EMBRYOLOGY OF THE GENUS *PSIDIUM*

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THE genus *Psidium* belonging to the family Myrtaceæ, probably represents a very ancient group of plants. *Psidium guajava* L., a native of tropical America, is almost naturalized in many parts of India and is chiefly cultivated for its fruits. In Assam, the leaves and barks of the plant are employed in dyeing and in Bengal and Uttar Pradesh, for tanning. The wood of *Psidium* is close-grained and takes on a beautiful polish.

Psidium cujavillus Brum. resembles *P. guajava* and is known more recently as another form of the latter (*Psidium cujavillus* Brum. f. Ind. 114 = *P. guajava* L., *Ref. Index Kewensis* Pt. II, 1946; p. 640). However, there is a marked difference in size of the leaves and fruits between the two species. The leaves of this plant are small and measure 1·4″ × 0·5″ while those of *P. guajava* are 5″ × 2″. The fruits are also smaller. *P. cujavillus* flowers twice a year, once in the month of April and then in the month of September under local climate.

Although several members of the family are well represented in the tropics and in European conservatories, their embryology have not been investigated extensively. Moreover, the family has an added interest in that some of the species show polyembryony (Tiwary, 1926; Pijl, 1934; Johnson, 1936). Investigations were, therefore, undertaken in order to explore the nature of embryo development and the extent of polyembryony if any, present in the genus *Psidium*.

MATERIAL AND METHODS

Materials of *Psidium guajava* L. were collected from an orchard in Allahabad and that of *P. cujavillus* Brum. from the botanical gardens of Banaras Hindu University, Varanasi, in the years 1952 and 1955 respectively by one of us (S. K. R.). Fixations were done between 9 and 11 a.m. in the fields in formalin-acetic-alcohol after trimming off the ovary wall so as to facilitate penetration of the fixing fluid. The customary procedures of infiltration and imbedding were followed. Sections were cut at 10–20 μ thick depending upon the stage of development. The slides were stained both in Haidenhain's iron-alum hæmatoxylin and a combination of safranin and fast green.

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Observations

Ovary and ovules.—The ovary is inferior and four to five-celled and contains an indefinite number of ovules borne on projecting axile placentæ. Embedded in the superficial layers of the thick ovary wall are numerous lysigenous oil glands. "Grit" cells and druses are also found scattered irregularly in the pericarp.

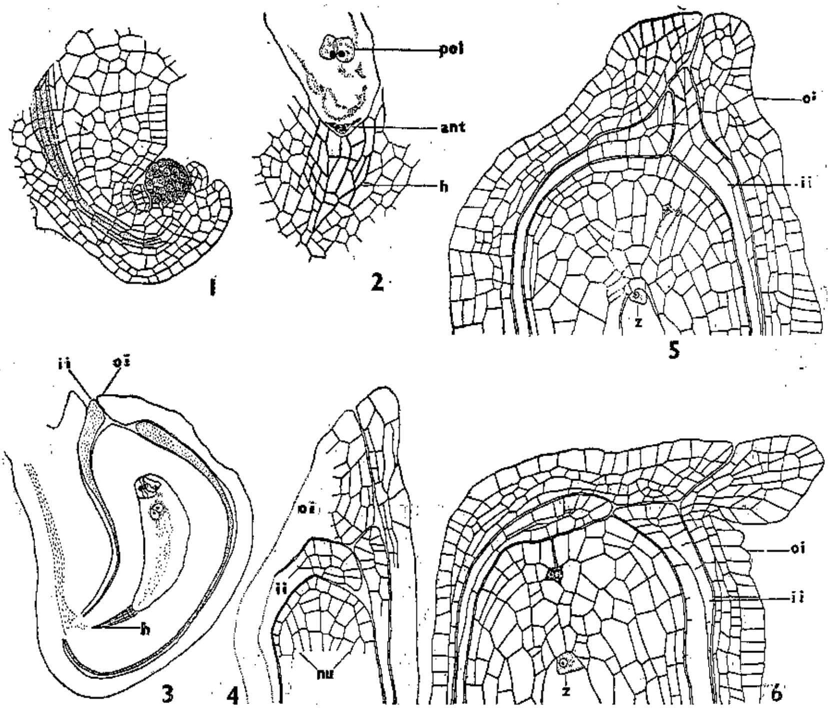
The ovules are anatropous and bitegminal with a conspicuous bend at the chalazal region towards the raphe (Text-Figs. 1, 3, 34). Owing to compression by mutual pressure of the large number of ovules some remain "hemi-anatropous" as in *Psidium cujavillus* (Text-Figs. 35, 36).

