Anatomy and fruit development in *Schinopsis balansae* (Anacardiaceae)

by

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Abstract


*Schinopsis balansae* Engl. is a dioecious tree; reproduction is by woody samaras containing a single seed. Fruit set is high, even in isolated trees, empty fruits are frequent and account for a high proportion of the total fruit production. To describe the anatomy and the ontogeny of seeded and seedless fruits, flowers and fruits at different development stages from bagged and pollen exposed flowers were studied. The development and the anatomy of the pericarp in seeded and parthenocarpic fruits did not differ. It consisted in an exocarp formed *sensu lato* from the external epidermis of the ovary and some layers of the underlying parenchyma. The mature mesocarp was constituted by a sclerified tissue and lysigenous channels. The fruit is of Anacardium type: the endocarp presented three sclerenchymatic and a crystaliferous layer. The endocarp development was also *sensu lato* because it was formed from the epidermis and the hypoderms of the carpel. This organization agrees with the only species described in the genus: *S. haenkeana* Engl. *Schinopsis balansae* is able to produce parthenocarpic fruits in the absence of pollination. Empty fruits from free exposed flowers presented embryos arrested at different stages, although total absence of an embryo was the most common condition. Parthenocarpy seems to be a plesiomorphic trait within the Anacardiaceae, but regardless of its origin, the maintenance of empty fruit production in a wind-dispersed samara is difficult to explain. Whether parthenocarpy has an adaptive value, or is an evolutionary constraint remains unclear in *Schinopsis*.

Keywords: dioecy, dry fruits, fruit ontogeny, parthenocarpy, agamospermy, samaras, quebracho, empty fruits, pericarp.

Introduction

*Schinopsis balansae* Engl. is a dioecious tree that grows in Argentina, Bolivia, Paraguay and Brazil, and is the dominant species of Argentine forests called “quebrachales”. Although most species of the Anacardiaceae family has drupaceous fruits, many of them adapted to different dispersers, *S. balansae* fruits are woody samaras with a single oblong wing
and an ovoid seminiferous portion containing a single seed.

Due to timber logging its populations have suffered a marked reduction, and part of the area formerly covered by the species has been devoted to farming. Despite the economic, ecological and social importance of the species, a basic knowledge on its biology is still lacking. *Schinopsis* reproducen only by means of seeds and so, to determine the factors constraining fertile fruit production, how the samara develops, and whether or not pollination is necessary, is of fundamental importance for developing strategies for the conservation of this species.

Wannan & Quinn (1990) studied the fruit of 29 genera in the Anacardiaceae. They recognized two basic types of endocarp: the Spondias-type, which is composed of a mass of irregularly oriented sclerenchyma and the Anacardium-type, characterized by a lignified outer epidermis and discretely layered and includes palisade like sclereids. *Schinopsis baenkoeana* Engl was the only species studied by Wannan & Quinn (*loc. cit.*) and has been described and classified as Anacardium-type. So far the anatomic structure of the fruit in *S. balansae* remains unknown.

Empty fruits are frequent and can account for almost half the total fruit production (Alzugaray, 2005). Fruit set is striking since before maturity the red samaras are easily observed on pistillate individuals. Notably, isolated trees can also yield huge amounts of fruit as well as plants growing in dense populations, and these observations led us to the hypotheses that fruit development may be independent of pollination, and/or that embryo abortion does not prevent fruit formation.

Atypical fruit production is frequent in the Anacardiaceae: there are cases of parthenocarpy (development of fruit without fertilization) and fruit development following embryo abortion. For instance, Grundwag & Fahn (1969) observed post-fertilization embryo abortion in *Pistacia vera* L., and Shuraki & Sedgley (1994) found that in *P. vera* funicle degeneration was the most common cause to empty seeds. More recently, Polito (1999) found that vascular transport to ovules is blocked at the placenta or in the funicle in *P. vera*. Some authors have explained the paradox of inviable fruits from an evolutionary viewpoint, suggesting that parthenocarpy can reduce viable seed predation (Traveset, 1993; Verdú & Garcia-Fayos, 1998, 2002). Other studies on sterile seeds in the Anacardiaceae occur in the genera *Anacardium* L., *Mangifera* L., *Rhus* L., and *Spondias* L. (Peebles & Hope, 1937; Purseglove, 1968; Young, 1972; Crane, 1975; Janzen, 1985; Chung & Waller, 1986; Von Teichman & Robbertse, 1986).

The aim of the present research was:
1) To describe the anatomy and ontogeny of the pericarp.
2) To determine if fruit development is unrelated to fertilization, and if so, to compare pericarp development in both seeded and seedless fruits.

**Material and methods**

Pistillate and staminate individuals growing in natural populations located at “Las Gamas” station, near Vera, Santa Fe province, Argentina, were studied. Material from cultivated specimens in Corrientes City, Argentina, was also studied. Vouchers of the specimens are deposited in the Herbarium of Instituto de Botánica del Nordeste (CTES), Argentina.

