

EMBRYOLOGY AND SYSTEMATIC POSITION OF *THELIGONUM* LINN.

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Theligonum cynocrambe is a monoecious herb. The male and female flowers are apetalous and are borne in small sessile cymes. In each male flower there are about 20 stamens. There is a single carpel with an erect ovule. The fruit is a nut. The anther is 4-lobed. It consists of an epidermis, fibrous endothecium, one middle layer and secretory tapetum with uninucleate cells. Cytokinesis is simultaneous. The tetrads are tetrahedral or isobilateral. The pollen grains are 6-porate and are shed at the 3-celled stage. The ovule is campylotropous, unitegminal and tenuinucellar. The hypodermal archesporial cell functions directly as the megaspore mother cell. The development of the female gametophyte conforms to the Polygonum type. There are three antipodal cells which sometimes persist after fertilization and become 2 to 4-nucleate. Due to degeneration of the cells of the nucellus, the mature embryo sac comes directly in contact with the integument. The endosperm is Nuclear. The mature embryo is horseshoe-shaped and dicotyledonous. In the ripe seed the testa shows the presence of starch grains. Two regions are demarcated in the pericarp—the outer zone with elongated cells, some of which contain bundles of raphides, and an inner zone of thick-walled cells forming a hard shell around the seed. The genus *Theligonum* differs from the members of the Urticaceae, Phytolaccaceae and Euphorbiaceae in many embryological features. The creation of a separate family, the Theligonaceae, is therefore fully justified. This does not show any relationship with either the Haloragaceae or the Hippuridaceae, but is closely associated with the Phytolaccaceae and Amaranthaceae.

INTRODUCTION

The genus *Theligonum* comprises three species, namely *T. cynocrambe*, *T. macrantha* and *T. japonica* (Engler 1964). These are distributed in Atlantic Islands, Mediterranean region, China and Japan (see Hutchinson 1959). There are conflicting views regarding the systematic position of this genus. Bentham and Hooker (1862–1883) included it in the family Urticaceae. Warming (1895) assigned it to the Phytolaccaceae. Strasburger (1904) related it to the Euphorbiaceae. However, in 1829 Dumortier (quoted in Takhtajan 1959) raised it to the rank of a family, the Theligonaceae. Later Poulsen (1894) supported this viewpoint.

The relationships of the family Theligonaceae are also controversial. Baillon (1873), Eichler (1878), Poulsen (1894) and Takhtajan (1959) placed

this family, along with the Phytolaccaceae, in the order Centrospermales. Hallier (1912), Ulbrich (1933) and Engler (1964) suggested its alliance to the family Haloragaceae. On the basis of the ovule structure, Schneider (1914) advocated its relationship with the genus *Hippuris* (Hippuridaceae). Hutchinson (1959) included the Theligonaceae in the order Chenopodiales, next to the Amaranthaceae.

As far as the embryology of *Theligonum* is concerned, Schneider (1914) found the pollen of *T. cynocrambe* to be 6-porate and the embryo sac of the Polygonum type. Dahlgren (1916) described the ovule as unitegminal and tenuinucellar. Woodcock (1929) traced the origin of the integument from the terminal portion of the nucellus. However, according to Schnarf (1931) the ovule in *T. cynocrambe* is bitegminal and crassinucellar and the development of the embryo sac is of the Polygonum type. Mauritzon (1934) interpreted the endosperm to be Cellular whereas Schnarf (1931) considered it to be Nuclear.

The present investigation was undertaken to elucidate some of these controversies especially with regard to the ovule and endosperm. The systematic position of *Theligonum* as well as the family Theligonaceae have also been discussed.

MATERIAL AND METHODS

Professor P. Maheshwari obtained the material of *Theligonum cynocrambe* through the courtesy of Professor Th. Eckardt of the Institut für systematische Botanik, Berlin, Germany, and very kindly passed on to us for investigation. Later, he himself collected some material from the Botanical Gardens at Berlin in 1961. We extend our grateful thanks to them.

Formalin-acetic-alcohol was used for fixation. The material was dehydrated and infiltrated through alcohol-xylool grades and imbedded in paraffin wax of 56-58° C. m.p. For a study of the embryogeny, the fruits were trimmed from the sides as well as from the top and bottom without injuring the seeds and then passed through alcohol-xylool series. Longitudinal and transverse sections of buds, flowers and fruits were cut between 5 and 16 microns. Sections were stained either with safranin or iron alum-haematoxylin with fast green as a counterstain. Soaking the imbedded seeds in a solution suggested by Gifford (1950) facilitated sectioning.