The ovule originates as a nucellar primordium which soon grows in size. The inner integument is differentiated first followed closely by the outer one when the ovule begins to curve (Text-Fig. 1). The two integuments overtop the nucellus and together form the micropyle which may be somewhat zig-zag (Text-Figs. 4–6, 35, 36). In *Psidium guajava* the inner integument does not cover the entire nucellus but stops short at some distance away from the summit of the nucellus. On the funicular side, however, it grows normally and together with the portion of the outer integument from the opposite side, forms the micropyle (Text-Fig. 3). Occasionally the inner integument alone forms the micropyle, as in *P. cujavillus* (Text-Fig. 37). Of the two integuments the inner is always two-layered and thin-walled except at its tip where it may be more-layered (Text-Figs. 4–6, 34–36). The outer integument may vary in thickness. The nucellus which is massive develops a hypostase when the ovule is ripe (Text-Figs. 2 *h*, 3 *h*). Between the hypostase and the lower boundary of the nucellus occur several layers of thin-walled cells.

The vascular supply normally terminates at the chalazal region, but in exceptional cases it extends to the base of the hypostase as in *Psidium cujavillus* (Text-Fig. 42).

Megasporogenesis and female gametophyte.—The archesporial cell differentiates in the third or fourth layer of the nucellus and directly functions as the megaspore mother cell (Text-Fig. 7). It enlarges and becomes deep-seated by continued divisions of the wall cells. The first division results in the formation of two dyad cells of unequal size, of which the micropylar one may degenerate before the second division is completed (Text-Fig. 8) or both the dyad cells may undergo the normal second meiotic division (Text-Fig. 9). Thus a linear row of three or sometimes four cells may result (Text-Fig. 10). The chalazal cell alone is functional (Text-Fig. 11). It enlarges, becomes densely protoplasmic and begins to divide. The embryo-sac passes through the usual two-, four-, and eight-nucleate stages (Text-Figs. 12–14). The free nuclei organize themselves into a normal female gametophyte. The mature embryo-sac shows two pear-shaped synergids, an egg cell, two polar nuclei and three antipodal cells (Text-Figs. 15, 38). The synergids may or may not possess well-defined hooks. Abundant starch grains are present in the embryo-sac at the time of fertilization or soon after (Text-Figs. 17, 19). By the time the zygote divides, they are however depleted.

Abnormal embryo-sacs were also observed. In one instance in *Psidium guajava*, the antipodal cells closely resembled an egg apparatus while the latter simulated the antipodals; the polar nuclei lay wide apart Text-Fig. 16). Another embryo-sac showed what appeared to be two



TEXT-FIGS. 1–6. *Psidium guajava* (*ant*, antipodal; *h*, hypostase; *ii*, inner integument; *oi*, outer integument; *nu*, nucellus; *pol*, polar nuclei). Fig. 1. L.s. young ovule showing initiation of integument. Fig. 2. Portion of chalazal part of ovule showing hypostase (*h*), degenerating antipodals (*ant*) and the polar nuclei (*pol*). Figs. 3, 4. L.s. ovules showing anomalous formation of micropyle; note the prominent hypostase below embryo-sac in Fig. 3. Figs. 5, 6. Zig-zag micropyle as seen in longisections. Figs. 1, 4–6, × 52. Fig. 2, × 72. Fig. 3, × 31.

pairs of polar nuclei besides the normal antipodal cells and an egg apparatus (Text-Fig. 17). In another instance three polar nuclei and two unorganized small nuclei were present at the micropylar end. The antipodal cells in such embryo-sacs were ephemeral (Text-Fig. 39).

