MORPHOLOGY AND ANATOMY OF THE DEVELOPING FRUIT OF *MACFADYENA UNGUIS-CATI* (L.) A.H.GENTRY, BIGNONIACEAE

Morfología y anatomía del fruto en desarrollo de *Macfadyena unguis-cati* (L.) A.H.Gentry, Bignoniaceae

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RESUMEN

Macfadyena unguis-cati es una liana que produce frutos similares a legumbres, con dos carpelos y semillas aladas. La diversidad en el tipo de cápsula y los pocos estudios en las semillas de Bignoniaceae motivaron la ejecución de este trabajo. Se analizaron las flores y frutos en distintas fases de desarrollo, según las técnicas usuales de anatomía vegetal. El fruto es una cápsula con dehiscencia septífraga asociada con dehiscencia septicida. En preantesis se forma un meristema en la región media del mesofilo del ovario. La semilla se origina de un óvulo anátropo, unitegumentado, tenuinucelado y endotelial. La semilla madura es exotestal y presenta poco endosperma y un embrión recto.

Palabras clave: Bignoniaceae, desarrollo, fruto, liana, *Macfadyena*, semilla

ABSTRACT

Macfadyena unguis-cati is a species of liana that produces pod-like fruits consisting of two carpels and bearing winged seeds. This study was motivated by the diversity of capsule types and the few seed studies conducted in Bignoniaceae. Flowers and fruits in different development stages were structurally analyzed, following standard plant anatomical techniques. The fruit is a capsule with septifragal dehiscence associated with a septicidal dehiscence. During pre-anthesis, a meristem is formed in the middle region of the ovary mesophyll. The seed originates from an anatropous, unitegmic, tenuinucellate and endothelial ovule. The mature seed is exotestal and presents little endosperm and a straight embryo.

Key words: Bignoniaceae, development, fruit, liana, *Macfadyena*, seed

INTRODUCTION

In Bignoniaceae, fruit characters can help in tribal and generic identification (Barroso *et al*. 1999). In fact, a key for the identification of Brazilian and exotic Bignoniaceae genera, based on their fruits is available (Barroso *et al*. 1999). However, classifying plants on the basis of their fruits and without a good understanding of their ontogeny can lead to erroneous assessments of homology and classification. For example, classifying Lauraceae fruits as drupes or Fabaceae

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(Leguminosae) fruits as indehiscent follicles represent some of those mistakes (Souza 2003; Souza *et al*. 2003).

Species of Bignoniaceae generally present capsular fruits with variable structure and dehiscence type (Barroso *et al*. 1999). Fruits of *Tabebuia ochracea* (Cham.) Standl. and *T. chrysotricha* (Mart. ex DC.) Standl. have been erroneously considered as siliquas because of the presence of a seminiferous column and two valves at the time of dehiscence. However, Costa (2003) and Souza *et al.* (2005) demonstrated that these fruits actually represent loculicidal capsules.

As far as seeds of Bignoniaceae are concerned studies have focused mainly on their ontogeny. According to Corner (1976), Bignoniaceae seeds originate from anatropous or hemianatropous, unitegmic and tenuinucellate ovules. In addition, they vary in size, frequently possess membranous or corky wings, and are exotestal and exalbuminous. Additional studies on the seeds of Bignoniaceae taxa are those of Alves (1975) on *Jacaranda brasiliana* (Lam.) Pers., Beltrati & Picollo (1979) on *Distictella mansoana* (Bureau) Urb., Mehra & Kulkarni (1985) on several species of Bignoniaceae, Souza (1993a) on *Tabebuia caraiba* (Mart.) Bureau, Costa (2003) on *Tabebuia ochracea* and Souza *et al*. (2005) on *Tabebuia chrysotricha*.

There is an ongoing debate in the literature regarding the fruits of *Macfadyena* DC. While Sandwith & Hunt (1974) consider their fruits as septicidal capsules, Barroso *et al*. (1999) consider them as septifragal capsules.

The main goal of the present study is to contribute for the understanding of fruits and seeds of *Macfadyena* DC. (Bignoniaceae), through detailed morphological studies of the developing pericarp and seeds of *Macfadyena unguis-cati* (L.) A.H.Gentry.

MATERIAL AND METHODS

Two specimens of reproductive materials of *Macfadyena unguis-cati* (L.) A.H.Gentry were collected at the Universidade Estadual de Maringá (UEM), in Brazil (state of Paraná). Voucher materials were deposited at the UEM Herbarium, collection number: R.C. Oliveira 10646.

