# The structure of the seed of *Mangifera indica* L. and notes on seed characters of the tribe Mangifereae (Anacardiaceae)

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The anatropous, unitegmic and pachychalazal mango ovule develops into the campylotropous, pachychalazal seed. The undifferentiated seed coat is of dual origin, developing from the integument as well as from the pachychalaza. The term 'peritesta' is suggested for the peripheral, band-like integumentary part of the seed coat. The major saddle-shaped chalazal part is associated with a tanniniferous hypostase. The discussion includes the anatomy and histo-chemistry of the seed. The pachychalazal seed with undifferentiated seed coat probably characterizes the tribe Mangifereae. After consideration *inter alia* of seed characters, it is concluded that, according to generally accepted criteria, the Anacardiaceae is phylogenetically one of the more advanced forest families.

Die anatrope, unitegmiese en pagichalasale saadknop van die mango ontwikkel tot 'n kampilotrope, pagichalasale saad. Die ongedifferensieerde saadhuid is van tweeledige oorsprong en ontwikkel uit die integument sowel as die pagichalasa. Die term 'peritesta' word voorgestel vir die periferale bandagtige integumentêre deel van die saadhuid. Die grotere saaltjievormige chalasale deel is met 'n looisuurhoudende hipostase geassosieer. Die bespreking sluit in die anatomie en histochemie van die saad. Dit blyk dat die pagichalasale saad met ongedifferensieerde saadhuid kenmerkend mag wees vir die tribus Mangifereae. Na oorweging van o.a. saadeienskappe, kan volgens algemeen-aanvaarde kriteria afgelei word dat die Anacardiaceae filogeneties een van die meer gevorderde woud families is.

Keywords: Anacardium, hypostase, mango, pachychalaza, peritesta

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# Introduction

The family Anacardiaceae is chiefly tropical and subtropical in its distribution and includes about 70 genera with c. 600 species (Hou 1978). Although the general structure of the seed of the Anacardiaceae has been investigated by several authors (e.g. Weber 1907; Juliano & Cuevas 1932; Ziesak 1972; Mitra & Mehrotra 1980), the seed coat as such, according to the assessment of Corner (1976), had received detailed attention in only a few species. The senior author of the present paper has since researched the ontogeny of the seed coat in three species of the Anacardiaceae (tribe Spondieae) indigenous to South Africa, viz. Lannea discolor (Sonder) Engl. (von Teichman 1988), Sclerocarya birrea (Richard) Hochst. subsp. caffra (Sonder) Kokwaro (von Teichman 1988), and Harpephyllum caffrum Bernh. ex Krauss. (von Teichman & van Wyk 1988) and described the raphechalazal seed and undifferentiated seed coat occurring in these taxa.

Mangifera indica L., of the tribe Mangifereae, family Anacardiaceae, is a species with significant economic importance and one that ranks fifth in production among all fruit trees (Corbineau et al. 1986). However, low fruit-set has been encountered in some South African cultivars. This has recently led to various studies aimed at possible solutions to the problem, e.g. pollination studies by de Wet et al. (1986) and an investigation of the mango ovule by Robbertse et al. (1986) which also highlighted the broad taxonomic importance of ovule and seed structure. However, as no adequate description of the mango seed and seed coat has been published before and also because of its relevance to the fruit industry, it was decided to provide this relevant information. This information is also needed to elucidate the phylogenetic position of the mango. In this report the structure of mainly the mature seed coat of Mangifera indica is described as well as that of some other species of the tribe Mangifereae (sensu Engler 1892).

# **Materials and Methods**

Ovules and fruit in various stages of development of the mango cultivars Haden, Sensation and Zill were collected on the Lisbon Estate, eastern Transvaal. Free-hand sections were prepared from fresh material and cleared with Herr's clearing fluid (Herr 1982).

For the preparation of semi-thin sections of the mature seed coat and cotyledons, the material was fixed in 2,5% glutaraldehyde in 0,1 mol dm<sup>-3</sup> buffer consisting of Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> (Coetzee & van der Merwe 1985). For better preservation of tanniniferous substances 0,5% caffeine was added to the fixative according to the recommendation of Mueller & Greenwood (1978). The material was dehydrated, infiltrated and embedded in purified glycol methacrylate (GMA) according to the method of Feder & O'Brien (1968). The composition of the monomer mixture, procedures of the periodic acid-Schiff reaction (PAS) and counter staining with toluidine blue O were previously described in detail (von Teichman 1987).

