

DEVELOPMENT OF OVULE AND SEED-COAT OF *DICHAPETALUM MOMBUTTENSE* ENGL. WITH NOTES ON OTHER SPECIES

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SUMMARY

The ovule primordium of *Dichapetalum mombuttense* seems to be trizonate. The ovule is tenuinucellate and both integuments are of dermal derivation.

During seed development the whole inner integument and a part of the outer integument are resorbed.

The outer integument is vascularized. The seed-coat is exotestal and consists of a differentiated outer layer of the outer integument and crushed remains of the middle layers. The endosperm is initially nuclear, later to become one cell layer thick.

The systematic position of Dichapetalaceae is discussed.

1. INTRODUCTION

The tropical family Dichapetalaceae (Chailletiaceae) is very natural and consists of the three woody genera *Dichapetalum*, *Tapura* and *Stephanopodium* with 120, 25 and 9 species, respectively.

According to VAN TIEGHEM (1901) the Dichapetalaceae are unitegmic, but in 1903 he described the ovule of *Dichapetalum toxicarium* (G. Don) Baill. as bitegmic, with a thick inner integument, resorbed nucellus and without clearly discernible endothelium. SCHNARF (1931), referring to VAN TIEGHEM (1903), described the ovule of *Dichapetalum* as bitegmic and tenuinucellate, whereas DAVIS (1966) dispatched the family as embryologically unknown. Both NETOLITZKY (1926), referring to VAN TIEGHEM (1901), and CORNER (1976), referring only to NETOLITZKY, as a consequence considered the ovule to be unitegmic. So far no description of the seed-coat development or even of the mature seed-coat anatomy had been given. The relationships of the family are uncertain and there has been a wide divergence of opinion as to the relationship of the Dichapetalaceae (PRANCE 1972). Although HEIMSCH (1942) states that the Dichapetalaceae share certain anatomical characters with families of Linales, Polygalales, and Malpighiales, according to METCALFE & CHALK (1950) the scanty anatomical evidence is not sufficient to define the taxonomic position of the family. The places of the Dichapetalaceae in the most important classification systems have been listed by BRETLEER (1973) and PRANCE (1972).

The following relationships have been suggested:

Euphorbiaceae (especially with the Phyllantoideae): ENGLER (1896); ENGLER & KRAUSE (1931); BESSEY (1907); WETTSTEIN (1935); GUNDERSEN (1950);

TAKHTAJAN (1969) and THORNE (1976);
Geraniales (next to *Meliaceae*): BENTHAM & HOOKER (1862);
Geraniales (*Trigoniaceae*, *Malpighiaceae*): BRETELER (1973);
Thymelaeales: MELCHIOR (1964) and DAHLGREN (1977);
Celastrales (*Icacinaceae*, *Celastraceae*): CRONQUIST (1968);
Polygalales (*Malpighiaceae*): PRANCE (1972);
Linaceae: HALLIER (1923);
Rosales (near *Rosaceae* and *Calycanthaceae*): HUTCHINSON (1969).

According to PUNT (1975, 1976) the palynological data are inconclusive as regards the relationships of the *Dichapetalaceae* with other families. In his scheme of pollenmorphological relationship arranged according to evolutionary trends, the *Dichapetalum bangii* type must be considered the most primitive in the family and this type resembles the *Tapura amazonica* type more closely than any other pollen type of the genus *Dichapetalum*. It is assumed that the pollen grains of these types have remained primitive. The other pollen types of the genus *Dichapetalum* and of the whole genus *Stephanopodium* are more advanced than those of the species with the *Dichapetalum bangii* type of pollen grains.

But little chemodiagnostic research was carried out on the family (HEGNAUER, 1966). Some species are very poisonous, owing to the presence of the toxic compound fluoroacetic acid in parts of the plant or in the seeds (WARD et al. 1964).

This study was undertaken in order to give the first complete description of the development of ovule and seed coat of representatives of the *Dichapetalaceae*.

2. MATERIALS AND METHODS

Spirit material of the following species was received from Dr. F. J. Breteler, Wageningen:

Dichapetalum bangii (F. Didr.) Engl.,
Tapura africana Oliv., *T. ivorensis* Breteler,
T. bouquetiana Hallé & Heine, and *T. amazonica* Poepp. & Endl.

In addition material of the following species was collected in the hothouses of the Department of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen:

Dichapetalum mombuttense Engl.,
D. oblongum (J. D. Hooker ex Benthams) Engl.,
D. madagascariense Poir.,
D. cymulosum (Oliv.) Engl. and *D. pallidum* (Oliv.) Engl.

