EMBRYOLOGICAL INVESTIGATIONS ON *BIXA ORELLANA* LINN.*

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Bixa orellana alone constitutes the family Bixaceae. The endosperm is of the Nuclear type. Cell formation starts from the micropylar end. The reserve food in the endosperm is starch. The embryogeny does not conform to any set type. After fertilization there is incurving of integuments in the chalazal region. The outermost layer of the inner integument forms the palisade-like layer and the cells of the inner hypodermal layer become hourglass-shaped. In a mature seed the outer integument is soft and full of dye cells, whereas the inner integument is multilayered and hard. The available embryological data show that Bixaceae have affinities with Cistaceae and Cochlospermaceae and almost none with Flacourtiaceae, Frankeniaceae, Tamaricaceae and Tiliaceae.

INTRODUCTION

Bixa orellana Linn. is a monotypic genus of the family Bixaceae. In India *Bixa* is grown mostly in the South as an ornamental and a hedge plant. Its mature seeds yield a red dye known as '*Arnotto*', which was previously used for colouring cotton and silk. Since the dye is not fast, it is at present employed for colouring edible materials, ointments, polishes, hair oils, etc.

The family Bixaceae has been put in the order Parietales by some taxonomists (Lawrence 1951; Rendle 1952); others assign it to the order Bixales (Johansen 1950; Hutchinson 1959) or to the Violales (Engler 1964). Johansen employed embryological characters, while Hutchinson based his grouping mainly on external morphology. Engler (1964) felt that if the Guttiferales are removed from the Parietales, the remaining taxon, i.e. Violales, forms quite a homogeneous and natural group.

This investigation was undertaken because there is no detailed work on the embryology of Bixaceae. On the basis of the present findings, a comparison of the Bixaceae with the allied families has been made.

MATERIAL AND METHODS

The material of *Bixa* was collected from the Indian Agricultural Research Institute, New Delhi, during October–February, 1964–65. Formalin-acetic-

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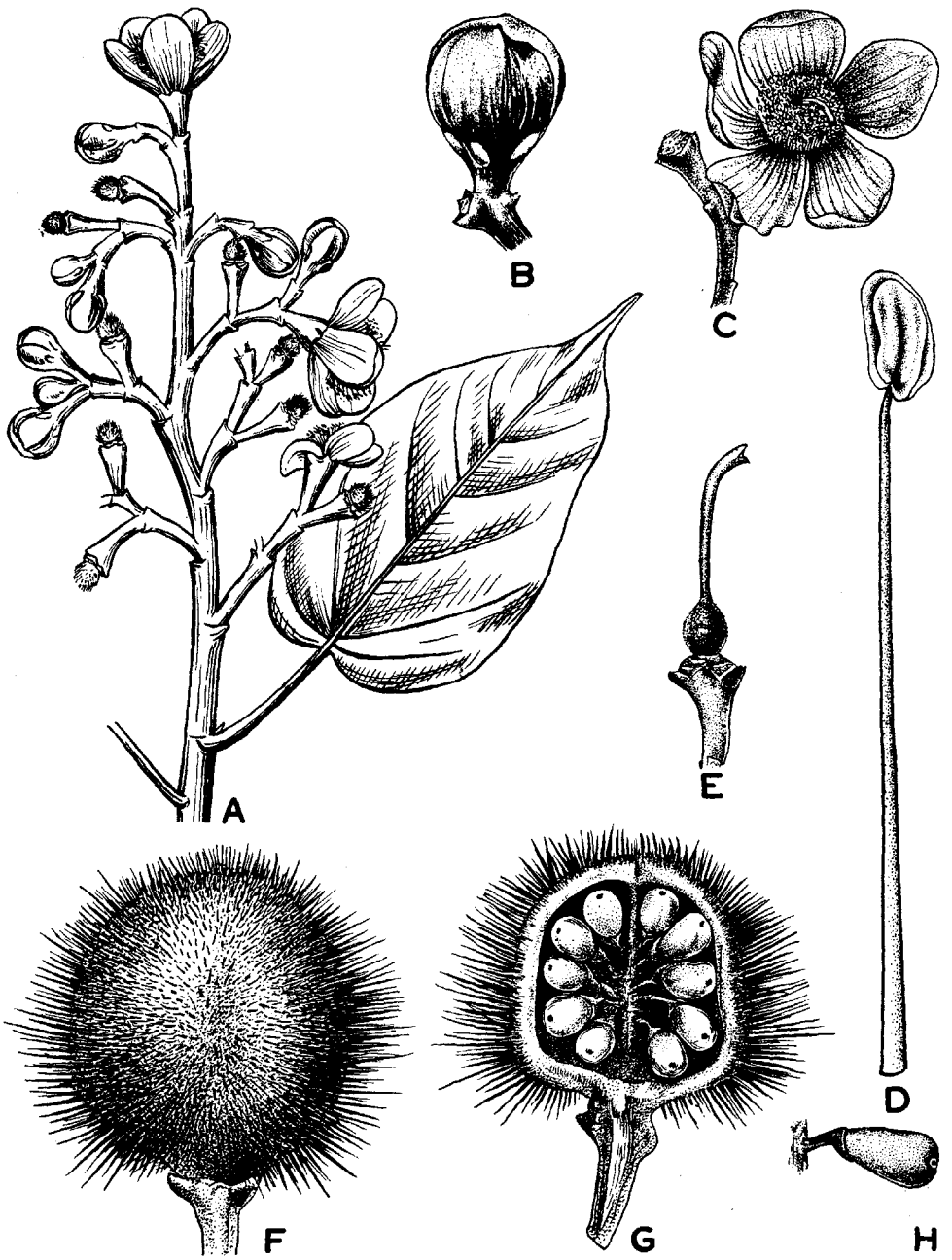

FIG. 1 A–H. *Bixa orellana*, floral morphology. A, twig with inflorescence. ×34. B, bud showing gland-like structures at the base of sepals. ×1·0. C, flower. ×3·4. D, stamen. ×5·9. E–F, young and mature fruits. ×68. G, one fruit valve showing the placenta bearing two rows of seeds. ×68. H, mature seed with a collar-like outgrowth at the micropylar end. The white, circular zone at the chalazal end represents thickened area. ×73.

alcohol was used for fixation and 70 per cent alcohol for preserving the material. Mature fruits were also fixed in Nawaschin's modified solution (Johansen 1940). Dehydration and embedding were done in the usual way. Sections were cut between 6 μ and 32 μ. Hard seeds were soaked in glycerine-alcohol mixture (30 cc of pure glycerine, 20 cc of 50 per cent alcohol), after embedding, for 15 days or so. Safranin-fast green combination was used for staining.

Observations

Floral morphology

Bixa orellana has two forms: one with white flowers and green capsules, and the other with pink flowers and brownish-red capsules. The following description is based only on the latter. The inflorescence is a panicle (Fig. 1 A). The buds are somewhat asymmetrical (Fig. 1 B). The flowers (Fig. 1 C) are bisexual, regular and hypogynous. There are usually five concave, distinct, imbricate sepals with gland-like structures at their base. The sepals fall off as soon as the bud opens. The five petals are obovate, distinct, imbricate and dotted (the dots representing the dye cells). The numerous free stamens develop centrifugally. The anthers are basifixed, dithecous and horseshoe-shaped (Fig. 1 D). The ventral arm of the anther (i.e. the one with filament) is shorter than the dorsal one. The filaments are long and extremely thin (Fig. 1 D). There is a single vascular trace to the anther (Fig. 2 A, B) which terminates in a small hook.

