

DEVELOPMENT OF OVULE AND SEED-COAT IN *AVERRHOA* (OXALIDACEAE) WITH NOTES ON SOME RELATED GENERA

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SUMMARY

The ovule primordium of *Averrhoa* is trizonate; both integuments originate dermally and are initially mainly 2-layered. The inner integument becomes 5- to 6-layered; its innermost layer is at first endothelium-like. The outer integument is multiplicative. Most cells of the seed coat are crushed. The crystal-bearing endotesta and the fibrous exotegmen constitute the mechanical layers of the seed. The outer walls of the seed epidermis have wall thickenings of a pectic nature covered with a rather thick cuticle. The seed of *Sarcotheca* is similar to that of *Averrhoa*. The ruminations are more pronounced and correspond with differences in the dimensions of the adjacent exotegmic cells. The smaller seed of *Dapania* is somewhat different from those of *Averrhoa* and *Sarcotheca*, but also has an oxalidaceous nature. *Averrhoa*, *Sarcotheca* and *Dapania* have to be retained in the Oxalidaceae.

1. INTRODUCTION

Recently the taxonomic position of the traditionally oxalidaceous genera *Averrhoa*, *Sarcotheca*, *Dapania*, *Lepidobotrys* and *Hypseocharis* has been discussed. Some authors assigned them to the families Averrhoaceae, Lepidobotryaceae and Hypeocharitaceae, thus separating them from the herbaceous Oxalidaceae sensu stricto. HUTCHINSON (1973) placed the Averrhoaceae among the Rutales (mainly because of their woody habit) and *Sarcotheca* (= *Connaropsis*) and *Dapania* in the Lepidobotryaceae among the Malpighiales. CHANT (1978) places *Averrhoa* together with *Sarcotheca* in the Averrhoaceae. Especially the views of Hutchinson are untenable, because of the close affinity between *Averrhoa*, *Sarcotheca* and *Dapania* (KNUTH 1931; VELDKAMP 1971), also expressed in the typical oxalidaceous seed-type of *Averrhoa* (CORNER 1976). The woody genus *Averrhoa*, probably of Indomalaysian origin, comprises two species, *Averrhoa bilimbi* and *A. carambola*, up to 12 m tall trees. The zoochorous, berry-like fruits are used for human consumption, but apart from some use as ornamentals the family Oxalidaceae is of little economic importance. Data relating to the anatomy and histology of the ovules and seeds of representatives of Oxalidaceae (and "Averrhoaceae") were summarized by NETOLITZKY (1926), DAVIS (1966) and CORNER (1976).

The embryology and seed anatomy of *Averrhoa* are only partially elucidated while that of *Sarcotheca* and *Dapania* are completely unknown. VENKATESWARLU (1936) found in *Averrhoa carambola* a crassinucellate, bitegmic anatropous

ovule, both linear and T-shaped tetrads, and the development of the chalazal megaspore into an 8-nucellate embryo sac of the *Polygonum* type. THATHACHAR (1942) confirmed these findings and in addition described nuclear endosperm formation. The mechanical layers of the seed coat in *Averrhoa* are mainly the endotesta (with crystal cells) and the fibrous exotegmen. NARAYANA (1970) refers to THATHACHAR (1942) erroneously by combining the name of *A. carambola* with a figure of the seed coat of *Biophytum sensitivum*. DAVE et al. (1975) and KUMAR (1975) studied the pistil and fruit wall of *A. carambola* and NARAYANA (1966) the floral anatomy of some Oxalidaceae. The above mentioned uncertainty concerning the true affinities of the traditionally oxalidaceous genera may only be cleared up when more embryological, anatomical and chemotaxonomical evidence has become available. For this reason the present study of the structure and development of ovules and seeds of *Averrhoa*, *Sarcotheca*, and *Dapania* was undertaken.