Fixation was done in FPA, CRAF or Allen-Bouin mixtures. Sections were made by means of standard microtome techniques and by hand. The following stains were used for specific tests:

Phloroglucinol-HCl, Sudan IV, ruthenium red and IKI.

The SEM observations were made with a Cambridge Stereoscan Mark 2a. The objects stuck on Scotch thin copper-foil with conducting adhesive, were mounted with Demetron silver-conducting paint on the specimen stub. The material

was scanned fresh or fixed without (*fig. 5A*) or (*figs. 5B, C, d*) after 3 minutes of sputter-coating with gold-palladium.

A high voltage of 10 KV was consistently used. The photomicrographs of seeds of *D. cymulosum* were made with a Zeiss-Tessovar, in combination with a Sunpak GX8R ring light, on 35 mm film.

3. RESULTS

3.1. Gynoecial morphology

The representatives of the family Dichapetalaceae have a 2-3-(4)-loculed, syncarpous ovary with two anatropous ovules in juxtaposition in each locule. Not more than one ovule per locule develops into a seed, so that the fruit is a 1-3-(4)-seeded, fleshy drupe.

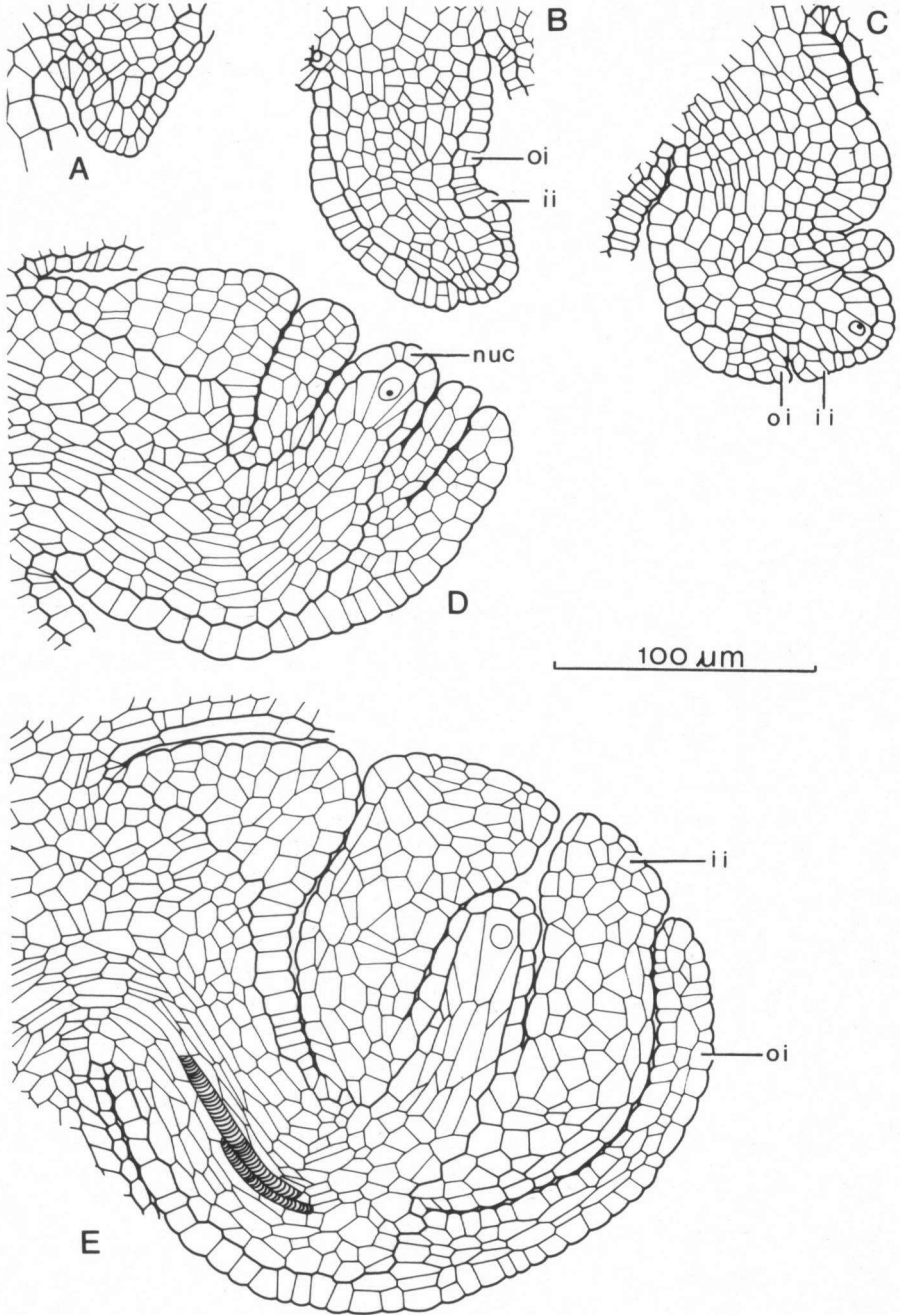
The seed germination is always hypogeal and the cotyledons remain enclosed in the endocarp.