The gynoecium is bicarpellary and syncarpous. The ovary is unilocular with two parietal placentae bearing numerous ovules. The fruit is a loculicidal, echinate, two-valved, globose capsule. The soft and pointed outgrowths on the ovary wall enlarge and become stiff at maturity (Fig. 1 E–G). The number of ovules per ovary may be 50 or more, but all of them do not develop further. The mature seeds show a cap-like, funicular outgrowth (Fig. 1 H) and are covered with red dye.

Some abnormal anthers have also been observed (*see* Fig. 2 C–H).

Microsporangium, microsporogenesis and male gametophyte

Serial transections of anther reveal different configurations (Venkatesh 1956). In the middle the thecae of both the arms are united and hence eight microsporangia are seen. Most of the diagrams have been made from this region. A transection of a young anther (Fig. 3 A) shows a single hypodermal archesporial cell (Fig. 3 B) in each of the eight lobes. The archesporial cells undergo periclinal division. The layer of parietal cells gives rise to three or four wall layers, the innermost of which functions as the tapetum (Fig. 3 C, D). The sporogenous cells divide repeatedly and ultimately form the microspore mother cells (Fig. 3 E, F).

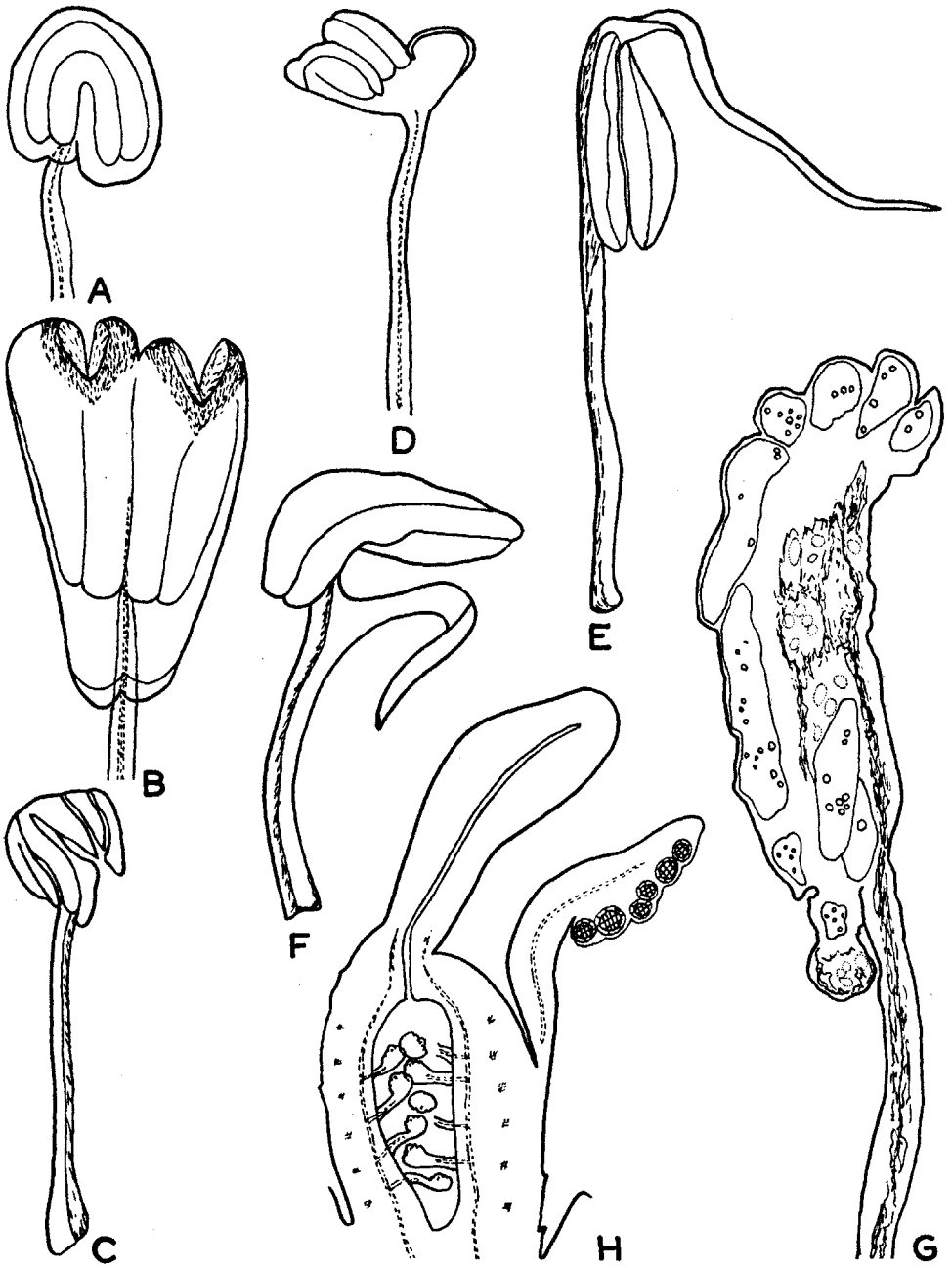

FIG. 2 A–H. *Bixa orellana*, normal and abnormal stamens. A–B, normal stamens; C–G, abnormal stamens. A, young stamen. × 17·4. B, dehisced anther in front view. Endothecial thickenings are present only around the slits. × 17·4. C–D, stamens with peculiarly bent thecae. × 4·4. E–F, thick and large stamens with a sterile appendage. × 4·4. G, l.s. stamen with several microsporangia. The vascular trace extends almost to its tip. × 17·4. H, l.s. carpel with a stamen arising from the ovary wall. × 13.

In a mature anther the epidermal cells become papillate (Fig. 3 I, J). Dehiscence takes place by two slits at the bend of the thecae and endothecial thickenings are confined around this area (Fig. 3 I, K). The thickenings develop as radial bands (Fig. 3 K) which are often fused towards the inner tangential walls of the cells (Fig. 3 J). The middle layers elongate (Fig. 3 G, H), get compressed and finally disappear at the time pollen grains are shed. When the sporogenous cells are multiplying the tapetum becomes 2-layered at places, but its cells are mostly uninucleate (Fig. 3 D, L). During meiotic divisions in the microspore mother cells the tapetal cells enlarge and become binucleate. Their inner tangential and radial walls get covered with 'Ubisch' granules (Fig. 3 H, L), and frequently the cytoplasm shows striations (Fig. 3 H). The tapetum is of the secretory type.

Mucilage appears between the cytoplasm and the cell wall of mother cells at the prophase. Several darkly stained bodies often appear in the cytoplasm. However, their number decreases in later stages (Fig. 3 M–Q). At metaphase II the spindles may be arranged side by side (Fig. 3 Q) or crosswise. At the end of meiosis II some nucleus-like structures are visible (Fig. 3 R, S), which are probably derived from laggard chromosomes (Fig. 3 O). Wall formation is the simultaneous type. Tetrads are mostly tetrahedral (Fig. 3 T), sometimes decussate. Young pollen grains are vacuolated (Fig. 3 U). The vegetative nucleus is often exceptionally large (Fig. 3 V). The pollen grains are tricolporate and are shed at the 2- or 3-celled stage. Pollen grains with as many as five nuclei have been observed (Fig. 3 W–Z).

Megasporangium

The ovules are bitegminal, anatropous and crassinucellar. As the ovular primordia elongate the integuments start differentiating. The outer integument overgrows the inner and both of them form the micropyle, which becomes narrow and zigzag. The derivatives of the nucellar epidermis form a cap, which remains distinct for some time.