OBSERVATIONS

1. *External morphology*

T. cynocrambe is a monoecious succulent herb. The leaves are borne alternately in the upper region of the stem (Fig. 1A) and opposite in the lower part. The flowers are unisexual and apetalous and are grouped in small sessile cymes. The calyx of the male flower is bipartite (Fig. 1B) and 5-nerved. There are 12-20 stamens which have short filaments and long

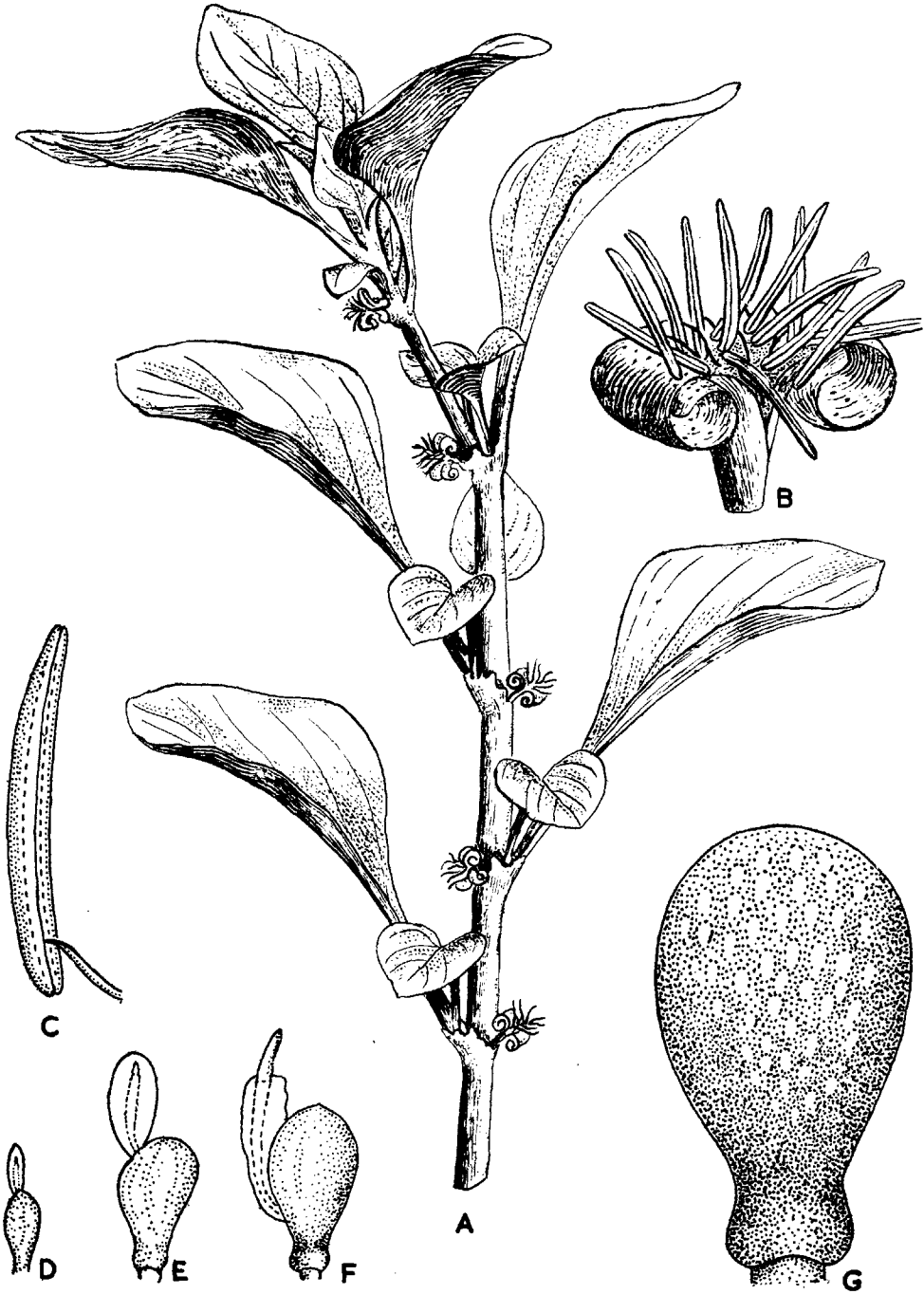


FIG. 1. External morphology. A, twig showing alternate phyllotaxy and solitary flowers. $\times 2.5$. B, male flower. $\times 8$. C, stamen with short filament and long anther. $\times 20$. D-F, young and old pistillate flowers. $\times 8$. G, fruit. $\times 8$.

anthers (Fig. 1B, C). The female flower bears an oblique tubular calyx which in the young stage appears to be situated near the tip of the ovary but subsequently acquires a lateral position (Fig. 1D-F) due to unequal enlargement of the latter. There is a single carpel with an erect ovule. The fruit is a nut (Fig. 1G).

2. *Microsporangium, microsporogenesis and male gametophyte*

The transverse section of a young anther shows a uniformly parenchymatous tissue which soon becomes 4-lobed. A multicelled hypodermal archesporium differentiates in each lobe. At the microspore mother cell stage four wall layers are discernible (Fig. 2A, D). The tapetum is glandular and uninucleate and is surrounded by one middle layer, endothecium and epidermis. At the uninucleate stage of the pollen grains (Fig. 2B, E), the epidermis develops cuticle and the middle layer begins to disorganize. At the time of dehiscence the epidermis shows papillate cuticle, its cells protrude and lend a wavy contour to the anther (Fig. 2C, F). The endothecium develops fibrous thickenings, and the tapetum disorganizes (Fig. 2F).

Cytokinesis of the microspore mother cells is simultaneous. Tetrahedral or isobilateral tetrads are formed (Fig. 2G, H). The microspores separate from each other and develop a thick exine and a thin intine. The pollen grain has six germ pores. Its nucleus divides to form a large vegetative and a small generative cell (Fig. 2F). The generative cell divides to form two oval sperm cells. The mature pollen grain is 3-celled and is packed with starch grains (Fig. 2I).