3.2. The young ovule

The ovule primordium exhibits an irregular cell arrangement, but seems to be 3-zonate. In such a build-up the ovule is initiated by periclinal divisions in the corpus which initially remains surrounded by the only anticlinally dividing cell layers subdermatogen and dermatogen (*fig. 1A*). The cells of the subdermal layer stretch radially but never divide periclinally in the nucellar part of the ovule, so that they remain clearly discernible for a considerable length of time (*figs. 1B-E*). One of the cells of the subdermal layer develops directly into the archesporium cell. Elsewhere some cells of this layer divide periclinally, for instance under the sites where the integuments originate. The nucellus remains rather small and is later completely resorbed by the developing embryo sac (*e.s.*) before the ovule is full-grown. In *Dichapetalum oblongum* the megaspore tetrad is linear and the chalazal megaspore becomes the functional one.

The initiation of the inner integument (*i.i.*) takes place in a zone of about 3 dermal cells broad concomitantly with that of the outer integument (*o.i.*), see *fig. 1B*. The *i.i.* develops as a symmetrical ring wall and is totally of dermal derivation (*figs. 1C-E*). At an early developmental stage the *i.i.* is 2 to 3 cells thick but it soon increases in width by periclinal divisions in all cells lying below the outer dermal cells (*figs. 1E* and *2A*). It ultimately becomes 6 to 8 cells thick (*figs. 1E* and *2A, B*) and accumulates an appreciable quantity of starch.

The *o.i.* originates as an asymmetrical ring wall by periclinal and oblique divisions in a zone of more than 3 cells broad, so that its zone of attachment is rather broad right from the beginning (*figs. 1C, D*). The *o.i.* is completely of dermal origin and initially 2 to 3 but later 3 to 5 cell layers thick (*figs. 1C, D, E*, and *2A*). It remains shorter than the *i.i.* and is relatively large on the raphe side because the anatropous curving is delayed. The ovule primordium starts bending at an early stage but it progresses so slowly that the micropyle has already been formed by the time the ovule is still hemi-anatropous.



In *Tapura africana* the cells of the inner layer of the i.i. stretch somewhat in the radial direction and resemble an endothelium to some extent.

3.3. The full-grown ovule

The full-grown ovule is bitegmic, anatropous and tenuinucellate (*fig. 4E*). The nucellus is completely resorbed and the archesporous cell functions directly as the megasporocyte. The inner layer of the starch-containing i.i. have likewise disappeared with the exception of those at the micropyle (*fig. 2B*). The o.i. is 4 to 5 cell layers thick and remains shorter than the i.i. The funicular obturator consists of short, pluricellular trichomes rich in cytoplasm. This obturator lies directly against the o.i. at the raphe side and is not clearly discernible in the younger developmental stages.

The micropyle is formed by the i.i. alone and is somewhat oval to slit-like.

The raphe contains an already somewhat differentiated amphicribal vascular strand and the narrow chalaza contains an appreciable quantity of provascular tissue. Of the two ovules in each ovary chamber not more than one may develop into a seed, the other ovule degenerating soon.

The ovules of *Dichapetalum oblongum*, *Tapura africana* and *T. bouquetiana* are also bitegmic and resemble those of *Dichapetalum mombuttense* rather closely.

3.4. Development of seed and seed-coat

The shape of the developing seed becomes more oblong after fertilization has taken place (*fig. 4E*). The scantily developed endosperm is initially nuclear and in the mature seed remains only one cell layer thick (*figs. 2B-E, 3A*). At the outer surface of the endosperm a well-defined cuticle develops. The i.i. is gradually resorbed by the developing endosperm and ultimately disappears completely (*figs. 2B-E*). During the process of resorption of the i.i. maceration of the cells commences whilst in the peripheral parts cell divisions still proceed (*figs. 2B, C*).