Megasporogenesis and female gametophyte

Generally only one archesporial cell differentiates in an ovule (Fig. 4 A). The sporogenous cell enlarges and functions as the megaspore mother cell (Fig. 4 B). At times two or more sporogenous cells are present. They may lie one above the other, diagonally or side by side. In a twin of mother cells (Fig. 4 C) one may divide earlier or both may divide simultaneously to form twin tetrads (Fig. 4 F). The tetrads are usually linear, at times T-shaped. Generally it is the chalazal megaspore which functions (Fig. 4 E), but in some preparations the micropylar megaspore had also enlarged (Fig. 4 D, E), and in still others both the chalazal and the epichalazal megaspores had developed up to the 2-nucleate embryo sac stage (Fig. 4 G).

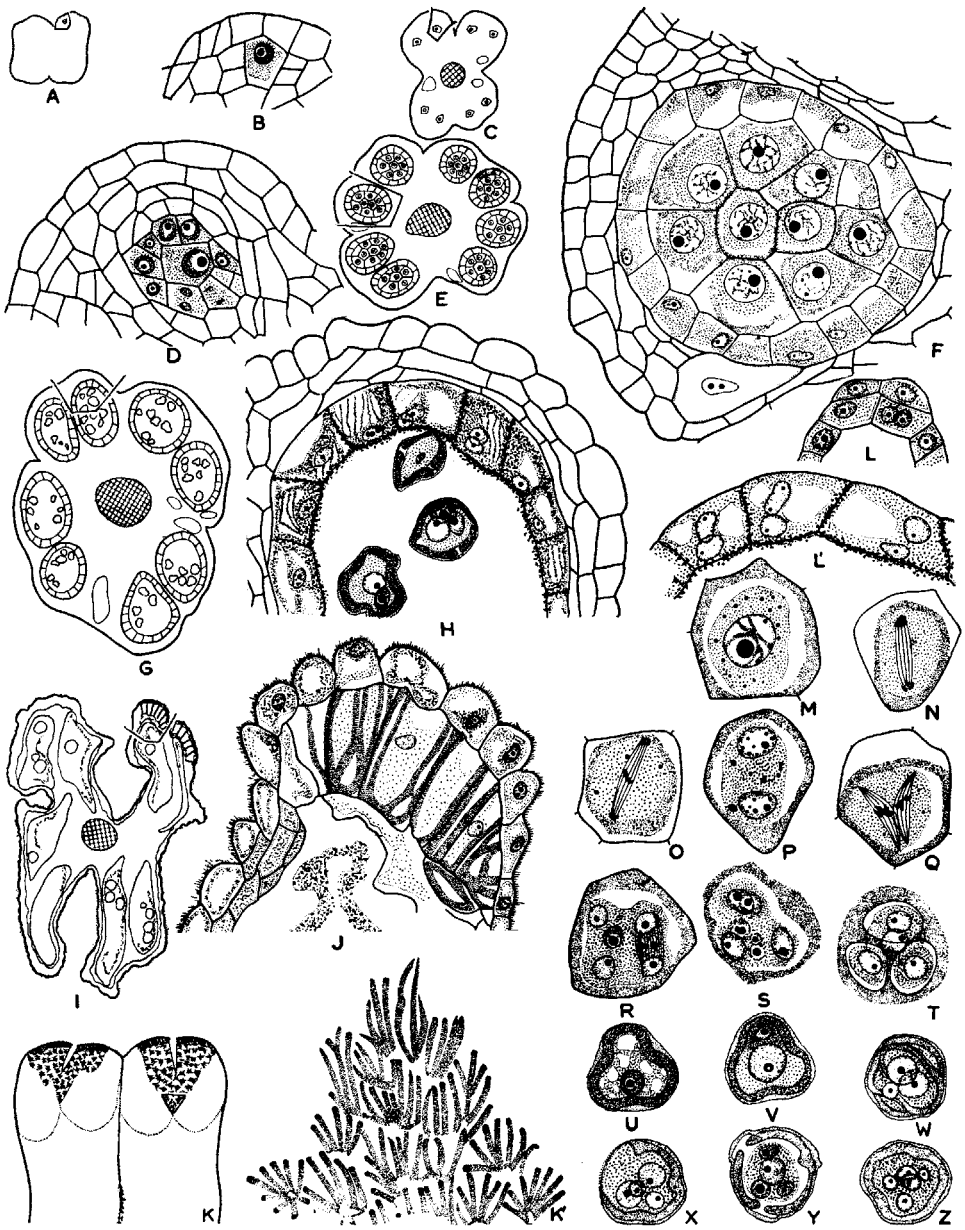

FIG. 3.

The nucleus of the functional megaspore divides thrice to form the Polygonum type of embryo sac (Fig. 4 H, I). Some embryo sacs with extra pairs of polar nuclei or extra cells at the micropylar end have also been observed. The embryo sac increases greatly in size after its organization (cf. Fig. 4 I, J). The egg may be apical or lateral in attachment, and contains a number of starch grains. The synergids are hooked, have basal vacuoles and often show filiform apparatus. One of the synergids collapses after fertilization, while the other enlarges and persists till the early proembryo stages. It resembles the zygote and its products, and can, therefore, be mistaken as a component of the proembryo. The antipodal cells usually degenerate before the entry of pollen tube. The polar nuclei come to lie in the middle of the embryo sac (Fig. 4 J).

Pollination and fertilization

At the time of pollination the two stigmatic flaps are wide open and copious liquid is secreted in which the pollen grains germinate. Fertilization is porogamous and syngamy is completed later than triple fusion. The pollen tubes persist till the massive nuclear endosperm stage.

Endosperm

The endosperm is of the Nuclear type (Fig. 5 A–D). The nuclei are at first evenly distributed in the peripheral cytoplasmic lining, but later more nuclei aggregate towards the micropylar end. The early nuclear divisions are usually synchronous, but afterwards they are not so (Fig. 5 E). In one preparation the endosperm showed considerable variation in nuclear size (Fig. 5 F).

After hundreds of nuclei are formed the cytoplasm becomes vacuolated and cell formation proceeds from the micropylar end downward (Fig. 5 G, H).

FIG. 3 A–Z. *Bixa orellana*, microsporangium, microsporogenesis and male gametophyte. A, outline of a young anther in cross-section. ×91. B, portion marked in A enlarged to show the archesporial cell. ×637. C, cross-section of slightly older anther. ×91. D, enlarged view of the portion marked in C to show the wall layers and differentiating tapetum. ×637. E, cross-section of anther at the microspore mother cell stage. ×75. F, microsporangium enlarged from E. ×487. G, cross-section of anther with young pollen grains. ×75. H, magnified view of the portion marked in G. The tapetal cells show cytoplasmic striations and 'Ubisch' granules. ×487. I, section of anther at the time of dehiscence. ×88·4. J, enlarged view of the portion marked in I. ×378. K, upper portion of a mature anther showing the extent of endothecial thickenings (diagrammatic). ×40·9. K′, portion marked in K enlarged to show the pattern of thickenings. ×378. L, portion of tapetum at the sporogenous cell stage. ×637. L′, binucleate tapetal cells at the young pollen grain stage. 'Ubisch' granules are present on the inner tangential and radial walls. ×637. M–Q, microspore mother cells at different stages of meiosis. In O laggard chromosomes are seen. ×637. R–S, microspore mother cell after the completion of meiosis. Some additional nucleus-like bodies have also been organized. ×637. T, tetrahedral tetrad. ×637. U–V, uni- and binucleate pollen grains. ×637. W–Z, abnormal pollen grains with supernumerary nuclei. ×637.