2. MATERIALS AND METHODS

Developmental stages of flowers of *Averrhoa carambola* Linné were collected in the Botanical Garden of the University of Amsterdam; flowers and developing and mature seeds of *A. bilimbi* Linné were gathered in the hothouse of the department of Plant Taxonomy and Plant Geography of the Agricultural University, Wageningen. Dry seeds of the following species were obtained from the collection of the Rijksherbarium Leiden: *Dapania grandifolia* Veldkamp: Nooteboom 1627 (Malaya, 1970). *Sarcotheca laxa* (Ridl.) Knuth: FRI 111647 (Malaya, 1968); FRI 20374 (Malaya, 1971); T & P 662 (Malaya, 1980). This dry material was softened by one overnight stay in 10% ammonia solution after which it was transferred to 70% ethanol or to a normal butyl alcohol series for infiltration with metacrylate. The material of *Averrhoa* was sectioned by standard microtome techniques in paraffin wax or methacrylate. For specific staining solutions of phloroglucinol-HCl, Sudan IV, ruthenium red and JKJ were used. The SEM studies of in- and external parts of seeds, after cutting, fragmentation or peeling of certain layers, were carried out with a Cambridge Stereoscan mark 2a and ISI DS 130 after gold-palladium sputtering.

3. RESULTS

Averrhoa has a syncarpous ovary with 3–7 superposed pendulous ovules in each of the 5 locules. The berry-like fruit contains numerous seeds (VELDKAMP, 1971).

3.1 Ovule ontogeny of *Averrhoa carambola*

The ovule primordia are trizonate (*fig. 1A*). Only a single cell of the subdermal layer of the nucellar apex differentiates into an archesporium which divides into a parietal cell and a megaspore mother cell (*fig. 1B-D*). The megaspore tetrad

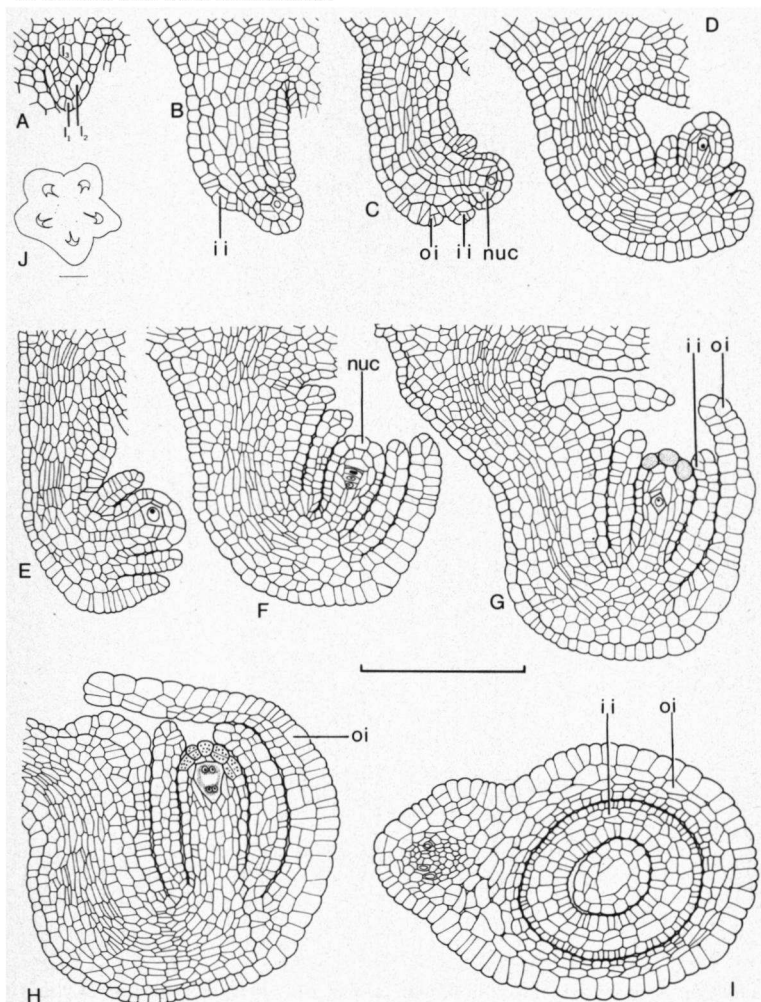


Fig. 1. *Averrhoa carambola*; A-H: longitudinal section of developing ovules; I: cross section of mature ovule; J: cross section of ovary with ovular primordia.

l_1 , l_2 and l_3 : dermal layer, subdermal layer and corpus, respectively. nuc = nucellus; i.i. = inner integument; o.i. = outer integument. The bar indicates 100 μ m.

