

# Ovule and seed characters of *Balanites aegyptiaca* and the classification of the Linales–Geraniales–Polygalales assembly

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## SUMMARY

*Balanites aegyptiaca* (L) Del. has a trizonate ovule primordium and an elongated bitegmic, crassinucellate and anatropous ovule. The dermal inner and outer integument are about 4–5-layered and the inner layer of the inner integument becomes slightly endothelial. After fertilization the nucellus and subsequently the inner integument are resorbed. The outer integument ultimately becomes about 30-layered. The cells of the inner layer of the outer integument divide once periclinally. The vascularized seed-coat consists of parenchyma cells with scattered, lignified sclerenchyma elements. Ovule and seed characters support the recognition of a separate family Balanitaceae and a relationship with an enlarged order Linales.

*Key-words:* ovule, seed, taxonomic relationships, *Balanites aegyptiaca*, Zygophyllaceae, Linales.

## INTRODUCTION

*Balanites* is the only genus in the monotypic family Balanitaceae. The genus comprises about 28 species but according to Maksoud & Hadidi (1988) Sands has proposed to reduce this number to only about nine species, seven of which occur in Africa and the remaining two in Asia; see also Sands (1983). They are thorny shrubs or trees with fleshy edible fruits. *Balanites aegyptiaca* is distributed from Tanzania and Guinea through the Sahara, Egypt, Yemen and Iran to India and Burma. Sands recognizes five varieties of *Balanites aegyptiaca* which vary as regards to the colour of the leaflets, the pubescence, the length of the spines and the number of flowers per corymb as well as the size and shape of the drupe. The species, which occurs in arid zones, grows very slowly and has a slow fruit development.

The literature on the embryology has been summarized by Davis (1966), and that on seed-coat anatomy by Corner (1976). The ovule of *Balanites* is anatropous, bitegmic and crassinucellate with an upwards directed micropyle. The multicellular archesporium consists of 1–4 cells, only one of which undergoes meiosis and develops into a linear tetrad. The chalazal megaspore develops into a functional embryo-sac of the Polygonum type (Nair & Jain 1956). The fruit is a fleshy, oily drupe of plum size with a brown sticky pulp enclosing a bony stone which contains one large, yellow, ovoid seed about 15 mm long.

The chlorophyllous acuminate embryo is ovoid with two thick oblong planoconvex cotyledons and a short hypocotyl. The nuclear endosperm has become completely resorbed (Corner 1976). The vascularized seed-coat consists of parenchyma cells, which may contain single or cluster crystals and scattered mesotestal sclerotic cells (Vaughan 1970). The seed contains about 50% oil and 20% crude proteins. Chapman *et al.* (1992) suggest that the seeds are toxic.

Reproduction is through seeds and budding from stumps and roots (Parameswaran & Conrad 1982). According to Chapman *et al.* (1992) the endozoochorous dispersal of the seeds of *Balanites wilsoniana* by elephants significantly increases the probability of a successful germination. These animals are vital to the dispersal of the seeds, and it is suggested that there is a highly interdependent plant–animal seed dispersal system in this species.

The genus *Balanites* was first treated as a member of the Simaroubaceae (Bentham & Hooker 1862), then as the monogeneric subfamily Balanitoideae of the Zygophyllaceae (Sleumer 1931; Cronquist 1981) and currently as the monogeneric family Balanitaceae. This family is grouped under either Geraniales (Takhtajan 1973; Dahlgren 1983; Dahlgren 1989), Malpighiales (Hutchinson 1973) or Linales (Thorne 1992a,b). Of the modern authors only Cronquist (1981) still treats *Balanites* under Sapindales. The Geraniales are, at least by Dahlgren (1983, 1989) and Takhtajan (1973), placed in the superorder Rutanae or Rutiflorae and by Thorne (1992a,b) under the superorder Geraniaeae. On the ground of epidermal characters, Vyayalakshmi Sarma & Shanmukha Rao (1991) advocate the subfamily status for *Balanites* within the Zygophyllaceae. Phytochemically, *Balanites* shows affinities with certain taxa of Zygophyllaceae (Hegnauer 1973, 1990), but evidence from the leaf vascularization, the floral morphology and the pollen structure still favours a very isolated position of *Balanites* (Maksoud & Hadidi 1988). On wood anatomical grounds, Parameswaran & Conrad (1982) favoured the family status for *Balanites* but Fahn *et al.* (1986), based on the same characters preferred the subfamily status within the Zygophyllaceae. From this it appears that the opinions concerning the relationships of *Balanites* are very diverse, but most authors place the Balanitaceae in the vicinity of the Zygophyllaceae or at least assume some degree of relationship with that family.