The persistent o.i., which is poor in starch, is multiplicative and becomes about 20 cells thick after fertilization has taken place. When it is 6–7 cell layers in width here and there star-shaped crystals originate and its inner layer develops into a clearly distinct structure (*fig. 2C*) which is no longer recognisable in later developmental stages. In peripheral layers of the o.i. procambial strands develop which later form an extensive system of vascular bundles in the testa, and the outermost layer becomes rich in tannic substances (*fig. 2D*). Also the o.i. is gradually resorbed from the inside and starts degenerating at that side (*figs. 2E, 3A*). In later developmental stages the remaining tissue becomes spongy and

Fig. 1 A-E: *Dichapetalum mombuttense*.

L.s. of developing ovules.

In all figures: cot = cotyledon; end = endosperm; nuc = nucellus; ii = inner integument; oi = outer integument; pvs = provascular strand.

All measures indicate 100 μ m.

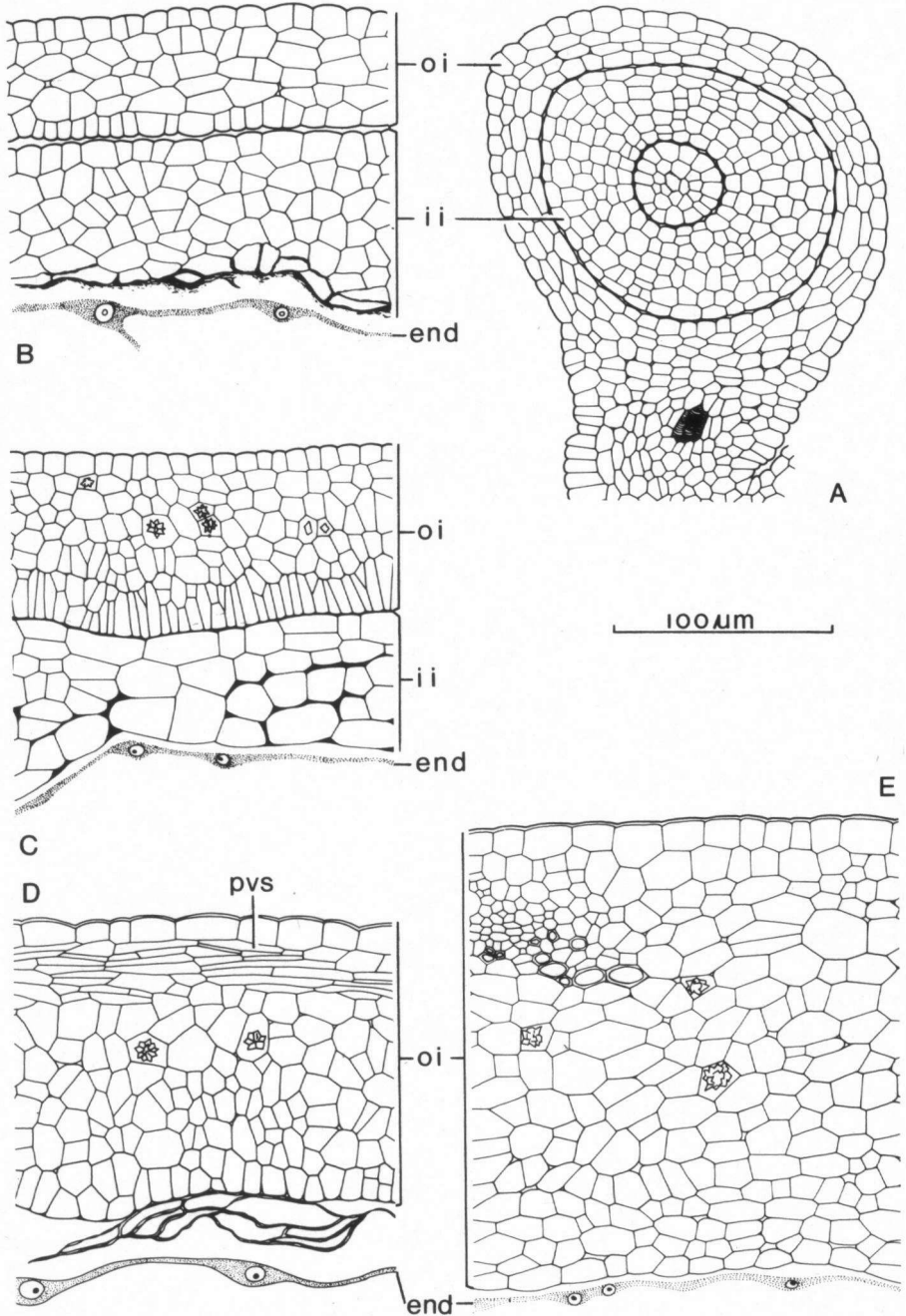


Fig. 2. *Dichapetalum mombuttense*.
 A: cross section of immature ovule; B-D: l.s. of developing seed-coat; E: t.s. of developing seed-coat.