is linear and the chalazal megaspore develops into the embryo sac (e.s.). The parietal tissue is 1-2 cells high. Shortly after the tetrad has been formed the apical, dermal nucellar cells enlarge, their cytoplasm becoming granular and more densely stainable (fig. 1G). Later most of these cells divide periclinally (fig. 1H). During further development the nucellus elongates rather strongly. The inner integument (i.i.) initiates before the outer integument (o.i.) as a complete ring-wall (fig. 1B, C). The ring-wall of the o.i. is only weakly developed at the raphe side. Both integuments arise from 2-3 celled dermal primordia

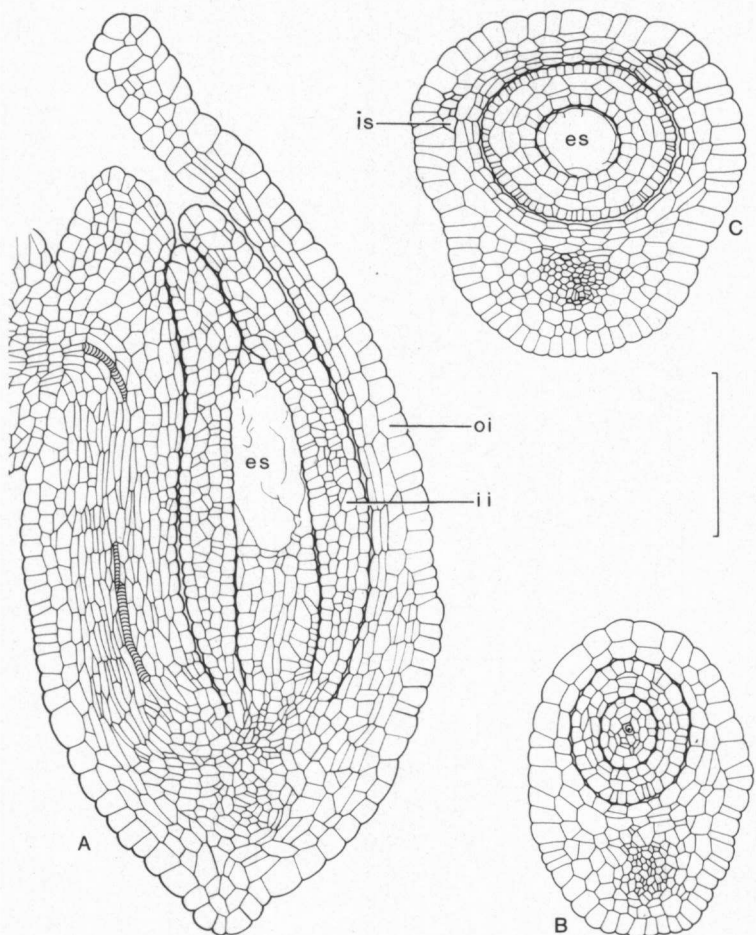


Fig. 2. *Aversrhoa bilimbi*; A: longitudinal section of mature ovule; B and C: cross section of young and mature ovule. e.s. = embryo sac; i.i. = inner integument; o.i. = outer integument; i.s. = integumentary strand. The bar indicates 100 μ m.

(fig. 1B, D) but soon are mainly 2-layered. When the i.i. has reached to the level of the nucellus top (fig. 1F, G) it has become 3-layered, mainly by periclinal divisions in its inner layer (see also fig. 2B, *A. bilimbi*). Finally the i.i. becomes 4- to 5-layered by subsequent divisions of the middle layer, but sometimes also of the inner layer (fig. 1H, I). The o.i. overgrows the i.i. and its top is bent over against the raphe, so that the exostome is situated next to the funicle (fig. 1H, see also fig. 2A). The exostome and endostome canals are not continuous but run zig-zag-wise (fig. 2A). When the e.s. is four-nucleate the cells of the inner layer of the o.i. have divided once and these divisions continue, so that the o.i. is multiplicative.

