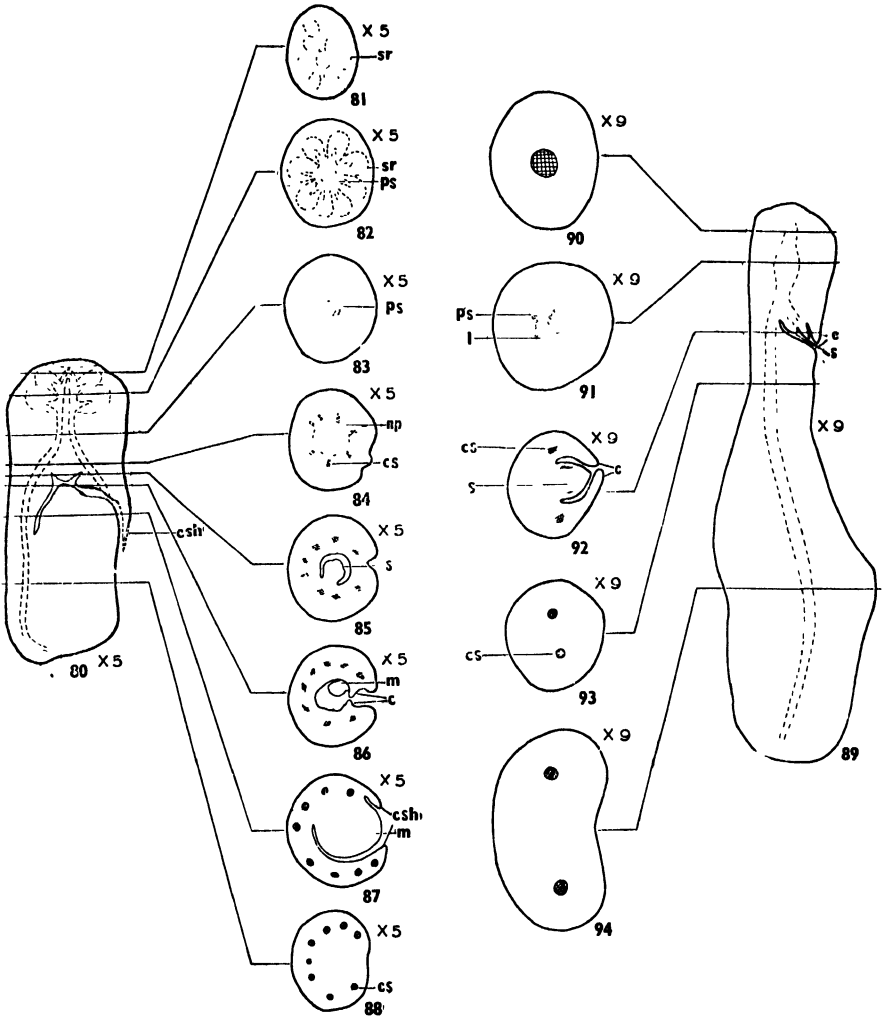
In both the types, the lateral shoot apex arises at the juncture of the tier *m* and the cotyledon. A few cells surrounding it develop into a circular coleoptile* (Figs. 60, 70-72, 85, 86, 89, 92). In case of *Costus*, as the embryo reaches maturity, the shoot apex will shift itself towards the centre due to unilateral growth of the cells behind the shoot apex and that of the cotyledon which forms a mound (Figs. 61-64, 86, 87). This results in an innovation and deeping of it into a groove (Figs. 63, 64, 85-87). Finally, the shoot apex will be situated opposite the cotyledonary mound (Figs. 63, 64, 85, 86). During the growth of the embryo, a cotyledonary sheath is formed arching the mouth of the groove and the margins of the cotyledonary mound (Figs. 61-63, 80, 87). A similar condition has been reported by Taylor (1957) in *Zostera maritima*.

Secondary root primordia (8-12 in *Costus* and only 4 in others) arise precociously, distal to the root tip followed by the differentiation of primary procambial strand and the cotyledonary strands (8 in *Costus* and only 2 in other members) which appear to diverge from the primary procambial strands at the nodal plate near the shoot apex (Figs. 61-64, 71, 72, 81-88, 90-94). In *Costus*, the mature embryo is cylindrical and its cells store fat, whereas in other members the embryo is long and tapering without any storage in the cells. A tendency for adventive embryony is observed in *Elettaria*, *Hitchenia* and *Zingiber*.

Seed. The mature seeds are hard and the cylindrical embryo occupies the entire length of the endosperm (Figs. 97, 102). Starch filled nucellus persists as perisperm forming the bulk of the seed (Figs. 97, 102, 103). The seed development is interesting due to the presence of aril, collar, lid and the chalazal tissue (Figs. 95-106).

* The term "coleoptile" has in the past been more or less restricted to the Gramineae. In the present study this term has been employed for the zingiberaceous embryo since this structure appears to be analogous to that in Gramineae.

The aril — also called "arillode" (Planchon, 1845) — is formed from the free end of the outer integument and that of the funicular side. It is in the form of a soft parenchymatous mound in *Costus* (Figs. 95, 96), a thin sheath in *Elettaria* (Figs. 101, 102), but lobed in *Hitchenia* (Fig. 106), whereas in *Zingiber* it is pink and sweet smelling with the fringes at the chalazal end (Figs. 104, 105). Secondary arillar outgrowths were observed in the young ovules of *Hitchenia* (Figs. 43, 44). Perhaps presence of such outgrowths prompted Humphrey (1896) to consider the aril as a "double structure" in *Amomum*, *Elettaria* and *Alpinia*.



FIGS. 80-95—(*c*, coleoptile; *cs*, cotyledonary strand; *csh*, cotyledonary sheath; *m*, mound; *np*, nodal plate; *ps*, procambial strand; *s*, shoot apex; *sr*, secondary root): Figs. 80-88. *Costus spiciosus*. Fig. 80. L. s. mature embryo. Figs. 81-88. Transections of mature embryo at levels marked in Fig. 80. Figs. 89-94. *Zingiber macrostachyum*. Fig. 89. L. s. mature embryo. Figs. 90-94. Transections of mature embryo at levels marked in Fig. 89

The role of aril is not clear. It has been described as third integument (Maheshwari, 1950) and nutritive sheath (Raju, 1956). Neither of the roles seems to be applicable to the members of Zingiberaceae. As Humphrey (1896) surmised, it might have some role in the dehiscence of fruit and in the dispersal of seeds.

A circular collar develops from the outer integument due to periclinal divisions of the cells (Figs. 95-97, 102, 105). The nucellus also grows upward on either side of the collar and facilitates deeper growth of it. The statement of Humphrey (1896) and Boehm (1931) that the inner integument contributes to the collar is not acceptable.

The outer integument also contributes to a well developed micropylar lid in *Costus speciosus* (Figs. 97, 98). It consists of polygonal cells with pitted thickenings and develops 2-3 layers removed from the inner epidermis. Along the outer border, there are thin walled cells which give way to the lid during the germination. The lid is of quite a different constitution in *Elettaria cardamomum* (Figs. 102, 103). The thickened cells of the inner epidermis (excepting a few cells along the margin of the collar) and those filled with refractive material above it together form a lid, as reported by Humphrey (1896) and Berger (1958). On the contrary, Mauritzon (1936) has reported its origin from the inner integument in *Alpinia*, *Roscoea* and *Costus igneus* which may be reinvestigated, on the basis of present findings. The function of lid is perhaps protection and it is absent in other members.

Another feature of interest is the chalazal tissue that persists below the nucellus where the vascular strand terminates (Figs. 97, 99, 100). It consists of radially elongated cells of epidermis and hypodermis filled with refractive material and the inner group of cells densely cytoplasmic (Fig. 100). The innermost cells that belong to nucellus also contain refractive material which perhaps acts as hypostase in the seed (Figs. 97, 99). Such a chalazal tissue is present in *Costus*, less developed in *Elettaria* (Fig. 102), but is absent in other members studied. According to Humphrey (1896) *Globba* shows the beginning of the tissue, *Amomum* and *Elettaria* are intermediate in this character and

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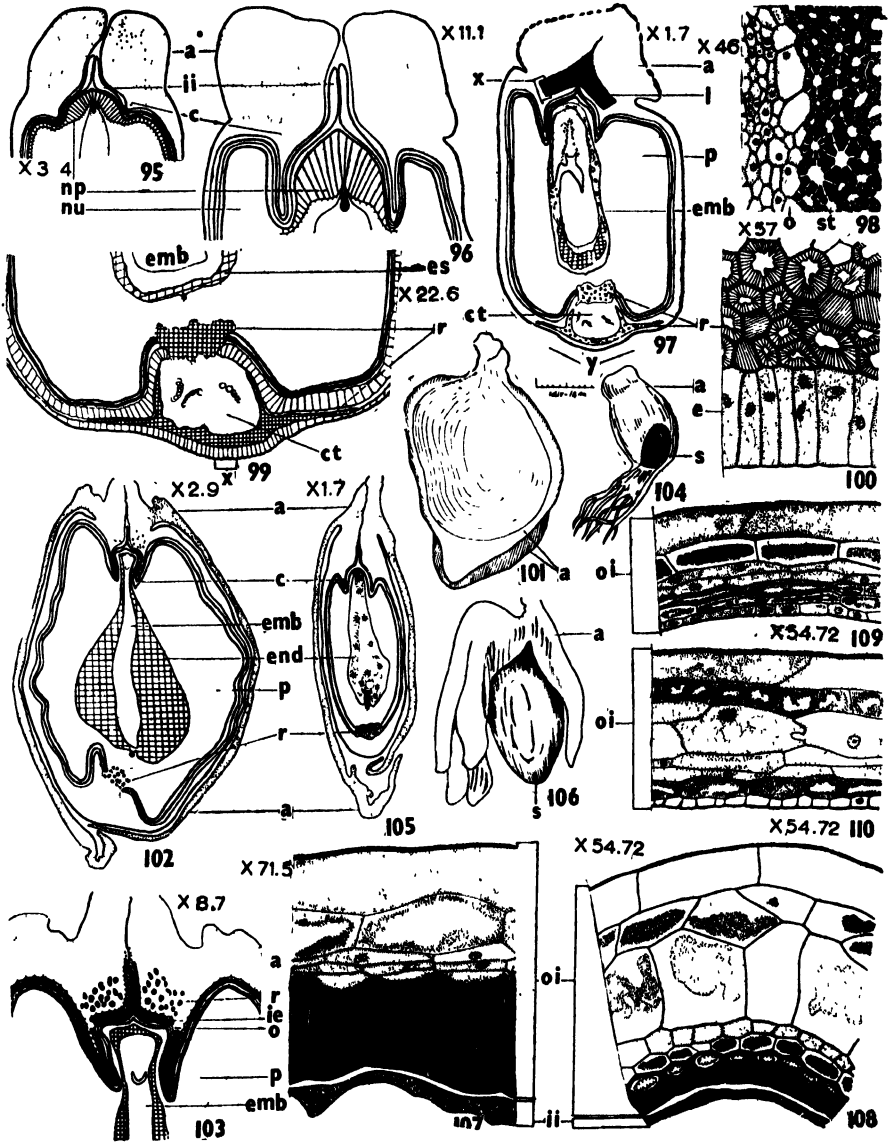
FIGS. 95-110—(*a*, aril; *c*, collar; *ct*, chalazal tissue; *e*, epidermis; *emb*, embryo; *end*, endosperm; *ie*, inner epidermis; *ii*, inner integument; *l*, lid; *np*, nucellar pad; *nu*, nucellus; *o*, opening; *oi*, outer integument; *p*, perisperm; *r*, cells with refractive material; *s*, seed; *st*, stone cells): Figs. 95-100. *Costus speciosus*. Figs. 95, 96. L. s. micropylar part of ovules showing the development of aril and collar from the outer integument. Fig. 97. L. s. seed showing the lid. Fig. 98. Magnification of portion marked *x* in Fig. 97. Fig. 99. Enlargement of the region marked *y* in Fig. 97. Fig. 100. Portion marked *x* in Fig. 99 enlarged to show the deposition of refractive material in the cells. Figs. 101-103. *Elettaria cardamomum*. Fig. 101. Mature seed enclosed within the thin veil-like aril (diagrammatic). Fig. 102. L. s. mature seed (reconstructed). Fig. 103. Micropylar part of the seed enlarged to show lid-like organization in the outer integument at the micropyle. Figs. 104-105. *Zingiber macrostachyum*. Fig. 104. Mature seed with a thick sheath of an aril terminating in quill-like fringes (diagrammatic). Fig. 105. L. s. of a young seed. Fig. 106. Mature seed with the lobed aril (diagrammatic). Fig. 107. *Costus speciosus*. Portion of the seed coat of a mature seed. Fig. 108. *Elettaria cardamomum*. Portion of the seed coat. Figs. 109, 110. *Hitchenia caulina* and *Zingiber macrostachyum* respectively. Magnified portions of the seed coat

the maximum development is reached in *Costus speciosus*. He presumes that the tissue aids in germination, whereas Schachner (1924) considers it as a "tunnel" for the transport of food material to the endosperm and embryo. In the author's opinion the latter view is a plausible one.