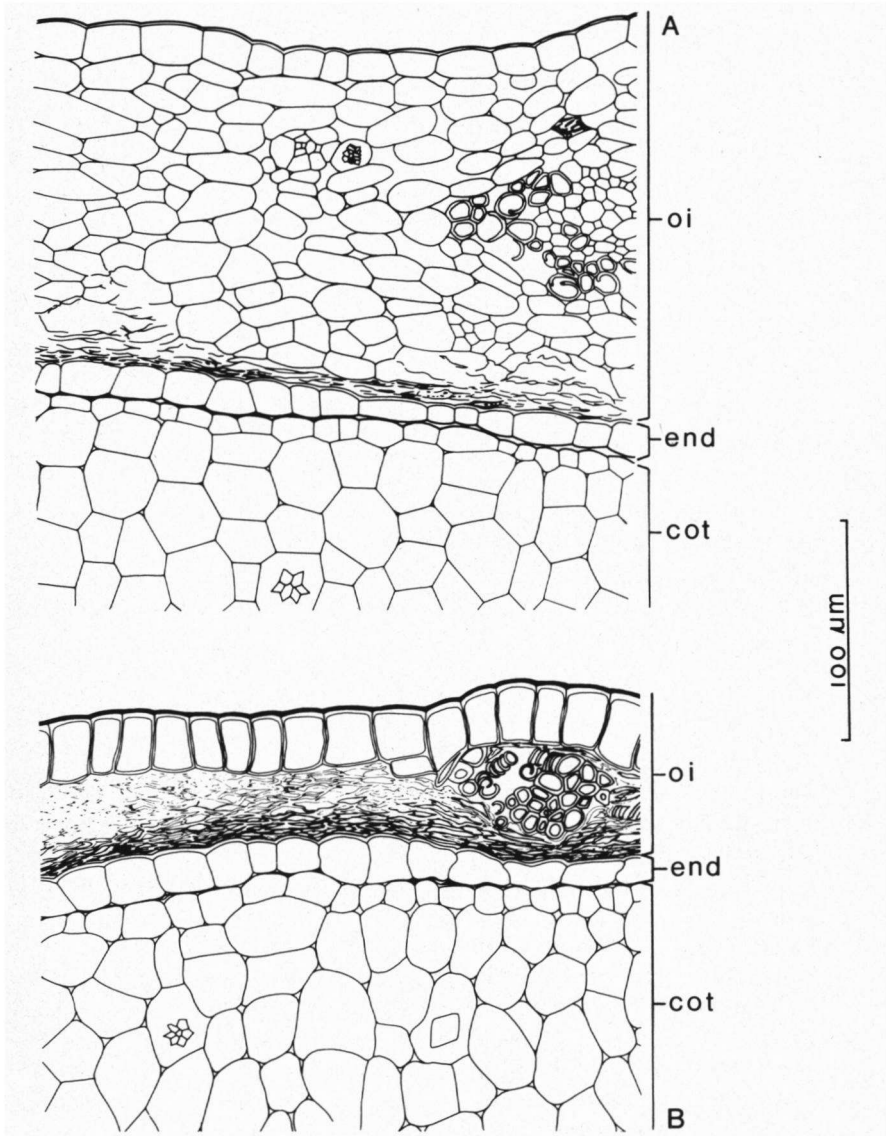


Fig. 3. *Dichapetalum mombuttense*, t.s. of seed-coat.
 A: shortly before maturity; B: fully matured seed-coat.

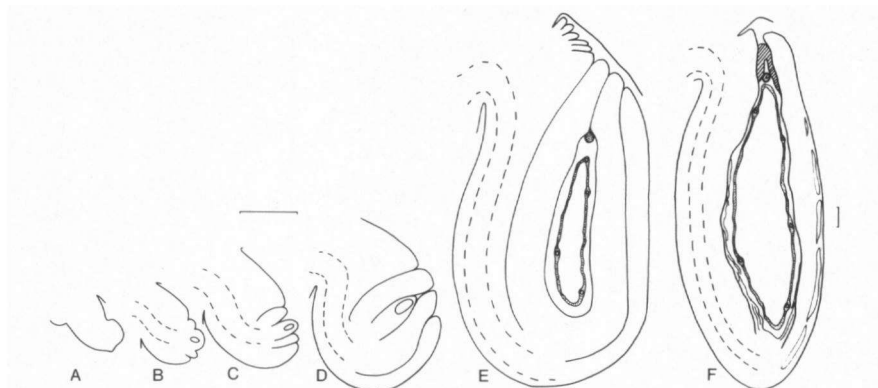


Fig. 4. Anatropous development of ovules and young seeds of *Dichapetalum mombuttense*. 4E: shortly after fertilization.

contains large intercellular cavities (fig. 3A). In *Dichapetalum bangii* this loosely built tissue in the o.i. even resembles an actinenchyma.