## MATERIALS AND METHODS

Developmental stages of flowers and fruit of *Balanites aegyptiaca* (L) Del. were collected as liquid material by J. J. de Wilde 4791 in Ethiopia and by F. Bouman s.n. in Curaçao (Klein Sint Joris).

Sections were made with a Reichert Semi-Dünnschnitt microtome with glass knives after embedding in glycol metacrylate. The sections were stained with the periodic acid Schiff's reaction and counterstained with aqueous toluidine blue. Phloroglucinol-HCl, Sudan 4, Ruthenium red, IKI and Nigrosine solutions were used for the identification of lignins, lipids, pectins, starch and proteins, respectively.

For scanning electron microscopy (SEM), dry seeds were gold- or gold/paladium sputtercoated for about 2.5 min and studied on an ISI DS/30.

## RESULTS

### *Balanites ovule development*

The ovary is mostly five-loculed and each locule contains one erect ovule hanging from the axis below the apex of the locule. The ovule primordium is trizonate (Fig. 1a). The

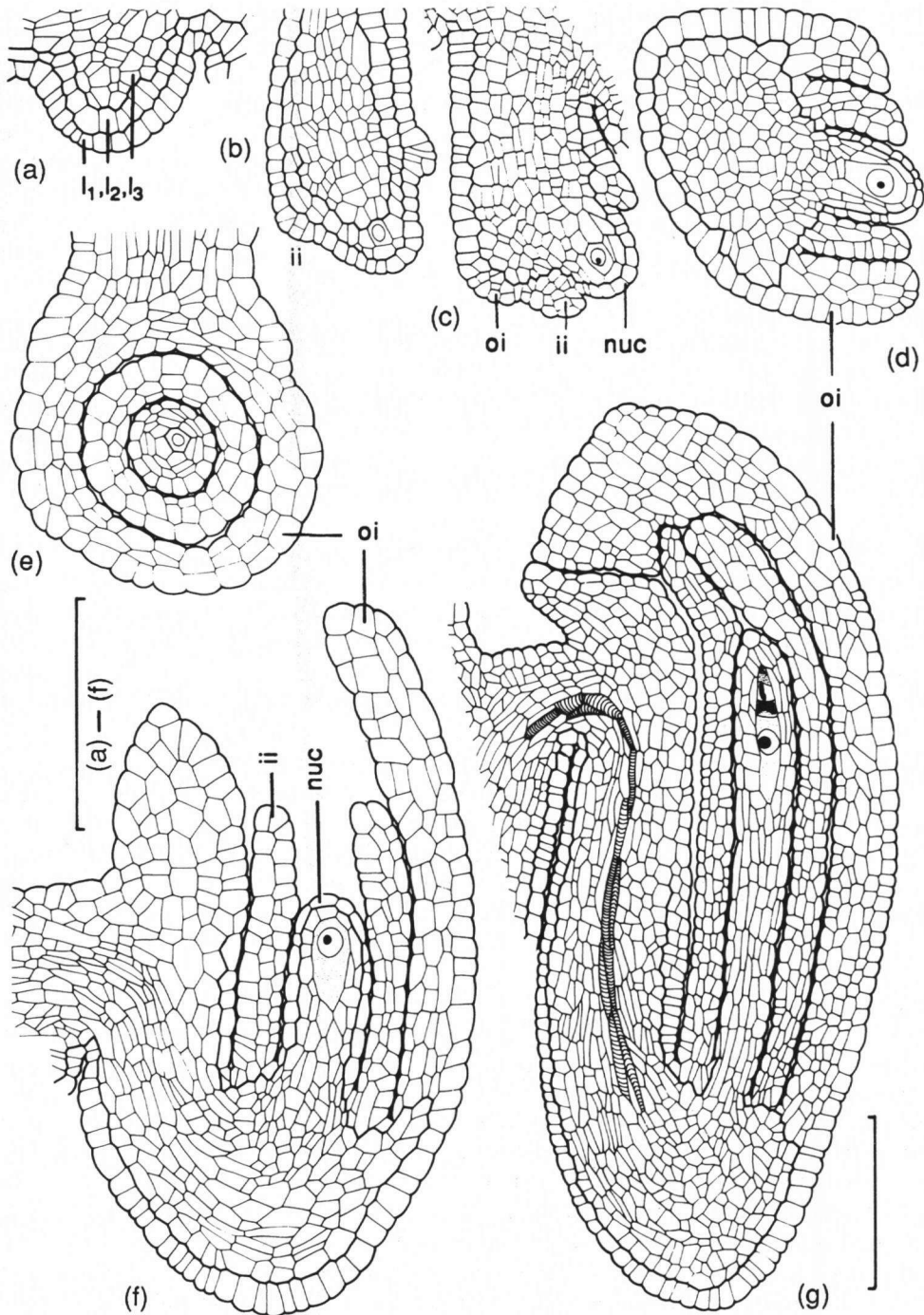


Fig. 1. *Balanites aegyptiaca*. Ovule development: (a) ovule primordium; (b), (c), (f) and (g) longitudinal sections of developing ovules with (g) a strongly elongated nucellus with megaspore tetrad; (d) and (e) transmedian and cross-section of developing ovules.  $l_1, l_2, l_3$ : dermal layer, subdermal layer and corpus, respectively. nuc, nucellus; ii, inner integument; oi, outer integument. Scale bars 100  $\mu$ m.

subdermal archespire, which may be multicellular, differentiates and already cuts off a parietal cell at the time of the initiation of the inner integument (Fig. 1b). The nucellus becomes slender and very elongated (Fig. 1d–g). Meiosis takes place when both integuments have grown around the