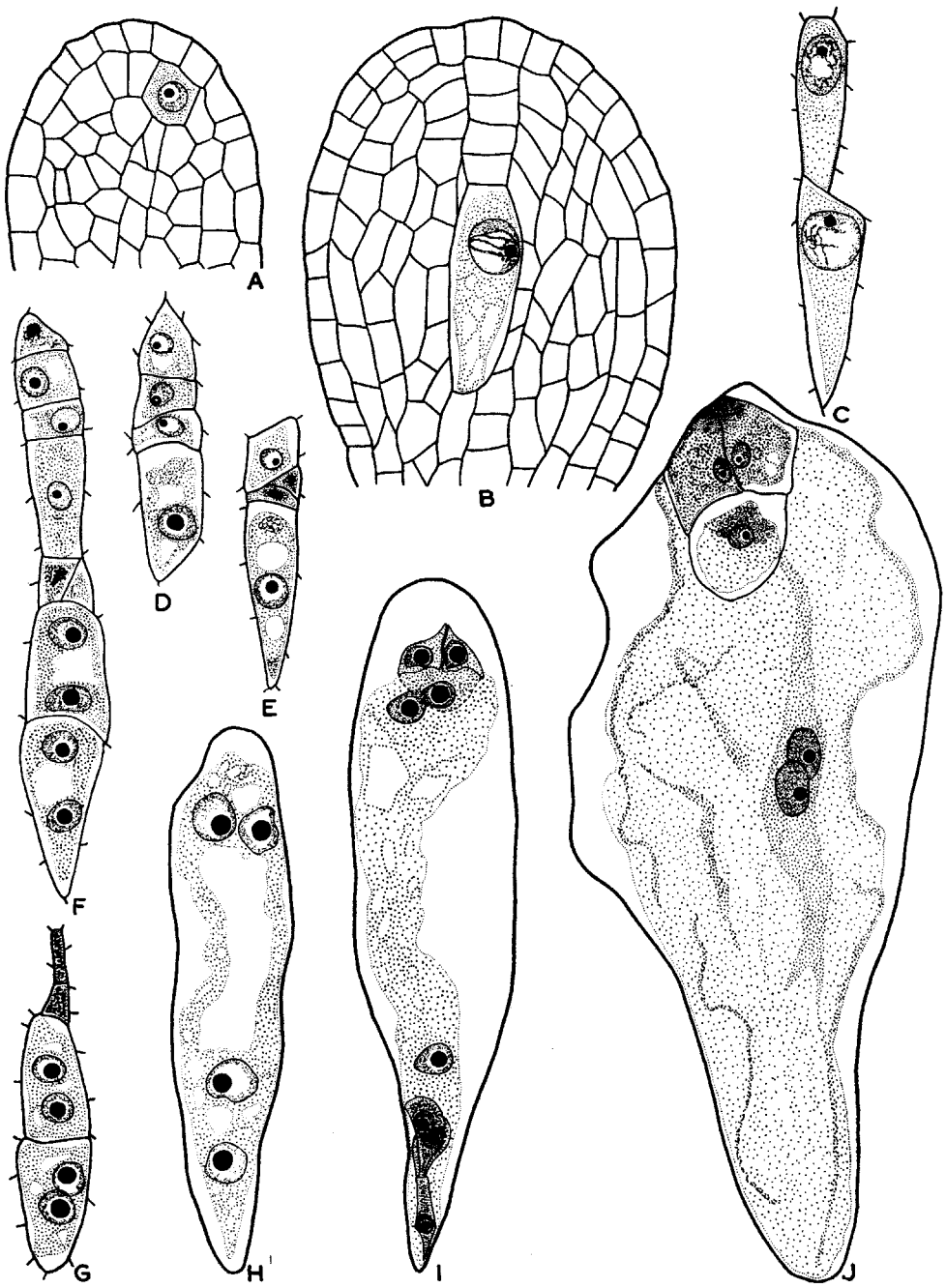

FIG. 4.

Divisions in the first formed cells result in a massive tissue (Fig. 5 I), which eventually fills the entire central space. The outer one or two layers of endosperm comprise small cells (Fig. 5 J), and excepting these all others store starch at maturity (Fig. 5 K).

Embryo

The zygote undergoes a period of rest which lasts till a large number of endosperm nuclei have been formed. It then divides transversely to form two cells (Fig. 6 A, B). In a subterminally or laterally attached zygote the first division can be mistaken for a vertical one. Both the cells of the two-celled proembryo divide vertically (Fig. 6 C–G). Further divisions in the four-celled proembryo do not follow any set pattern and result in a globular mass in which a few cells are at times larger than the rest (Fig. 6 H–M). Further differentiation of the globular embryo (Fig. 6 N, O) is quite normal (Fig. 6 P–R).

Development of seed

At the megaspore mother cell stage both the integuments comprise 3–4 layers. The cells of the inner integument are densely cytoplasmic whereas those of the outer are vacuolated. By this time, dye cells* start developing in the middle layer(s) of the outer integument.

The post-fertilization changes are as follows:

Funicular base.—A circular outgrowth (*fo*) of parenchymatous cells arises from the funiculus (Fig. 7 B, C) and it looks like a small cap at the tip of seed (Fig. 1 H).

Chalaza.—At maturity the chalaza shows a depressed circular band (16–20 cells in width) of thick-walled cells (*thc*) around the central, tannin-filled raised area (Figs. 1 H; 7 H). The chalazal tissue (*ct*) below the embryo sac (*tes*) shows different zones.

Nucellus.—The cells above and below the embryo sac become slightly thick-walled and constitute the epistase and hypostase (*h*) respectively. The

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* Dye cells occur in all the floral parts.

FIG. 4 A–J. *Bixa orellana*, megasporogenesis and female gametophyte. A, l.s. upper part of ovular primordium with an archesporial cell. ×668. B, l.s. nucellus with megaspore mother cell. Some cells of the nucellar epidermis have divided periclinally. ×668. C, twin megaspore mother cells. ×668. D, linear tetrad with enlarged chalazal megaspore. ×668. E, same, with two degenerated megaspores. ×668. F, twin tetrads. The lower tetrad is T-shaped and two of its megaspores have formed 2-nucleate embryo sacs. ×668. G, linear tetrad; the two lower megaspores have enlarged and become binucleate. ×668. H, four-nucleate embryo sac. ×668. I, organized embryo sac. The antipodal cells have dense contents. ×668. J, same, at the time of fertilization. The antipodal cells have degenerated and disappeared. The synergids show filiform apparatus and the egg contains starch grains. ×511.