3. *Megasporangium*

The ovular primordium arises from the placenta as a tiny protuberance (Fig. 5A). The integument originates from the base of the nucellus shortly after the differentiation of the archesporium. The curvature of the ovule is initiated at the megaspore mother cell stage and continues until the formation of the mature gametophyte (Fig. 5A-C) so that it becomes campylo-tropous. It is unitegminal and tenuinucellar.

4. *Megasporogenesis and female gametophyte*

The archesporium is unicelled and hypodermal (Fig. 3A) and directly functions as the megaspore mother cell (Fig. 3B). The meiotic divisions in the megaspore mother cell result in a linear tetrad (Fig. 3C) of which the chalazal megaspore functions (Fig. 3D). Two divisions of the nucleus of the functional megaspore lead to the 4-nucleate gametophyte (Fig. 3E). The next division produces eight nuclei (Fig. 3F) which organize into an egg apparatus, two polar nuclei and three antipodal cells (Fig. 3G). The nucellus

begins to degenerate at the 4-nucleate stage of the embryo sac and becomes completely obliterated during the maturation of the gametophyte which then

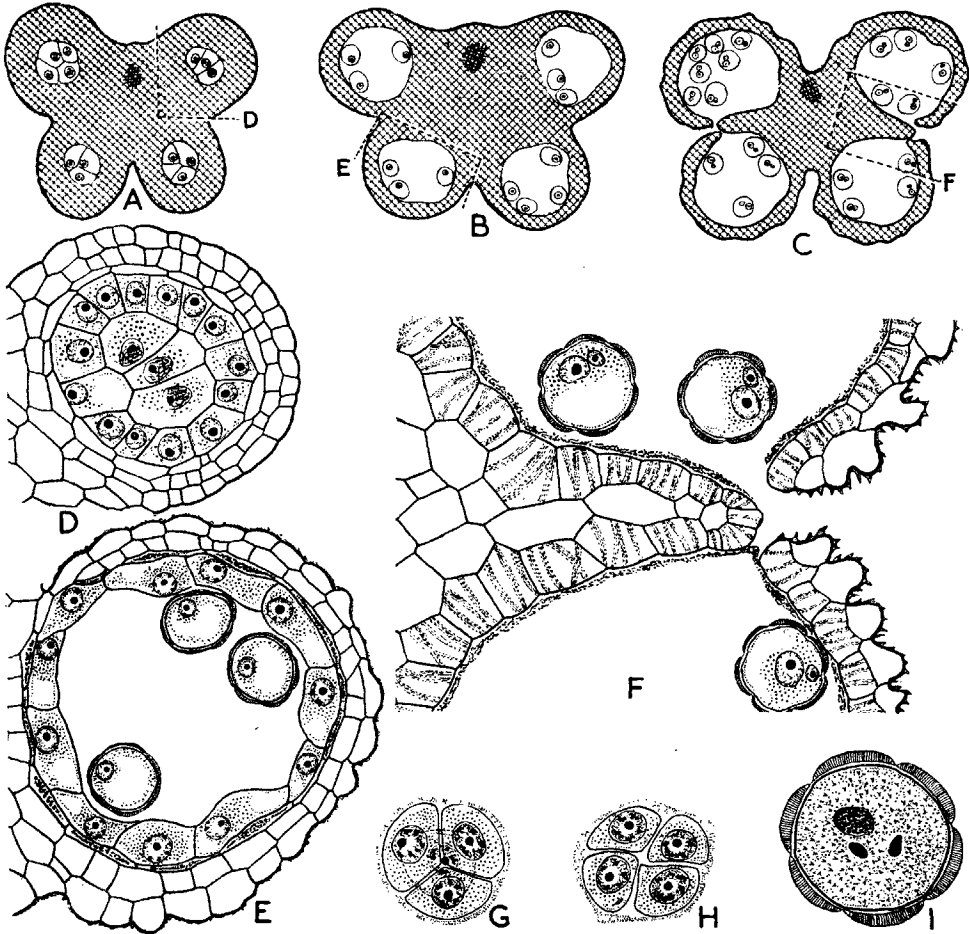


FIG. 2. Microsporangium, microsporogenesis and male gametophyte. A-C, diagrams of cross sections of anthers at various stages of development. A, $\times 200$; B, C, $\times 136$. D, enlarged view of portion marked D in A to show microspore mother cells and wall layers. $\times 568$. E, magnified view of section marked E in B to show the disintegrating middle layer, tapetum and uninucleate pollen grains. $\times 482$. F, wall layers of a dehisced anther enlarged from region F marked in C; epidermis is sinuous and endothecium shows fibrous thickenings. $\times 568$. G-H, tetrahedral and isobilateral tetrads. $\times 900$. I, 3-celled pollen grain. $\times 900$.