nucellar top to form the micropyle (Fig. 1f, g). The megaspore tetrad is linear and the chalazal megaspore develops into the embryo sac (es). The inner integument (ii) is initiated somewhat earlier than the outer integument (oi) (Fig. 1b, c). Both integuments are of dermal origin and are initiated as circular rims of about 3–4 cells thick and both remain initially 3–4-layered (Fig. 1c–f). The oi at the raphal side remains much shorter than at the antiraphe. At the time of meiosis the oi starts to become much thicker than the ii. During its development the ovule becomes much elongated longitudinally.

In the material from Curaçao, one abnormal, downwards pointing ovule was found. Also, the development of the ovules in this material may differ, and then the middle layers of the ii divide strongly, so that it becomes very thick. As a result, the es is compressed and the development of the ovule seems to become abnormal. In particular, the nucellus and the integuments of these ovules contain many starch grains. Also, the ovary is hairy, in contrast to the smooth ovaries of the Ethiopian material.

#### *The fully developed ovule*

The ovule is bitegmic, anatropous and crassinucellate. Figures 1g and 2a show the ovule shortly before and after maturity. Not more than about two layers of parietal cells were observed. The strongly longitudinally elongated nucellus is largely resorbed (Fig. 2a), its base excepted (cf. Fig. 2b). In the top of the es the surrounding ii develops wall ingrowths. The inner layer of the 4–5-layered ii has more or less assumed an endothelial character with radially stretched and plasm-rich cells. The oi has become about five-layered. Both integuments partake in the formation of the zig-zag micropyle.