In parts of the xylem of the vascular strands rhexogenic cavities develop (figs. 3A, B). In the tegumentary bundles the xylem and phloem parts are collaterally arranged.

No structure resembling a hypostase was observed in any stage of development.

3.5. The mature seed

The remaining cells of the fully matured seed-coat are compressed into a paper-thin layer, only the much enlarged tanniferous cells of the epidermis and a part of the xylem elements remaining intact (fig. 3B).

From the periphery towards the inside the following layers of the seed-coat can be distinguished in a transverse section:

(1) A cuticle reacting with Sudan IV.

(2) An outer layer (exotesta) consisting of tanniferous cells with thickened walls. The outer cell wall, which is the thickest, is not lignified but contains pectic substances and especially its peripheral part reacts with Sudan IV. In surface view these cells are not individually discernible (fig. 5A). The cuticle is relatively smooth but appears to be finely striate by the impressions of the sclereid endocarp cells. The vascular bundles also render the thickness of the seed-coat uneven. In the young and not yet fully mature and squashed seed-coat the outer cells can be individually recognized in the SEM photograph (fig. 5B).

(3) A layer of compressed cells with scattered groups of xylem elements of the vascular strands. In cross sections elements with helical wall thickening can be observed (fig. 5C) although also some elements with a reticulate type of wall thickening may be present.

(4) An endosperm layer only one cell thick, separated from the seed-coat proper by a clearly discernible cuticle reacting positively with Sudan IV.

The cotyledons of the embryo contain intercellular spaces and a few crystals of various shapes including stellate ones.

The mature seed of *Dichapetalum mombuttense* is ellipsoid and somewhat acuminate at both ends, about 2 cm long and 1 cm in diameter, and of a dull to shiny, light brown colour. The raphe bundle is clearly recognizable and divides at the chalaza into a number of branches running over the antiraphe in the direction of the micropyle. The whole seed-coat is covered with the finer ramifications of the often anastomosing vascular bundles.

The large and straight embryo is rich in starch and poor in lipids, epi- and hypocotyl together are about 1 mm long. The cotyledons are planoconvex.

The seed-coats of *D. pallidum*, *D. cymulosum*, *D. madagascariense* and *Tapura africana* have about the same structure as that of *D. mombuttense*; those of *D. cymulosum* and *D. madagascariense* also have a rather smooth surface (figs. 5D, E) in which the individual cells are not clearly recognizable (figs. 5D, E, F). Since in *D. cymulosum* the outer layer of the seed-coat is not or hardly tanniferous, the vascular bundles appear as a light-coloured nervation pattern against the green background formed by the cotyledon which contains chlorophyll in its peripheral layers. Below the about 1 mm wide micropyle a space is present which contains the pubescent radicle. The vascular bundle pattern does not correspond with the ovular development since some bundles run directly from the hilum along the micropyle to the antiraphal side (figs. 5E, F).

The seed-coat of *D. bangii* is thicker and more solid than those of the other species studied. This is partly attributable to the fact that during the ontogeny of the seed the outer epidermis becomes pluri-layered, so that the mature seed-coat consists of several layers of thick-walled and tanniferous cells instead of only one as in the other species. The innermost layer of the testa consists of a fairly thick layer of flattened cells with vestiges of vascular bundles. The testa also contains scattered lignified and pitted sclereids. In surface view the testa is black, and somewhat rugose on account of the small variations in the number and the direction of the periclinal divisions in the ultimately plurilayered epidermis.

Tapura africana has a dark-brown seed-coat with relatively large, tanniferous dermal cells which are individually recognizable in surface view (figs. 6A, B). The endosperm is also formed by only one cell layer.