Fertilization.—Fertilization is porogamous. The pollen tube is often seen to discharge X-bodies inside the embryo-sac (Text-Fig. 19). Syngamy and triple fusion occur simultaneously (Text-Fig. 18). The two polar nuclei do not fuse to form the secondary nucleus prior to fertilization. Following discharge of the sperms into the embryo-sac, fertilization of one of the polars occurs (Text-Fig. 18) before triple fusion is completed. The zygote undergoes a period of rest during which two nucleoli of unequal sizes are observed within the nucleus of the zygote indicating syngamy (Text-Figs. 18, 20).

Endosperm.—The primary endosperm nucleus divides earlier than the zygote (Text-Figs. 19, 20). The free nuclei arrange themselves round a central vacuole (Text-Fig. 43). Accumulation of endosperm nuclei imbedded in a dense mass of cytoplasm at the chalazal end also occurs. The nuclei increase in number and may undergo repeated fusion among themselves to form large nuclei containing several nucleoli. Cell-wall formation starts from the micropylar end gradually proceeding downwards.

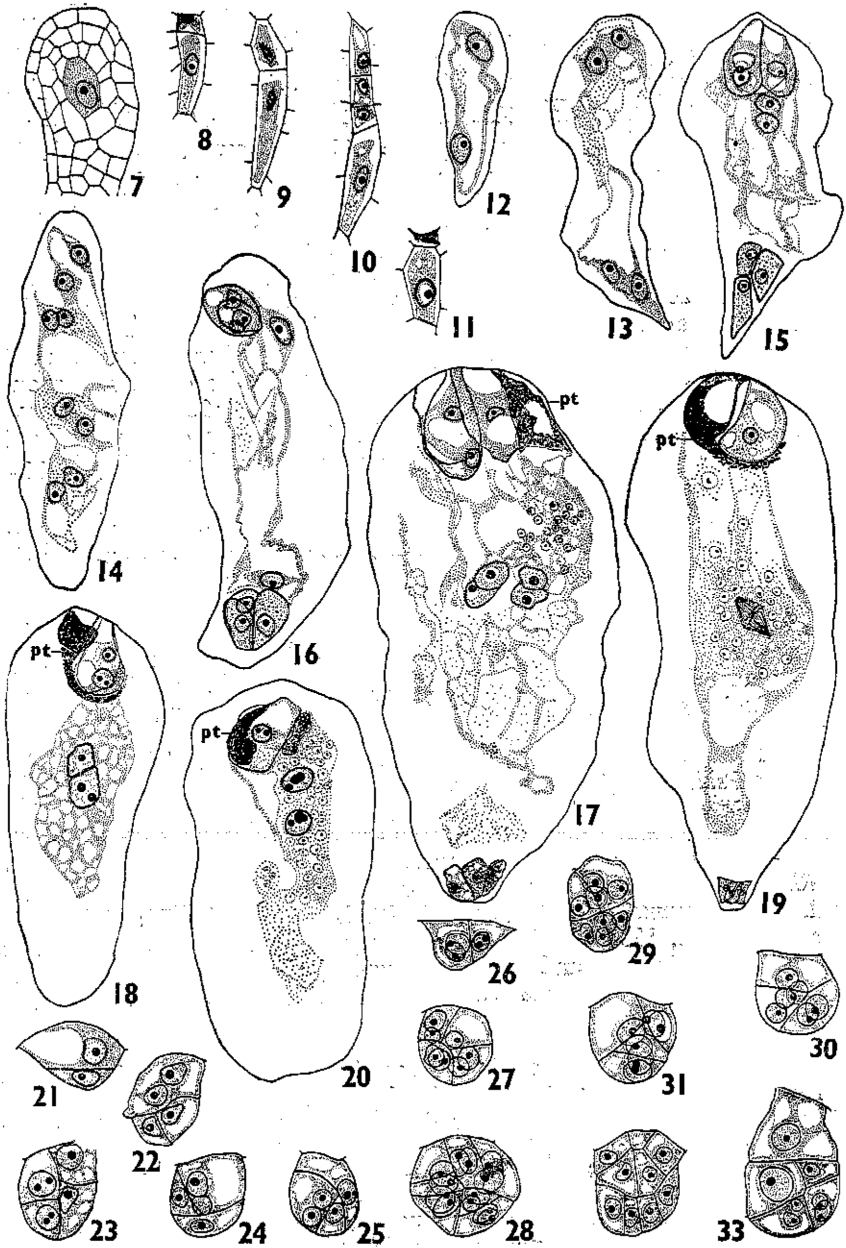
Embryo.—In *Psidium* the sequence of division of the cells in the initial stages of embryogeny is extremely irregular. The first division of the zygote is more often transverse (Text-Fig. 21) than longitudinal (Text-Fig. 26). The resultant cells again divide at right angles to the first plane of division or parallel to it. Cell divisions beyond the octant stage were difficult to follow. Text-Figures 22 to 25 show variations of cell divisions during development of the proembryo. Rarely the arrangement of the cells may be more or less regular (Text-Fig. 31). But due to divisions occurring in all planes, the eight-celled proembryo shows an irregular arrangement of its cells (Text-Figs. 27–30). Text-Figures 32 and 33 show young globular embryos in which no definite arrangement of cells nor a well-defined suspensor can be made out. However *P. cujavillus* shows a more regular arrangement of the cells in the embryo than *P. guajava* (Text-Fig. 41); and a minute suspensor may be organized by which the embryo remains attached to the micropylar part of the embryo-sac (Text-Figs. 40, 41).