3.2 The mature ovule of *A. carambola* and *A. bilimbi*

The full-grown ovule of *A. carambola* and *A. bilimbi* is anatropous, bitegmic and crassinucellate (fig. 11, 2A). The nucellus is longitudinally stretched and the upper part is resorbed by the e.s., some dermal cells at the top excepted of which the remains persist. Both integuments are 4- to 6-layered. The inner layer of the i.i. is endothelial in character and contains tannins. Also in the outer layer of the o.i. tannins begin to form. The o.i. is much longer than the i.i., but the micropyle is formed by both. The exostome is triangular to slit-shaped and the endostome more or less isodiametric. The provascular strands of the funicle and raphe already show differentiated elements. Around the funicular bundle tannins begin to form. There is no obturator. The ovule development of *A. bilimbi* is similar to that of *A. carambola* (fig. 2B, C).

3.3 Seed development of *A. bilimbi*

The endosperm is nuclear. The subsequent wall formation takes place from the periphery of the e.s. inwards and becomes abundant, the nucellus being resorbed in the process. A cuticle reacting with Sudan IV develops between seed coat and endosperm. The i.i. becomes ultimately about 6 cell layers thick (see also CORNER 1976, II: p. 375). The cells of the middle layers soon are crushed. The inner layer of the i.i. with its endothelial character develops into a tannin layer consisting of tangentially flattened, thin-walled cells. The outer layer forms a fibrous exotegmen with longitudinally oriented and in cross section narrow cells provided with reticulate wall thickenings (fig. 3A, B).

The o.i. is multiplicative and may become up to 20 cell layers thick (fig. 3A-B). The cells of the inner layer early contain crystals and the inner tangential cell walls show some slight wall thickenings. This situation persists for a rather long time until shortly before maturity, when these cells are rather strongly stretched in radial direction and their inner tangential walls and the basal parts of their radial walls develop substantial, stratified wall thickenings. As a result the remaining lumen containing the crystal is restricted to the opposite part of the cell (fig. 3C). The cells of the middle layers of the o.i. divide repeatedly. In the o.i. of the mature ovule 3-6 vascular bundles start to differentiate (fig. 2C). In cross sections of the o.i. groups of small provascular elements can be observed below the outer epidermis. In the basal part of the o.i. these cells (fig. 2A) appear already stretched longitudinally. The testal bundles in later stages contain differentiated vascular elements. The mesophyll-cells attain a rather large size and are rounded with many intercellular spaces between them. Especially the inner layers are slightly thick-walled, mainly by pectic substances, and show wide pits. At the inside of the mesophyll-layer groups of smaller cells develop (fig. 3A, B) that exert a greater resistance against pressure and are less easily crushed when these immature seeds are subjected to desiccation. As a result the dry, immature seeds show a type of rumination reminiscent to the rumination of mature seeds of *Oxalis* and *Biophytum* (fig. 4A).

On the whole the endosperm and the i.i. are not affected by the rumination, but in the micropylar part of the seed the ruminations are more pronounced.