Seed Coat. The seed coat is formed by the outer integument, whereas the inner integument degenerates (Figs. 107-110). The hardness to the seed coat is afforded by the thick walled outer and inner epidermis. In *Costus* and *Elettaria* the inner epidermal cells are thickened with granular deposition, but in the latter member a crystalline mass is observed which has been



identified by Netolitzky (1926) and Berger (1958) as silica (Figs. 107, 108). In *Hitchenia* and *Zingiber* the hardness is due to thickened walls of the inner epidermis and also perhaps due to refractive material present in the middle layers (Figs. 109, 110).

SYSTEMATIC POSITION

The status of the subfamilies, Zingiberoideae and Costoideae, is a taxanomic puzzle. The available data from various fields have shown that the division made by Schumann (1904) on the basis of morphology, is well justified. But in Costoideae, only *Costus* has been investigated in detail and how far some of these conclusions can be applied to the subfamily as a whole can only be revealed by further research. However, Tomlinson (1956) opines that the results obtained with *Costus* are equally applicable to its related genera.

Embryologically, Costoideae is distinct from Zingiberoideae in having an unilayered sporogenous cells and fibrous thickening in the anther, coenocytic and persistent basal apparatus, fat storage in endosperm, Caryophyllad type of embryogeny, mature embryo of different nature, bulbous aril, micropylar lid and a well developed chalazal tissue.

On the basis of vegetative anatomy and other evidences, Tomlinson (1956) advocated that Costoideae forms distinct and natural group and suggested that it should be given a family rank because of its unique and advanced features. In the author's opinion, however, this suggestion is inappropriate. As far as embryology is concerned, although there are a few unique features, numerous characters that are common to both the subfamilies namely, single anther with equal number of wall layers, secretory tapetum, isobilateral tetrad, two-celled pollen grains at the time of shedding, Polygonum type of embryo sac development, presence of hypostase and epistase in the ovule, Helobial endosperm, persistent perisperm, aril, collar and host of similar embryological characters, speak for an underlying basic similarity between the two groups and bind them in a single family.

Hutchinson's (1934) suggestion that all the members allied to *Costus* may be grouped into a tribe Costeae, amounts to degradation of the subfamily to the tribal level. The emphasis of Raghavan & Venkatasubban (1943) to raise the status of the genus *Costus* to a tribe or a subfamily, on the basis of chromosome morphology, is an extreme view. However, data on other genera of the subfamily Costoideae are required for determining the rank of *Costus*. Till then it seems best to retain the existing rank, Costoideae.

SUMMARY

The anther wall consists of 6-8 layers with a secretory tapetum. The spore tetrads are isobilateral. In *Costus* and *Elettaria* extranuclear bodies are present in the sporogenous cells, even in the anther wall layers in the latter member. Pollen grains are shed at two-celled stage. Exine has warty thick-

enings with a furrow in case of *Costus*, but a pore in *Zingiber*, whereas in other members, it is smooth.

The ovules are anatropous, bitegmic and crassinucellate, arranged on axile placenta. The micropyle is formed by the inner integument. The ovules show characteristic nucellar pad, hypostase and epistase. Development of the embryo sac follows Polygonum type.

The pollen grains germinate on the stigmatic surface, but are interstylar in *Elettaria* and the division of the generative cell takes place in the pollen tube only. Fertilization is porogamous. Syngamy and double fertilization occur in close succession.

Endosperm is Helobial. The basal apparatus is multinucleate and persistent in *Costus*, whereas it degenerates at two-nucleate stage in others.

Embryo development follows Caryophyllad type in *Costus* and Asterad type in other members. Tendency for adventive embryony was noticed in *Elettaria*, *Hitchenia* and *Zingiber*.

In the mature seed starchy perisperm persists and the micropylar aril and collar develop from the outer integument. The aril is bulbous in *Costus* and only a sheath in others. *Costus* is characterized by its prominent micropylar lid and the chalazal tissue which are less developed in *Elettaria*, but absent in others. The seed coat is formed by thickened outer and inner epidermal layers of the outer integument only. In *Elettaria*, a crystalline substance is deposited in the cells of the inner epidermis.

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In Vitro Culture of Nucelli and Embryos of *Citrus aurantifolia* Swingle

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The usefulness of nucellar embryos, especially in *Citrus*, is well known (*see* Maheshwari, 1950), and of late nucelli and nucellar embryos have also been cultured *in vitro* (Maheshwari & Ranga Swamy, 1958; Ranga Swamy, 1958a, b, 1961). This paper deals with the effect of a few chemicals on the growth of the nucelli and embryos of *Citrus aurantifolia* (lemon).

MATERIAL AND METHOD

The material for these experiments was obtained from lemon plants growing in an orchard in Panipat, about 40 miles from Delhi. The plants flowered in March-April and cultures of the nucelli and embryos were started in June-July, 1959. Dissections were carried out under sterile conditions under a stereoscopic microscope. The young fruits were cut transversely, slightly above the equatorial region. The ovules were squeezed out of the cut fruit on a slide and sectioned into halves by a sterilized scalpel after which the nucelli were excised from them. Those containing adventive globular embryos were cut transversely. The micropylar halves were further slit longitudinally to expose the embryos prior to inoculation. It was not possible to distinguish the zygotic embryo from nucellar embryos under a dissecting microscope. However, in all cases the proembryo situated at the extreme micropylar end (presumably the zygotic embryo) was removed as a regular procedure. In all the explants, some endosperm nuclei were seen adhering to the wall of the embryo sac.

Some globular embryos (0.051–0.102 mm.) were also excised and cultured separately.

White's modified nutritive medium (*see* Ranga Swamy, 1961), jelled with 0.8 per cent Difco Bactoagar, was used in all the experiments. This basal medium was supplemented with casein hydrolysate (200, 400, 600, or 800 ppm), coconut milk (40 per cent by volume, autoclaved), gibberellic acid (1, 5 or 10 ppm), indoleacetic acid (5 or 10 ppm) and yeast extract (200,

500 ppm) with a view to studying their effects. The *p*H of the medium was adjusted to 5.8 after dissolving the agar.

The cultures were maintained at a temperature of 25° ± 1° C., and at a relative humidity of 50 to 60 per cent in diffused light (2 to 10 foot – candles). Fifty cultures were run for each experiment. But in the experiment with the medium containing casein hydrolysate (400 ppm) 120 cultures of the micropylar halves of nucelli were maintained.

OBSERVATIONS

The chalazal halves of the nucelli showed no response on any of the media. They remained quiescent for about three months then turned brown and ultimately degenerated.

On the basal medium, the micropylar halves of nucelli bearing 1 to 4 adventive globular embryos (Fig. 1) showed a slight enlargement, but the embryos did not differentiate further. Some of the embryos turned slightly green while the others remained yellow. The addition of indoleacetic acid (5 or 10 ppm) had no marked effect. In media containing gibberellic acid (1, 5 or 10 ppm) the embryos sometimes turned deep green in colour. When autoclaved coconut milk (40 per cent by volume) was added to the basal medium, some embryos developed deep green cotyledons but exhibited varied forms. They callused slightly and ultimately produced roots.

In yeast extract (200 or 500 ppm) medium, the adventive globular embryos grew to maturity. In some cultures, however, the embryos callused 45 days after inoculation while the nucellar tissue always turned brown.

The globular embryos attached to the micropylar half of the nucellus showed varied responses when reared on different concentrations of casein hydrolysate.

When the micropylar halves of nucelli were cultured in 200 ppm of casein hydrolysate, only about 15 per cent of the cultures showed callusing of the adventive globular embryos while in another 15 per cent only small protuberances were formed from some areas on embryos. The rest of the cultures did not respond in any visible form.

On a medium containing 400 ppm of casein hydrolysate, the nucelli showed a variety of responses. In about 50 per cent of the cultures, the adventive globular embryos enlarged and proliferated 40 days after inoculation. The proliferated mass was whitish, spongy and soft (Fig. 2). The callus consisted of uninucleate parenchymatous cells having numerous starch grains. After another month, rounded or elongated bodies, designated here as pseudobulbils, appeared on the surface of callus. These developed singly or in groups, were light green or pale yellow in colour and gave rise to embryos possessing either normal (Fig. 3) or aberrant cotyledons. Sometimes, the embryos showed a massive 'suspensor' (Fig. 5). As the embryos developed from the callus the concentration of starch in its cells decreased. Embryos which grew from these calli had no starch grains.

The young pseudobulbils showed an outer region of large parenchy-

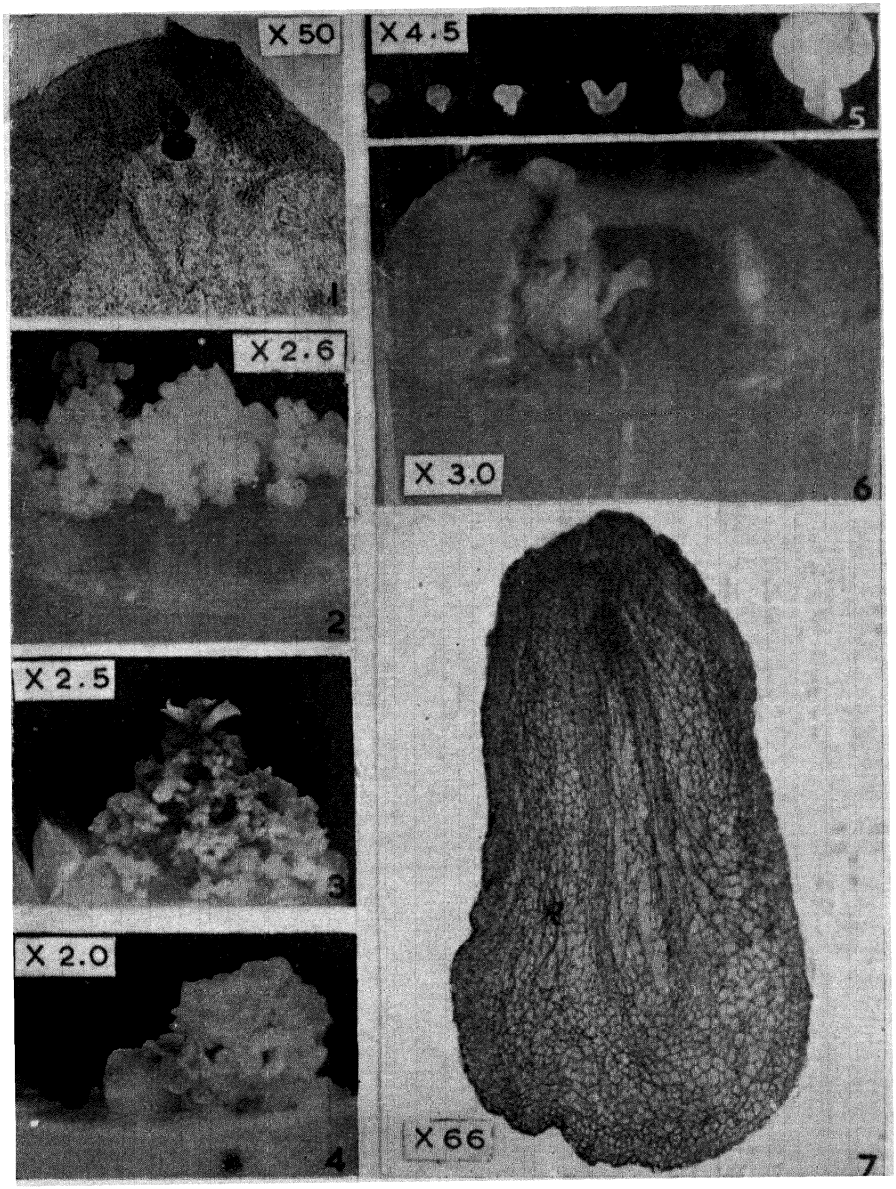

Fig. 1. Whole mount of the micropylar part of nucellus showing adventive embryos. Fig. 2. Three-month-old culture of micropylar half of nucellus bearing callusing adventive embryos (basal medium + casein hydrolysate—400 ppm). Fig. 3. Embryo-like structures differentiating from pseudobulbils. Fig. 4. Culture showing a mass of embryos differentiating from pseudobulbils (3 months after inoculation). Fig. 5. Stages in the differentiation of embryos from pseudobulbils; note the suspensor-like structures in four of them. Fig. 6. A fasciated seedling obtained from adventive globular embryo attached to the micropylar half of the nucellus. Fig. 7. L. s. differentiating pseudobulbil showing well developed provascular strands

matous uninucleate cells and a median provascular strand of narrow, elongated uninucleate and richly cytoplasmic cells. Further growth of the pseudobulbils followed no particular pattern but finally they acquired the form of typical dicotyledonous embryos. The cotyledons were well supplied with provascular strands (Fig. 7). Some of the embryos again started proliferating and produced an unorganized mass of tissue resembling the original callus. Germination of these embryos has not been achieved so far.

In about 25 per cent of the cultures, adventive embryos of globular form either produced masses of tissue which later differentiated into a number of embryos (Fig. 4) or the globular embryo itself continued its growth germinating to produce well-developed roots, although shoot growth was suppressed (Fig. 6). In the remaining cultures, the embryos did not show any marked response.

In the present study the nucellus as such did not respond to any of the treatments, for nucelli from which the proembryos had been excised did not grow *in vitro*.