In none of the hundreds of ovules examined was polyembryony ever noticed. Germinated seeds also never showed more than one seedling per seed. It appears therefore, that in the common species of guava, polyembryony does not occur nor in the species, *Psidium cujavillus.*

Discussion

Variations in arrangement of the ovules within the ovary point towards a close similarity between members of the Myrtaceæ and Onagraceæ (Johansen, 1929). The ovules of Myrtaceæ are anatropous but owing to their crowded condition may lie with their micropyle facing upwards. Such "orthotropous" or "hemi-anatropous" ovules are met with in *Psidium cujavillus.* A casual occurrence of orthotropous ovules finds its parallel in *Nesaea* of the Lythraceæ (Joshi and Venkateswarlu, 1936) and *Sonneratia* of the Sonneratiaceæ (Venkateswarlu, 1937).

The ovules are bitegminal and the micropyle is zig-zag, a feature also characteristic of the ovules of Lythraceæ (Joshi and Venkateswarlu, 1935*a*; 1935 *b*) and Melastomaceæ (Subramanyam, 1942). In *Psidium guajava* the inner integument occasionally fails to overtop the nucellus. The rim of the outer integument in such cases develops close to the inner one to form the narrow micropyle. Mauritzon (1939) has shown more or less



TEXT-FIGS. 7-33

TEXT-FIGS. 7–33. *Psidium guajava* (*pt*, pollen tube). Figs. 7–15. Stages in development of female gametophyte. Fig. 16. Abnormal embryo-sac; the antipodals simulate an egg apparatus. Fig. 17. Embryo-sac showing two pairs of polar nuclei and starch grains. Fig. 18. Syngamy and triple fusion. Fig. 19. Division of primary endosperm nucleus; observe X-bodies in the vicinity of the egg cell. Fig. 20. Embryo-sac showing zygote and two endosperm nuclei formed by division of primary endosperm nucleus. Fig. 21. Two-celled proembryo. Figs. 22–26. Four-celled proembryos. Figs. 27–33. Proembryos formed by irregular planes of division. All, ×145.

similar deviations in *Myrceugenia apiculata* and *Orthostemon sellowianus*, both of the Myrtaceæ. A general agreement in structure of the ovule in the plants under investigation with those of Lythraceæ, Melastomaceæ, Rhizophoraceæ, Onagraceæ and Combretaceæ has been observed. However, a marked difference between the nucelli of Myrtaceæ and Combretaceæ occurs. In Combretaceæ the nucellar epidermis at the upper part divides tangentially forming a "nucellar cap", a structure not present in Myrtaceæ. Tiwary (1926) and Pijl (1934) have, however, used the term in their investigations on *Eugenia*, obviously meaning the micropylar portion of the nucellus, an usage which is rather inappropriate.