FIG. 3. Megasporogenesis and female gametophyte (*ant*, antipodal cells; *dn*, degenerating nucellus; *e*, egg; *pn*, polar nucleus; *s*, synergid; *sn*, secondary nucleus). A, longitudinal section of young nucellus showing archesporial cell. $\times 623$. B, megaspore mother cell. $\times 762$. C, linear tetrad. $\times 762$. D same; the chalazal megaspore is functional. $\times 762$. E-F, 4- and 8-nucleate female gametophytes. $\times 762$. G-H, organized embryo sacs; the polar nuclei have fused to form secondary nucleus in H. $\times 762$. I-M, antipodal cells showing variation in the number of nuclei. $\times 1192$.

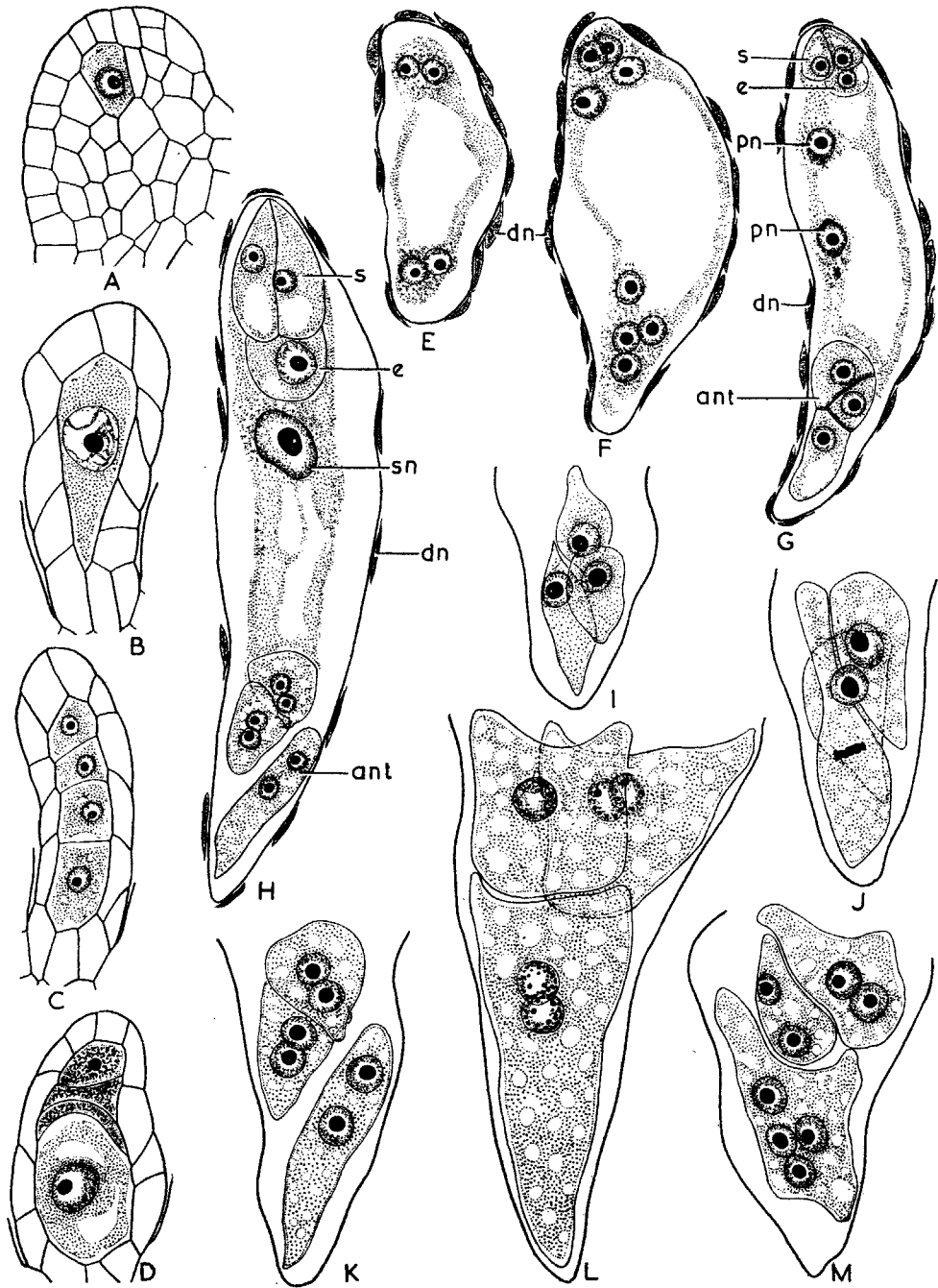


FIG. 3

comes to lie in direct contact with the integument. The polar nuclei fuse in the upper part of the embryo sac. The synergids are ephemeral. Frequently the nuclei of the antipodal cells divide to form 2- to 4-nucleate cells (Fig. 3H-M). Rarely they enlarge considerably and show high vacuolation and prominent nuclei (Fig. 3L).

5. *Endosperm*

Both the synergids disorganize soon after fertilization. Usually the antipodal cells appear healthy even up to the quadrant stage of the proembryo (Fig. 4B-D) but subsequently they degenerate. In a few embryo sacs they degenerate soon after fertilization (Fig. 4A).

The primary endosperm nucleus lies just below the zygote (Fig. 4A). It divides to form two, four (Fig. 4B) and eight free nuclei (Fig. 4C). When the proembryo is at the quadrant stage, the entire endosperm becomes cellular (Fig. 4D). Subsequent divisions lead to the formation of a massive, curved endosperm (Fig. 4E). Its cells are thin-walled, highly vacuolate and show starch grains (Fig. 4F). Although a few cells of the endosperm around the embryo lose their contents and disintegrate, most of them persist in the mature seed.