SUMMARY AND CONCLUSIONS

In *Citrus microcarpa* Ranga Swamy (1958a) observed that the micropylar halves of nucelli with 1-3 proembryos, when cultured on a medium containing casein hydrolysate (400 ppm), produced a continuously growing callus tissue showing rounded, ellipsoidal or elongated structures which he termed "pseudobulbils." Later he (Ranga Swamy, 1958b) added that "apart from the proliferation of nucellar cells, the pseudobulbils may in part also originate from callusing of proembryos."

The present study on *Citrus aurantifolia* shows that the nucellus does not proliferate *per se* but it is the proembryos (of adventive origin) which show a variety of responses even on the same medium. Maximum variations were observed in a medium supplemented with 400 ppm casein hydrolysate. Some adventive embryos merely enlarge and do not grow further while some others grow into seedlings. Sometimes they gave rise to spherical bodies which later differentiated into embryos. Most of these proembryos proliferated to produce a whitish callus. The role of amino acids in such a behaviour is obscure since the medium containing casein hydrolysate was sterilized by autoclaving and some of the amino acids may have been destroyed or modified by heat (*see* Nitsch & Nitsch, 1957).

The micropylar halves of nucelli from which proembryos had been excised, showed no response to any treatment. The behaviour of the chalazal halves which normally lack the adventive embryos, resembled that of the embryo-free micropylar halves. When the longitudinal halves of nucelli containing 1 to 2 proembryos (in the micropylar part) were cultured, there was no callusing of the nucellus but only proembryos developed. When proembryos were excised and cultured separately, they did not callus but only enlarged and exhibited slight lobing. It may, therefore, be concluded

that while nucellus induces the proliferation of the proembryos in close contact with it, it does not itself proliferate or form any pseudobulbil.

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In Vitro Growth of Achenes of *Ranunculus sceleratus* L.

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The artificial rearing of excised ovary has been found very useful in understanding the effect of pollination, fertilization and vegetative parts on the developing fruit. The pioneer attempt in this direction was made by LaRue (1942), who obtained rooting of pedicels of excised ovaries in several plants. Nitsch (1949, 1951, 1952) employed this technique to understand various problems concerning fruit development. In tomato, he was able to get parthenocarpic fruits by supplementing the medium with growth substances and also reported that the mode of fruit development in artificial medium is essentially similar to that in Nature. Jansen & Bonner (1949) and Leopold & Scott (1952) also grew excised ovaries of tomato in test tubes successfully.

During the last decade fruitful results have been obtained with ovaries of wheat (Rédei & Rédei, 1955), *Fragaria* and *Pisum* (de Capite, 1955), *Althaea* (Chopra, 1958), *Iberis* (Maheshwari & Lal, 1958), *Linaria* (Sachar & Baldev, 1958), *Tropaeolum* (Sachar & Kanta, 1958), *Zephyranthes* (Sachar & Kapoor, 1959) and *Aerva* (Murgai, 1959).

The present study summarizes the authors' observations on the *in vitro* culture of ovaries of *Ranunculus sceleratus*.

MATERIAL AND METHODS

The plants of *Ranunculus sceleratus* were grown in the University Botanical Garden. The flowers were tagged on the day of anthesis, and the ovaries were excised three and six days after pollination* and were sterilized with a 15 per cent decanted solution of bleaching powder for 25–30 minutes. Thereafter, the ovaries were washed repeatedly with sterile water.

The basic medium was the one used by the senior author, in a previous investigation (*see* Sachar & Kanta, 1958). The medium was jelled with Difco bacto agar (0·7 per cent) and the *p*H was adjusted at 5·8.

* Young ovaries (i.e. at anthesis, and one and two days after pollination) were also cultured but they gave a poor response.

The medium was supplemented with indoleacetic acid (2 or 5 ppm), kinetin (0·5 ppm), gibberellic acid (7·5 ppm), casein hydrolysate (100, 500 or 1000 ppm) and autoclaved coconut milk (5, 10, 15, 20 or 25 per cent) from unripe fruits. For every treatment 50 ovaries were inoculated and three replicates were maintained.

In vitro and *in vivo* growth of fruits was compared. The fruits were sectioned at 10–15μ and stained in Heidenhain's haematoxylin and counter-stained with fast green. Unless otherwise mentioned, all observations recorded here are from ovaries inoculated six days after pollination.

OBSERVATIONS

Growth Pattern In Vivo. The plant is an annual, which flowers from February to April. The bisexual flowers are pentamerous with indefinite number of stamens and carpels. The carpels are free and are spirally arranged on an elongated torus. Each carpel consists of a flattened ovary with a short style and indistinguishable stigma. The ovary is unilocular and contains a marginal hemianatropous ovule.

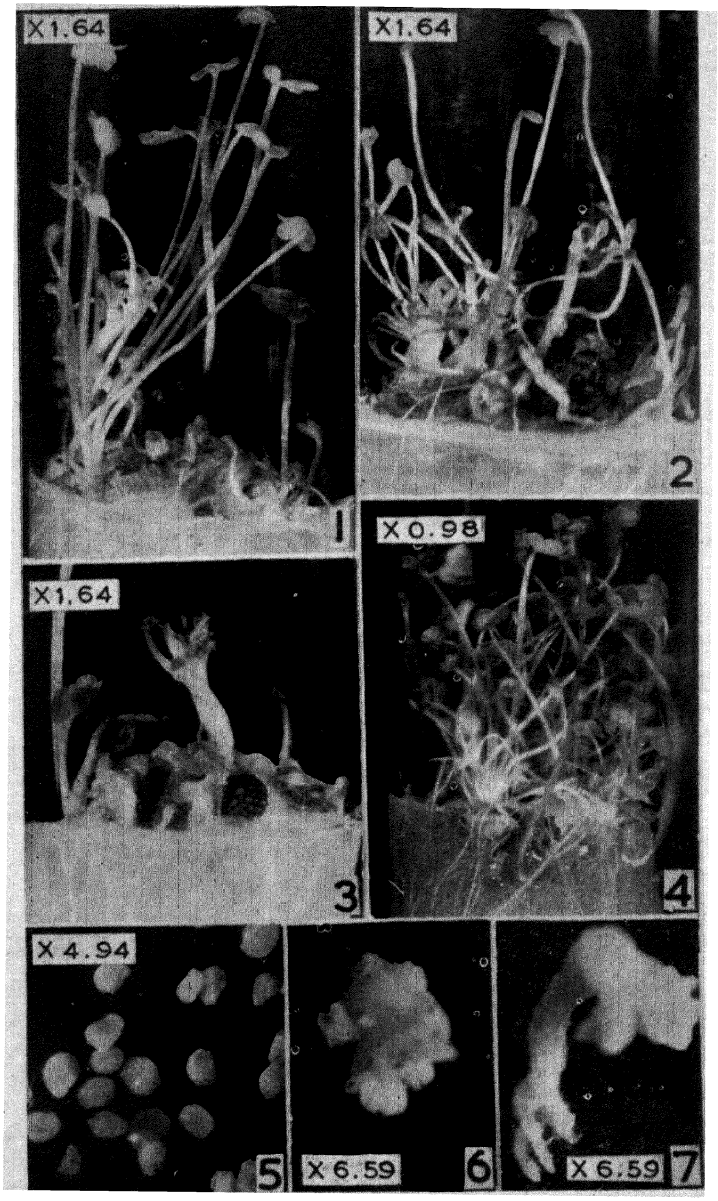
Pollination occurs on the day of anthesis, and the ovaries continue to grow for two weeks. Usually 90 per cent of the achenes are fertile and show differentiated embryos. At maturity the fruit becomes light brown and the achenes are shed individually.

The ovary wall is six-layered on the day of pollination and becomes eight- or nine-layered after two weeks. The two innermost layers become stony. The fruit is an etaerio of achenes.

Growth Pattern In Vitro. Ovaries cultured in Nitsch's basic medium (NB) supplemented with vitamins and glycine (NBV), showed slight increase in size (Table 1). When planted three days after pollination, only a few achenes developed normally while the remaining shrivelled. However, when cultured six days after pollination, all the achenes gave a plumpy appearance but only a few of them were fertile.

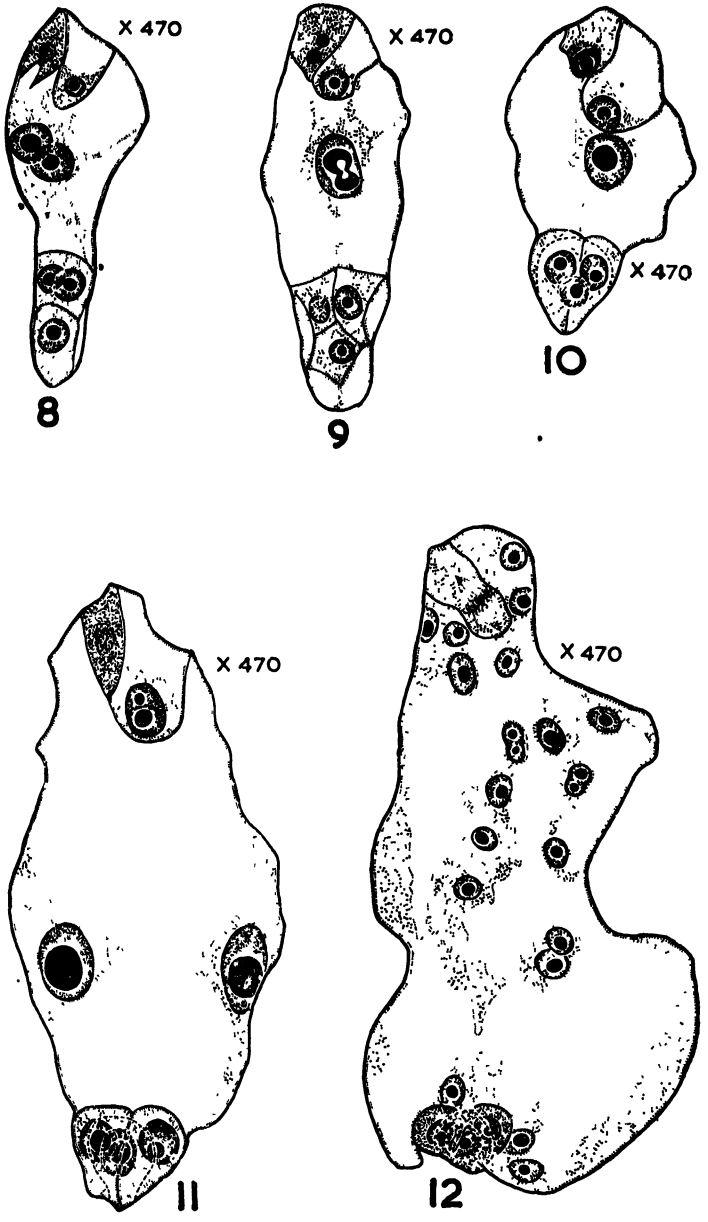
TABLE 1—FRUIT GROWTH IN THE FIELD AND IN DIFFERENT NUTRIENT MEDIA

Treatment	Av. size of 10 fruits (14-day-old) Length mm.	Breadth mm.
Control *in vivo*	9·00	3·00
Nitsch's basic (NB)	5·5	3·7
NBV	5·5	3·7
NBV + IAA 2 ppm	7·7	3·4
NBV + IAA 5 ppm + kinetin 0·5 ppm	6·5	4·0
NBV + casein hydrolysate 100 ppm	5·0	3·2
NBV + casein hydrolysate 500 ppm	5·5	3·3
NBV + casein hydrolysate 1000 ppm	5·7	3·4
NBV + gibberellic acid 7·5 ppm	8·0	3·8
NBV + coconut milk 20%	5·0	3·5



FIGS. 1-7 — *In vitro* GROWTH AND GERMINATION OF ACHENES OF *Ranunculus* : Fig. 1. *In vitro* germination of achenes (14-week-old) in NBV + casein hydrolysate (1000 ppm) inoculated six days after pollination. Fig. 2. Same, in NBV + casein hydrolysate (100 ppm). Fig. 3. A germinating achene in the centre of the culture tube shows fasciation of stem (NBV + casein hydrolysate 1000 ppm). Fig. 4. Profuse germination of achenes in NBV + casein hydrolysate (500 ppm) (14-week-old culture). Fig. 5. Mature achenes reared on NBV + casein hydrolysate (500 ppm); some of them produce aberrant embryos. Figs. 6, 7. Callusing of embryos in NBV + casein hydrolysate (500 ppm). In Fig. 7, three root primordia are seen to arise from the radicular end of the embryo

Fruit size was not influenced by the addition of casein hydrolysate. Nevertheless, the achenes were fertile and after seven weeks they germinated



FIGS. 8-12—DEVELOPMENT OF EMBRYO SAC *in vivo* : Figs. 8-10. Mature embryo sacs, showing stages in the fusion of polar nuclei. Fig. 11. Embryo sac showing syngamy and two endosperm nuclei. Fig. 12. Embryo sac with free endosperm nuclei and a dividing zygote, the antipodal cells continue to persist at the basal end

in situ. The seedlings grew vigorously and produced plantlets inside the test tubes (Figs. 1, 2 & 4). In some cultures the young plants showed fasciation of the stem (Fig. 3). Although germination of achenes occurred in all the three concentrations used, 500 ppm seemed to be the best for the growth of the plantlets (Figs. 1-4). A few ovaries rooted from the pedicels and the roots were often green in colour.