The materials were fixed in FAA 50% and later transferred into alcohol 70%, following the protocol of Johansen (1940). Materials of different developmental stages of ovary, flowers and fruits were embedded in paraffin and sectioned (cross and longitudinal sections) in a rotation microtome also following the technique described by Johansen (1940). Sections were stained in astra blue and safranin (Kraus & Arduin 1997). Developing fruits were analyzed in freehand sections stained using the same techniques.

Mature fruits were macerated according to Jeffrey's Method (Johansen 1940). Specific microchemical tests were done for lipid substances (Sudan III) (Rawlins & Takahashi 1952), starch (iodine-potassium iodide test) and lignin (phloroglucin test) (Berlyn & Miksche 1976).

Illustrations were prepared using an optical microscope (Wild M20) equip-

ped with a reflex camera. Photographs were taken with the digital camera and subsequently prepared using the software Image Pro-Plus, version 4.0 (Media Cybertecnics). All samples were prepared on the same micrometric scale.

RESULTS

Fruit morphology and anatomy

1. Flower

The fruit originates from the bisexual flower, with 5 fused green sepals, 5 fused yellow petals (Fig. 1a, b), an ovary with 2 fused carpels and 2 locules, and axile placentation. The base of the ovary is completely surrounded by a nectary (Fig. 1b), which consists of an epidermis, secretory parenchyma and only phloem, as conductive tissue (Fig. 2a). The ovary has an uniseriate outer epidermis (Fig. 2b), with cuboid or tabular cells and stalked scales. The mesophyll is parenchymatous. In pre-anthesis, the middle portion of the mesophyll differentiates in a meristem, whose cells undergo cellular divisions on several planes, forming a tissue composed of small and thin-walled cells (Fig. 2b). The inner epidermis is uniseriate (Fig. 2b), glabrous and with tangentially elongated cells. The ovary septum is composed of a glabrous epidermis with single-layer prismatic cells and parenchymatous tissue.

Ovary vascularization, that stays in the fruit (Fig. 2c), consists of dorsal, ventral/marginal and lateral bundles. The bundles are collateral, except the lateral one (located in each carpel closest to the septum), which is amphicribral (Fig. 2c).

2. Fruit Development

Very early in fruit development, the corolla, stamens, style and stigma undergo abscission. The calyx remains (Fig. 1c, e), and in the phase of earlier maturation, dries and is shed. The fruit grows considerably, taking on the aspect of a very long green pod (Fig. 1e).

In the young fruit the exocarp is very similar to the outer epidermis of the ovary (Fig. 2d). The mesocarp originates from the ovary mesophyll and is formed by parenchyma and middle tissue with small polyhedral cells (Fig. 2d). The inner mesocarpic parenchyma possesses wider cells than the outer tissue and tangentially elongated subendocarpic cells (Fig. 3b). The endocarp or inner epidermis remains glabrous (Fig. 2d) and its cells also undergo tangential elongation (Fig. 3b). Abscission tissue is observed in either dorsal carpellary region (Fig. 2c, 3b). In the septum, the epidermis (from prismatic cells in the ovary) assumes an irregular aspect (Fig. 3a, b). The number of lateral vascular bundles of the pericarp and septum increases considerably.

The exocarp is epidermal, and the trichomes disappear in the immature fruit (Fig. 2d, 3b). The mesocarp presents four regions: 1) An outer parenchymatous region (Fig. 2d), with cells that vary in size and shape, which contain chlo-