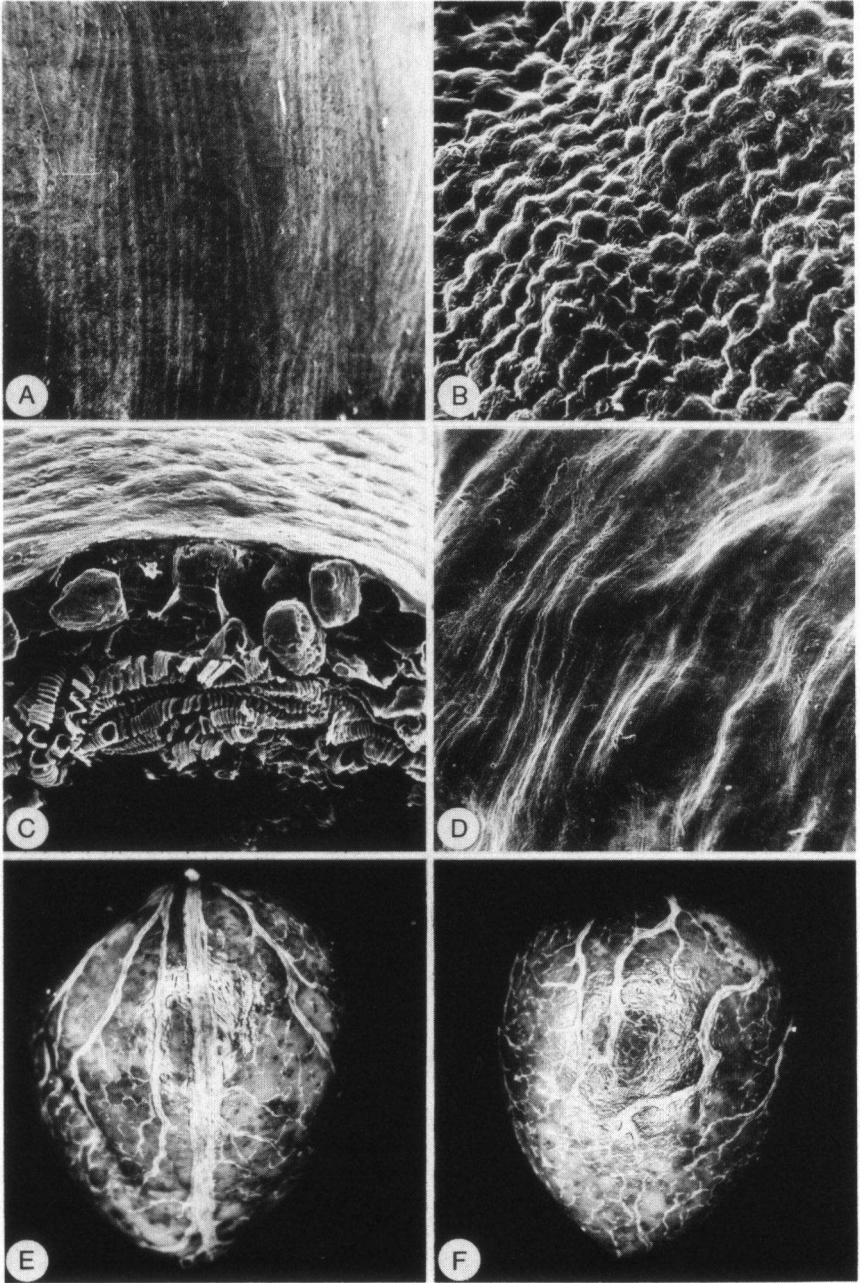
The cotyledons of *D. cymulosum*, *D. guineense*, *D. madagascariense*, *D. bangii* and *Tapura africana* are rich in starch but poor in lipids.

4. DISCUSSION

The Dichapetalaceae apparently all have a trizonate ovule primordium, a well-vascularized, bitegmic ovule, and a one-layered nuclear endosperm.

In addition the members of this family exhibit a number of clearly derived features, namely the tenuinucellate condition, the early resorption of the nucellus, and the completely dermal derivation of the o.i.

The seed-coat is structurally not very complex. The i.i. is completely resorbed, the o.i. partially so and ultimately becoming compressed. Only the outer layer of