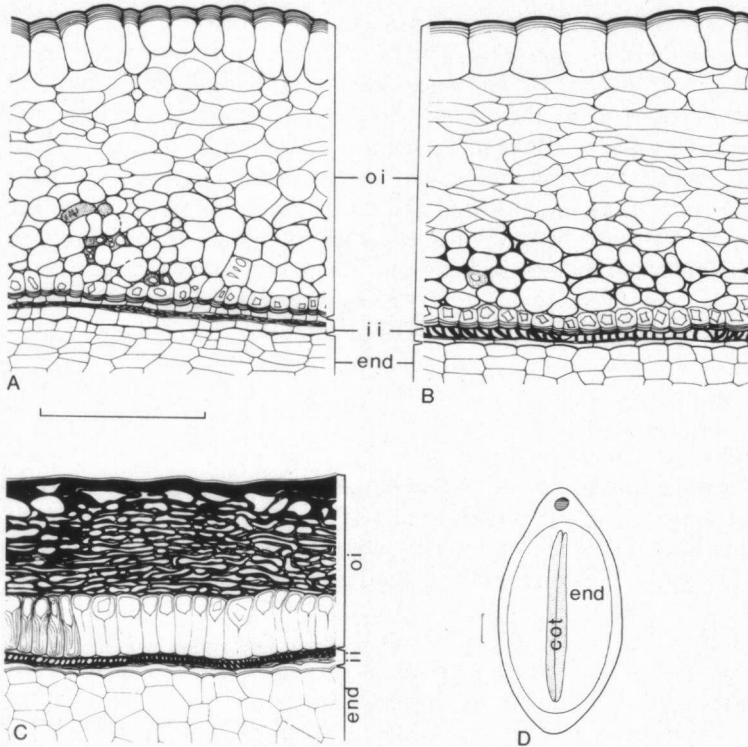


Fig. 3. *Averrhoa bilimbi*; A: longitudinal section of developing seed coat; B and C: cross section of developing and mature seed coat; D: cross section of mature seed. cot = cotyledon; end = endosperm; i.i. = inner integument; o.i. = outer integument. The bars indicate 100 μ m.

At an early stage of development the outer tangential walls of the outer cell layer of the o.i. become thickened and covered by a substantial cuticular layer. When the immature seed is allowed to dry-out the cuticle becomes wrinkled by the shrivelling of the seed coat (fig. 4C).

3.4 The mature seed of *A. bilimbi* and *A. carambola* and some notes on the seeds of *Sarcotheca* and *Dapania*

The seeds of *A. bilimbi* and *A. carambola* are similar. From the periphery going inwards the seed coat of *A. bilimbi* consist of the following parts: (fig. 3C, D)

1. The exotesta has a distinct cuticular layer, containing fatty substances, that covers the thickened pectic outer walls of the exotestal cells (fig. 4D). These cells are compressed and, therefore, in sections, cannot be readily recognised.
2. The crushed middle layers of the o.i., containing many starch grains. Their cell walls are slightly thickened by pectic substances. Because these cells are crushed the mature seed coat shows only slight rumination or none at all (fig. 4B).