The raphal bundle is well developed and consists of vascular xylem elements with net-shaped wall thickenings and oblique separation walls. The chalaza is relatively large and contains a few separated procambial strands. The ovule is strongly longitudinally elongated. The tip of the oi and the stylar channel stain more densely which may be related with pollen tube conduction.

#### *Seed development and mature seed*

After fertilization only one of the five ovules develops further, the other ones degenerate. At first the overall shape of the ovule does not change much (Fig. 2a), i.e. it remains longitudinally elongate and becomes rich in starch grains. Soon a thin layer of nuclear endosperm is formed, but the mature seed is exendospermous. Shortly after fertilization the ii is crushed and resorbed from the inside, its top remaining intact longest. During this process some of its cells swell and collapse, discharging their content into the es. A few remains of the ii can still be found on the inside of the seed-coat (Fig. 2c). The oi develops further and starts to thicken mainly by periclinal divisions in its middle layers. A small number of the outer cells may divide periclinally once (Fig. 2b and c). Ultimately, the seed-coat becomes about 30 cells thick. The seed-coat consists of more or less isodiametrical parenchyma cells with scattered lignified sclerenchyma elements (Fig. 3b, and e) which also react somewhat positively with Ruthenium red. The shape of these sclerenchyma cells may approach that of adjoining xylem elements. The cells of the inner layer of the seed-coat divide about once periclinally and the arising two inner cell layers consist of small and thin-walled cells in comparison with the other seed-coat