Ovaries planted in NBV supplemented by IAA (2 ppm) produced larger fruits than those reared on the basic medium (Table 1). The achenes developed better on the lower part of the torus. Only one or two seeds germinated *in situ* after 6-7 weeks and the seedlings were also no match to the ones obtained in casein hydrolysate medium.

The basic medium was also fortified with kinetin (0·5 ppm) in combination with IAA (5 ppm). In this medium fruit growth was retarded and the fruits were smaller in length than those reared in IAA alone (Table 1). None of the achenes germinated.

Gibberellic acid (7·5 ppm) induced the elongation of fruit more than any other chemical (Table 1). Mature embryos were present only in a few achenes and no germination was observed. The addition of autoclaved coconut milk did not promote fruit growth more than that in the basic medium (Table 1) and there was also poor germination of achenes. After 10-12 weeks, if the achenes were transplanted from the coconut milk medium to a casein hydrolysate medium, they gave rise to vigorous seedlings.

Effect of Age of Ovaries on Fruit Growth. The age of ovaries, at the time of inoculation, greatly influenced the growth of fruits. When planted on the day of anthesis or 1-3 days after pollination they produced only small fruits. Best results were obtained when older ovaries (six days after pollination) were tested.

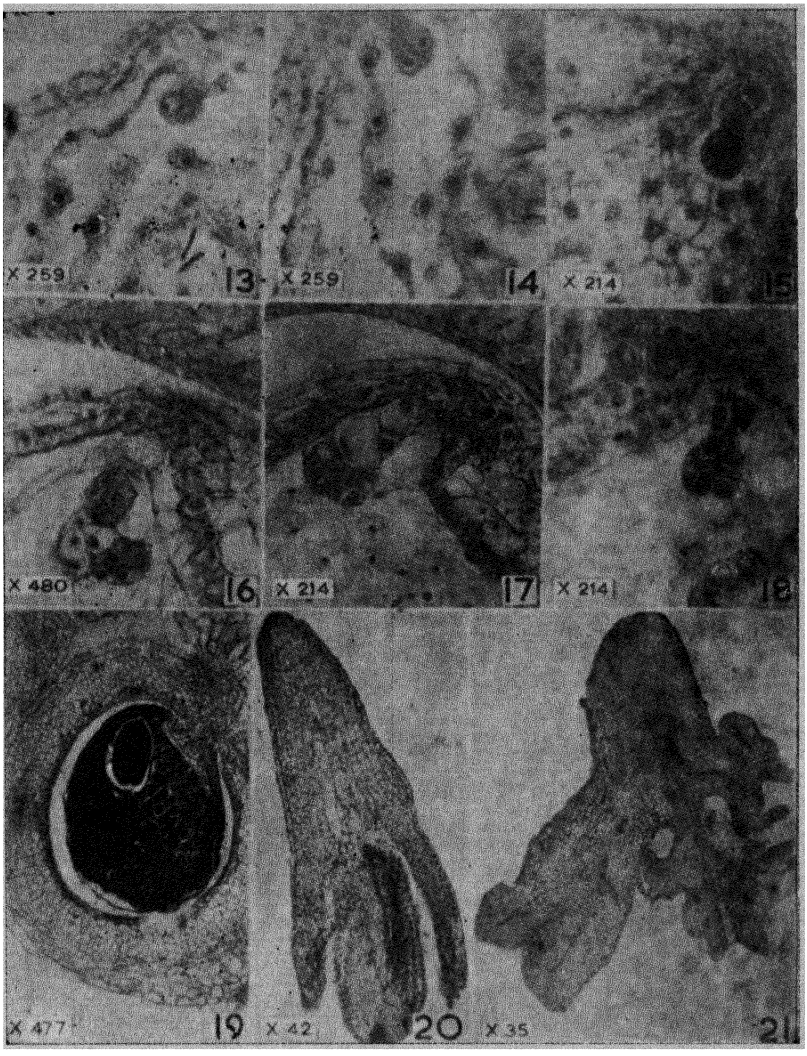
Although prolific germination of achenes took place in casein hydrolysate, ovaries inoculated three and six days after pollination showed a marked difference in germination. In the former, germination was much delayed and seedlings produced were never so vigorous as those formed in the latter.

In Vivo Development of Achenes. On the day of pollination most of the ovules contained mature embryo sacs with the usual organization. On the same day polar nuclei fused to form a secondary nucleus (Figs. 8-10).

Fertilization occurred 24-28 hr after pollination. The development of endosperm started before the division of the zygote (Fig. 11). The endosperm nuclei divided rapidly and by the time the zygote underwent the first division, 19-24 nuclei were formed (Fig. 12). Thirteen to 14 days of pollination the achenes showed mature embryos and the endosperm almost filled the cavity of the achene. Soon after this, shedding of the achenes started.

Histological Study of Cultured Achenes. At the time of inoculation, the ovules usually showed zygotes or rarely 2-celled proembryos (3 days after pollination), or 2- to 7-celled proembryos (6 days after pollination) (Figs. 13, 14).

After a week's growth in Nitsch's basic medium about 5% of the achenes showed heart-shaped embryos and a normal endosperm. The remaining



FIGS. 13-21— *In vitro* GROWTH OF EMBRYO AND ENDOSPERM : Fig. 13. L. s. ovule at the time of inoculation, showing a zygote and a few endosperm nuclei (3 days after pollination). Fig. 14. Same, showing three-celled proembryo and free endosperm nuclei (6 days after pollination). Fig. 15. Seven days' growth of proembryo and endosperm tissue reared on NBV + gibberellic acid (7·5 ppm). Fig. 16. Abnormal proliferation of proembryo grown on NBV + IAA (2 ppm). The endosperm tissue has completely aborted (7-day old). Fig. 17. Proembryo with excessively enlarged suspensor cells and degenerating endosperm nuclei from two-week-old culture (NBV + Kinetin 0·5 ppm + IAA 5 ppm). Fig. 18. Proembryo showing signs of cleavage, but such embryos fail to grow as the endosperm has already aborted (NBV + casein hydrolysate 500 ppm, 2-week old). Fig. 19. L. s. seed showing normal development of the endosperm and embryo on NBV + casein hydrolysate 500 ppm (2-week old). Figs. 20, 21. L. s. embryos isolated from cultures reared on NBV + casein hydrolysate 500 ppm showing three cotyledons and formation of accessory buds respectively

achenes either shrivelled after attaining an early globular stage of the embryo, or failed to advance beyond the stage of inoculation (6 days after pollination). Usually the upper achenes showed rapid degeneration. Ovaries cultivated 3 days after pollination were devoid of embryo and endosperm. Rarely 1-2 achenes in the lower part of the torus contained weakly developed embryos and scanty endosperm.

When IAA (2 ppm) was incorporated in the basic medium, about 19 per cent achenes showed mature dicotyledonous embryos and well formed cellular endosperm. The remaining achenes showed abortive seeds with degenerating embryos and without any endosperm. A combination of IAA (5 ppm) and kinetin (0·5 ppm) was found to have a retarding effect on th development of the achenes. About 96 per cent of the achenes showed complete abortion and became shrunken. The remaining 4 per cent of the achenes, however, showed heart-shaped embryos and the endosperm was also well developed.

On NBV + gibberellic acid medium only 4 per cent achenes showed mature embryos. A peculiar feature was that one cotyledon was much more developed than the other. In certain achenes the proembryo grew up to globular stage (Fig. 15) but eventually degenerated. Rarely twin embryos were also observed.

Addition of casein hydrolysate to the basic medium proved to be the best for the development of endosperm and embryo. About 65-70 per cent of the achenes, produced mature embryos (Fig. 5). The rest showed all the stages of embryos starting from the zygote to the heart-shaped, with free nuclear endosperm. Usually these achenes showing early stages of embryogeny failed to attain maturity.

The achenes, inoculated six days after pollination took more or less the same time for producing differentiated embryos as those under *in vivo* conditions (12-14 days). The development of embryo was considerably delayed (21-25 days) if the ovaries were inoculated three days after pollination.

Usually degeneration of the embryo was preceded by that of the endosperm tissue. Often the embryos showed morphological disfiguration (Figs. 16-18). Casein hydrolysate seems to promote the normal growth of endosperm and in turn that of the embryo (Fig. 19).

As regards the size of the embryo, there was a distinct improvement over that of the control *in vivo* (15·2μ). The maximum average size (24·4μ) was attained in the medium which was fortified with gibberellic acid. In Nitsch's basic medium the embryo grew to 20·2μ. There was slight increase (21·9μ) in NBV + IAA medium. On adding kinetin + IAA the growth of the embryo was distinctly inhibited (18·1μ). In NBV + casein hydrolysate there was no significant difference from that of the basic medium (Fig. 22).

Budding of Embryos. Mention may also be made of some aberrant embryos produced in a medium containing casein hydrolysate or coconut milk (Figs. 6, 7). The growth of such embryos was quite normal up to the stage of differentiation. Thereafter, they projected out rupturing the wall of the achenes which were still attached to the fruit. Such embryos continued

meristematic activity, but failed to produce normal seedlings. Externally the embryos appeared to be a mass of irregular tissue (Fig. 6). The place of maximum activity was the region of hypocotyl which became enormously swollen. Several accessory buds appeared from this zone (Fig. 21). Simultaneously the radicular end grew and gave rise to several root primordia (Fig. 7). Such embryos did not develop any chlorophyll even when kept in cultures for many days. The embryos usually remained dwarf but sometimes the root system grew normally. A few embryos showed three cotyledons but these were devoid of any accessory buds or root primordia (Fig. 20).

SUMMARY AND CONCLUSIONS

The ovaries of *Ranunculus sceleratus* were excised three and six days after pollination, and planted in the following nutrient media: (a) Nitsch's basic, (b) Nitsch's basic + vitamins and glycine (NBV), (c) NBV + IAA 2 ppm, (d) NBV + IAA 5 ppm + kinetin 0·5 ppm, (e) NBV + gibberellic acid

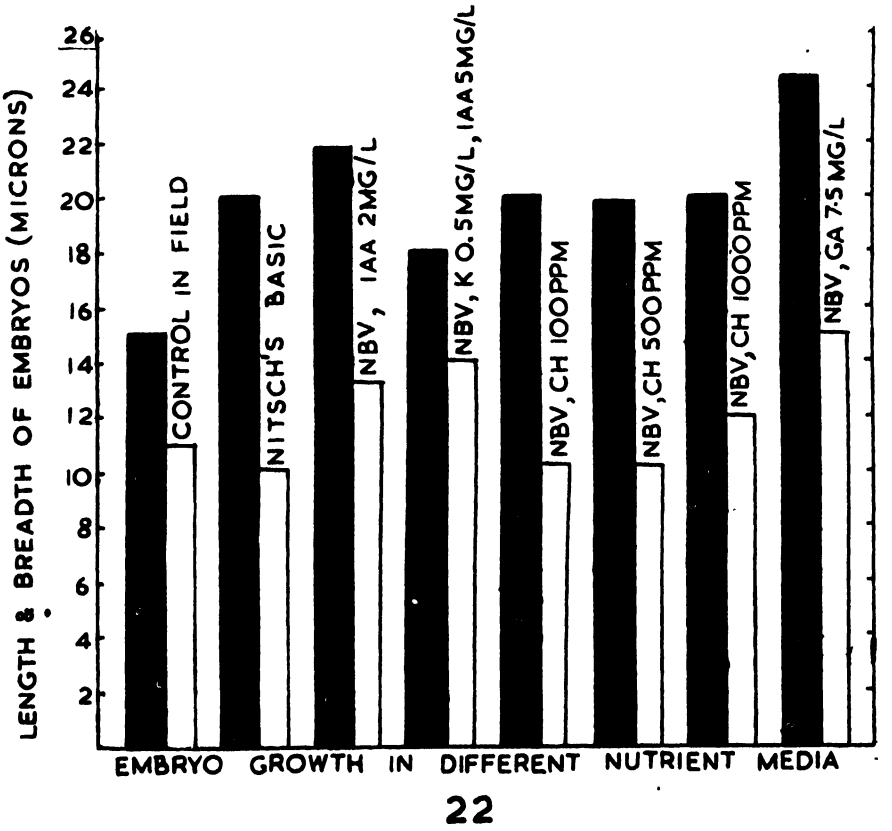


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FIG. 22—HISTOGRAM SHOWING GROWTH OF EMBRYOS IN DIFFERENT NUTRIENT MEDIA. BLACK BANDS REPRESENT THE LENGTH AND WHITE BANDS DENOTE THE BREADTH OF THE EMBRYOS

7·5 ppm, (f) NBV + casein hydrolysate 100, 500, 1000 ppm, and (g) NBV + coconut milk 5, 10, 15, 20, 25 per cent. Of all the supplements, gibberellic acid was found to be the best for producing large-sized fruits, and the results were quite comparable to those in nature.