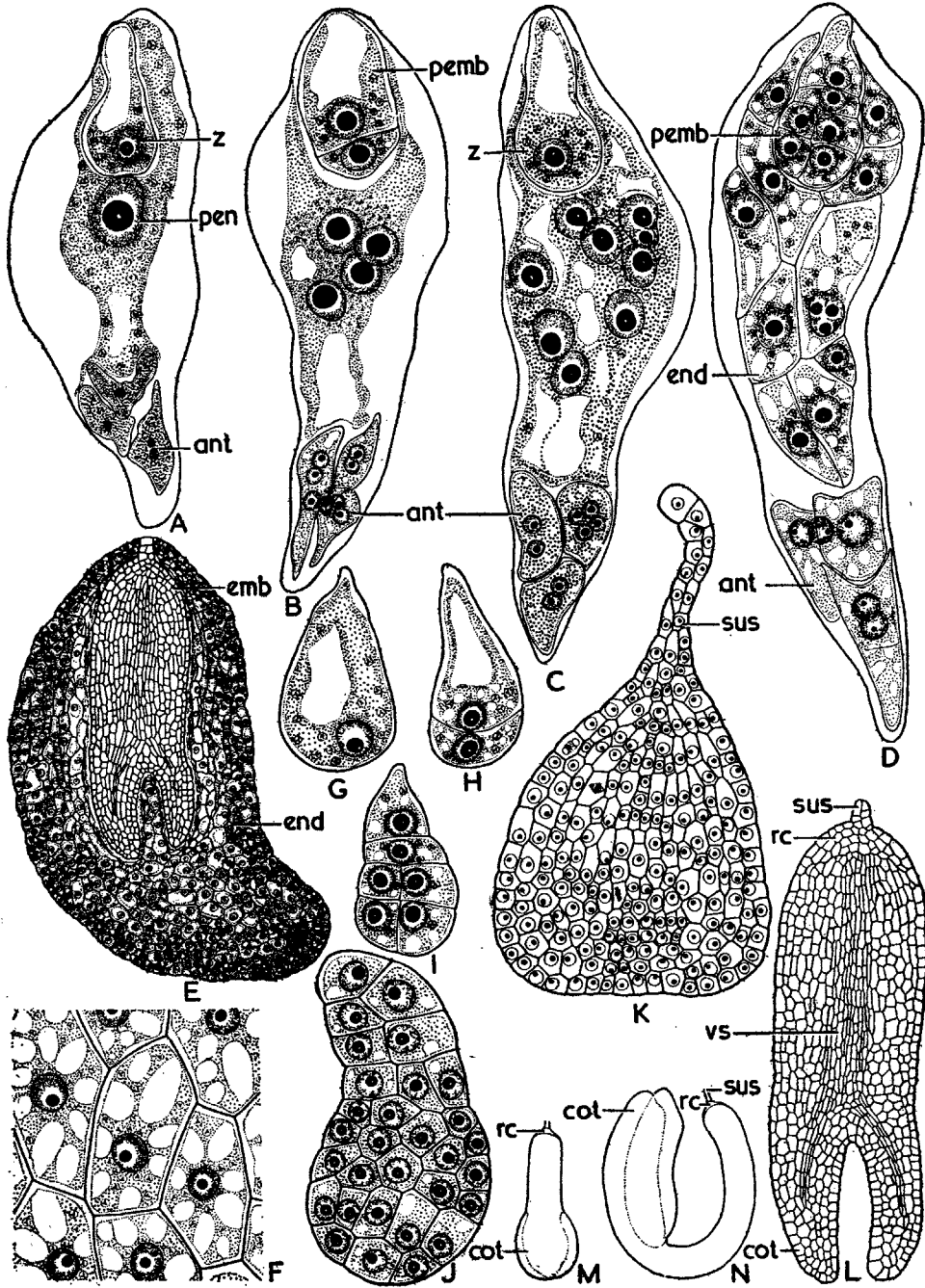
6. *Embryo*

The zygote (Fig. 4G) divides transversely and results in a small terminal and a large basal cell (Fig. 4H). Transverse divisions occur in both these cells. Vertical divisions in the derivatives of the terminal cell result in a quadrant (Fig. 4I). Further longitudinal and transverse divisions in the tiers of the quadrant and their derivatives result in the formation of a globular, heart-shaped, and finally a dicotyledonous (Fig. 4J-N) embryo. The mature embryo is dicotyledonous and horseshoe-shaped (Fig. 4N).