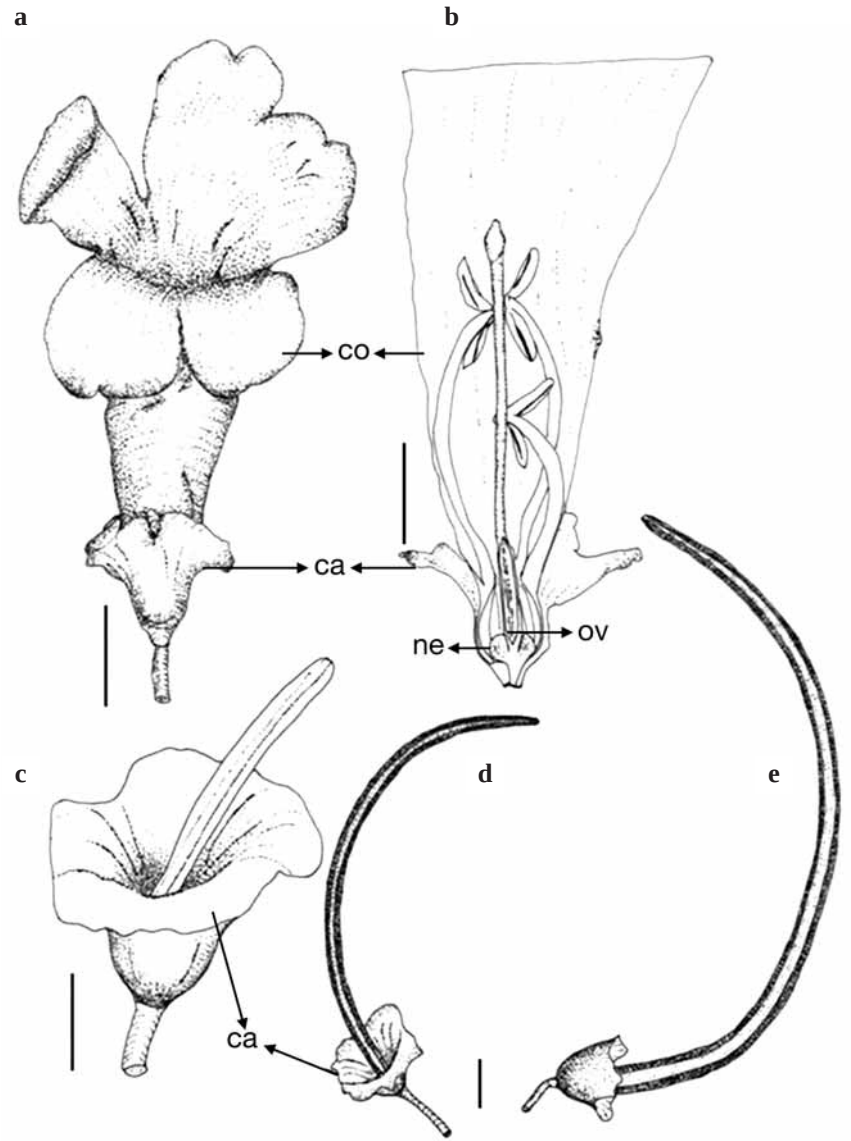

Fig. 1. Flower and fruit of *Macfadyena unguis-cati*. **a.** External flower morphology. **b.** Longitudinal section of flower showing stamens, ovary and nectary. **c, d, e.** Fruits at different stages of development. ca = calix; co = corolla; ne = nectary; ov = ovary. Scale = 1 cm (a, b, d, e), 0,5 cm (c).

roplasts; 2) A middle region (Fig. 2d, 3d), that consists of one to four layers of thin-walled, fibrous cells; 3) An inner parenchymatous region (Fig. 2d, 3b, d), that has large cells where small lateral bundles occur; and 4) A subendocarpic region (Fig. 3b) with one or two layers of elongated, thick-walled cells. The endocarp (Fig. 2d, 3b, d) presents elongated cells and a thin cell wall. The septum is composed of an epidermis and parenchyma with vascular bundles of variable size (Fig. 3a). At the junction of the septum and carpel wall (Fig. 3a), weak tissue characterized by loosely-arranged parenchymatous cells occurs.