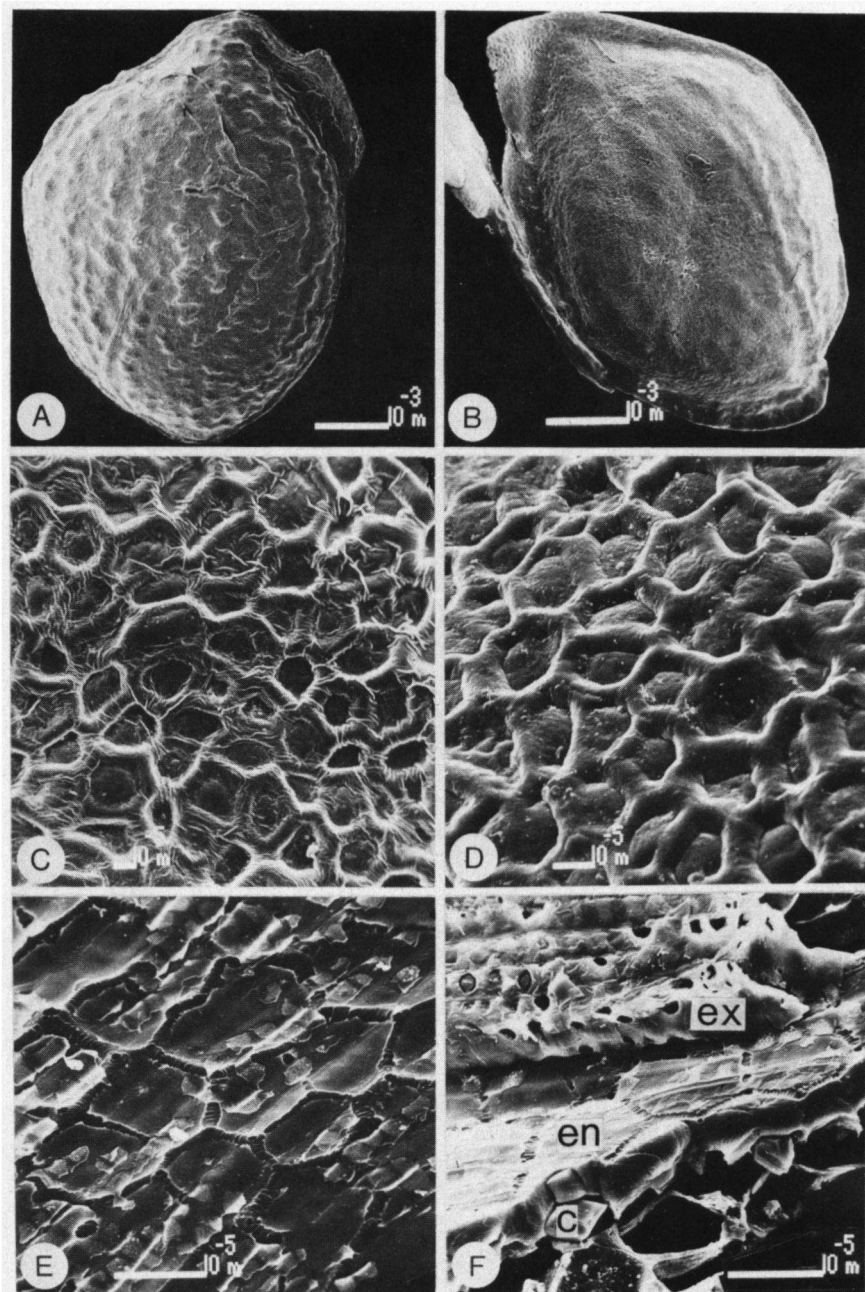


Fig. 4. *Averrhoa bilimbi*, SEM photomicrographs of seed. A, B: an immature and a mature seed; C, D: an immature and a mature seed; seed coats in surface view; E: endotesta after removal of exotegmen, showing adhering exotegmic wall parts, striation caused by contact with exotegmic cells and rupture of primary walls; F: endotesta (en) with crystals (c) partly covered by attached exotegmic cells (ex).

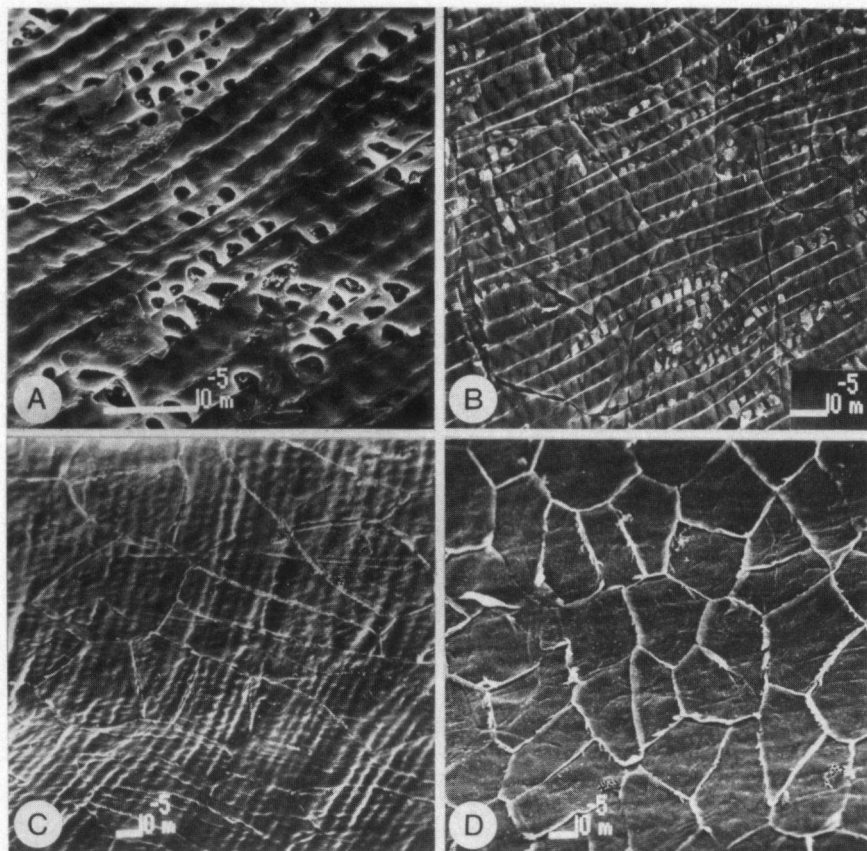


Fig. 5. *Averrhoa bilimbi*. SEM photomicrographs of seed. A: exotegmen in surface view, showing reticulate wall thickenings; B: compressed middle layers of the i.i. in outside surface view showing zones of contact with exotegmic cells and parts of their walls; C: crushed middle layers of the i.i. seen from the inside with imprints of exotegmic cells still visible; D: inner pigment layer of i.i. in surface view.