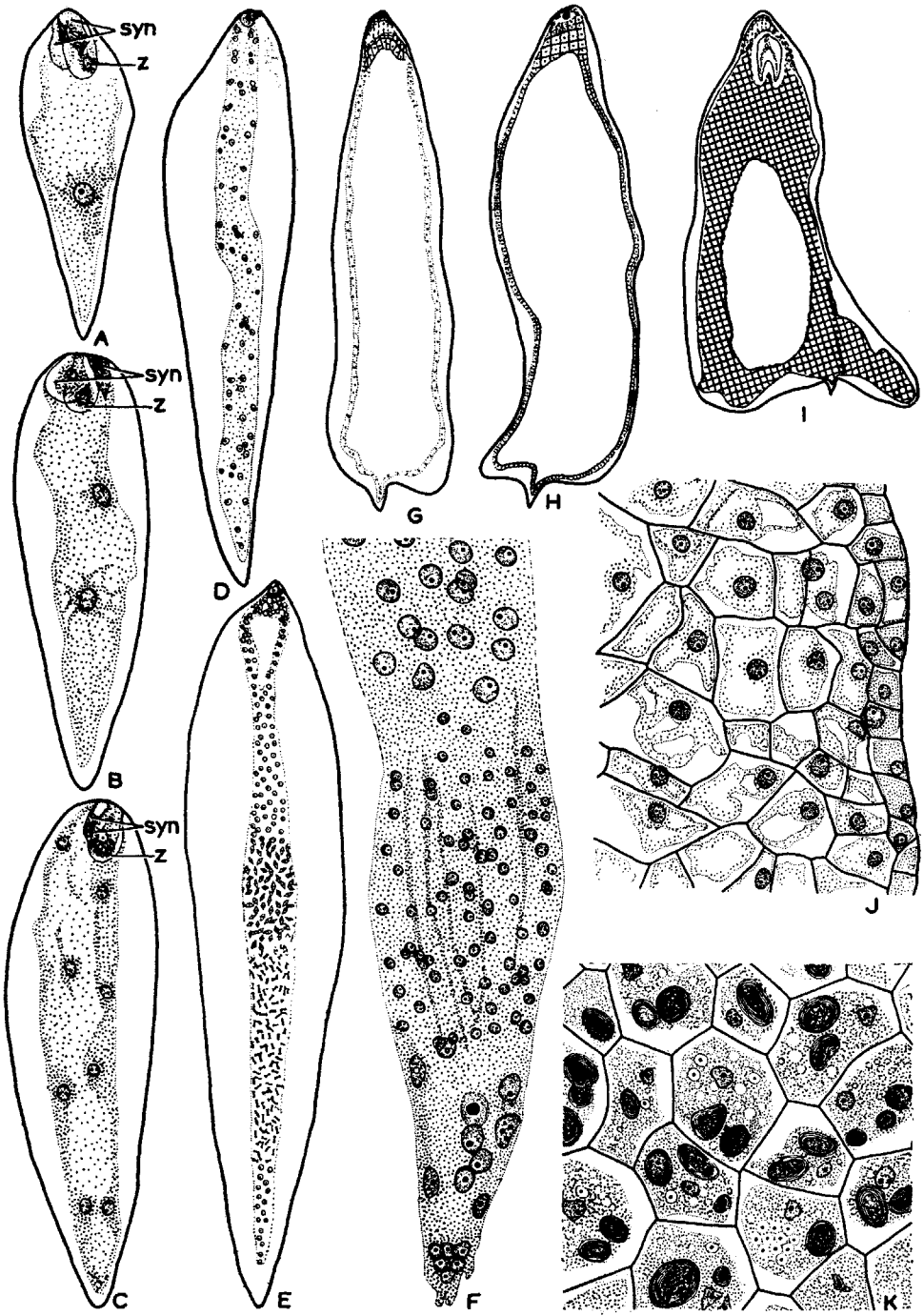

FIG. 5.

cells of nucellar epidermis in the chalazal region elongate radially and undergo repeated anticlinal divisions. Later, periclinal divisions also occur and generally it becomes difficult to distinguish their derivatives (*dne*; Fig. 7 D–G). In the developing seed, remnants of some nucellar cells (*mu*) are seen at the tip, but ultimately only a few nucellar cells (*pnc*) other than those of the hypostase persist (Fig. 7 H).

Outer integument.—While the outer epidermis (*oloi*) does not alter much, more and more of dye cells (*dc*) differentiate in the middle layers. The dye cells become multinucleate (Fig. 8 A–F) and vacuolated. The nuclei at times show fusions. The vacuoles coalesce and the nuclei are pushed to the peripheral layer of cytoplasm (Fig. 8 G). In a mature seed almost all the cells of middle layers are converted into dye cells. Finally, the nuclei degenerate and the cells become filled with the dye* (Fig. 8 H).

The cells of the inner epidermis in the chalazal region (*iloi*) elongate and get obliquely oriented. At the early globular embryo stage these cells assume a S-shape configuration along with the palisade-like layer of the inner integument (Fig. 7 E–H).

Inner integument.—During the early stages of embryo development the integument becomes 7–8 layered, at places even thicker. The order and behaviour of the various layers during subsequent stages are as follows:

(i) Cells of the outer epidermis (*olii*) enlarge radially to form a palisade-like layer (*plii*). Except for some cells in the chalaza, the rest develop thickenings (Fig. 7 G, H). Cell enlargement as well as the thickenings start simultaneously from the micropylar and chalazal ends and progress towards the middle. Within the individual cells thickenings start from the outer side and extend towards the inside (Fig. 7 G). The contents of such cells are gradually lost.

(ii) The outer hypodermal layer (*ohii*) remains almost unchanged (Fig. 7 E–H).

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* The dye gets lost during processing and is not, therefore, observed in prepared slides.

FIG. 5 A–K. *Bixa orellana*, endosperm (*syn*—synergid; *z*—zygote). A, embryo sac showing primary endosperm nucleus and zygote. One of the synergids has degenerated while the other has enlarged. ×200. B–C, embryo sacs with 2 and 8 endosperm nuclei. Note the enlarged synergid. ×200. D, sixty-four-nucleate endosperm. ×102. E, non-synchronous divisions in the endosperm nuclei. The zygote has not yet divided. ×55·6. F, chalazal portion of an endosperm showing variation in the nuclear size. ×407. G, embryo sac showing initiation of cell formation in the endosperm at the micropylar end. Some of the endosperm cells have got detached from the tip of embryo sac. ×24·5. H, same, cell formation has extended up to the chalazal end. ×24·5. I, advanced stage of cellular endosperm, the central region has not yet been filled. ×17·9. J, enlarged view of peripheral portion of endosperm at the young cotyledonary embryo stage. ×407. K, portion of endosperm from a mature seed. The cells contain abundant starch grains. ×437.