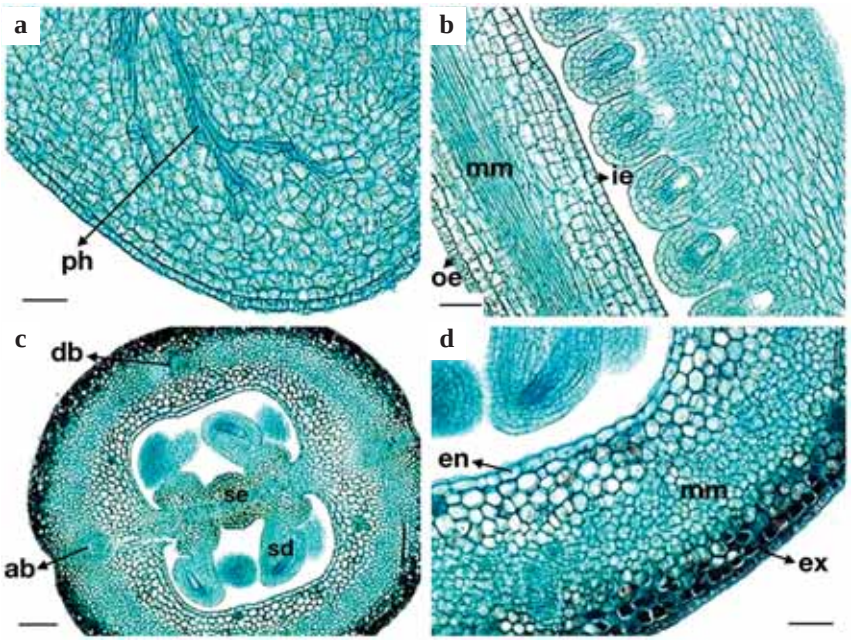

Fig. 2. Structure of *Macfadyena unguis-cati*. **a.** Nectary. **b.** Ovary. **c, d.** Young fruits. ab = amphicribral bundle; db = dorsal vascular bundle; en = endocarp; ex = exocarp; ie = inner epidermis; mm = middle mesophyll/mesocarp; oe = outer epidermis; ph = phloem; sd = young seed; se = septum. Scale = 50 µm (a, b, c), 100 µm (c).

3. Mature fruit

Fruit is a dry capsule, with the pericarp opening by two longitudinal splits and isolating a persistent septum.

The peridermic exocarp (Fig. 4a, 5a) has few phellem layers, phellogen and uniseriate phelloderm. The outer mesocarp (Fig. 4a, 5a) is collenchymatous and has sclereids. The middle mesocarp (Fig. 4a, 5a) is sclerenchymatous and composed of thick-walled, and lignified, pitted fibers. The inner mesocarp (Fig. 4a, 5a) is collenchymatous and parenchymatous, with elongated or isodiametric cells of variable size; it may present cells with bipyramidal, laminar or irregular crystals. The subendocarpic mesocarp (Fig. 5a) consists of two to four layers of elongated

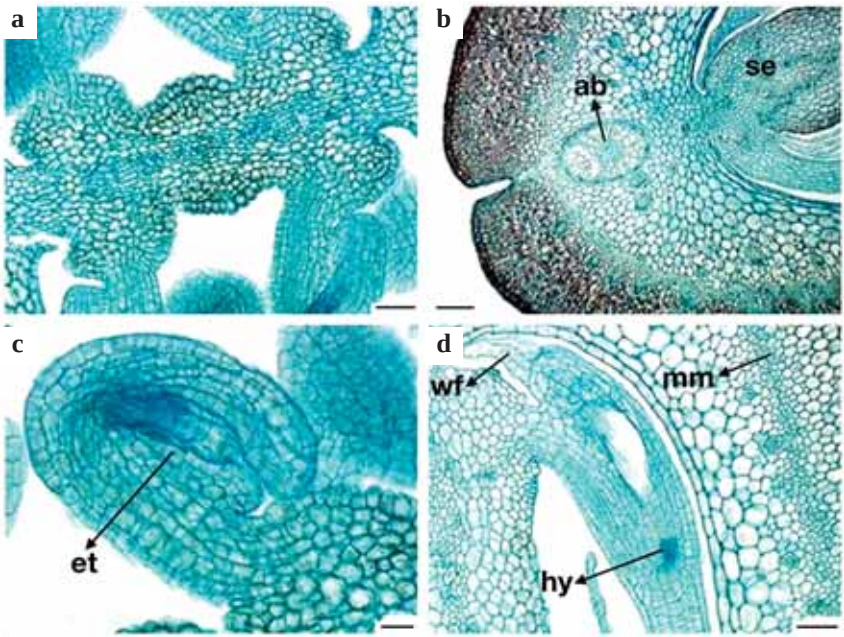

Fig. 3. Fruit and seed structure of *Macfadyena unguis-cati*. **a.** Septum of young fruit. **b.** Young fruit. **c, d.** Young seeds. ab = lateral amphicribral bundle; et = endothelium; hy = hypostase; mm = middle mesocarp; se = septum; wf = wing in formation. Scale = 50 μm (a, d), 100 μm (b), 20 μm (c).

cells arranged longitudinally, transversally or obliquely in the fruit. The subendocarpic mesocarp possesses either thin-walled cells or macrosclereids of thick, lignified and pitted cell walls. The endocarp (Fig. 4a, 5a) has fiber-like cells with thickened and non-lignified walls. Endocarpic cells may be arranged longitudinally, transversally or obliquely in the pericarp.