In *Psidium*, the hypostase in later stages projects into the embryo-sac to form a "postament," as observed in *Cuphea lanceolata* (Mauritzon, 1934).

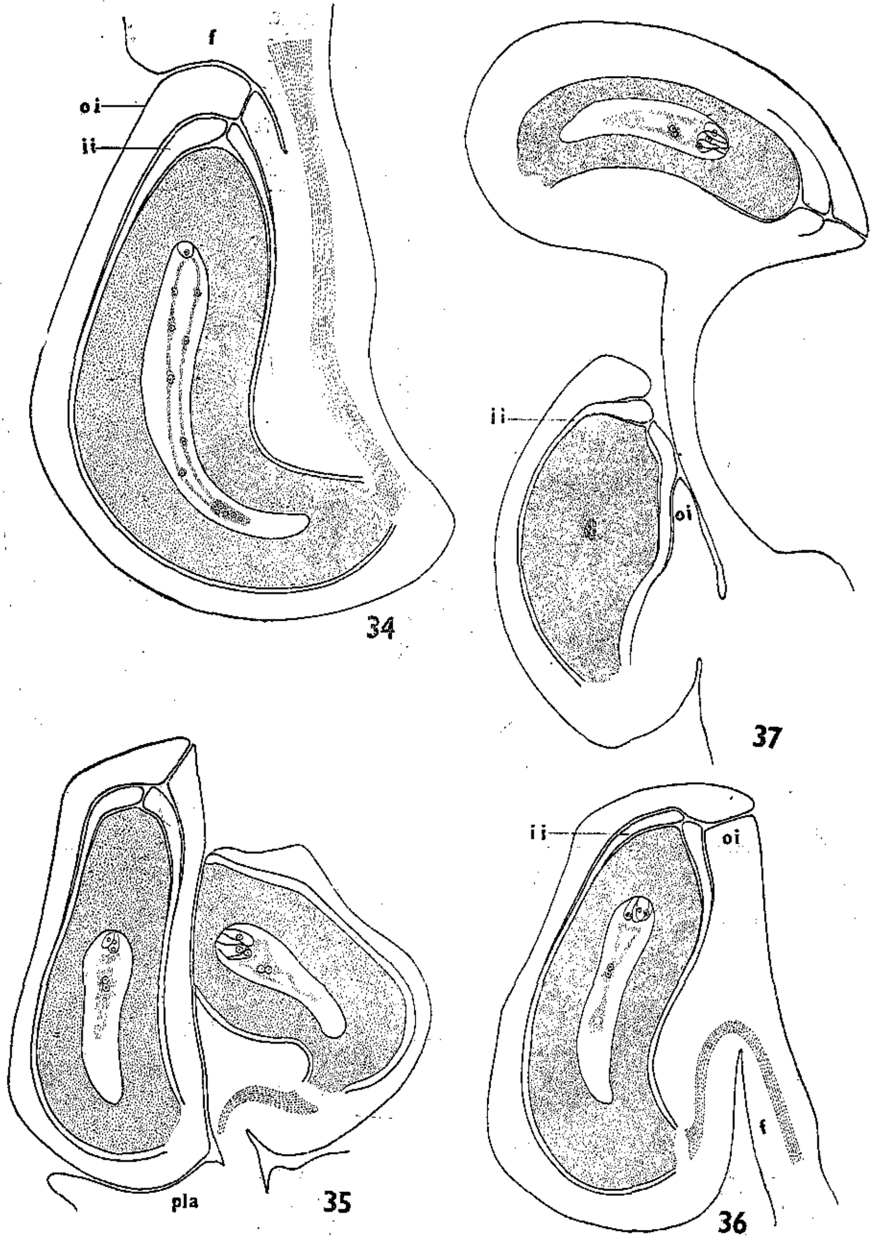
An unusual feature in the genus is the differentiation of the archesporial cell in the fourth layer below the nucellar epidermis and which functions directly as the megaspore mother cell. King (1947) working on *Punica granatum* has reported a similar development.