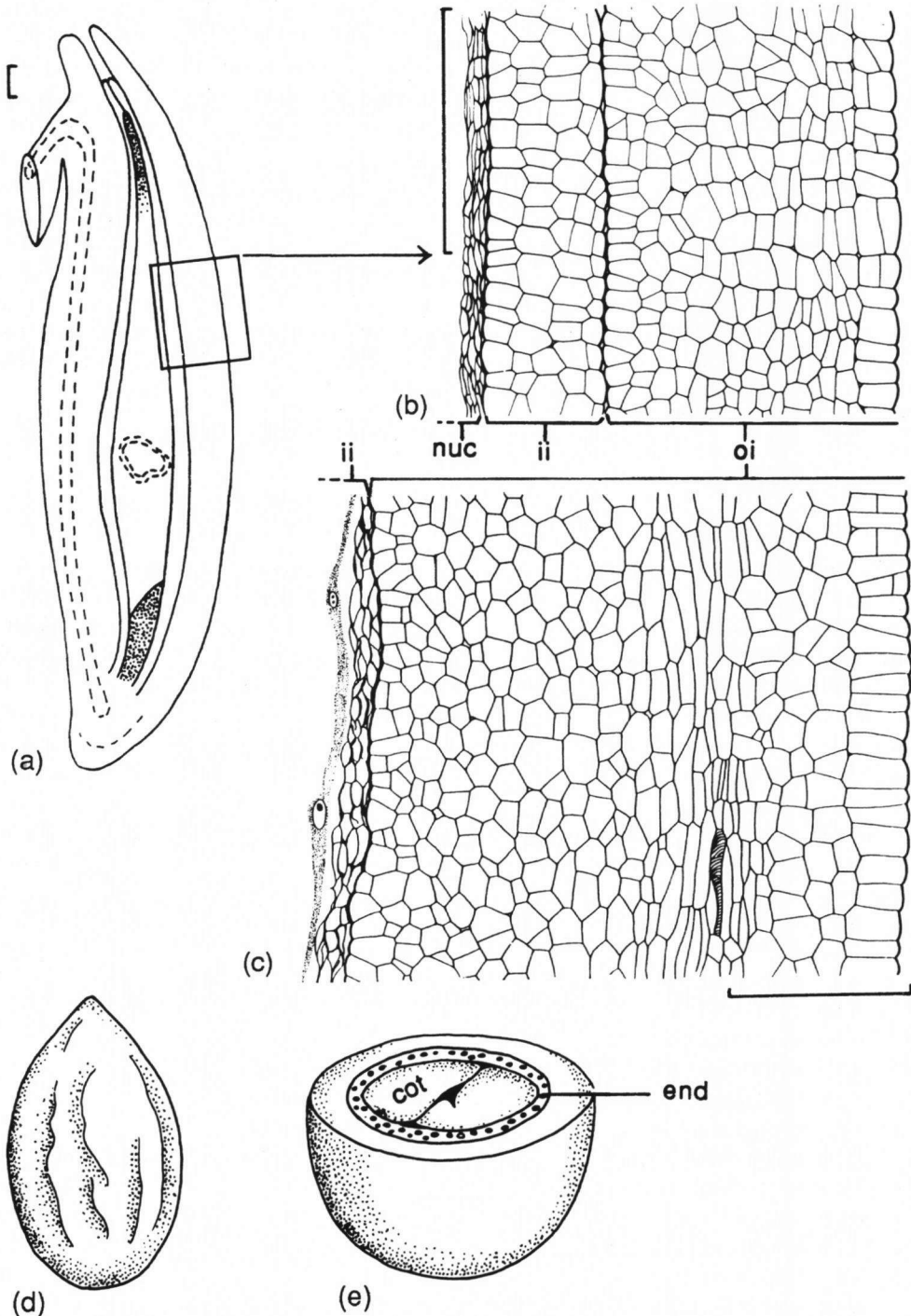


Fig. 2. *Balanites aegyptiaca*. Seed development: (a) and (b) longitudinal sections of an early developing seed, outline and detail, respectively, showing developing seed-coat and nucellar remnants; (c) longitudinal section of developing seed-coat with crushed remnants of ii, starting differentiation of vascular bundle (by periclinal divisions) and nuclear endosperm; (d) and (e) mature seed and cross-section of fruit with cotyledons of embryo within endocarp. cot, cotyledon; nuc, nucellus; ii, inner integument; oi, outer integument; end, endocarp. Scale bars 100  $\mu$ m.

cells (Figs 2b, c and 3a, b). The outer cells are isodiametrical, relatively large and may incidentally become slightly sclerotic (Fig. 3a, c).

Soon after fertilization procambium elements, which gradually develop into a network of postchalazal testal bundels, differentiate in the outer peripheral middle layers (Figs 2b, c and 3a). Most of these bundles run from the chalaza to the micropyle with smaller anastomosing branches between them. The vascular bundles consist of phloem on the outside, composed of thin-walled cells with sieve-plates, and a xylem part on the inside composed of elements with ring- spiral-, net-shaped, or pitted wall thickenings (Fig. 3a and d). The youngest elements are provided with ring or spiral thickenings. The seed-coat adheres strongly to the endocarp.

The mature embryo measures about  $15 \times 10$  mm, has slightly thickened and pitted walls and is rich in fatty substances and proteins and poor in starch. The shape of the ovule and that of the mature seed differ considerably (Fig. 2a and d), which is probably mainly caused by the shape of both the locule and the short, thick embryo.