The epidermis and subepidermis of the septum (1-3 layers) (Fig. 4a, 5a) is constituted by sclereids of variable shapes. Most of the septum (Fig. 4a, 5a) is formed by parenchyma with thin-walled cells of variable sizes and shapes. The cells have bipyramidal, laminate and short raphide crystals. There are collateral vascular bundles (Fig. 4a), accompanied by fibers in the phloem margin, located at the central region of the septum. The septum presents a constriction in the contact zone with the wall of the fruit. Here there is a very fragile parenchyma that facilitates the breaking of the septum when the fruit opens up.

Each carpel is vascularized by several collateral bundles: one dorsal, many laterals and one ventral (Fig. 4a). The vascular supply of the seed is derived from the ventral bundle, which occurs in the septum. In the fruit wall, in the region of the septum, there is a large amphicribral bundle (Fig. 4a). All of the carpel bundles have cambium and secondary xylem and phloem.

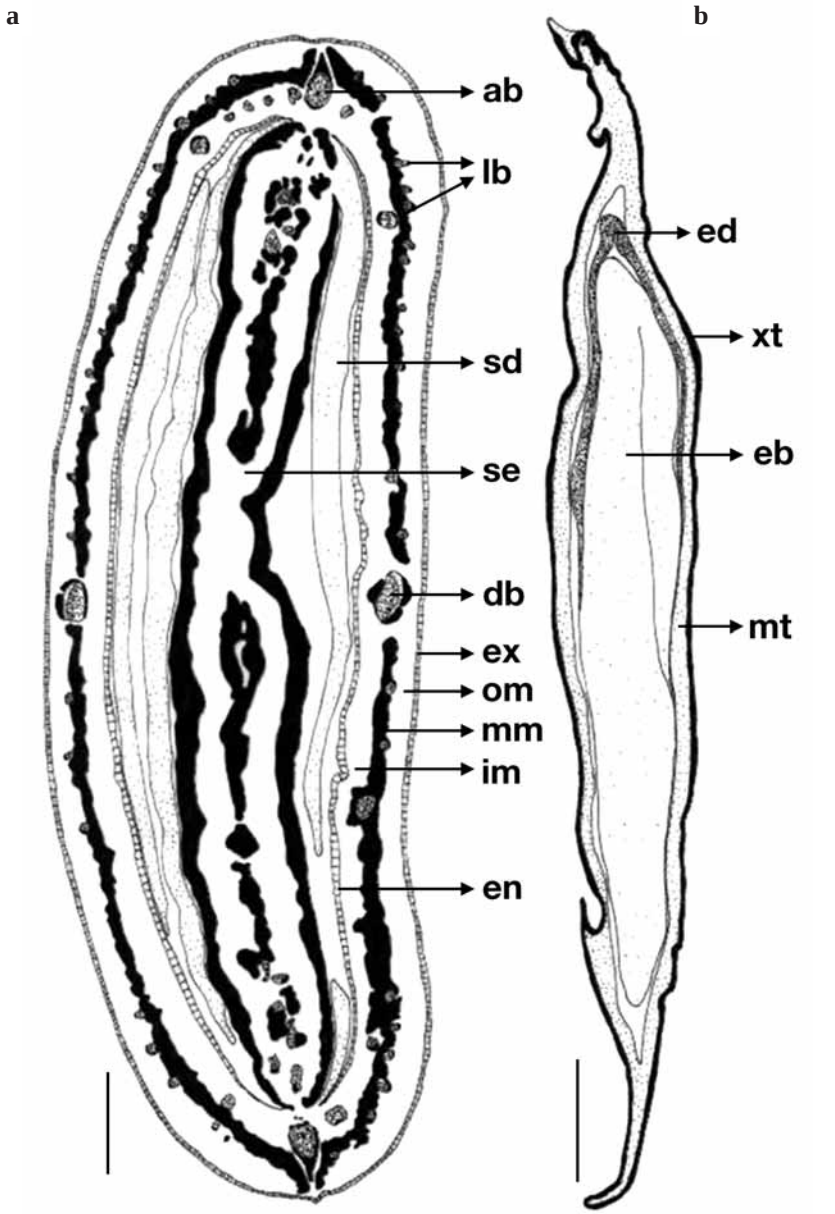

Fig. 4. Diagrams in cross and longitudinal sections of *Macfadyena unguis-catis*. **a.** Mature fruit; **b.** Seed. ab = lateral amphicribal vascular bundle; db = dorsal vascular bundle; eb = embryo; ed = endosperm; en = endocarp; ex = exocarp; im = inner mesocarp; lb = lateral vascular bundle; mm = middle mesocarp; mt = mesotesta; om = outer mesocarp; sd = seed; se = septum; xt = exotesta. Scale = 1 mm.