When ovaries were inoculated three days after pollination they behaved in slightly different manner from those inoculated six days after pollination. In the former, the growth was considerably delayed, and the fruits did not attain the full size. While ovaries cultured younger than three days after pollination did not give any encouraging results.

At the time of inoculation, the embryo sac showed a zygote or rarely a 2-celled proembryo (three days after pollination) and 2- to 7-celled proembryo (six days after pollination). In the basic medium, 95 per cent of the achenes were completely sterile, while the rest showed mature dicotyledonous embryos with well developed endosperm. More or less similar results were obtained when the basic medium (NBV) was supplemented by IAA, kinetin, or gibberellic acid. However, if the basic medium was supplemented by casein hydrolysate or coconut milk, the fertility of achenes shot up to 65-70 per cent. In casein hydrolysate medium the achenes germinated *in situ* and produced miniature plants. Some of the achenes on this medium produced aberrant embryos which developed accessory buds on the swollen hypocotyl. Such embryos became enormously large due to excessive meristematic activity, but were chlorophyll-less and failed to germinate normally.

In the various culture media the size of the embryos was larger than that of the controls *in vivo*. The largest embryos were obtained in the gibberellic acid medium.

It gives the authors great pleasure to express their thanks to Professor P. Maheshwari for his encouragement and keen interest.

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Formation of Male Gametes in the Pollen Tubes of Some Crop Plants

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Pollen grains in angiosperms are shed either at the 2-or the 3-celled stage. In all those cases where shedding takes place at the 2-celled stage, i.e. after the formation of a vegetative nucleus and a generative cell, the latter divides in the pollen tube at any time during its growth towards the ovules in the ovary (*see* Maheshwari, 1950). In these plants, therefore, it becomes increasingly difficult to study the division of the generative cell since the pollen tube is very narrow, often takes a tortuous course in the style and cannot be cut medianly in microtome sections or dissected out easily. Mainly due to these reasons our knowledge of male gamete formation in such plants where the mitosis of the generative cell takes place in the pollen tube is very meagre. However, pollen culture technique can be profitably employed to study the details of male gamete formation and the behaviour of the male cells and the vegetative nucleus in the pollen tube.

MATERIALS AND METHODS

Pollen grains of 14 species of plants belonging to 10 genera distributed over 6 families were cultured :

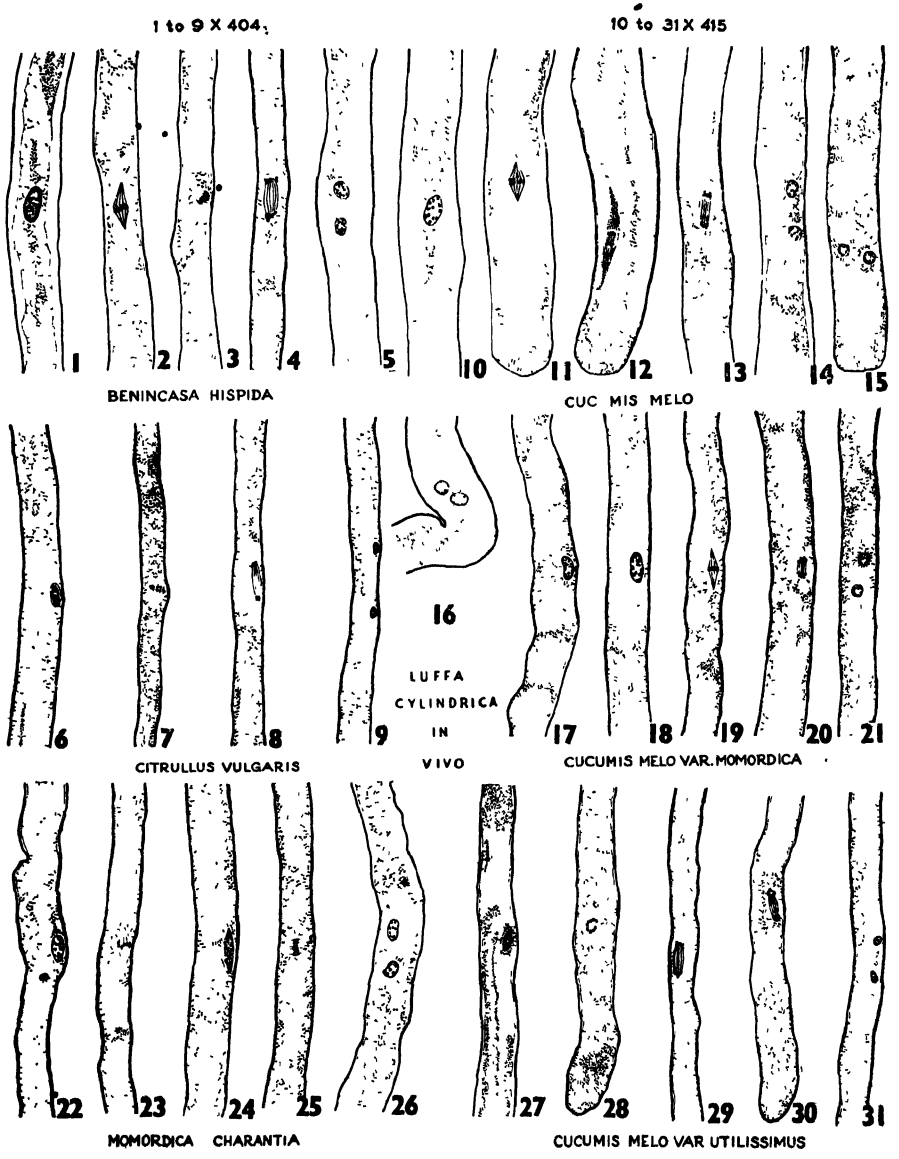
Benincasa hispida, *Citrullus vulgaris*, *Cucumis melo*, *C. melo* var. *momordica*, *C. melo* var. *utilissimus*, *Momordica charantia* (Cucurbitaceae), *Capsicum annuum*, *Solanum melongena*, *S. tuberosum* var. *phulwa* (Solanaceae), *Dolichos lablab* (Leguminosae), *Gossypium herbaceum*, *G. hirsutum* (Malvaceae), *Carica papaya* (Caricaceae) and *Citrus microcarpa* (Rutaceae).

Pollen were cultured in sugar-agar or sugar-agar-boric acid media by the hanging drop technique (*see* Vasil, 1960). The cover glasses containing growing pollen tubes on agar films were fixed in acetic acid-alcohol (1:3) and were subsequently stained, dehydrated and cleared in the following series :

Propionocarmine ——→ 45 per cent Propionic acid ——→ Propionic acid ——→ Propionic acid + *tert.*-Butyl alcohol (1 : 1) ——→ *tert.*-Butyl

alcohol ——→ *tert.*-Butyl alcohol + Xylol (1 : 1) ——→ Xylol (mounted in canada balsam).

Propionocarmine was invariably found to be better than acetocarmine. In some cases hematoxylin-fast green combinations were also used.



FIGS. 1-31—PORTIONS OF POLLEN TUBES SHOWING THE GENERATIVE CELL, ITS DIVISION AND FORMATION OF MALE GAMETES *in vitro* (EXCEPT *Luffa cylindrica*, Fig. 16, WHICH IS FROM *in vivo*) IN *Benincasa hispida* (Figs. 1-5), *Citrullus vulgaris* (Figs. 6-9), *Cucumis melo* (Figs. 10-15), *Cucumis melo* var. *momordica* (Figs. 17-21), *Momordica charantia* (Figs. 22-26) AND *Cucumis melo* var. *utilissimus* (Figs. 27-31)

OBSERVATIONS

Within 5-20 minutes of the sowing in the culture medium the pollen grains put out the tubes which elongated to different lengths depending on the medium, the plant species and various other factors like the *p*H and temperature. Generally the nuclei in the pollen grain did not move out immediately after the germination of the pollen and in most cases they moved only after the tube had attained a length of about 1000–3000μ. In *Solanum tuberosum*, however, the generative cell elongated from 8 to 29μ soon after the germination of the pollen grain and moved out of the grain into the tube (Figs. 32-35).

In most of the plants studied the vegetative nucleus degenerated early and was not traceable in the pollen grain even at the time of its germination except in *Dolichos lablab* (Figs. 51-53), *Gossypium herbaceum* and *G. hirsutum* (Figs. 55-59) where it could be clearly seen even after the formation of the male gametes.

After the pollen tubes attained a particular length (about 1000μ in most plants) almost all the contents of the pollen tube, its cytoplasm, the vegetative nucleus if it was still traceable and the generative cell, moved to the tip portion of the tube. The nuclei were generally present within about 200μ from the extreme tip of the tube.

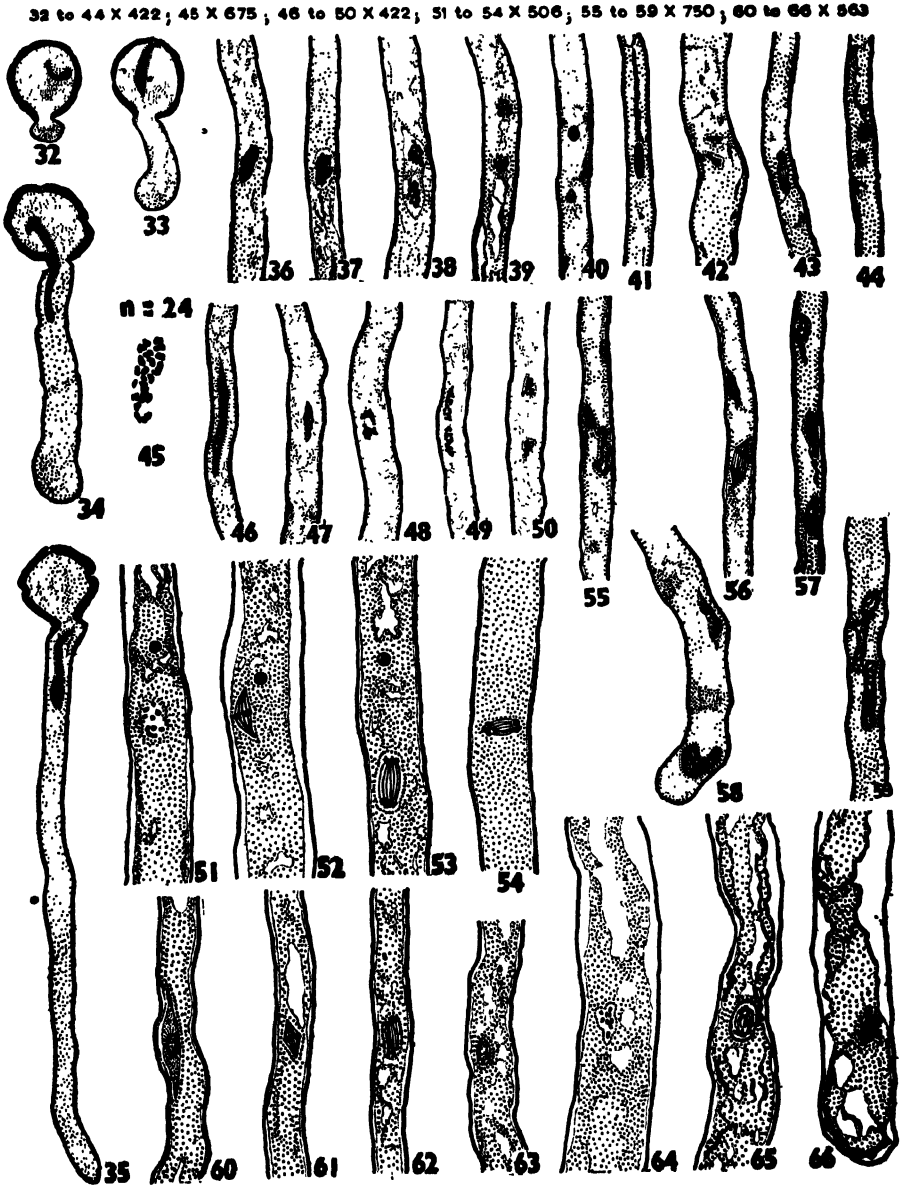
The generative cell showed a thin hyaline area around its nucleus which represented the male cytoplasm reduced to its barest minimum. It had no visible vacuoles and food particles, a fact which is responsible for the very hyaline nature of the male cytoplasm.