3. The endotestal cells, which are stretched, and have layered pectic wall thickenings (fig. 4E, F).
4. The exotegmen consisting of short and unligified fibres that seem to be somewhat tracheidal (reduced?) (fig. 4F, 5A).
Their length is rather variable but they are mostly 100–200 μm long. The exotegmen is multilayered at the base of the i.i. and near the endostome.
5. The isodiametric, compressed cells of the middle layers of the tegmen (fig 5B, C) with impressions of the exotegmen.
6. The isodiametric, tangentially flattened cells of the inner pigment-layer (fig 5D).
7. The rather copious endosperm and rather large embryo with small, flat cotyledons (fig. 3D). Both are rich in oils and have no starch.

The complex raphal bundle is amphicribal. A few postchalazal bundles run in the direction of the micropyle. The seed coat contains no lignins, the vascular bundles excepted. No hypostase and no chalazal tannin could be found.

The mature seed of *A. bilimbi* is dark brown and measures about $7 \times 5 \times 3$ mm (fig. 4B). The mature seed of *A. carambola* is somewhat longer than that of *A. bilimbi* and measures about $10 \times 5 \times 3$ mm. No vascular bundles were observed in the seed coat of *A. carambola*. The surface of both seeds is somewhat slippery when moist, which is probably caused by adhering remains of the fruit wall, or, as observed in fresh seeds of *A. carambola*, by substances produced by the raphal and hilar regions of the seed. The seed of *Sarcotheca laxa* externally is similar to that of *A. bilimbi* and *A. carambola*, but differs in some respects as to its architecture. The ruminations are more pronounced and can, also in the mature seed, be observed from the outside. Apparently there is also a localised, stronger mitotic activity in the o.i. Between the zones of mitotic activity the exotegmic cells are strongly stretched radially, so that the exotegmen appears undulate at the outside.

The embryo and endosperm of *Sarcotheca laxa* resemble those of *Averrhoa*.

The seeds of *Dapania grandifolia* are smaller and more circular in cross section as compared with those of *Averrhoa* and *Sarcotheca*. CHAUVEL (1903) reported the existence of an aril, but in our sections we could not detect such a structure. Possibly the colourful and fleshy surface of the fresh seed is a sarcotesta. The seed coat shows an endotesta with crystals and a well-developed exotegmen. No ruminations could be detected. The embryo has flat cotyledons.

4. DISCUSSION

The ovules and seeds of *Averrhoa* show a great deal of resemblance to those of *Oxalis* and *Biophytum* (BOUMAN 1974) (see also table 1).

The main differences between the ovules and seeds of *Averrhoa* and those of *Oxalis* and *Biophytum* lie in the simpler construction of the latter and their specialized type of dispersal. *Oxalis* and *Biophytum* have tenuinucellate ovules and the i.i. remains 3-layered. In the inner layer of the o.i. not so many divisions take place, so that the outer integument remains thinner, even only 3-layered in *Biophytum*. In this genus the ruminations of the seed coat are exclusively caused by the differential, radial stretching of the cells of the middle layer of the o.i., in *Oxalis* and *Averrhoa* by an increased mitotic activity of groups of cells in the middle layers of the o.i. The mature seeds of *Averrhoa* are hardly or not ruminant, in contrast to those of *Oxalis* and *Biophytum*, (BAHADUR et al., 1983). It appears that the simpler ovules and seeds of the herbaceous genera *Oxalis* and *Biophytum* are derived from the more complex and original seeds of arborescent progenitors, like those of *Sarcotheca*.

The seed structure of *Sarcotheca* agrees rather closely with that of *Averrhoa*. The function of the ruminations in *Averrhoa* and *Sarcotheca* is not clear, but in *Oxalis* and *Biophytum* they possibly prevent the shifting of the ejected part of the seed in respect of the overlying tissues, during active dispersal. *Dapania-*

Table 1. Comparison of ovule and seed characters of *Averrhoa*, *Oxalis* and *Biophytum*, and *Linum*.