7. *Seed coat*

At the megaspore tetrad stage in the ovule, the integument consists of seven or eight layers of thin-walled, vacuolate cells with prominent nuclei

FIG. 4. Endosperm and embryo (*ant*, antipodal cells; *cot*, cotyledons; *emb*, embryo; *end*, endosperm; *pemb*, proembryo; *pen*, primary endosperm nucleus; *rc*, root cap; *sus*, suspensor; *vs*, vascular supply; *z*, zygote). A, embryo sac showing zygote, primary endosperm nucleus and degenerating antipodal cells. $\times 660$. B-C, 4- and 8-nucleate endosperm; antipodal cells are 2-, 3- or 4-nucleate. $\times 660$. D, embryo sac showing cellular endosperm at the proembryo stage; the cells contain starch. $\times 540$. E, endosperm at the dicotyledonous stage of the embryo. $\times 70$. F, a few cells of mature endosperm enlarged to show large vacuoles and starch grains. $\times 473$. G, zygote. $\times 660$. H, 2-celled proembryo. $\times 660$. I-K, stages leading to the formation of globular and heart-shaped embryos. I-J, $\times 660$; K, $\times 256$. L-M, stages in the differentiation of cotyledons. L, $\times 113$; M, $\times 20$. N, mature curved embryo. $\times 20$.



(Fig. 5B, F). By the time the mature embryo sac is organized, the cells of the inner layers divide periclinally and add to its thickness (Fig. 5C, G). Except for the enlargement and vacuolation of the cells and accumulation of starch grains in them, there are no marked post-fertilization changes in the integument (Fig. 5D, H).

8. *Pericarp*

Prior to fertilization, the ovary wall is made up of 8–10 layers of homogeneous parenchymatous cells (Fig. 5A, E). Subsequent increase in the number of layers appears to be due to repeated periclinal divisions in the cells situated next to the inner epidermis. By the time the integument differentiates in the ovule, the inner epidermal cells elongate tangentially and show dense cytoplasm and prominent nuclei (Fig. 5B, F). At the organized embryo sac stage two regions are demarcated in the ovary wall—the outer zone of large cells with vacuolated cytoplasm and an inner region of compactly arranged, small, thick-walled cells (Fig. 5C, G).

After fertilization the cells of the inner zone increase in number and their walls are further thickened. The cells of the outer zone elongate, and some of them contain bundles of raphides (Fig. 5D, H). Concomitant with these changes, the cells of the basal part of the young fruit undergo repeated divisions and result in a pulpy, knob-like structure (Fig. 5D). The mature fruit is a brownish black nut with hard shell around the seed contributed by the inner zone of the pericarp.

DISCUSSION

Embryology—Schnarf (1931) stated that in *Thebigonum cynocrambe* the ovule is bitegmal and crassinucellar. However, the present study shows that the ovule is unitegmal and tenuinucellar. According to Woodcock (1929) the integument develops near the tip of the nucellus. But our observations reveal that its origin is from the base.

Schneider (1914) noted the ephemeral nature of the antipodal cells in *T. cynocrambe*. Although in some embryo sacs the antipodal cells degenerate early, in the majority of them they persist till the early stages of the endosperm and even become 4-nucleate.

Mauritzon (1934) recorded the development of the endosperm as Cellular while Schnarf (1931) suggested that this might be of the Nuclear type. We also find that it is Nuclear.

In the centrospermal members the perisperm constitutes the main food storage tissue in the seed. However, it is absent in *T. cynocrambe* and instead it is the endosperm that persists.

Systematic position—As mentioned earlier, various views have been expressed regarding the taxonomic assignment of *Thebigonum*. On the basis of