## DISCUSSION

### *Relationships of the Balanitaceae*

The ovule of *Balanites* is anatropous and longitudinally elongate with a slender nucellus. On the basis of inadequate drawings, Nair & Jain (1956) concluded that the ovule is campylotropous and Davis (1966) calls it hemitropous, but these statements are incorrect. The micropyle is somewhat pushed sideways against the funicle by the locule wall but the main parts of the ovule, nucellus and embryo are straight. The ovule characters, especially the slender nucellus, the multi-layered ii and the endothelium, point to a relationship with the Linales alliance. The families of this alliance are, generally speaking, characterized by crassinucellate ovules, a stretched, but not too large nucellus, a more or less secondarily thickened ii, an endothelium and a seed-coat with a fibrous exotegmen (Boesewinkel 1980, 1985a,b, 1987; Boesewinkel & Geenen 1980; Boesewinkel & Venturelli 1987). These characters are not found in the families of Geraniales and Polygalales. The resorption of the ii and the vascularized seed-coat without definite mechanical layers (see also Vaughan 1970) point to a derived condition of *Balanites*. The postchalazal vascular bundles in the seed-coat probably function in the necessary transport of nutrients to the large embryo which accumulates an appreciable quantity of fats and proteins. The scattered mesotestal sclerenchyma cells may prevent a complete crushing of the seed-coat.

It is known (Corner 1976) that the seed-coat of large overgrown seeds has often lost its original character or that it is degenerated, so that it is difficult or impossible to draw conclusions regarding relationships from the seed-coat texture alone.

*Balanites* has drupes, which is also the case in the representatives of some families of the Linales, such as Humiriaceae and Erythroxylaceae which, however, have often retained their exotegmic character. In the Geraniales and Polygalales drupes are not a common occurrence. Hill (1937) has concluded that at the time of germination the drupes of *Balanites*, *Canarium* (Burseraceae) and *Saccoglottis* (Humiriaceae) open in about the same way with shutters.

The large seed of *Irvingia gabonense* is provided with a seed-coat, very similar to that of *Balanites* (Corner 1976). Its fruit is also a drupe. However, in the ovule of *Irvingia* the outer layer of the ii consists of longitudinally elongated, plasm-rich cells which resemble