<i>Averrhoa</i>	<i>Oxalis</i> + <i>Biophytum</i>	<i>Linum</i>
crassinucellate	tenuinucellate	crassi/tenuinucellate
nucellus resorbed early	nucellus resorbed early	nucellus resorbed early
i.i. initially 2-layered	i.i. initially 2-layered	i.i. initially 2-layered
i.i. finally 4- to 5-layered by divisions in middle layer	i.i. finally 3-layered	i.i. finally strongly multiplicative by divisions in middle layer
endothelium developing into inner pigment layer	endothelium developing into inner pigment layer	endothelium developing into inner pigment layer
middle layers crushed	middle layer crushed	middle layers crushed
fibrous exotegmen	fibrous exotegmen	fibrous exotegmen
multiplicative o.i. by divisions in inner layers	multiplicative o.i.	o.i. 2- to 3-layered by divisions of inner layer
endotestal crystal-layer	endotestal crystal-layer	no endotestal crystal-layer
ruminate seed	ruminate seed	no ruminant seed
zoochory	autochory	mucilage seeds

seeds are not ruminated, but the seed coat anatomy also appears to be oxalidaceous. There is also a good deal of correspondence between the other embryological characters of *Oxalis*, *Biophytum* and *Averrhoa* (DAVIS 1966). The e.s. is of the *Polygonum* type and the embryogeny of the Asterad type. The endosperm is nuclear. The 1–2 middle layers of the anther become crushed. The cells of the glandular tapetum become 2–4 nucleate and simultaneous cytokinesis follows meiosis in the microspore mother cells.

From all these corresponding characteristics one may conclude that *Averrhoa*, *Sarcotheca*, *Dapania*, *Oxalis* and *Biophytum* are closely related and that the last two genera are derived, herbaceous representatives of a single and essentially woody family. Hutchinson's classification of his *Averrhoaceae* under the Rutales and of *Sarcotheca* and *Dapania* under *Lepidobotryaceae* (Malthighiales) is unacceptable. The recognition of a separate family of the *Averrhoaceae* is also unwarranted and would render the classification unduly complicated.

It has often been assumed that the Oxalidaceae are related to the Linaceae (BOUMAN 1974; CORNER 1976). The anatomically more complicated and primitive ovules and seeds of *Averrhoa* (thicker integuments, crassinucellate) may well provide more conclusive evidence for this viewpoint. The ovules and seeds of *Averrhoa*, *Linum* and to a lesser extent also *Erythroxylum* (BOESEWINKEL 1980a, b, BOESEWINKEL & BOUMAN 1984 pp. 575–579) share many characteristics (table 1). The main differences between the ovules and seeds of *Averrhoa* and *Linum* are:

1. The thinner i.i. of *Averrhoa*, as compared to that of *Linum* (a more massive i.i. indicates a derived condition).
2. Only in *Averrhoa* is an endotestal crystal layer.
3. The thicker o.i. of *Averrhoa*, as compared to that of *Linum*. In Linaceae the contributes of the inner integument exceed that of the o.i., whereas in *Averrhoa* the outer integument is more strongly developed than the i.i., and it

is even vascularized in *A. bilimbi* (so as to provide an adequate quantity of nutrients to the rather large seed?).

4. The absence of ruminations in the seed of Linaceae.

According to VAN WELZEN & BAAS (1984) the relationships of the Lepidobotryaceae are obscure. They belong either to the Oxalidaceae or to the Linaceae complex. A family status of the Lepidobotryaceae is not supported by their leaf anatomy. OLTMANN (1971), concludes that the Lepidobotryaceae are palynologically closely related to the Oxalidaceae, but they are also akin to Linaceae (Hugoniaceae). HEIMSCH (1942), on the other hand, states that the xylotomic characters do not indicate a close relationship between Oxalidaceae and Linaceae. Also according to OLTMANN, *Hypseocharis* takes an intermediate position between Oxalidaceae and Geraniaceae. BEHNKE (1982) suggests a relationship of Oxalidaceae with Connaraceae on the ground of the occurrence of P-type sieve-element microplastids in representatives of both Oxalidaceae and Connaraceae. See also the discussion by VELDKAMP (1971: 152, 153). The construction of the seed coat of Connaraceae with endotestal crystal cells and a fibrous exotegmen does not contradict this assumption.

5. ACKNOWLEDGEMENTS

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