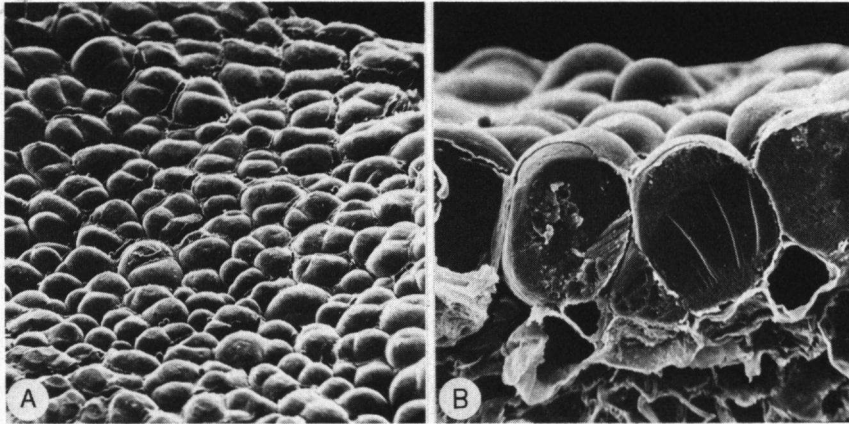


Fig. 6. *Tapura africana*. SEM photomicrographs of immature seed-coat.

A: seen from above ($\times 200$); B: t.s. of peripheral part of seed-coat with cells filled with tannin ($\times 500$).

the o.i. differentiates into a tanniferous exotesta with thickened cell walls. The embryo is large and surrounded by the thin endosperm. The relatively simple texture of the seed-coat is associated with the indehiscent type of fruit, a fleshy drupe, the endocarp providing the protective layer for the seed.

The dichapetalous seeds are relatively large. The extensive network of vascular bundles in the seed-coat is most probably connected with the size of the seed and the embryo. The vascular strands run in the periphery of the o.i., the cells of which are only compressed during the final stages of seed maturation, so that the vascular tissues remain functional for a long time.

It is striking that in *Tapura africana* the cells of the innermost layer of the i.i. stretch periclinally and are somewhat reminiscent of an endothelium before they are resorbed by the developing e.s. This behaviour of the cells of the innermost i.i. layers differs from that in Linaceae and Geraniaceae in which this layer remains intact during the oogenesis and the later stages ultimately to become a tanniferous cell layer (BOESEWINKEL & BEEN 1979). In the post-fertilisation phase the inner layer of the o.i. of *Dichapetalum mombuttense* develops into a layer with a distinct structure which may be a vestige of an endotesta. In the relatively thick seed-coat of *D. bangii* (considered to be primitive by PUNT 1975) diffuse, lignified sclerenchym cells are present. The question whether these cells represent the remains of a former mesotesta is altogether speculative.

Fig. 5: SEM photomicrographs (A, B, C and D) and photomicrographs (E and F).

A: fully matured seed-coat of *Dichapetalum mombuttense* (approximately $\times 75$); B: immature seed-coat of *D. mombuttense* ($\times 250$); C: t.s. of immature seed-coat of *D. mombuttense* ($+ 350$); D: fully matured seed-coat of *Dichapetalum madagascariense* ($\times 75$); E, F: raphal and antiraphal sides of seeds of *D. cymulosum* showing vascular bundles in seed-coat (approximately $\times 5$).

The pluri-layered epidermis as found in *D. bangii* may conceivably be used as a diagnostic character to separate certain sections of the genus. It is especially the absence of a well-defined structure in the mature seed-coat that renders a comparative morphological analysis based on seed-coat characteristics not very reliable for the recognition of relationships of the Dichapetalaceae with other families – compare CORNER (1976: Vol. I, 240): “It is this simplification (in plain exotestal and overgrown seeds) which renders so difficult the assessment of exotestal families devoid of other details of the seed-coat”.

Although most families at one time or another supposed to be related to the Dichapetalaceae are classified in the orders 9–15 of CRONQUIST's (1968) Subclass Rosidae, the views of taxonomists are so diverse and controversial that they do not help us much. As far as the embryological data and the seed-coat anatomy have been recorded, all taxa of suggested affinity are bitegmic and crassinucellate (Celastraceae and Linaceae partly tenuinucellate). Often remnants of the nucellus remain discernible in the mature seed (Euphorbiaceae, Thymelaeaceae, Polygalaceae and Malpighiaceae) and a markedly differentiated seed-coat is always present which contains at least vestiges of the i.i. and often a clearly differentiated exotegmen. For a better substantiated judgment additional, and more detailed, information concerning ovule and seed-coat structure of taxa of the Malpighiales and Polygalales must have become available: especially some representatives of the Malpighiaceae and Polygalaceae have drupaceous fruits (BOESEWINKEL, 1980).

On the basis of the (at present still fragmentary) knowledge of the taxonomy, morphology, anatomy, palynology and embryology of the Dichapetalaceae and their putative allies the conclusion may be drawn that the Dichapetalaceae constitute a well-defined and isolated taxon whose phylogenetic relationships are difficult to assess.

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