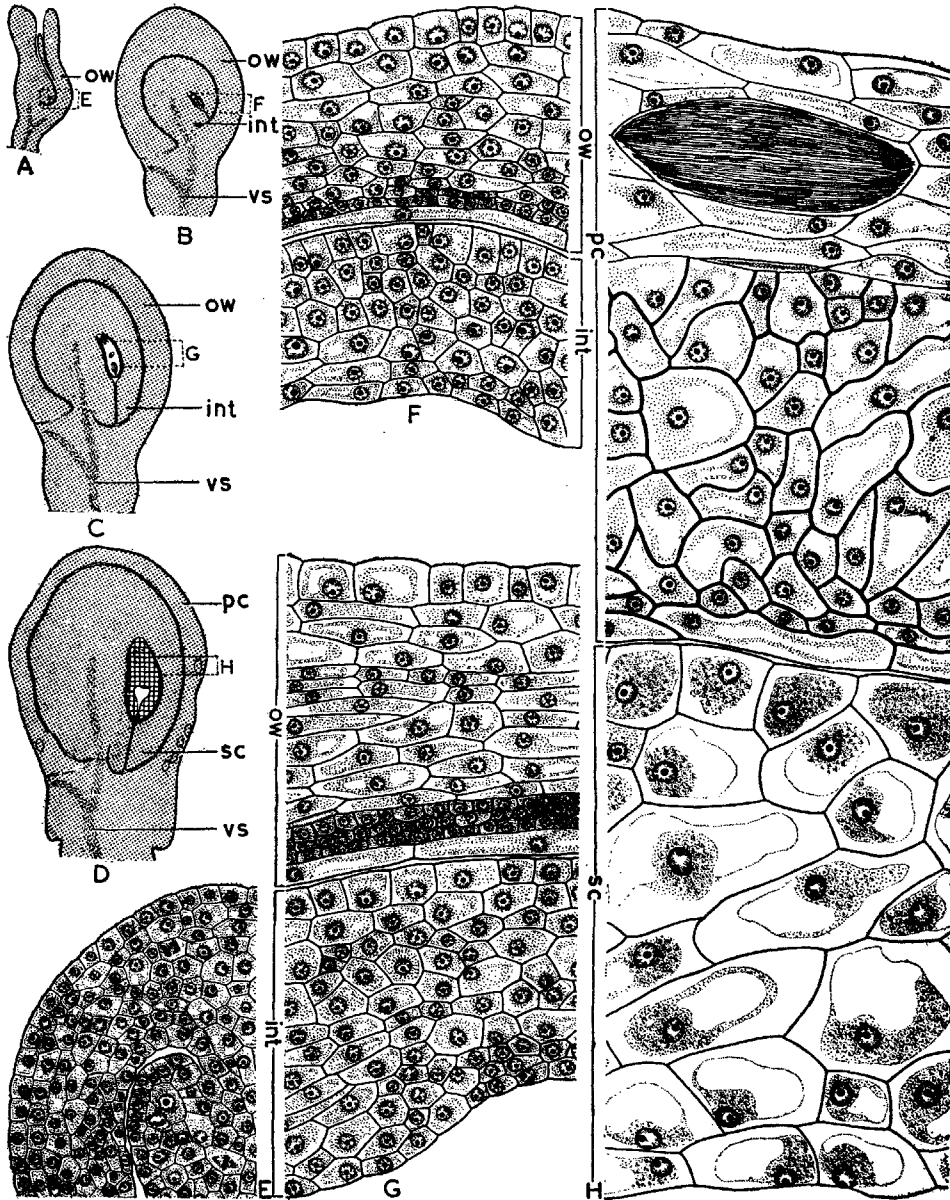


FIG. 5. Seed coat and pericarp (*int*, integument; *ow*, ovary wall; *pc*, pericarp; *sc*, seed coat; *vs*, vascular supply). A-D. l.s. of young flower and fruits (diagrammatic). A, $\times 49$; B, $\times 54$; C, $\times 41$; D, $\times 18$. E, portion marked *E* in A magnified to show the ovary wall at megaspore mother cell stage. There are six to eight layers of parenchymatous cells. $\times 350$. F, portion marked *F* in B enlarged to show the ovary wall and integument at the megaspore tetrad stage. The former is 10 or 11 cells thick whereas the latter contains about seven layers of parenchyma. The cells of the inner epidermis of ovary wall have elongated tangentially. $\times 442$. G, portion marked *G* in C magnified to show increase in the number of cell layers of ovary wall and integument at the mature embryo sac stage. $\times 442$. H, portion marked *H* in D enlarged to show the pericarp and seed coat at the heart-shaped stage of embryo. The pericarp consists of two regions—the outer of thin-walled and the inner of thick-walled cells. $\times 442$.

floral characters Bentham and Hooker (1862-1883), Warming (1895) and Strasburger (1904) included it in the families Urticaceae, Phytolaccaceae and Euphorbiaceae respectively. However, Dumortier (1829; *quoted in* Takhtajan 1959) raised it to the status of a family—*Theligonaceae*. This view was later supported by Poulsen (1894) because of the presence of raphides in all the parts of the plant body. The morphological and embryological characters of *Theligonum*, Phytolaccaceae, Urticaceae and Euphorbiaceae are compared in the following Table (for literature *see* Cooper 1933; Crété 1947; Engler 1964; Erdtman 1952; Gregson 1963; Johansen 1950; Johri and Kapil 1953; Kajale 1944; Kapil 1961; Lawrence 1951; Metcalfe and Chalk 1950; Narayana and Jain 1962; Schnarf 1931; Singh 1954; Venkateswarlu and Rao 1963; and present work).

	<i>Theligonum</i>	Phytolaccaceae	Urticaceae	Euphorbiaceae
Habit	Herbs	Herbs, shrubs or trees	Herbs, shrubs or small trees	Herbs, shrubs or trees
Inflorescence	Cymose	Cymose or racemose	Cymose, head or reduced to a single flower	Racemose, cymose or cyathium
Flower	Unisexual, calyx in male bipartite, in female tubular, petals absent	Bisexual or unisexual, sepals 4 or 5 connate, petals absent	Unisexual, no differentiation into calyx and corolla, perianth 4 or 5-partite or connate	Unisexual, di-, mono-, or achlamydeous, pentamerous, perianth in two whorls
Androecium	Stamens 12-20	Stamens 4 or 5	Stamens 4 or 5	Stamens 1-cc
Gynoecium	Pistil one, unilocular, uniovulate, inferior	12 to 4-carpelled, multilocular, 1 ovule in each locule, superior	Monocarpellary, unilocular, uniovulate, superior or inferior	Tricarpellary, trilocular, 1 or 2 ovules in each locule, superior
Seed	Albuminous perisperm absent, embryo curved	Albuminous, perisperm present, embryo curved	Albuminous, perisperm present, embryo straight	Albuminous, perisperm present, embryo straight or curved
Fruit	Nut	Utricle or achene	Achene or drupe	Capsule, rarely berry or drupe
Anther tapetum	Secretory, cells uninucleate	Secretory, cells 2- or 3-nucleate	Secretory, cells uninucleate	Secretory, cells uni- or multi-nucleate
Pollen	6-porate, suboblate, 3-celled	3-colpate, suboblate, 3-celled	Porate, suboblate, spheroidal, 2-celled	2 to 10-colporate, oblate, 2- or 3-celled