Lipids were stained for 10 min with a saturated solution of sudan black B in 70% ethanol. To stain lignified cell walls, sections were mounted in saturated phloroglucinol in 20% HCl. Protein staining was done with acid fuchsin and amido black 10B (Bullock *et al.* 1980). Starch was tested for with the PAS reaction and with potassium iodide-iodine (Jensen 1962). To localize callose, sections were mounted in a solution of 0,1% w/v water-soluble aniline blue in 0,067 M phosphate buffer at pH 8,5. Pretreatment with toluidine blue O was done to eliminate autofluorescence of the lignin and of the aniline blue non-specifically bound to cell walls (Smith & McCully 1978). Sections were examined with a Reichert Univar microscope equipped with epifluorescence optics.

# **Results and Discussion**

#### Structure of the ovule

In their discussion of the seed coat in general, Boesewinkel & Bouman (1984) point out that structures that seem identical at maturity may sometimes arise along different ontogenetic pathways. The true nature of any seed coat can therefore only be interpreted if the structure of the ovule is known and the ontogeny has been investigated. The relevant details concerning the mango are provided here.

#### S. Afr. J. Bot., 1988, 54(5)

According to Davis (1966), the ovule of the Anacardiaceae and therefore by implication also that of the mango, is anatropous, uni- or bitegmic and crassinucellar; endosperm is of the nuclear type. Robbertse et al. (1986) confirmed the anatropous nature of the basal mango ovule and found that it was unitegmic due to integumentary shifting, resulting in the inner integument remaining rudimentary. The chalazal tissue accommodates the greater part of the nucellus whereby a pachychalaza is formed. According to Corner (1976) such a pachychalazal ovule develops from basipetal, intercalary growth in the area where the chalaza adjoins the integument. Although Robbertse et al. (1986) did not mention whether or not they had observed a hypostase, it is evident from their figure illustrating a longitudinal section of the mango ovule (their Figure 3B) that a tanniniferous hypostase is associated with the extensive chalaza. This pachychalazal nature of the ovule (Figure 1A) obviously has a significant effect on the eventual structure of the mature seed and seed coat.



**Figure 1** Diagrammatic illustrations of longitudinal sections of the pachychalazal ovule in the ovary (A) and fruit with an intact, mature seed (B). Parallel lines in B represent position of Figure 3. Ovary wall — ow, ponticulus — p, style base — st, short integument — i and dotted, funicular knee — k, nucellus — n, pachychalaza — pc, vascular tissue — vt, endocarp — endo, exocarp — exo, micropyle — m, mesocarp — meso. The seed, which is bending around the ponticulus is shown with chalazal seed coat (chsc), funicle (f) and integumentary seed coat (isc and dotted). Arrows indicate direction of meristematic growth.

#### Development of the seed and seed coat

During the development of the seed, the raphe becomes attached to the pericarp at the ponticulus (Figure 1A & B). Since the antiraphe develops much more extensively than the raphe, the seed becomes curved or campylotropous (*sensu* Corner 1976). The pachychalaza of the ovule including the hypostase, undergoes extensive radial expansion resulting in the formation of a large saddle-shaped pachychalaza (Figure 1B). The study of young seed material revealed the presence of an active plate meristem, with mainly anticlinal divisions developing near the antiraphal base of the integument and causing the rapid growth of the integument at the antiraphal periphery (Figure 1B). At maturity the seed is therefore also pachychalazal according to the definition of Corner (1976).

## Mature seed coat

Two regions can be distinguished in the mature seed coat: the first is a thin, fragile, narrow, light brown band or girdle (Figure 2) extending from the micropylar end around two thirds of the circumference of the seed and covering the convex antiraphal side. This region is at its broadest, i.e. 10 to 15 mm, near the micropyle. It consists of about 15 to 20



Figure 2 Parts of the fibrous endocarp, seed coat (shown from inner surface) and the fibrous funicle (f). Integumentary part of seed coat (black arrow heads) surrounding chalazal part and mark left by the edge of the smaller cotyledon (white arrow head) are also illustrated. Scale bar = 10 mm.

squashed cell layers containing single, scattered, little distorted tanniniferous parenchyma cells causing the light brown colour of this part. No endosperm remnants are found on the inner surface of this region. The second, and by far the largest, region of the seed coat (Figures 2 & 3) is the dark brown, thick, chalazal part. This part originates from the pachychalaza of the ovule, while the narrow girdle covering the antiraphal part originates from the single short integument. The seed coat is thus of dual origin.