Meiosis in the megaspore mother cell is normal. The upper dyad cell in *Psidium guajava* may show a belated division as observed in *Cuphea* (Mauritzon, 1934). The chalazal megaspore always functions.

In structure the egg apparatus is typical and resembles those of *Rhizophora* (Cook, 1907; Mauritzon, 1939), *Peplis* (Mauritzon, 1934), *Lawsonia*, *Ammannia*, *Lagerstroemia* and *Woodfordia* (Joshi and Venkateswarlu, 1935, 1936), *Orthostemon*, *Decaspermum*, *Melaleuca*, *Callistemon*, *Tristania*, *Kunzea* and *Leptospermum* (Mauritzon, 1939), *Leandra*, *Osbeckia* and *Melastoma* (Subramanyam, 1942, 1948), *Punica* (King, 1947) and others.

Mature embryo-sacs of *Psidium* show abundant starch grains as reported also in *Lawsonia inermis* (Joshi and Venkateswarlu, 1935). Shortly after fertilization the contents diminish and by the time the zygote divides, they are absent. X-bodies similar to that reported in *Sonneratia* (Venkateswarlu, 1937) were encountered in *Psidium guajava*.

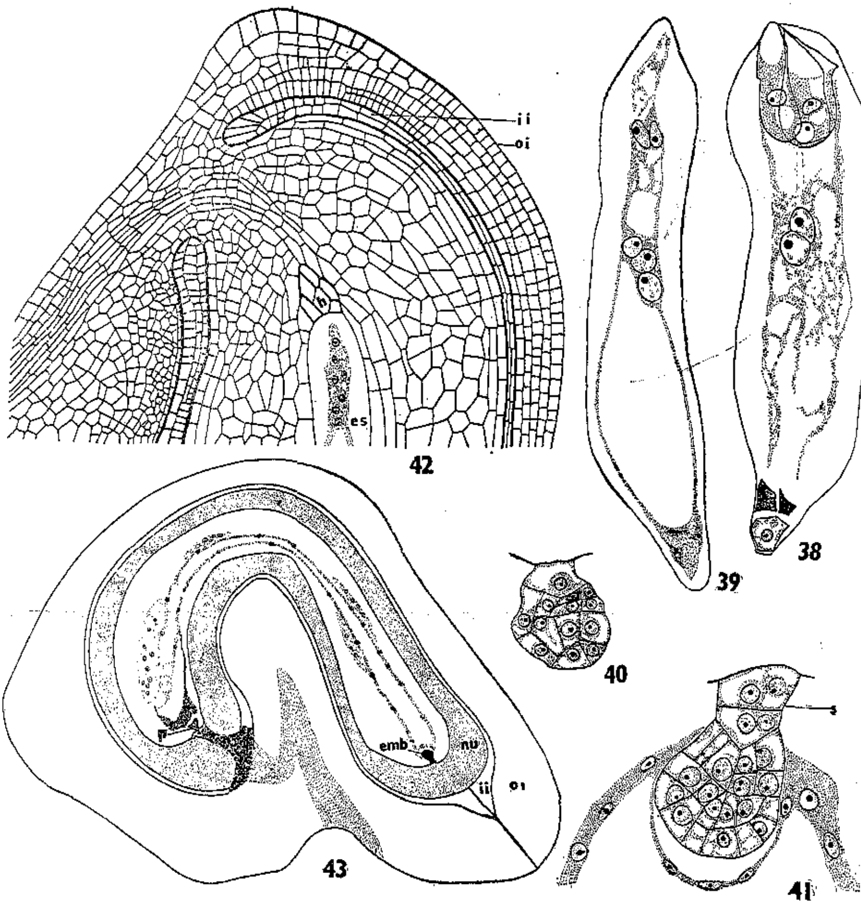
A few abnormalities in the structure of the mature embryo-sac like, reversed polarity, supernumerary polar nuclei and unorganized nuclei in the embryo-sac have been observed. Such abnormalities have been noted in the allied plants like, *Woodfordia* (Joshi and Venkateswarlu, 1935), *Zauschneria latifolia* (Johansen, 1931) and others.