	<i>Theligonum</i>	Phytolaccaceae	Urticaceae	Euphorbiaceae
Ovule	Unitegmina1, tenuinucellar, campylotropous	Bitegmina1, crassinucellar, campylotropous	Bitegmina1, crassinucellar, orthotropous	Bitegmina1, crassinucellar, anatropous
Female archesporium	1-celled	1 to many-celled	1-celled	1 to many-celled
Embryo sac	Polygonum type, antipodal cells contain 1-4 nuclei and may persist	Polygonum type, antipodal cells uninucleate and ephemeral	Polygonum type, rarely antipodal cells many and persistent	Mono-, bi-, or tetrasporic, antipodal cells uninucleate and ephemeral
Endosperm	Nuclear, haustorium absent	Nuclear, haustorium absent	Nuclear, chalazal haustorium rarely present	Nuclear, chalazal haustorium rarely present
Embryo	Chenopodiad type	Onagrad type	Asterad type	Crucifer, Piperad, Solanad, or Chenopodiad type
Poly-embryony	Absent	Nucellar polyembryony present	Somatic parthenogenesis, apogamy occur	Nucellar polyembryony present
Mechanical tissue other than xylem	Absent	Present	Present	Present
Raphides	Present	Present	Absent	Absent
Hairs	Unicellular, non-glandular	Unicellular, non-glandular	Mostly multicellular, glandular	Mostly multicellular, glandular
Stomata	Rubiaceous	Ranunculaceous or rubiaceous	Ranunculaceous or cruciferous	Cruciferous, ranunculaceous or rubiaceous
Chromosome number	n = 10	n = 9	n = 7, 12, 13	n = 7-11, 12

It is evident that *Theligonum* differs from the Urticaceae and Euphorbiaceae in many important features like the structure of the flower, androecium, pollen, gynoecium, ovule, embryo, seed and fruit; in the presence of raphides, unicellular non-glandular hairs; and the chromosome number. Hence, any relationship between *Theligonum* and these two families is unwarranted.

Theligonum also stands apart from the Phytolaccaceae in the structure of the flower, androecium and fruit, in the possession of unitegminal, tenuinucellar ovules, and in the absence of mechanical tissue other than xylem (see Metcalfe and Chalk 1950) in the plant body. It is clear, therefore, that this genus cannot be accommodated even in the Phytolaccaceae. Its elevation to the rank of a separate family, the Theligonaceae, is therefore justified.

Taking into consideration certain resemblances in the structure of the carpel and pollen and the presence of tannin, Hallier (1912) and Ulbrich (1933) placed the Theligonaceae next to the Haloragaceae. Engler (1964) also included it along with the Haloragaceae in the order Myrtiliflorae. On the basis of the tenuinucellar and unitegminal ovule in the Theligonaceae, Schneider (1914) suggested an alliance with the genus *Hippuris* (Hippuridaceae). Poulsen (1894) and Takhtajan (1959) assigned the Theligonaceae to the order Centrospermales near the Phytolaccaceae. Hutchinson (1959) adopted a similar view but kept it near the Amaranthaceae.

The Theligonaceae resemble the members of the Haloragaceae in having secretory type of anther tapetum, 3-celled pollen grains, inferior ovary, 1-celled female archesporium and Polygonum type of embryo sac. However, the family stands apart from the Haloragaceae in possessing a cymose inflorescence, unisexual flowers, unilocular and uniovulate carpel, 6-porate pollen grains, unitegminal, tenuinucellar and campylotropous ovules, 1- to 4-nucleate antipodal cells, Nuclear type of endosperm, Chenopodiad type of embryogeny and in the basic number of chromosomes. Similarly the Hippuridaceae differ from the Theligonaceae in the presence of bisexual flowers, a single stamen, anatropous ovules, Cellular endosperm, straight embryo with suspensor haustorium and drupaceous fruit. Therefore, it is not justified to give the Theligonaceae a position either near the Haloragaceae or the Hippuridaceae (for literature see Engler 1964; Erdtman 1952; Johansen 1950; Lawrence 1951; Schnarf 1931; Sethi 1964).

The Theligonaceae differ from the centrospermalean families, particularly the Amaranthaceae and Phytolaccaceae, in the structure of the flower, tapetum and ovule, and in the absence of a perisperm in the seeds and mechanical tissue in the plant body; but these families also show several common features like: (1) mono-carpellary ovary with solitary ovule; (2) secretory type of anther tapetum; (3) 3-celled pollen grains; (4) Polygonum type of embryo sac; (5) Nuclear endosperm; (6) albuminous seeds with curved embryos; (7) presence of raphides; (8) unicellular non-glandular hairs; and (9) rubiaceous stomata (for literature see Bakshi 1952; Bhargava 1956; Crété 1947; Engler 1964; Erdtman 1952; Joshi 1936; Joshi and Kajale 1937; Kajale 1940, 1944, 1954; Metcalfe and Chalk 1950; Narayana and Jain 1962; Schnarf 1931; Souèges 1937). Therefore, the proper place for the Theligonaceae is in the order Centrospermales near the families Phytolaccaceae and Amaranthaceae.

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* Not seen in original.