The chalazal part is smooth on the outside and unevenly rugged on the inside due to the presence of rigid vascular bundles. On the shallow, concave, raphal side it is about 1 mm thick, relatively tough and consists of an outer



**Figure 3** Drawing of a transverse section of the seed coat. The chalazal part (ch) represents only 40% of the total — the missing 60% indicated with the dotted line, while the integumentary part (i) is illustrated in full. Vascular bundles — vt, hypostase — hy and endosperm remnants — end. The area between the two small arrows is enlarged in Figure 4. Scale bar = 300  $\mu$ m.

thin-walled more or less squashed parenchymatous chalazal tissue with scattered groups or single tanniniferous cells (Figure 4). Numerous amphicribral, anastomosing vascular bundles on the inner side form a wickerwork, resulting in this region of the seed coat being relatively tough. Longitudinal sections of vascular bundles reveal tracheoids (sensu Schmid 1986) with helical secondary wall thickenings. An extensive thin-walled, parenchymatous hypostase of about 10 cell layers is also present (Figures 3 & 4). The tanniniferous contents of the cells cause the dark brown colour of this region. According to Corner (1976) the hypostase typically consists of a few layers of small, angular, contiguous cells without intercellular cell spaces, which become brown and suberized or lignified but lack differentiation in form. The mango hypostase agrees with this description. In the maturing mango seed the cell walls are impregnated with lignin and lipids, probably suberin or cutin. If cutin is present this would be in accordance with the findings of Bouman (1984), who mentioned various kinds of wall impregnations in the hypostase, including cutin. We found that these walls also consist mainly of cellulose. According to Tiwari (1983) the hypostase wall impregnations of Torenia fournieri Lind. also contain cellulose, as well as callose and pectin. However, in the mango seed coat no callose was found.

Other authors who have superficially investigated the mango seed coat structure include Juliano & Cuevas (1932) who noted slight lignification; and Lindner (1971) who mentioned three types of stone cells, both authors describing the outer layers of the seed coat. The latter author also mentioned an inner tannin-containing layer which he referred to as 'braune Haut'. This is probably the first reference to the tanniniferous hypostase described in the present paper.

In the mango, the pachychalaza is therefore associated with an extensive tanniniferous hypostase, an association



Figure 4 Transverse section of the chalazal seed coat. The chalazal parenchyma (ch), the vascular bundles (vt) and tanniniferous cells (tc) are illustrated. The hypostase (hy) with thin cell walls, nucellus and endosperm remnants (end) are also shown. Scale bar =  $100 \ \mu m$ .

also noted by Corner (1976) for other species with pachychalazal seed or seed with a more or less extensive chalaza.

However, the integumental part of the mature mango seed coat seems to differ distinctly from the integumentary part of similar previously described pachychalazal seeds (vide Boesewinkel 1981). The seed coat of e.g. Trichilia grandifolia Oliv. (Boesewinkel 1981), originating from a bitegmic pachychalazal ovule is also of dual origin but the integumentary part occupies approximately the lower onethird or micropylar area and is more or less restricted to the original position of the integuments. In the mango the integument expands peripherally after fertilization through intercalary meristematic activity. Anticlinal divisions occur and the integumentary part gradually expands and eventually virtually surrounds the chalazal part as a narrow band or 'peritesta', a term that could be created in analogy with 'perichalaza' sensu Corner (1976). This description of a 'peritesta' is probably the first to be published, but this type of integumental seed coat may be of much more general occurrence.

Boesewinkel & Bouman (1978) described the pachychalazal seed of *Glycosmis* cf. *arborea* (Roxb.) DC., a member of the Rutaceae, and striking similarities can be noted with respect to the mango: the unitegmic condition of the ovule, derived through integumentary shifting; the pachychalazal proliferation and nature of the chalazal part of the seed coat and the single, large exalbuminous seed that occurs in an animal-distributed fruit.

# Embryo

The embryo of the mango has inter alia two cotyledons of unequal size, the edge of the smaller one causing a noticeable indentation on the inside of the seed coat (Figure 2). The characteristic impression of the rough, chalazal part and narrow, smooth, integumentary part of the seed coat on the cotyledon surface is illustrated in Figure 5. The cotyledons consist of a starchless parenchymatous epidermis, an inner and outer storage parenchyma and numerous amphicribral vascular bundles occurring in close association with schizogenous secretory ducts. Besides protein bodies and oil in elaiosomes, the cells of the storage parenchyma contain simple starch grains. The latter are described by Wuersch & Hood (1981) as being ellipsoidal, 8 to 20  $\mu$ m in diameter, some with dimples in the outer surface, apparently due to the tight packing of the protein bodies and the grains.

The mature seed of other species of the tribe Mangifereae As mentioned in the introduction, detailed seed-related ontogenetic studies have been undertaken by the senior author on species related to the Mangifereae. These, in conjunction with the present study on the mango, provide the background for the interpretation of research by other authors.