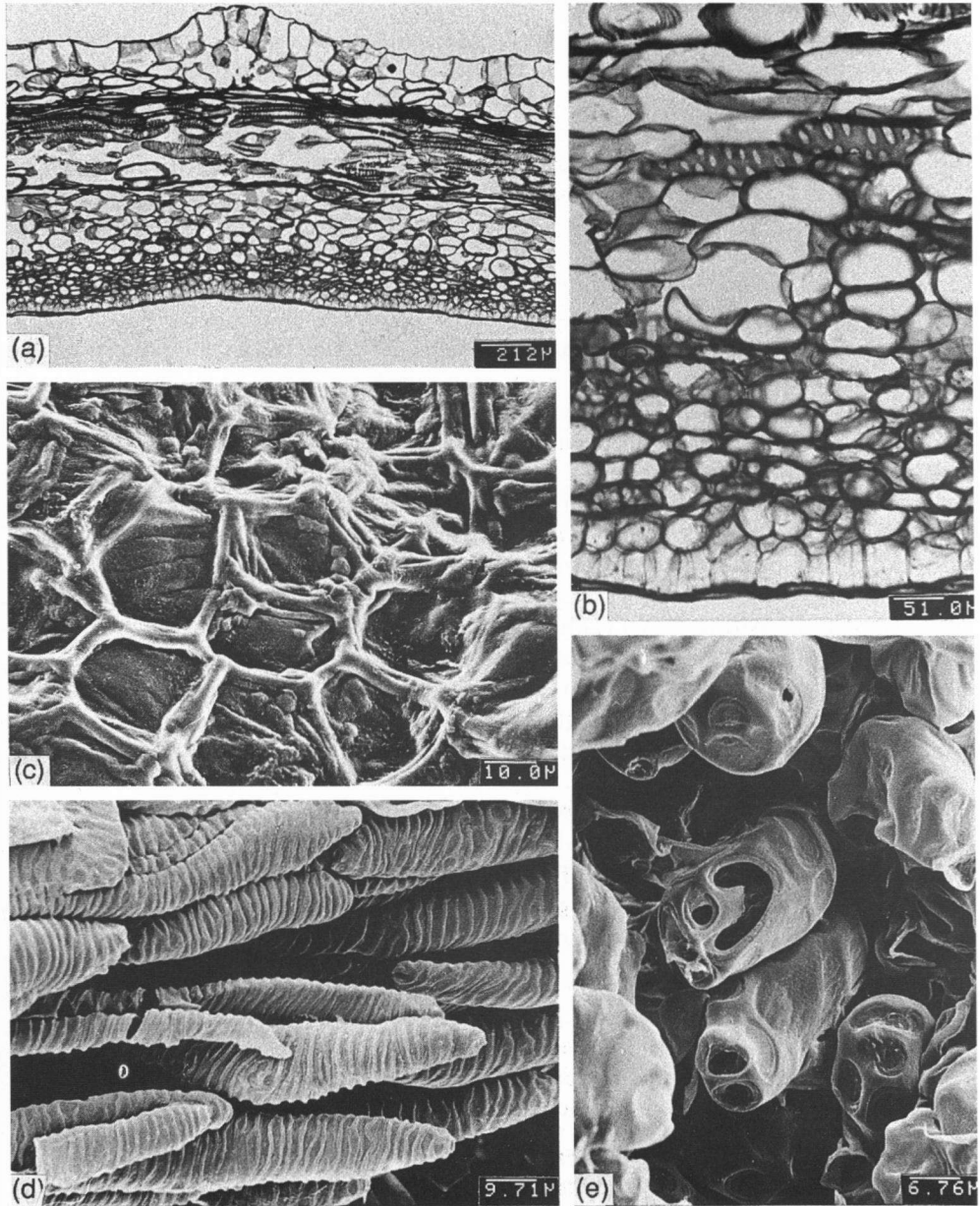


Fig. 3. *Balanites aegyptiaca*. Mature seed-coat: (a) and (b) length sections of seed-coats with vascular bundle and detail of inner part with pitted sclerotic cells, respectively; (c)–(e) SEM photographs of seed-coat surface, spiral elements of testal vascular bundle and pitted sclerotic cells, respectively.

an early stage of a developing exotegmen (Boesewinkel, personal observation). So a grouping of *Irvingia* together with the exotegmic *Klainedoxa* under Linales seems justified from evidence of the seed-coat anatomy (see also Thorne 1992a,b).

A very close relationship of *Balanites* with the Zygophyllaceae is not sustained by seed anatomy because the seed-coat of the latter family is, generally speaking, characterized

by an endotestal crystal layer and a lignified endotegmen. In *Balanites* the ii disappears completely and, in contrast to the *Zygophyllaceae*, there is no endotestal crystal layer, while even the inner epidermis of the oi divides at least once during seed development. These facts support a separate family status for *Balanitaceae*. However, because the seed-coat of *Zygophyllaceae* is rather variable, the seed-coat anatomy of *Balanites* may not be decisive in rejecting a relationship with *Zygophyllaceae*. In summary, a relationship of *Balanitaceae* on the basis of ovule and seed characters with the *Linales* is most likely.

*Grouping of the Balanitaceae and other families in the orders Linales, Geraniales and Polygalales*

There is a broad consensus concerning the delimitation of the major families in the above-mentioned orders but there is much uncertainty as regards which families belong to what higher-level taxa. In general the *Linales*, *Geraniales* and *Polygalales* are considered to be related and grouped in the same higher level taxon mostly referred to as *Rutanae* (*Rosidae* by Cronquist 1981 and *Geranianae* by Thorne 1992a,b), but the systems differ in the composition of the orders and number of families (Hutchinson 1973; Takhtajan 1973; Cronquist 1981; Dahlgren 1983, 1989; Thorne 1992a,b). The main differences between the modern systems can be summarized as follows.