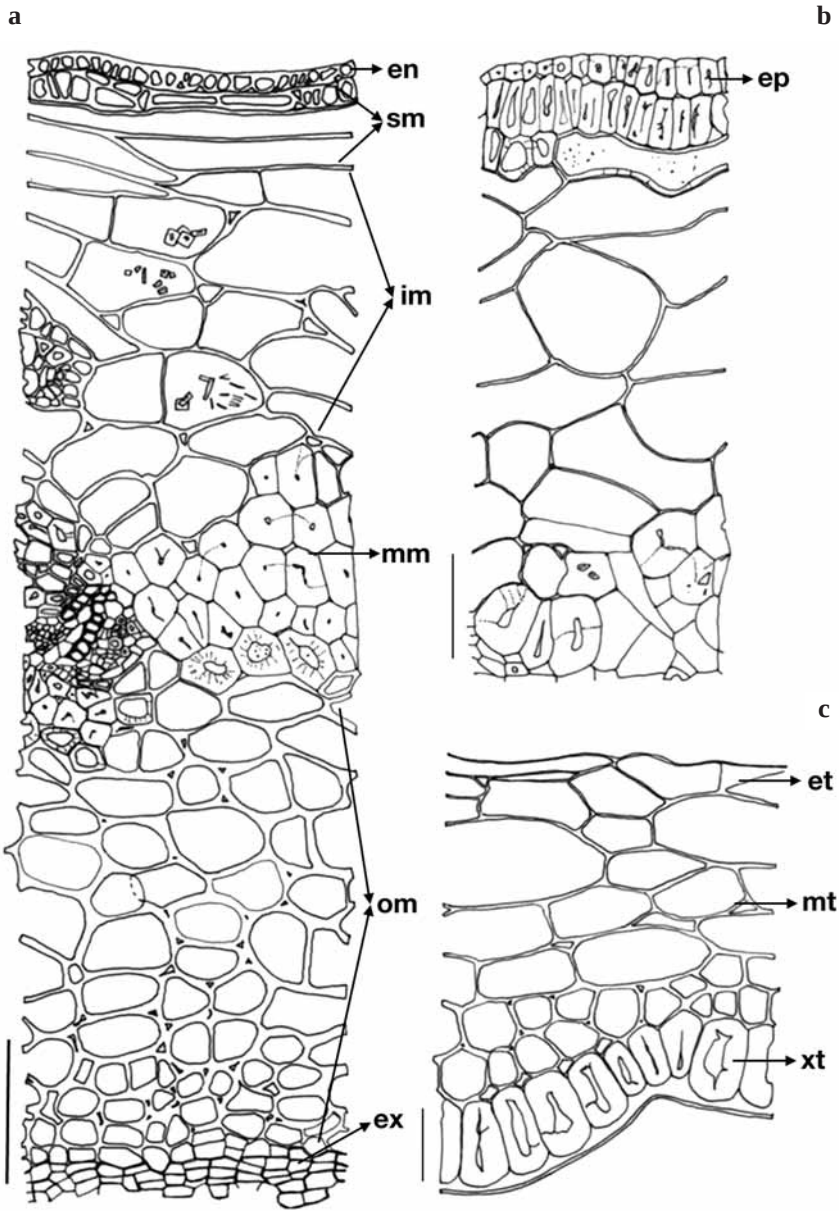

Fig. 5. Cross-sections of *Macfadyena unguis-cati*. **a.** Details of the mature pericarp. **b.** Septum. **c.** Testa. en = endocarp; ep = epidermis; et = endotesta; ex = exocarp; im = inner mesocarp; mm = middle mesocarp; mt = mesotesta; om = outer mesocarp; sm = subendocarpic mesocarp; xt = exotesta. Scale = 100 µm (a, b), 30 µm (c).