Communications on seed-related studies on other species of the Mangifereae include those on *Anacardium occidentale* L. (Weber 1907; Ziesak 1972), *Swintonia acuta* Engl. and species of *Gluta* L. (Hou 1978) as well as on *Buchanania lanzan* Spreng. (Mitra & Mehrotra 1980).

The account of Weber (1907) confirms the close relationship of the cashew nut with the mango which was already pointed out by Engler (1892). In both species the seed coat consists of an epidermis, a many-layered parenchymatous tissue, the inner part of which contains numerous ramifying chalazal vascular bundles and an inner layer, 10 to 15 cells thick, of brown cells with thicker walls. According to Weber (1907) the latter layer is responsible for the brown colour of the seed coat. This represents a hypostase. In the figures of the kidney-shaped cashew seed



Figure 5 The embryo with radicle (r), impressions of the narrow, smooth, integumentary part of the seed coat (isc) and rough chalazal part (chsc). Scale bar = 8 mm.

(Weber 1907, Tafel VI Figs 79 & 80) the 'Chalazastelle' is indicated. The seed of the cashew is doubtlessly also pachychalazal. The vascular bundles in the cotyledons are amphicribral (Weber 1907, Tafel VII Fig. 96) and closely associated with the schizogenous secretory ducts, while starch grains, aleuron grains and oil also occur.

Ziesak (1972), possibly unaware of the work by Weber, also studied the cotyledons of Anacardium occidentale. He states that the size of the simple starch grains, 1–8  $\mu$ m in diameter, facilitates the identification of cashew seeds in confectionery. In the vascular bundles delicate spiral and reticulate tracheids (sensu Schmid 1986) are found. On studying the detailed illustrations of Hou (1978, Figs 15g & 18g) of the embryos of *Gluta papuana* Ding Hou and G. sabahana Ding Hou respectively, one can conclude that these seeds are also pachychalazal. The same conclusion can be drawn regarding several species of Buchanania Spreng., viz. B. sessilifolia Bl., B. macrocarpa Laut. (Hou 1978), and B. lanzan Spreng. (Mitra & Mehrotra 1980) while the drawing of the embryo of the closely related Swintonia acuta Engl. (Hou 1978, Fig. 13j) indicates the occurrence of an extensive chalaza.

# Conclusions

As far as the evidence at hand allows one to draw some conclusions, there are indications that certain seed characters occur constantly in numerous genera of the Mangifereae, viz.

- (1) size relatively large,
- (2) the absence of endosperm,
- (3) an undifferentiated seed coat (*sensu* Corner 1976) which is of dual origin,
- (4) an extensive chalaza, and associated with it
- (5) a hypostase.

Our study, therefore, confirms the observations of Corner (1976) that an extensive chalaza is often closely associated with a tanniniferous hypostase.

As far as the phylogenetic position of *Mangifera indica* is concerned, this species exhibits various characters that may be defined as either primitive or advanced. Primitive characters include the woody habit and moist tropical habitat, and the anatropous ovule with nuclear endosperm. On the other hand, in the angiosperms unitegmy and a pachychalazal ovule are generally considered advanced characters (Corner 1976; Boesewinkel & Bouman 1978; Boesewinkel 1981). Therefore, the mango ovule is partly advanced. The mango seed also shows several advanced characters: it is exalbuminous and pachychalazal and the seed coat is undifferentiated, representing advanced simplification. Characters that, on superficial examination, may be considered primitive are the large seed size and recalcitrant viability. Recalcitrance generally occurs in large-seeded, woody species of the moist tropics (King & Roberts 1979) e.g. in Dipterocarpus Gaertn. (Tompsett 1987). However, Boesewinkel & Bouman (1978) reported that seed germination in Glycosmis cf. arborea, an advanced species of the Rutaceae, takes place immediately after fruit drop, indicating probable recalcitrance. Moreover, the large size of the mango seed is the result of extensive secondary development of the embryo, the integument and the strongly vascularized pachychalaza. The latter represents a type of derived specialization of the seed coat which probably plays an important role in the transport of nutrients to the large embryo.

In the mango the protective function of the seed coat is adequately taken over by the specialized sclerenchymatous endocarp and there is no necessity for a sclerenchymatous seed coat. According to Stebbins (1974), this transference of the protective function of the seed coat often occurs in groups with medium to large-sized seeds and he, as well as Boesewinkel & Bouman (1978), consider this large, oneseeded, animal-dispersed drupe a more effective and advanced fruit type.

Therefore, according to generally accepted criteria, our investigation of the mango ovule and seed indicates a general advanced phylogenetic condition. This, together with the information gathered from other genera, confirms the opinion of Corner (1976) that the Anacardiaceae can be regarded as one of the more advanced forest families.

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