The generative nucleus rarely showed a nucleolus (e.g. *Cucumis melo* var. *utilissimus*, Fig. 27). Mitosis of the generative nucleus was perfectly normal and spindle fibres were clearly seen during this period (Figs. 2-4, 7, 8, 11-13, 19, 20, 23-25, 28-30, 43, 52-54, 56, 61, 62, 65). In some cases they may not be so clearly visible unless special stains are used (Figs. 38, 42, 48, 49). During the early stages of the mitosis it was sometimes possible to count the number of chromosomes, e.g. in *Cucumis melo* (n = 12), *Cucumis melo* var. *momordica* (n = 12), *Dolichos lablab* (n = 10; *see* Fig. 51), *Solanum*

FIGS. 32-66—Figs. 32-35. *Solanum tuberosum* var. *phulwa*—stages in the emergence of the generative cell from the pollen grain into the tube; note the change in size. Figs. 36-44. *Capsicum annuum* N.P. 46A (Figs. 36-40) and *Solanum melongena* Long Purple I.C 1417 (Figs. 41-44) — parts of pollen tubes showing the generative cell, its division and formation of male gametes *in vitro*. Fig. 45. *Solanum tuberosum* var. *phulwa* — haploid chromosome complement (n = 24) from the generative nucleus. Figs. 46-50. Same, parts of pollen tubes enlarged to show the division of the generative cell and the formation of male gametes *in vitro*. Figs. 51-59. *Dolichos lablab* (Figs. 51-54), *Gossypium hirsutum* Indore-2 (Figs. 55-57) and *G. herbaceum* 1027-A.L.F. (Figs. 58-59)—parts of cultured pollen tubes showing generative cell, its division and male cells; vegetative nucleus can be seen in all tubes except in Fig. 54. Fig. 51 shows the haploid set of chromosomes of *Dolichos lablab* (n = 10). *Carica papaya* (Figs. 60-62) and *Citrus microcarpa* (Figs. 63-66)—parts of pollen tubes showing the generative cell, its division and male cells *in vitro*. Fig. 64 shows the haploid chromosome complement of *Citrus microcarpa* (n = 9)

tuberosum var. *phulwa* (n = 24; *see* Fig. 45) and *Citrus microcarpa* (n = 9; *see* Fig. 64).

In some plants from the very beginning the generative nucleus appeared in an early prophase (Figs. 1, 10, 22, 36, 63) while in others it was in a 'resting' condition (Figs. 6, 17, 27, 41, 46, 55, 58, 60). The spindles were normally organized lying parallel to the long axis of the pollen tube but may be obliquely



placed in some cases (Figs. 3, 25, 56). Rarely, the spindles may be placed at right angles to the long axis of the pollen tube (Fig. 54). Depending on the mode of arrangement of the spindles during mitosis, the male cells may be arranged parallel (Figs. 5, 9, 14, 21, 26, 31, 39, 40, 44, 50, 57, 59, 66) or at right angles (Fig. 15) to the long axis of the pollen tube. The mode of cytokinesis during the organization of the male cells could not be studied. The form, shape and size of the male cells formed *in vitro* was the same as observed *in vivo* (Fig. 16).

The time taken for the initiation of mitosis in the generative cell after the placing of pollen grains in the culture medium varies in the different plants studied.

Cucurbitaceae	Mitosis starts after 4 hr. Male cells seen after another 2 hr.
Solanaceae	Mitosis initiated and completed within 6-10 hr. after inoculation.
Leguminosae	Division started and completed within 3-5 hr.
Gossypium	Division s arted and completed within 30 minutes to 1 hr.
Citrus and *Carica*	Division initiated and completed within 12-24 hr.

In many cases profuse branching of the pollen tubes was seen, especially in the members of the family Solanaceae. In the various species studied from the Cucurbitaceae, more than one pollen tubes were often formed from a single pollen grain. In all such cases, whether the supernumerary pollen tubes arose due to branching or a polysiphonous habit, any one pollen tube or its branch may receive either a single or all the nuclei from the pollen grain. The pollen tube receiving the nuclei may not always be longest at the given time but more often it was this tube which elongated most. In *Solanum melongena* the generative as well as the vegetative nuclei were sometimes arrested near the pollen grain due to the formation of a callose plug but in spite of this the pollen tube which was thus made enucleate grew to about 4000 μ. The generative cell arrested near the pollen grain without much accompanying cytoplasm underwent a normal mitotic division and produced two male cells.

SUMMARY AND CONCLUSIONS

The vegetative nucleus when present takes a very poor stain due to a low DNA level. Usually, however, it is ephemeral and is untraceable when the generative cell is undergoing mitosis. Evidently, it has no impact on the division of the generative cell nor does it seem to control the growth of the pollen tube. In many plants it degenerates before the germination of the pollen grain or may remain in the pollen grain itself. The pollen tube grows normally even when the generative cell and the vegetative nucleus are arrested near the pollen grain due to the formation of a callose plug.

The male cells have often been reported to change their shape after their release in the embryo sac as well as during the time they are contained in the growing pollen tube. Sometimes the male cells in the course of their movement in the pollen tube may change their shape so markedly that it may be difficult even to recognize them. Observations made by Safijovska (1955) *in vivo* on the male cells of a number of species have led him to state that "sperms, as complete cells, are not in possession of any constant form. During their way in the pollen tube the microgametes can change their form sometimes very markedly under external influences and perhaps, from internal changes which take place in the generative plasm". In *Campanula americana*, *Scilla hispanica* var. *rosea*, *Hyacinthus orientalis* and *Tradescantia zebrina* the tips of the male cells frequently change their shape while moving in the pollen tube and quite often the ends become markedly stretched which further indicates their autonomous movement (Safijovska, 1955). The pressure of the cytoplasmic streaming and the narrowness of the pollen tube also affects the shape of the generative and the male cells as has been seen in *Galanthus nivalis* by Steffen (1953). The cytoplasm of the pollen tube is also under a high turgor and it may also have a decisive effect on the shape of the generative cell and the male cells as it has been the experience of the author with many plants that if the pollen tube bursts, the gametes which may have had an elongated form in the pollen tube immediately become round on being thrown out of the pollen tube. The disparity in the size of the two male gametes reported in many plants may be in some cases due to the different rates of the transformation of male gametes from one shape to the other.

The question whether the male gametes occur as naked nuclei or as distinct cells has also attracted considerable attention. The evidence at present, from *in vivo* and fixed material, however, supports the view that the male gametes are distinct and complete cells and have their own cytoplasm and a cytoplasmic sheath. The male cytoplasm is reduced to its barest minimum and is hyaline and clearer than the rest of the vegetative cytoplasm in the pollen grain or the pollen tube which is full of granular food contents. The male cytoplasm is much hardier than the vegetative plasm and remains healthy and does not show any signs of degeneration even several hours after the cytoplasm of the pollen tube has coagulated and degenerated.

A suggestion has often been made that the male gametes move passively along with the streaming of the cytoplasm in the pollen tube. If this were so, the gametes should keep on moving from the base of the pollen tube to its tip and back (if there are no callose plugs formed). In practice, however, the generative or the male cells are always found near the tip of the pollen tube and always at a more or less fixed distance from the tip depending on the species. This observation itself is sufficient to disprove the view that the male cells move passively. At present it is believed that the generative and the male cells have a power of autonomous movement (Safijovska, 1955; Vazart, 1958). Steffen (1953) has observed, however, passive as well as amoeboid movement of the generative and the male cells in *Galanthus nivalis.*

Grateful thanks are due to Professor P. Maheshwari and Dr B. M. Johri for their kind help and suggestions.

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Morphology and Embryology of *Lomatia* Br. with a Discussion on its Probable Origin

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The Proteaceae with 61 genera and about 1,400 species is mainly a southern hemisphere-family, the chief centres of distribution being Australia, New Caledonia, South America and South Africa. The genus *Lomatia* was placed by Brown (1810), Bentham (1870) and Engler (1898) in the tribe Embothrieae of the sub-family Grevilloideae. In his revised classification of the family the present author (Venkata Rao, 1957) placed it in the tribe Telopeae. Out of the 13 species of *Lomatia*, 6 are found in East Australia, 2 in Tasmania, 1 in New Caledonia, 1 in Tahiti and 3 in Chile, the various species being endemic in their respective regions. Though at present the genus is confined to the southern hemisphere, it apparently enjoyed a wider distribution in the past since fossils are reported not only from southern land masses (East Australia, Tasmania, South America and Antarctica) but also from different regions of northern hemisphere like North America, South of England, Italy and Switzerland (Kausik, 1943). Fossil specimens closely resembling *Lomatia* belonging to Cretaceous of Saxony are placed in an allied genus *Lomatites*. However, there is much controversy regarding the validity of northern hemisphere fossils not only of this genus but of the whole family (Good, 1953).

PREVIOUS WORK

The embryological work in the Proteaceae is limited to the study of a few species of three Australian genera, *Grevillea*, *Hakea* and *Macadamia* (Brough, 1933; Kausik, 1938-42) and the African genus *Brabeium* (Jordaan, 1946; Garside, 1946). Recently the embryological work in the family has been reviewed by the present author (Venkata Rao, 1960).

The present paper deals with the morphology and embryology of 6 species of *Lomatia* viz. *L. tinctoria* Br., and *L. polymorpha* Br. of Tasmania, *L. salaifolia* Br. of New South Wales, *L. dentata* Br., *L. hirsuta* Br. and *L. ferruginea* Br. of Chile.

MATERIAL AND METHOD

Material of the Tasmanian species was collected by the writer during his stay at Tasmania (1955 - 1957). Fixed material of *L. salaifolia* was kindly sent by Mr R. Carolin of Sydney University, Sydney, N. S. W., Australia. Herbarium specimens of the Chilean species were obtained from the National Herbarium, Chile. Fixed material of *L. ferruginea* was kindly put at the disposal of the author by Mr V. Garcia of Delhi University. Formalin-acetic-alcohol was used as the fixative. Customary methods of dehydration and embedding were followed. The herbarium material was soaked overnight, in distilled water, boiled in 2 per cent solution of sodium hydroxide for 2-3 minutes or left overnight in paraffin bath at 50° C., washed thoroughly and then treated like freshly fixed material. Whole mounts of endosperm were made by Kausik's (1938) method.

OBSERVATIONS

Bentham (1870) stated : "the structure and proportions of the flower and fruit are remarkably uniform in the Australian species (of *Lomatia*) leaving little for their identification besides their foliage which is extremely variable". The present studies show that this statement can be extended to the Chilean species also.

Of the Tasmanian species, *L. tinctoria* is a small shrub 2 to 3 feet high, which reproduces by subterranean rhizomes and therefore occurs gregariously. It ranges from sea level to about 2,500 ft altitude. *L. polymorpha* is a taller shrub, 10 to 12 feet high and occurs from about 1,500 to 3,500 ft altitude. The leaves of *L. tinctoria* are usually bipinnately compound though rarely they may be simple. In *L. polymorpha* (as the name indicates) the leaves are usually simple but extremely variable in shape being oblong, linear, lanceolate, entire or lobed even on the same plant. The same range of variation is noticed in the Chilean species : the leaves of *L. dentata* and *L. hirsuta* are simple while those of *L. ferruginea* are bipinnately compound (Figs. 1-4, 6).

The inflorescences are axillary or terminal panicles; they are lax in *L. tinctoria* (Fig. 1) and *L. ferruginea* (Fig. 3) while in *L. polymorpha* (Fig. 2) and *L. dentata* (Fig. 6) they are more dense. As in other genera of the Grevilloideae, a pair of flowers occurs in the axil of each bract (Fig. 13). Since the posterior sides of the flowers of a pair are away from each other (Fig. 18), it is evident that the flowers are not oriented in relation to the main axis but to a lateral axis which is completely suppressed (Fig. 19). The bract, therefore, belongs to the main axis and the bracts of the individual flowers are completely suppressed.

The flowers are pedicellate, zygomorphic, hermaphrodite and monochlamydeous. The apical portion of the bud is globular and termed as 'limb'; it remains curved in the bud (Figs. 5, 10, 12). The four tepals bear nearly sessile anthers at their terminal parts (Figs. 11, 12). The ovary is monocarpellary, stipitate and bears about 16 ovules placed in two imbricating

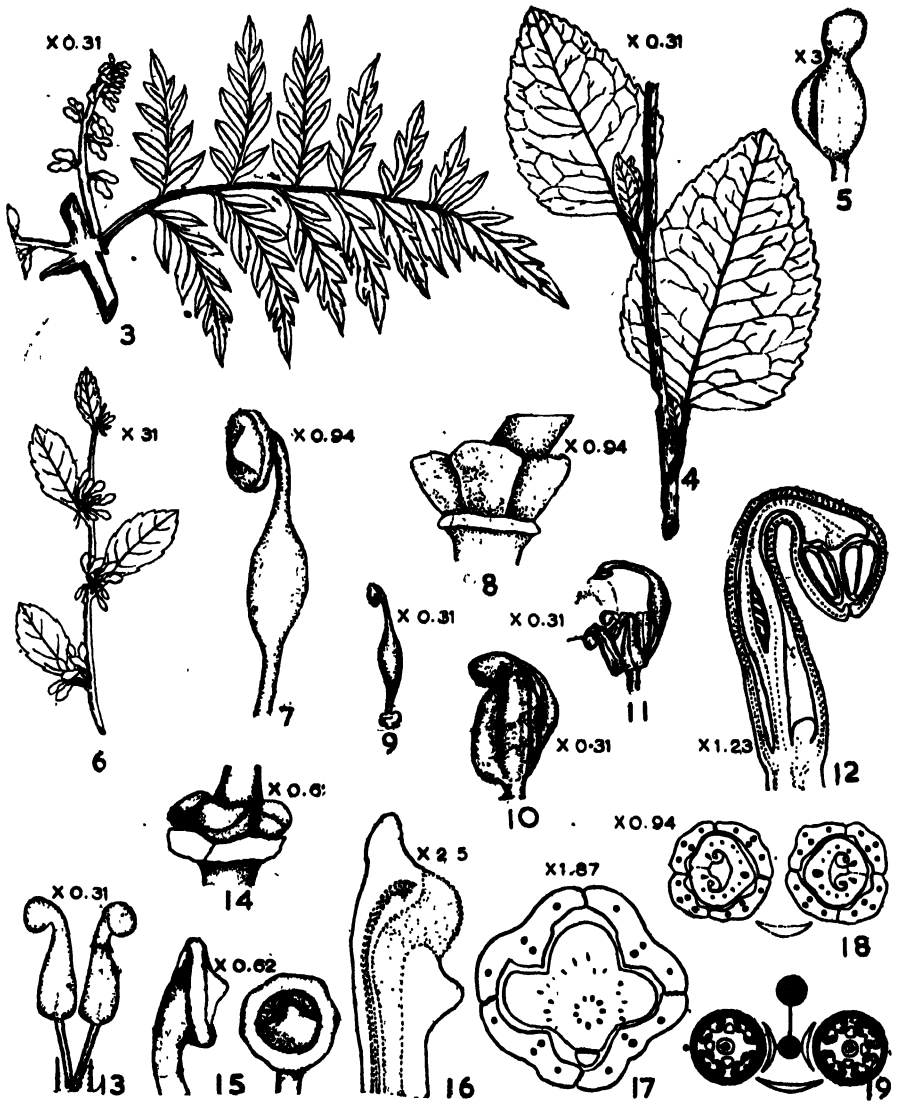
rows on marginal placenta. The style is curved and bears a lateral stigma situated in the centre of a discoid pollen collecting apparatus (Figs. 7, 9, 11, 15, 16). The nectary consists of 3 free alternitepalous lobes (Figs. 8, 14); in this feature *Lomatia* resembles the two other Australian genera *Austromuellera* and *Musgravea* which constitute the tribe Musgraveae (Venkata Rao, 1957). Presence of a small vestigial anterior lobe in *Lomatia tinctoria* (Fig. 17) indicates that probably the zygomorphic nectary has evolved due to the suppres-