TEXT-FIGS. 34–37. *Psidium cujavillus* (*f*, funicle; *ii*, inner integument; *oi*, outer integument; *pla*, placenta). Fig. 34. L.s. normal anatropous ovule. Figs. 35, 36. L.s. ovules showing 'hemi-anatropous' condition. Fig. 37. L.s. ovules, one showing a short funicle and the other, a long one; lower ovule appears sterile. **All, ×42.**

The endosperm is free nuclear to begin with, later becoming cellular. Soon after the formation of a large number of nuclei, some of them accumulate at the chalazal part of the embryo-sac, the rest forming a peripheral layer around a central vacuole in the embryo-sac as noted by Mauritzon (1939) in *Kunzea*, *Melaleuca* and *Callistemon*.

Embryogeny of *Psidium* differs markedly from that of *Myrtus* (Souèges, 1940), another member of the Myrtaceæ. While in *Psidium*



TEXT-FIGS. 38–43. *Psidium cujavillus* (*ii*, inner integument; *oi*, outer integument; *emb*, embryo; nu, nuclellus; *p*, 'Postament'; *s*, suspensor; *h*, hypostase; *es*, embryo-sac). Fig. 38. Mature embryo-sac. Fig. 39. Abnormal embryo-sac showing no organization of nuclei. Fig. 40. Young globular embryo. Fig. 41. Young embryo surrounded by free nuclear endosperm; a short suspensor (*s*) may be seen. Fig. 42. Chalazal part of ovule showing elongated conducting strand near the hypostase (*h*). Fig. 43. L.s. mature ovule showing curved embryo-sac and distinct 'Postament' (*p*). Figs. 38, 39. ×138. Figs. 40, 41, ×113. Fig. 42, ×29. Fig. 43, ×7.

it is extremely irregular from the very beginning, that of *Myrtus* follows the pattern of Ranunculaceæ, *i.e.*, the two juxtaposed cells of the four-celled proembryo are derived from the terminal cell and the two superposed lower cells from the basal cell of the two-celled proembryo. The juxtaposed cells by subsequent divisions give rise to the quadrant and octant stages. The middle cell represents the hypophysis and the lowest cell divides transversely to form a short suspensor (*see* Johansen, 1950). Unlike *Eugenia* (Tiwary, 1926; Pijl, 1934) *Psidium* does not show any trace of adventive embryony.

During development of the seed coat the inner integument is completely crushed out. The outer integument becomes many-layered by the tangential division of its cells. A few inner layers of the outer integument are flattened out and the cells of the remaining layers undergo lignification. These thick-walled cells constitute the hard testa. The family Lythraceæ which otherwise shows a close resemblance to the Myrtaceæ differs from the latter in the structure of the seed coat wherein both the integuments take part.

SUMMARY

The investigation deals with the embryology of two common species of guava, *viz.*, *Psidium guajava* and *P. cujavillus.*

Ovules are variously disposed in the ovary; it is generally anatropous save in a few cases of *Psidium cujavillus* where they are more or less "orthotropous" or "hemi-anatropous".

The ovules are bitegminal and crassinucellar; the micropyle may assume a zig-zag outline and is formed by both the integuments except in a few.

The vascular supply normally terminates at the chalazal end of the ovule; but in *Psidium cujavillus* it may sometimes traverse the nucellus and extend to the base of the hypostase.

The ovule when ripe develops a hypostase below the mature embryo-sac. The embryo-sac continues its downward growth around the hypostase which appears to project into the former forming the "Postament".