Seed morphology and anatomy

1. Ovule

The ovule (Fig. 2b) is anatropous, unitegmic, tenuinucellate and has a short funiculus. It presents endothelium, hypostase and a vascular supply without postchalazal branches. In the placentary region, the inner ovary epidermal cells are cylindrical, slightly papillate and have a large nucleus.

2. Developing seed

In the young seed (Fig. 3c, d), the only integument is formed by the outer epidermis of cuboid or tabular cells. The mesophyll is parenchymatous with few cell layers varying in size and shape. The inner epidermis is composed of small and tabular cells. The endothelium is composed of shortly prismatic cells that have relatively dense cytoplasm. The hypostase cells stand out for being thick-walled and having more intense coloration. In the embryo sac, the zygote and the degenerate synergid are observed. The vascular supply is as in the ovule.

The seed wing originates from epidermal cells that occur in the chalazal region. These cells undergo gradual elongation, and could undergo anticlinal and periclinal cellular divisions. The wing also develops less intensely in the micropylar region of the seed (Fig. 3d).

The immature seed has a seminiferous nucleus and a wing. The nucleus testa consists of the outer epidermis which is composed of elongated and narrow cells, and parenchymatous mesophyll. The number of strata in the nucleus mesophyll decreases gradually towards the wing. One to three strata of elongated cells form the wing in the hyaline portion.

3. Mature seed

The mature seed is alate (Fig. 6a, b) with a well-developed and straight embryo (Fig. 6c).

The seed is exotestal. The mechanical layer is formed by the epidermis (exotesta) (Fig. 4b, 5c) and subepidermis in the lateral region, and only by the epidermis towards the wing margin. The mechanical layer is composed of macrosclereids and fibers with thick, lignified and pitted walls. The mesotesta (Fig. 4b, 5c) is parenchymatous and constituted by thin-walled cells of variable size and shape. The endotesta or inner epidermis has thin-walled cells (Fig. 5c). The small endosperm (Fig. 4b, 6d) has little starch and a copious oily reserve in the cells. The embryo possesses cotyledons (Fig. 6e) with uniseriate epidermis and parenchymatic mesophyll with oily reserve.

The wing (Fig. 6f) in the hyaline zone has two layers or is only constituted by the epidermis, with thick-walled and non-lignified cells. Eventually, a third layer occurs in the wing of cells similar to the epidermal ones. Alternatively, a third layer represented by fibers can also occur.