FIG. 1 — A BRANCH OF *Lomatia tinctoria* WITH INFLORESCENCE

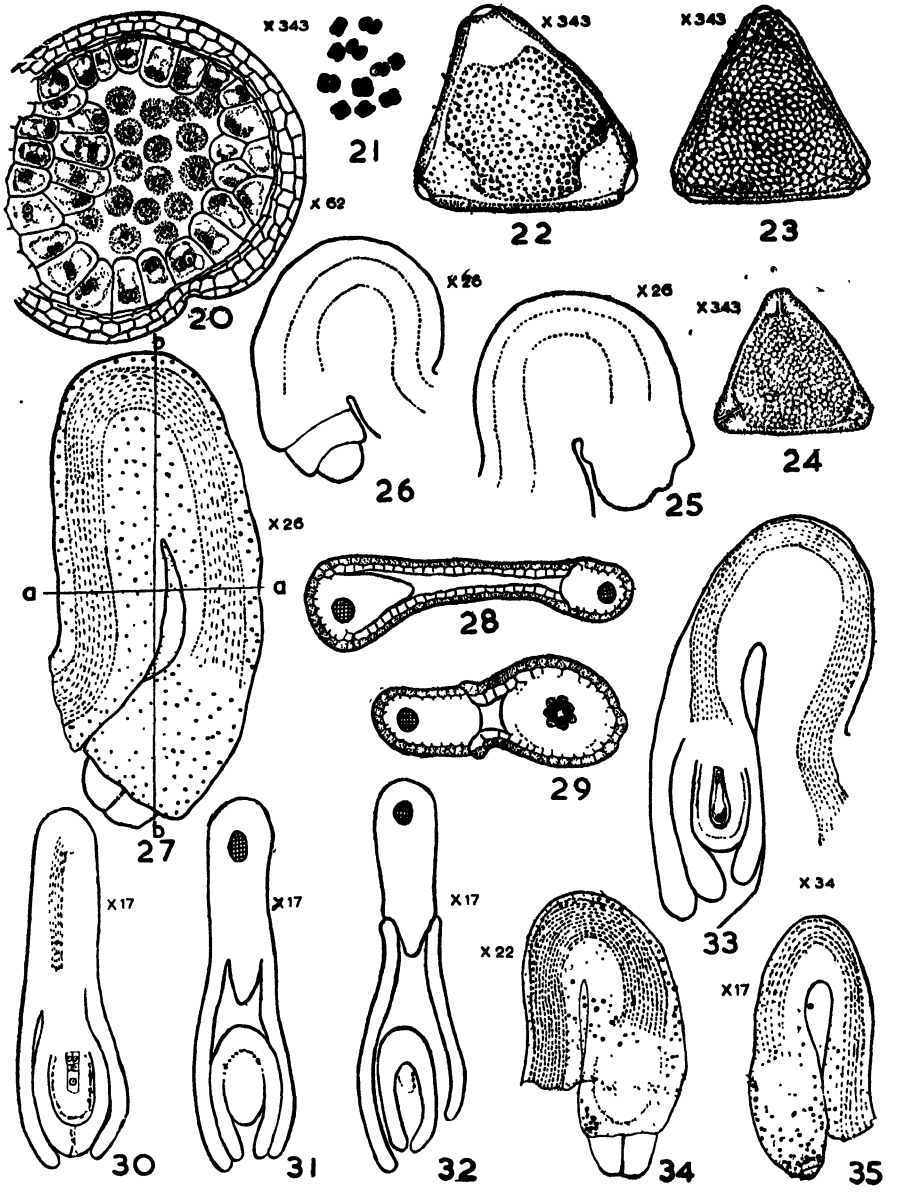
sion of the fourth lobe of the typically actinomorphic flowers found in the tribe Persoonieae (Venkata Rao, 1960).

Microsporogenesis and Male Gametophyte. The anthers are 4-locular. In *L. tinctoria* and *L. polymorpha* one or two rows of hypodermal archesporial cells differentiate at 4 places in the anther primordium. These cut off the primary sporogenous cells to the inside and the primary parietal cells to the outside. Due to further divisions in the parietal cells, the anther wall ultimately

FIG. 2 — A BRANCH OF *L. polymorpha* WITH INFLORESCENCE



FIGS. 3-19 — Fig. 3. Twig of *Lomatia ferruginea*. Fig. 4. Twig of *L. hirsuta*. Fig. 5 Flower bud of *L. hirsuta*. Fig. 6. Twig of *L. dentata*. Figs. 7, 8. Ovary and nectary of *L. dentata*. Figs. 9-11. Ovary, bud and open flower of *L. polymorpha*. Fig. 12. L. s. flower bud of *L. salaifolia*. Figs. 13-18. *L. tinctoria*. Fig. 13. A flower pair (bract removed). Fig. 14. Nectary. Fig. 15. Side and front views of pollen collecting apparatus and stigma. Fig. 16. L. s. stigma. Fig. 17. T. s. flower bud showing vestigial anterior lobe of the nectary. Fig. 18. T. s. flower pair; note the opposite orientation of the ovaries in the two flowers. Fig. 19. Diagrammatic representation of the relation of the flower pair to the suppressed lateral axis



FIGS. 20-35 — Fig. 20. T. s. of young anther lobe of *L. polymorpha*. Fig. 21. Diakinesis in *L. tinctoria* showing 11 bivalents. Figs. 22-24. Pollen grains of *L. salaifolia*, *L. polymorpha* and *L. hirsuta* respectively. Figs. 25-32. *L. polymorpha*. Figs. 25-27. Stages in the development of the ovule. Figs. 28, 29. Sections of ovule cut in the plane a-a shown in Fig. 27. Figs. 30-32. Sections of ovule cut in the plane b-b shown in Fig. 27. Fig. 33. Median longitudinal section of the ovule of *L. tinctoria* cut parallel to the flat side; note the absence of the outer integument on the side of the funicle. Fig. 34. Ovule of *L. hirsuta*. Fig. 35. Ovule of *L. dentata*

becomes 6-layered — the epidermis, the fibrous endothecium, 3 middle layers and the tapetum. In *L. tinctoria* the tapetum is 2-layered on the side of the connective (Fig. 20).

There is a secondary increase in the sporogenous cells. The meiotic divisions proceed normally and 11 bivalents are noticed at diakinesis in *L. tinctoria* (Fig. 21). The same number is reported by Lancaster (1952) in *L. salaifolia* (*see* Darlington & Wylie, 1955). Cytokinesis is brought about by furrowing at the end of the second division and mostly tetrahedral tetrads are produced. The pollen grains in all the 6 species studied are triangular, oblately flattened and triporate (Figs. 22-24). The germ pores are situated at the corners. The exine is granular or reticulately thickened. The intine is thicker in the region of the germ pores and protrudes slightly. The pollen grains are shed at the two-celled stage.

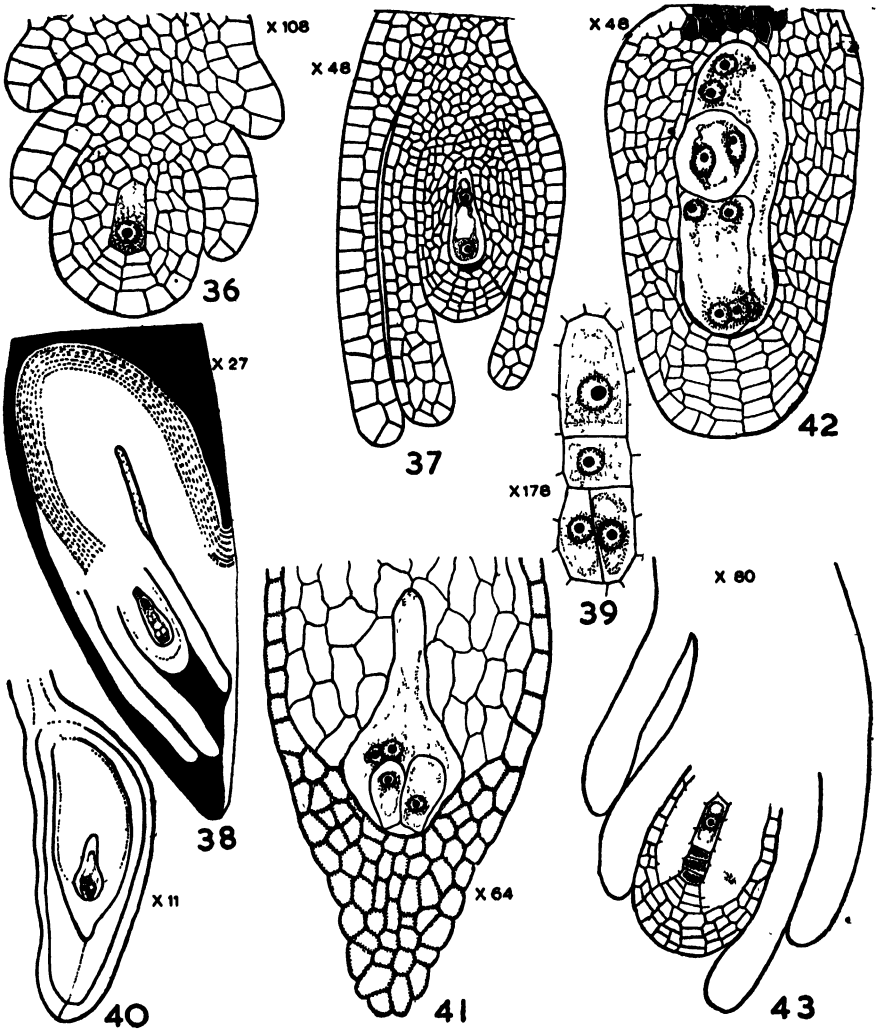
Ovule. In all the 6 species studied, the ovule is bitegmic and crassinucellate. It shows a characteristic and unique method of development. The ovule primordium arises transverse to the ovarian loculus (Fig. 25), and undergoes curvature during growth till in the mature condition the ovule lies parallel to the placenta (Fig. 38). The funicle elongates precociously and bends like a horse shoe (Figs. 26, 27) and finally becomes bent in the form of a hairpin in the mature ovule (Figs. 33-35). It develops into a samaroid wing in the mature seed (Figs. 56-59). The outer integument remains two-layered; tannin accumulates in the cells of its outer epidermis. The inner integument becomes 3-layered (Fig. 37), and covers the nucellus in such a way as to leave a wide micropyle. The outer integument ceases to grow along the line of contact with the funicle. From the chalazal region extensions of the outer integument arise and form flap like coverings on either side of the bent funicle. This structure becomes clear when sections of developing ovule and seed taken perpendicular to the long axis of the ovule, and also parallel and perpendicular to the flat side (Figs. 28-33) are studied.

The primary parietal cell cut off by the archesporial cell divides to form 5-6 layers of parietal cells. The cells of the nucellar epidermis also undergo periclinal divisions early in development of the ovule and form a nucellar cap (Figs. 37, 43). These cells accumulate some tannin and stand out distinctly from other nucellar cells (Fig. 41). Only the nucellar cap persists in the mature ovule. The developing embryo sac extends nearly up to the chalaza (Figs. 38, 42). As the ovule matures, the meristematic zone situated in the chalaza adds some tissue so that the antipodal end of the embryo sac becomes much removed from the chalaza (Fig. 40). As the seed develops the outermost 3-4 layers of nucellar cells become richly protoplasmic and get tangentially flattened; these cells persist along with the zone of richly protoplasmic cells in the chalazal region, even in the mature seed (Figs. 46, 47). The remaining cells of the nucellus are irregular, large, scantily cytoplasmic and get absorbed by the developing endosperm.