The archesporium differentiates in the fourth layer of the nucellus from top and functions directly as the megaspore mother cell. It becomes deep-seated owing to the formation of a large number of cover cells. Meiosis is normal and a linear row of triad or tetrad megaspores is formed of which the chalazal one functions.

The development of the embryo-sac is of the *Polygonum* type.

Abnormalities in the structure of the mature embryo-sac, namely, reversed polarity, supernumerary polar nuclei, unorganized embryo-sacs, etc., have been observed.

Fertilization is porogamous. Syngamy occurs normally. During triple fusion the male cell fuses with one of the polar nuclei before fusion with the second polar nucleus.

Starch grains are abundant in the ripe embryo-sac but soon after fertilization, are depleted.

The endosperm is free nuclear to begin with but becomes cellular later. A chalazal accumulation of endosperm nuclei is commonly observed in early stages. The free nuclei usually show random fusion amongst themselves and thus form giant nuclei containing several nucleoli.

Embryogeny is very irregular from the beginning and the sequence of cell divisions cannot be followed with accuracy. A suspensor is absent in *Psidium guajava* while in *P. cujavillus* a minute one may be organized. Polyembryony is completely absent in the genus.

The testa is very hard and formed by the lignification of the cells of the outer integument.

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REFERENCES

COOK, M. T. 1907. The embryology of *Rhizophora Mangle*. *Bull. Torrey bot. Cl.* **34**: 271–77.

JOHANSEN, D. A. 1929. Studies on the morphology of the Onagraceæ. I. The megagametophyte of *Hartmannia tetraptera*. *Ibid.* **56**: 285–98.

———. 1931. Studies on the morphology of Onagraceæ. V. *Zauschneria latifolia*. *Ann. N.Y. Acad. Sci.* 35: 1–26.

———. 1950. *Plant Embryology*. Chronica Botanica Co. Waltham, Mass., U.S.A.

JOHNSON, A. M. 1936. Polyembryony in *Eugenia Hookeri*. *Amer. J. Bot.* **23**: 83–85.

JOSHI, A. C. AND VENKATESWARLU, J. 1935. A case of reversed polarity in the embryo-sac. *Ann. Bot., Lond.* **49**: 841- 3.

———. 1935 *a*. Embryological studies in the Lythraceæ. I. *Lawsonia inermis* Linn. *Proc. Indian Acad. Sci.* **2 B**: 481–93.

———. 1935 *b*. Embryological studies in the Lythraceæ. II. *Lagerstroemia* Linn. *Ibid.* **2 B**: 523–34.

———. 1936. Embryological studies in the Lythraceæ. III. *Ibid.* **3 B**: 377–400.

KING, J. R. 1947. Development of ovule and megagametophyte in pomegranate. *Bot. Gaz.* **108**: 394–98.

MAURITZON, J. 1934. Zur embryologie einiger Lythraceen. *Acta Hort. gothoburg* **9**: 1–21.

MAURITZON, J. 1939. Contributions to the embryology of the orders Rosales and Myrtales. *Acta Univ. Lund.* **2**: 1–120.

PIJL, L. v. d. 1934. Über die Polyembryonie bei *Eugenia*. *Rec. Trav. bot. nêerl.* **31**: 113–87.

SUBRAMANYAM, K. 1942. Gametogenesis and embryogeny in a few members of the Melastomaceæ. *J. Indian bot. Soc.* **21**: 69–85.

———. 1948. An embryological study of *Melastoma malabaricum* L. *Ibid.* **27**: 11–19.

SOUÈGÈS, R. 1940. Embryogènie dès Myrtaceés. Dèveloppement de l'embryon chez le *Myrtus communis* L. *C.R. Acad. Sci. Paris* **210**: 548–50.

TIWARY, N. K. 1926. On the occurrence of polyembryony in the genus *Eugenia*. *J. Indian bot. Soc.* **5**: 125–36.

VENKATESWARLU, J. 1937. A contribution to the embryology of Sonneratiaceæ. *Proc. Indian Acad. Sci.* **5 B**: 206–23.