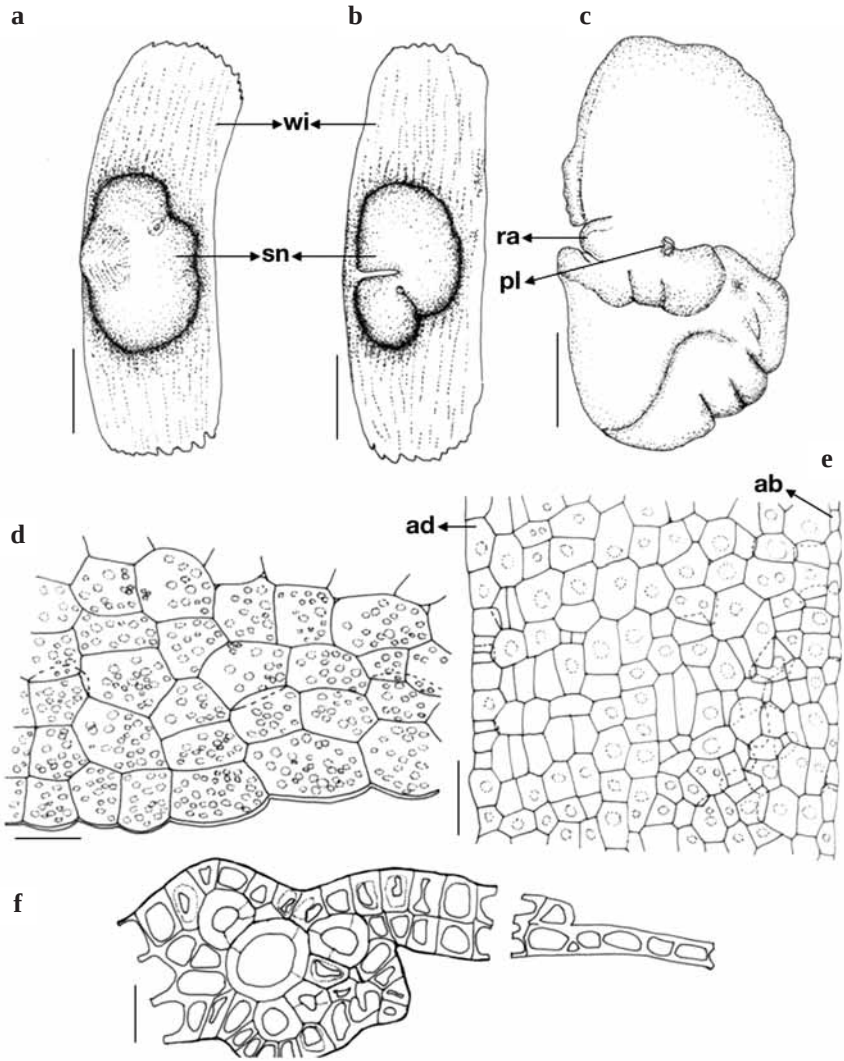

Fig. 6. Mature seed structure of *Macfadyena unguis-cati*. **a, b.** Seeds. **c.** Embryo. **d, e, f.** Details of endosperm, cotyledon and wing in longitudinal sections, respectively. ab = abaxial epidermis; ad = adaxial epidermis; pl = plumule; ra = radicle; sn = seminiferous nucleus; wi = wing. Scale = 6 mm (a, b), 3 mm (c), 20 µm (d), 50 µm (e), 30 µm (f).

DISCUSSION

Placentation in *Macfadyena unguis-cati* generally referred to as axile, is here considered an intermediate condition between axile and parietal placentation, basing on the observations by Costa (2003) and on the similarity of the placentation of *Macfadyena unguis-cati* with that observed in *Tabebuia* Gomes ex DC. species (Costa 2003; Souza *et al*. 2005).

Macfadyena unguis-cati has a floral nectary that remains for some time in the young fruit. There are countless recordings of floral nectaries that remain later in the fruit. They can be designated as postfloral or postnuptial nectaries (Schmid 1988), especially in Bignoniaceae (Elias 1983). Postfloral nectary secretion in the young fruit was not encountered in *Macfadyena unguis-cati*. However, fruit nectaries in Bignoniaceae are known to have secretion with function in the anti-herbivore mechanism (Costa 2003).

The endocarp or inner mesocarp of many Leguminosae and Rutaceae fruits can originate from an adaxial or ventral meristem (Souza 1984, 1993b; Souza *et al*. 2003). Different from the fruit of Leguminosae and Rutaceae, a middle meristem in the carpel that originates a sclerenchymatous middle mesocarp is formed in the capsules of *Macfadyena unguis-cati* and *Tabebuia*.

There are diverging opinions regarding the capsule type that occurs of *Macfadyena*. According to Sandwith & Hunt (1974), the capsules are septicidal and according to Barroso *et al*. (1999) they are septifragal. However, according to Hertel's classification (1959), fruits of *Macfadyena unguis-cati* fruit do not seem to fit either of these two types. In the septicidal capsule, the septum splits long-radially into two lamellas. In the septifragal capsule, the septum is divided into two portions, an internal one (close to the columella) and an external (close to the fruit wall). Hertel (1959) considers septifragal dehiscence a rare type, only found in some Ericaceae. Septifragal dehiscence usually takes place together with loculicidal or septicidal dehiscence. In the case of *Macfadyena unguis-cati*, septifragal dehiscence seems to be associated with a septicidal dehiscence.

The ovule and the young seed of Macfadyena *unguis-cati* presents an endothelium. This characteristic is common in families that show unitegmic and tenuinucellate ovules (Kapil & Tiwari 1978). There seems to be no doubt that the endothelium is a nutritive layer whose chief function is to serve as an intermediary for the transport of food materials from the integument to the embryo sac (Maheshwari 1971). On the other hand, in the mature seed of *Tabebuia chrysotricha* the endothelium seems to have a different function, i.e. to protect the embryo (Souza *et al*. 2005).

In this study, a structural characterization of the seed ontogenesis of *Macfadyena unguis-cati* allowed to record most of the seed characters previously described for the Bignoniaceae (Corner 1976). However, the seeds of *Macfayena unguis-cati* present little endosperm and, sometimes more than two cell layers in the hyaline wing.

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