Megasporogenesis and Female Gemetophyte. Usually there is a single archesporial cell in the ovule. After cutting off the primary parietal cell it functions as the megaspore mother cell (Fig. 36). Megaspore tetrads are usually

linear (Fig. 43); in one ovule of *L. polymorpha* an inverted T-shaped tetrad was noticed, where the micropylar megaspore was functioning (Fig. 39). The 3 antipodals are inconspicuous and ephemeral. The polar nuclei do not fuse before fertilization (Fig. 41). In one ovule of *L. polymorpha* three developing embryo sacs were noticed (Fig. 42).

Endosperm and Embryo. The development of the endosperm was



FIGS. 36-43 — Figs. 36-38. *L. tinctoria*: Fig. 36. L. s. ovule with megaspore mother cell. Fig. 37. Ovule with 2-nucleate embryo sac. Fig. 38. L. s. ovule with 8-nucleate embryo sac. Figs. 39-42. *L. polymorpha*. Fig. 39. Inverted T-shaped tetrad. Fig. 40. L. s. mature ovule. Fig. 41. Micropylar part of the nucellus from the above. Fig. 42. L. s. nucellus of an ovule with 3 developing embryo sacs. Fig. 43. L. s. young ovule of *L. salaifolia* with linear tetrad

studied in detail in the Tasmanian species. The primary endosperm nucleus undergoes free nuclear divisions. The developing endosperm digests some of the surrounding nucellar cells and lies loosely in the cavity so that it can be dissected out easily (Figs. 46, 47). The seed becomes flat as it grows. The endosperm in the micropylar region becomes cellular by the time the embryo is 20- to 30-celled. The cells immediately around the embryo are smaller and richly cytoplasmic as compared to those in the remaining part (Figs. 45, 48, 50). The endosperm in the chalazal part remains nuclear. The nuclei in this region are sparsely distributed and may form groups (Figs. 49, 51). The cellular part becomes wavy in outline due to the development of secondary haustorial lobes (Figs. 45, 52, 53). In addition to these, finger-shaped, 1-celled outgrowths arise all over the surface of endosperm and increase the absorbing area. These become absorbed as the endosperm grows. Since the growth of the nuclear part of the endosperm in the early stages is rapid and exceeds that of the seed, it becomes coiled and gets the characteristic vermiform appearance. As the seed grows, the nuclear part shrinks and becomes less and less conspicuous (Figs. 52, 53). Ultimately the whole of the endosperm gets absorbed by the developing embryo. The structure and development of the endosperm in *Lomatia* resembles that described in some Cucurbitaceae (Chopra & Agrawal, 1958).

The endosperm in the Chilean species *L. ferruginea* (Fig. 54) closely resembles the developmental sequence of the endosperm of the Tasmanian species.

The first division of the fertilized egg is transverse. Both the cells divide longitudinally and by further divisions give rise to a globular embryo which is devoid of a suspensor (Figs. 44, 45, 54). The embryo development keys out to the Penaea variation of the Asterad type (Johansen, 1950), as in other members of the family (Venkata Rao, 1960). The mature embryo shows foliaceous cotyledons with prominent basal lobes (Figs. 53-55).

Seed. Bentham (1870) stated "the fragile pellicle or powdery substance interposed between the seeds in *Lomatia* and *Telopea* appears to be epidermal production of the seed itself, but its real nature can scarcely be ascertained without observing it in the fresh state before and after maturity of the seed". The structure of the seed wing as also the presence of the powdery pellicle between the seeds are closely similar in the Tasmanian and Chilean species.

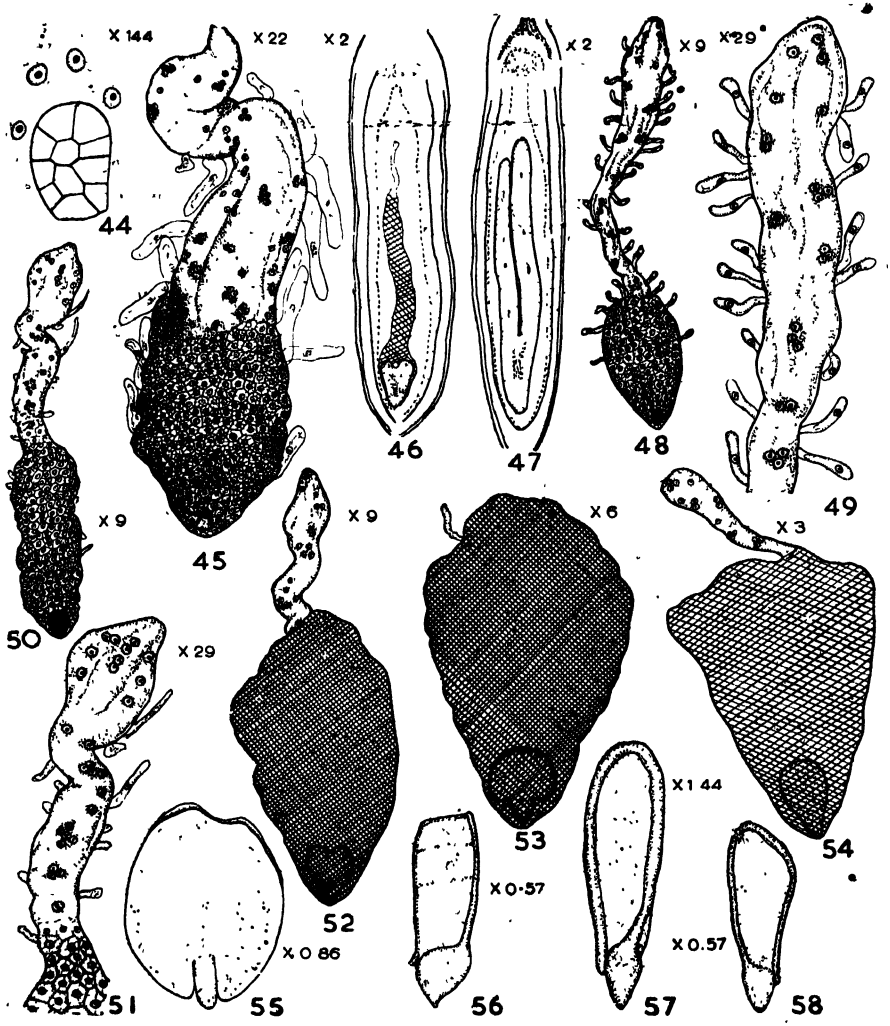
The development of the powdery substance was followed in the Tasmanian species. The membraneous coverings of the funicular wing, like the outer integument of which they are part, are 2 cells thick. As the seed matures, the protoplasts of the outer layer round up and the cell walls become fragile. The cells separate out easily forming the powdery pellicle. The cells of the inner layer become thick-walled and empty and appear like lattice and contribute to the firmness of the seed wing (Figs. 60, 61).

The outer integument remains 2-layered in the seed. The cells of the outer layer become somewhat papillate and stain deeply due to the presence of tannin. The cell walls of the inner layer become slightly thickened. The inner integument becomes 6 to 7 layered in the developing seed but only the

two innermost layers persist in the mature seed (Figs. 62, 63). The seed coats are therefore flimsy.

DISCUSSION

The apparently simple racemes of *Lomatia* as also those of other Grevilloideae are in reality condensed panicles in which the lateral branches are reduced and represented by pairs of flowers in bract axils. Similar reduction



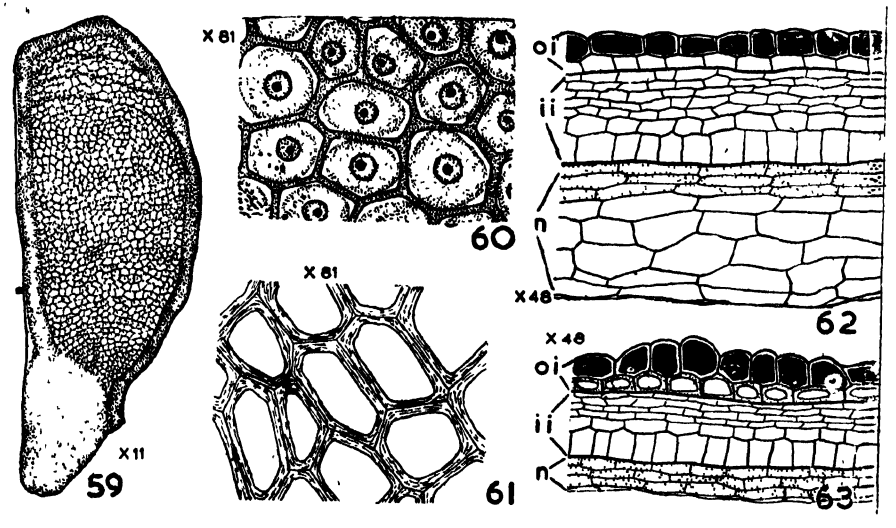
FIGS. 44-58 — Figs. 44-47. *L. polymorpha*: Fig. 44. Young embryo with some endosperm. Fig. 45. Endosperm and embryo dissected out from young seed. Figs. 46, 47. L. s. developing and mature seeds. Figs. 48-53. Stages in the development of endosperm of *L. tinctoria*. Fig. 54. Endosperm of *L. ferruginea* dissected from young seed. Fig. 55. Mature embryo of *L. hirsuta*. Fig. 56. Seed of *L. hirsuta*. Fig. 57. Seed of *L. dentata*. Fig. 58. Seed of *L. ferruginea*

of the lateral branches of a paniculate inflorescence is noticed in other angiospermous families. In the Amarantaceae reduction has resulted in the persistence of a single flower (Bakshi & Chhajlani, 1955). In the Palmae it led to the retention of three flowers at each node, the median being female and the laterals, male (Venkata Rao, 1958).

There is some controversy regarding the nature of the proteaceous flower, as to whether it is primitively monochlamydeous or monochlamydeous by reduction. The interpretation rests on the nature of the nectary. Haber (1959) regarded the nectary as reduced corolla and thought that the glandless flowers in some genera are monochlamydeous by reduction. The author, however, feels that the position of the annular or tubular nectary inner to the tepal stamen whorl (e. g., *Brabeium*, *Macadamia*) rules out the possibility of the nectary being homologous to the corolla (*see* also Venkata Rao, 1960).

Several genera of the sub-family Grevilloideae are characterized by winged seeds. A comparative study of the various genera shows that the seed wings are diverse in method of formation and structure. In *Grevillea* it is a membraneous outgrowth covering the body of the seed uniformly all round. In *Orites* and *Hakea* it is an outgrowth on the chalazal side into which the funicular vascular bundle does not extend. In *Stenocarpus* it is an outgrowth on the micropylar side. Only in *Telopea* and *Lomatia* (which belong to the tribe Telopeae), it is funicular. In these genera the funicle is not only elaborated but specialized to form the seed wing. In other genera the seed wing is membraneous; in the two above named genera it is stiff being made of thick-walled cells.

The Australian and Chilean species of *Lomatia* show close similarity in morphological and anatomical structure of the flower, development and



FIGS. 59-63 — (*oi*, outer integument; *ii*, inner integument; *n*, nucellus): Figs. 59-61. L. *tinctoria*: Fig. 59. Young seed. Fig. 60. Surface view of the young seed-wing. Fig. 61. Surface view of the wing of mature seed. Figs. 62, 63. *L. polymorpha*. Seed coats from developing and mature seeds

structure of the pollen, ovule, endosperm and embryo. The development and structure of the seed wing and even the powdery pellicle between the seeds are also quite similar.

The different species of *Lomatia* are disjunctly distributed on widely separated land masses. There are three possibilities which can account for such a distribution: (i) an independent (polyphyletic) origin of the different species on separate land masses; (ii) long range dispersal by birds or oceanic currents; and (iii) the origin and spread of the genus on a connected land mass which subsequently fragmented.

Regarding the first possibility it is difficult to imagine that the different species which show such a great uniformity in morphological, anatomical and embryological features could have evolved independently. Regarding the second, the winged seeds are adapted only for wind dispersal which necessitates continuous land. The possibility of long range dispersal by birds can be ruled out because the seeds are large and devoid of outgrowths which can make them attach to birds. Oceanic dispersal seems improbable since the seed coats are flimsy and the seeds lose their viability on being immersed in sea water even for a short time. Moreover, the greater concentration of species in Australia shows that this was the original home of the genus wherefrom dispersal and migration could have taken place. But oceanic currents in southern latitudes run from America towards Australia.

So it seems probable that the genus originated on a connected land mass which later fragmented. Such a continuous land mass (Pangaea or Gondwanaland) is visualized by Geologists. The existence of three other genera of the Proteaceae viz. *Gevuina*, *Oreocallis* and *Orites* and other angiospermous genera like *Drymis* and *Nothofagus* common between Australia and South America gives strong support to such a view. It is interesting to notice that fossils of *Lomatia*, *Drymis* and *Nothofagus* were found on Grahamland (Antarctica). This shows that the continent was habitable to plants in the past. Hill (1929) also believed that it served as a migratory route for circumpolar species.

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