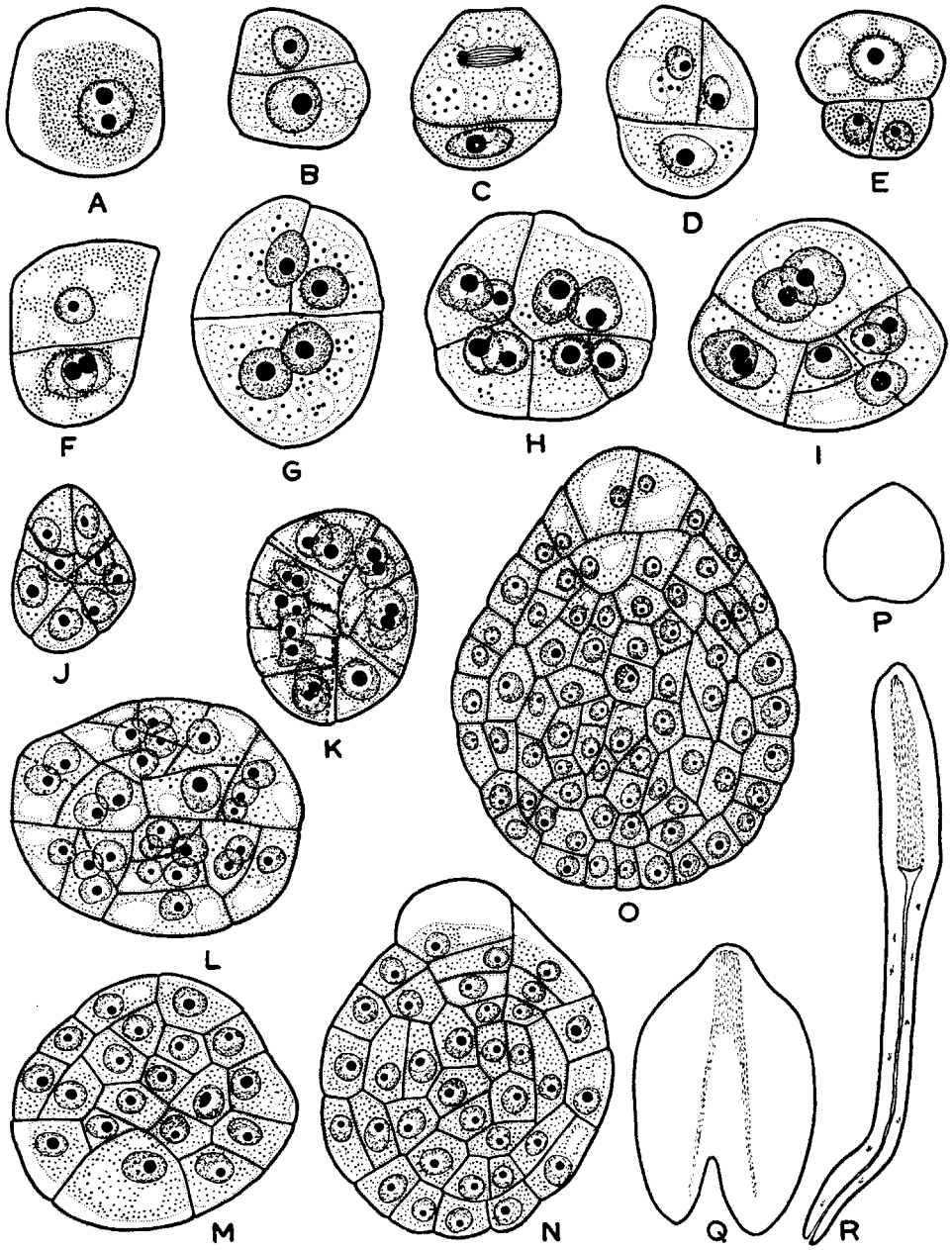

FIG. 6 A–R. *Bixa orellana*, embryo. A, zygote. ×868. B–C, two-celled proembryos; in C the nucleus in the basal cell is in division. ×868. D–G, three- and four-celled proembryos formed by a vertical division of the apical and basal cells of the two-celled proembryo. ×868. H–J, eight-celled proembryos. Note the difference in size and variation in the plane of divisions. ×865. K–O, young and old globular embryos. M and N show one enlarged cell each. K, M, ×868; the rest, ×704. P–R, diagrams of embryos (in l.s.) in later stages of development. P, Q, ×123; R, ×16.

(iii) The next 3–4 parenchymatous layers (*mlsii*) get greatly compressed, but towards the chalaza they cut off a number of derivatives (*dmlsii*; Fig. 7 D–H).

(iv) The cells of the inner hypodermal layer (*ihlii*; Fig. 7 G) develop thickenings and become hourglass-shaped (*hgc*; Fig. 8 H; *see also* Netolitzky 1926).

(v) Repeated divisions at the distal end of the inner epidermis (*ilii*) result in a massive tissue (*dilii*; Fig. 7 G, H).

Besides the above changes, various cell layers towards the micropylar end show radial elongation (Fig. 7 C) and there is a gradual deposition of tannin (*tfc*) in various parts of the seed (Fig. 7 A–H).

Pericarp

At the time ovular primordia are initiated, the ovary wall comprises 15–16 layers of homogeneous cells. During subsequent stages the number of layers increases and the cells enlarge, excepting those of the outer and inner epidermis. Dye cells are usually confined to a few layers below the outer epidermis. Mucilage cavities* develop inner to the vascular bundles and are initially arranged in more or less concentric rings. In the mature fruit tannin is deposited, especially in the cells surrounding the mucilage cavities and vascular bundles. Some of the inner layers of pericarp get detached and form a stiff membranous covering.

Ontogeny of mucilage cavities.—Certain cells in the pericarp enlarge and become densely cytoplasmic. They divide and redivide to form small groups of prominent cells. Later, degeneration starts in the central cells in each group and extends towards the periphery. The space thus formed enlarges and gets filled with mucilage.

Appendages on the fruit wall.—Two kinds of appendages occur on the fruit wall: (i) The vascularized outgrowths, which persist in the mature fruit as long stiff bristles and (ii) the non-vascularized appendages,† which occur on both sides of the pericarp and also on the vascularized appendages. The second type originates from the epidermal initials which enlarge and undergo longitudinal division. The resulting cells usually again divide longitudinally, at times transversely. Further divisions result in an expanded tip. Eventually, these appendages get detached from the ovary wall.

Discussion

The various orders in which Bixaceae have been placed vary in their composition. Some families like Cistaceae and Cochlospermaceae are common to all, whereas others like Tamaricaceae, Flacourtiaceae and Frankeniaceae are present in most of them.

* Mucilage cavities occur abundantly in almost all parts of the plant.

† These appendages also occur on other floral parts and on the inner side of cotyledons.