A separate order *Linales* or *Malpighiales* is recognized (Cronquist 1981, Hutchinson 1983; Dahlgren 1989) or not (Takhtajan 1973; Dahlgren 1983). The *Malpighiaceae* are mostly placed in *Polygalales* (also named *Malpighiales* by Thorne 1992a) but Hutchinson groups this family in a separate order *Malpighiales*. The families *Oxalidaceae*, *Balanitaceae*, *Zygophyllaceae* and *Tremandraceae* in particular are placed in different orders by the various authors (see Table 1).

*A classification based on ovule and seed-coat structure*

Corner (1976) tried to improve the classification by emphasizing the importance of seed-coat characters as indicators of relationships. He concluded that in the modern systems especially the *Geraniales* s.l. show a mixture of different seed-coat types and are therefore heterogenous. Corner distinguished four categories.

- (1) *Malpighiales* with fibrous exotegmen.
- (2) *Geraniales* with stellate exotegmic cells.
- (3) *Polygalales* with an endotesta.
- (4) *Balsaminaceae*, *Limnanthaceae* and *Tropaeolaceae* which are pachychalazal and/or more or less unitegmic.

According to Corner, the fibrous exotegmic seed is monophyletic. In the diagram of Dahlgren (1991) the largest concentration of taxa with a fibrous exotegmen indeed can be found in the *Rutales*–*Sapindales*–*Geraniales* assembly. But other taxa with exotegmic fibres such as *Violales*–*Caparales*, *Euphorbiales*, *Myrtales*, *Proteales*, *Papaverales* and *Aristolochiales* do not seem to be closely related. Dahlgren (1991) nevertheless considers, amongst Corner's seed-coat types, those with exotegmic fibres to be of particular phylogenetic significance, and remarks that its common occurrence in some families of, e.g. *Linales* should be regarded as important since these families have quite a number of characters in common.

The studies of the present author (Boesewinkel 1980, 1985a,b and 1987) strongly support most of Corner's views.





Accordingly, it seems preferable to arrange those families of the Linales, Geraniales and Polygalales with a fibrous exotegmen in a large order Linales. The term Linales is preferred over Malpighiales because the large campylotropous ovules and most of the seeds of Malpighiaceae are evolutionary derived and not characteristic for the other anatropous representatives of the Linales.

Families which (besides other ones) according to their fibrous exotegmen or ovule characters most probably belong in the Linales are Linaceae, Erythroxylaceae, Humiriaceae, Malpighiaceae, Trigoniaceae (Boesewinkel 1987), Vochysiaceae (Boesewinkel & Venturelli 1987), Tremandraceae (Boesewinkel, personal observation), Ixonanthaceae, Irvingiaceae, Oxalidaceae (Boesewinkel 1985), Zygophyllaceae (Corner 1976) and Balanitaceae (present paper).

Next to the exotegmic character, some of the above-mentioned families develop additional characteristic seed layers, e.g. the inner layer of the ii develops in *Linum* into a slightly thick-walled inner pigment layer and in Malpighiaceae and Zygophyllaceae as a thick-walled endotegmen.

Heimsch (1942) supported, on wood anatomical grounds, the relationship of a number of the above-mentioned families in the Linales. He considers the Erythroxylaceae, Malpighiaceae, Trigoniaceae, Vochysiaceae, Tremandraceae, Zygophyllaceae and Polygalaceae related to the Linaceae and Humiriaceae. On the basis of seed-coat anatomy the endotestal Polygalaceae are misplaced here. Several authors relate the Polygalaceae with families of the Linales. There is also palynological evidence for a common ancestry for the Zygophyllaceae, Oxalidaceae and Linaceae (Oltmann 1971).

The proposed classification on seed characters differs in the following features from recent classifications: The Linaceae, Oxalidaceae, Zygophyllaceae, Balanitaceae, Malpighiaceae, Vochysiaceae, Trigoniaceae, Tremandraceae, etc. are to be transferred to the Linales. As a result of these transfers the Linales become a large order and the Geraniales and Polygalales become much smaller (see Table 1).

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