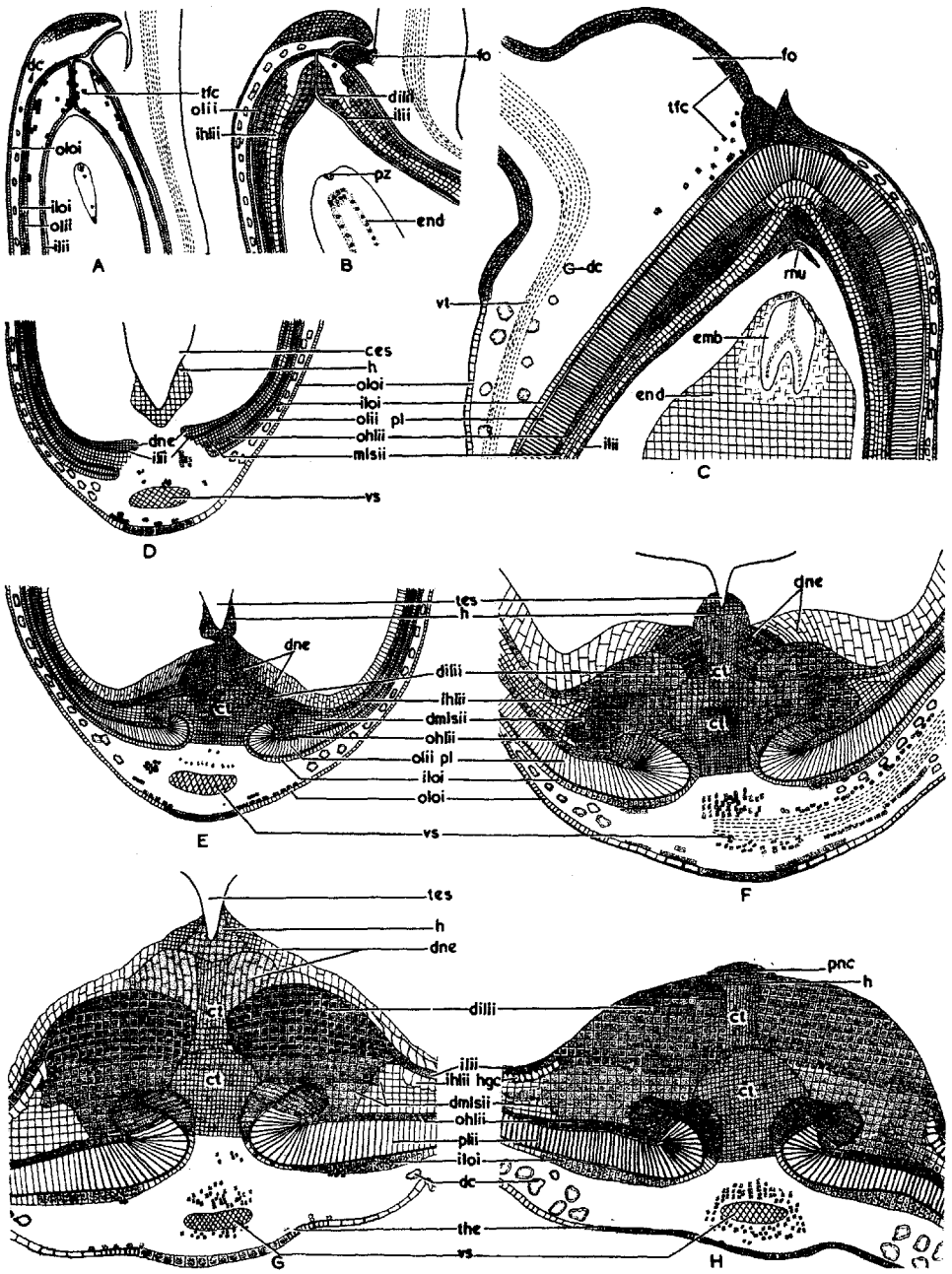

FIG. 7 A–H. *Bixa orellana*, seed coat (*ct*—chalazal tissue; *dc*—dye cell(s); *dilii*—derivatives of inner layer of inner integument; *dmlsii*—derivatives of middle layers of inner integument; *dne*—derivatives of nucellar epidermis; *emb*—embryo; *end*—endosperm; *fo*—funicular outgrowth;

The Bixales of Hutchinson (1959) include seven families, but embryological data are available only on four of them. A comparison of their characters have been made in Table I.

The above data indicate affinities of Bixaceae with Cistaceae and Cochlospermaceae. Seed structure in these families resembles, especially in the formation of palisade-like layer from the inner integument and the characteristic curvature of the integuments in the chalazal region. Erdtman (1952) also stressed the affinities of Bixaceae with Cistaceae and Cochlospermaceae on the basis of his palynological observations.

Of the seven families included in the Bixales of Johansen (1950), four have been included in Table I, the 5th, Samydaceae, has not been worked out, and the 6th, Canellaceae, has recently been removed to the Ranales on the basis of anatomical and embryological features (*see* Parameswaran 1962; Wilson 1965). The 7th family, Frankeniaceae, differs from Bixaceae in having the following characters (*see* Walia and Kapil 1965):

(*a*) sepals 5, united, coriaceous, tubular
(*b*) petals 5, free, clawed
(*c*) anthers 5, free or connate at the base
(*d*) absence of parietal cells in the ovule
(*e*) formation of micropylar endosperm haustoria
(*f*) Solanad type of embryogeny.

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h—hypostase; *hgc*—hourglass-shaped cells; *ihlii*—inner hypodermal layer of inner integument; *ilii*—inner layer of inner integument; *iloi*—inner layer of outer integument; *mlsii*—middle layers of inner integument; *ohlii*—outer hypodermal layer of inner integument; *olii*—outer layer of inner integument; *oloi*—outer layer of outer integument; *plii*—palisade-like layer of inner integument; *pnc*—persisting nucellar cells; *rnu*—remnants of nucellus at the tip; *tes*—tip of embryo sac; *tfc*—tannin-filled cells; *thc*—thickened cells of chalaza; *vt*—vascular trace; *z*—zygote). A, diagram of upper portion of an ovule at mature embryo sac stage. ×86. B, diagram of the upper portion of a young seed showing cell enlargement in various layers. Note the initiation of outgrowth at the base of funiculus. ×71. C, diagram of upper portion of a seed at early cotyledonary stage of the embryo. The tannin-filled layers and the palisade layer have undergone further elongation. The funicular outgrowth has enlarged considerably. ×37·8. D, diagram of lower portion of young seed. ×71. E, same, at a later stage. The derivatives of nucellar epidermis have enlarged. In the inner integument, the innermost and the middle layers have proliferated; the outermost layer shows radial extension and incurving along with the innermost layer of the outer integument. ×46. F, same, at early globular embryo stage. The cells of the palisade layer have enlarged further and the curvature of this layer has become more pronounced. In the curved region the cells of the inner epidermis of the outer integument have also enlarged. ×46. G, same, at late globular stage of embryo. In the inner integument, the derivatives of the inner epidermis and middle layers have increased in bulk; cells of the palisade-like layer have become thick-walled. ×46. H, diagram of the lower portion of a mature seed. The hypostase is seen with a few persisting nucellar cells above it. Cells of the inner hypodermal layer of inner integument have developed into hourglass-shaped cells and the cells of the palisade-like layer have become thickened. The epidermal cells in the circular depression at the chalaza have become thick-walled. ×34·4. (The stippled areas in all the diagrams represent tannin-filled cells).

TABLE I

Character	Bixaceae (Mauritzon 1936; Venkatesh 1956; and present work)	Cistaceae (Chiarugi 1925; Souèges, 1937; Kapil and Maheshwari 1964)	Cochlospermaceae (Schnarf 1931)	Flacourtiaceae (Gopinath 1946; Narayanaswami and Sawhney 1959)
Anther	Horseshoe-shaped, basifixed, dehiscence by slits	Basifixed, dehiscence lengthwise	Dehiscence by apical pores	Dorsifixed
Archesporium	Single-celled	Multicelled	—	—
Tapetum	Secretory, 'Ubisch' granules present	Secretory, 'Ubisch' granules absent	—	Secretory, 'Ubisch' granules absent
Gynoecium	2-carpellary with 2 parietal placentae	3–5-carpellary, the placentae meeting in the centre to form axile placentation	3-carpellary, axile placentation	—
Ovule	Anatropous	Orthotropous	Anatropous, but after fertilization it curves and the raphe comes to lie on the inner side of the bend	Orthotropous, arillate, after fertilization the tip of the ovule bends like a hook
Archesporium	Single-celled	Multicelled	—	Single- or multicelled
Embryo sac	Polygonum type	Polygonum type	—	Polygonum type with variation in the functional behaviour of gaspores. The embryo sac becomes extra nucellar after 4-nucleate stage
Endosperm	Nuclear	Nuclear	Nuclear	Nuclear
Zygote	Does not elongate	Does not elongate	—	Elongates up to half the length of embryo sac

TABLE I—(*concld.*)

Character	Bixaceae (Mauritzon 1936; Venkatesh 1956; and present work)	Cistaceae (Chiarugi 1925; Souèges 1937; Kapil and Maheshwari 1964)	Cochlospermaceae (Schnarf 1931)	Flacourtiaceae (Gopinath 1946; Narayanaswami and Sawhney 1959)
Embryogeny	Irregular	Solanad	—	—
Seed	Albuminous, reserve material starch	Albuminous, reserve material starch	Albuminous, reserve material oil	—
Seed coat				
(a) Palisade layer	Present	Present	Present	—
(b) Hourglass-shaped cells	Present	Absent	Absent	Absent
(c) Dye cells	Present	Absent	Absent	Absent
(d) Any other behaviour	—	—	—	In the upper region the inner layer of inner integument becomes nutritive in function

TABLE II

Character	Bixaceae	Tamaricaceae (Battaglia 1941; Johri and Kak 1954)
Ovule	Anatropous, micropyle is formed by both the integuments	Anatropous, micropyle is formed by the inner integument only
Seed	Chalazal end highly modified	Hairy outgrowths at the chalazal end
Female gametophyte	Monosporic, Polygonum type	Tetrasporic, all types
Synergid polyembryony	Absent	Present in *Tamarix* and *Myricaria*
Embryogeny	Irregular	Solanad type
Suspensor	Absent	Forms a massive structure
Seed	Albuminous	Exalbuminous

Another family which is often placed close to Bixaceae is Tamaricaceae. The important embryological features of these families are presented in Table II.

The above data reveal that Tamaricaceae have no affinity with Bixaceae.

Metcalfe and Chalk (1950) suggested that anatomically Bixaceae show close relationship with Cochlospermaceae and Tiliaceae. Embryological features of Bixaceae and Tiliaceae are compared in Table III.

The above data, though insufficient, do not indicate any relationship of Tiliaceae with Bixaceae.

It thus appears, as Engler (1964) has also remarked, that differences in these families may prompt someone to raise them to an ordinal rank. As a matter of fact some of the orders, viz. Cistales, Tamaricales, etc., already exist in the literature. My views are in agreement with those of Engler who points out that such a classification would be of little use in the study of the existing links.

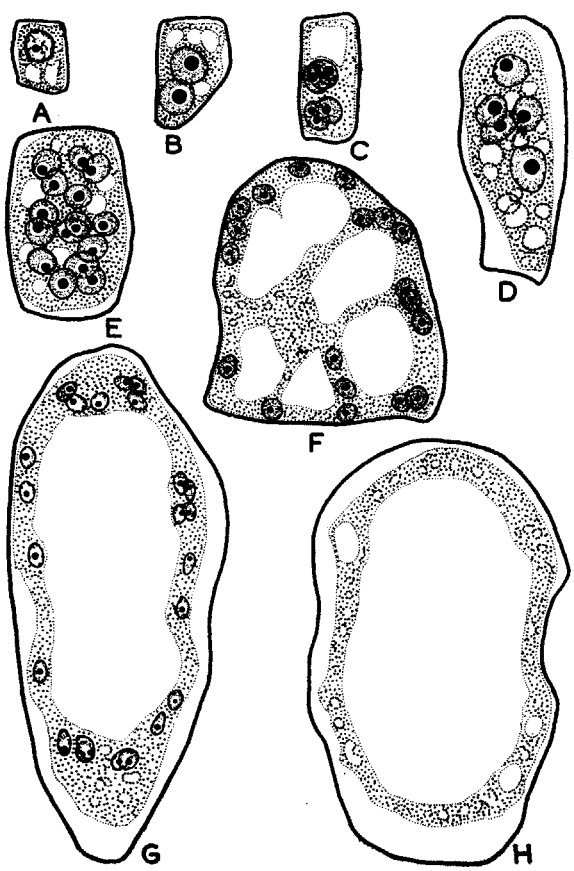

FIG. 8 A–H. *Bixa orellana*, development of dye cells. A–G, stages in the development of a dye cell. G, ×166; the rest, ×294. H, mature dye cell in which the nuclei have completely degenerated and disappeared. The dye has leached out during processing. ×166.

TABLE III

Character	Bixaceae	Tiliaceae (Banerji 1932; Venkata Rao and Sambasiva Rao 1952; Venkata Rao 1954; Bansal 1956)
Stamens	Numerous, all free	Numerous, members of the outer whorl absent or reduced to staminodes, monoadelphous or polyadelphous
Microsporangium	Single archesporial cell	Multicelled archesporium
Pollen grains	Round, 3-colporate, sexine thinner than nexine	Ellipsoidal, 3-colporate with sexine as thick as nexine
Megasporogenesis	Mostly single-celled archesporium	Multicelled archesporium, out of which only one functions
Obturator	Absent	Ovarian hairs constitute the obturator
Embryogeny	Irregular	Asterad type

As far as the grouping of families is concerned, the lack of sufficient embryological information prevents one from passing any conclusive remarks.

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REFERENCES

Banerji, I. (1932). The development of the embryo sac and fertilization in jute. *J. Indian bot. Soc.*, **11**, 228–240.

Bansal, P. K. (1956). The embryology of *Corchorus olitorius* L. and *C. capsularis* L. Abstr., 221, *Proc. 43rd Indian Sci. Congr.*, Agra, Pt. III.

Battaglia, E. (1941). Contributo all, embriologia della Tamaricaceae. *Nuovo G. bot. ital.*, N.S., **48**, 575–612.

Chiarugi, A. (1925). Embriologia delle 'Cistaceae'. *Nuovo G. bot. ital.*, N.S., **32**, 223–316.

Engler, A. (1964). Syllabus der Pflanzenfamilien (ed. H. Melchior). Vol. II. Berlin.

Erdtman, G. (1952). Pollen Morphology and Plant Taxonomy: Angiosperms. Stockholm.

Gopinath, D. M. (1946). Development of female gametophyte in *Flacourtia cataphracta* Roxb. and *F. ramontchi* Her. Abstr., 109–110, *Proc. 33rd Indian Sci. Congr.*, Bangalore, Pt. III.

Hutchinson, J. (1959). The Families of Flowering Plants. Vol. I. Dicotyledons. London.

Johri, B. M., and Kak, D. (1954). The embryology of *Tamarix* Linn. *Phytomorphology*, **4**, 230–247.

Johansen, D. A. (1940). Plant Microtechnique. New York.

———— (1950). Plant Embryology. Waltham, Mass.

Kapil, R. N., and Maheshwari, R. (1964). Embryology of *Helianthemum vulgare* Gaertn. *Phytomorphology*, **14**, 547–557.

Lawrence, G. H. M. (1951). Taxonomy of Vascular Plants. New York.

Mauritzon, J. (1936). Zur Embryologie einiger Parietales—Familien. *Svensk bot. Tidskr.*, **30**, 79–113.

Metcalfe, C. R., and Chalk, L. (1950). Anatomy of the Dicotyledons. Vol. I. Oxford.

Narayanaswami, S., and Sawhney, S. (1959). Microsporogenesis and embryo sac development in *Casearia tomentosa* Roxb. *Phyton, B. Aires*, **13**, 133–144.

Netolitzky, F. (1926). Anatomie der Angiospermen—Samen. Berlin.

Parameswaran, N. (1962). Floral morphology and embryology in some taxa of the Canellaceae. *Proc. Indian Acad. Sci.*, **55**, 167–212.

Rendle, A. B. (1952). The Classification of Flowering Plants. Vol. II. Dicotyledons. Cambridge.

Schnarf, K. (1931). Ein Beitrag zur Kenntnis der Samenentwicklung der Gattung *Cochlospermum*. *Öst. bot. Z.*, **80**, 45–50.

Souèges, R. (1937). Developpement de l'embryon chez l'*Helianthemum guttatum* Mill. *Bull. Soc. bot. Fr.*, **84**, 400–407.

Venkata Rao, C., and Sambasiva Rao, K. V. (1952). A contribution to the embryology of *Triumfetta rhomboidea* Jacq. and *Corchorus acutangulus* L. *J. Indian bot. Soc.*, **31**, 56–68.

Venkata Rao, C. (1954). Embryology of some Tiliaceae. Abstr., 141–142, *Proc. 41st Indian Sci. Congr.*, Hyderabad, Pt. III.

Venkataesh, C. S. (1956). The curious anther of *Bixa*—its structure and dehiscence. *Am. Midl. Nat.*, **55**, 473–476.

Walia, Karvita, and Kapil, R. N. (1965). Embryology of *Frankenia* Linn. with some comments on the systematic position of the Frankeniaceae. *Bot. Notiser*, **118**, 412–429.

Wilson, T. K. (1965). The comparative morphology of the Canellaceae. II. Anatomy of the young stem and node. *Am. J. Bot.*, **52**, 369–378.