In the natural population, inflorescences at flower bud stage were bagged with two bags: an internal plastic mesh -to keep the outer bag free from the flowers- and an external wax paper bag to impede pollen flow. Bagging was carried out during two flowering periods: 2004 and 2005. One group of flowers was left in the bags until fruit dispersal, when we determined: the number of abscised flowers, and undeveloped and mature fruits.

Subsets of the flowers and fruits were collected at different developmental stages from free exposed flowers, bagged flowers, and also from unbagged flowers of very isolated individuals. The material was fixed in FAA (5% formalin, 5% acetic acid, and 90% ethyl alcohol), dehydrated in Johansen's tert-butyl alcohol series and embedded in paraffin (Johansen, 1940). Transverse (TS) and longitudinal (LS) sections, 10-12 µm, were stained with a safranin-Astra blue combination (Luque & al., 1986). Histochemical tests included the FeSO4 (Ruzin, 1999) and IKI-H2SO4 methods for tannins (Jensen, 1962), and phloroglucinol for cellulose/lignin.

To check bagging efficiency and to detect pollen grain presence or germination, some bagged and free exposed flowers were fixed in FAA, stained with Aniline blue (Martin, 1949) and were observed using fluorescence microscopy. The observations, drawings and photomicrographs were made with a Leica DM LB2 microscope. For scanning electron microscopy (SEM) observations, fresh material was fixed in FAA, dehydrated in an acetone series, dried at critical point and coated with gold-palladium. The observations and micrographs were made with a JEOL LV 5800 at 20 kV.

**Results**

The flowers of *S. balansae* were imperfect, pentamorous, heterochlamydeous, with an apotropous...
ovule and an intrastaminal nectariferous disc. The gynoecium developed only in pistillate flowers, the ovary was superior, ovoid, unilocular and laterally compressed with three styles placed in the middle portion of the ovary: a central one and two lateral ones, with a capitate stigma each (Figs. 1 A, 2 A). Occasionally gynoecia possessed either two styles (11%) or only one ventral style (4%) (Fig. 1 B). The ovary contained a single anatropous ovule with sub apical placentation (Figs. 1 C, 4 A).

Stamens in pistillate flowers have been reduced to staminodes, with two thecae and longitudinal slits (Fig. 1 B). These staminodes were not functional: the sporogenous tissue and the endothecium were not present (Fig. 1 D). In staminate flowers the anther thecae were tetrasporangiate and contained pollen grains. The endothecium developed as a subepidermal layer readily distinguished from the epidermis by its bands of secondary wall (Fig. 1 E).

**Ontogeny of the ovary wall**

In a floral bud, the ovary wall consisted of an external uniseriate epidermis consisting of quadrangular cells with conspicuous nuclei (Fig. 2 B, e). The middle zone of the carpel was made up of several layers of
Fig. 2. Ontogeny of the ovary wall: A, pistillate flower; B, ovary in TS, previous to the ovule development; C, young ovary in transverse section, corresponding to the shaded area in diagram D; E, external epidermis and stomata; F, internal epidermis; G, mature ovary in transverse section. Abbreviations: c, secretory ducts; d, druses; e, external epidermis; h, vascular bundles; hip, hypodermis; i, internal epidermis; me, external mesocarp; mi, internal mesocarp; pr, procambial tissue. Scale bar: A = 1 cm; B, C, E-G = 10 µm; D = 0.1 mm.
parenchyma cells which were isodiametric and had thin walls; several strands of procambial tissue were present in the central portion of the carpel (Fig. 2 B, pr). The protoderm formed an internal unistratified epidermis; the sub epidermal layer was differentiated in hypodermis also consisting of only one layer (Fig. 2 B, i, hip).

During the development of the ovary (Figs. 2 C; 4 A, B), the external epidermal cells continued dividing anticlinally and then elongated radially forming a palisade of short cells of less than 12 µm high, with thin walls and a smooth cuticle (Figs. 2 C, e; 4 B, e). These cells, as seen from above, were polygonal and some anomocytic stomata were differentiated (Fig. 2 E). The number of cellular layers was higher in the middle zone of the carpel: cells remained with thin walls and without intercellular spaces. In the external part of the vascular bundles apparently lysigenous secretory ducts were formed (Fig. 2 C, h, c). Cells of the internal epidermis and the hypodermis divided periclinally, forming four layers (Figs. 2, C, hip, i; 4 B, hip, i). On superficial view, the inner epidermis presented polygonal cells without stomata (Fig. 2 F).

General cell size increased during anthesis, with an extensive tannin deposition. Druses appeared in the parenchyma exterior to vascular bundles (Fig. 2 G, me, d).

At this point, the following tissues were already differentiated through the ovarian cavity: a) a uniseriate external epidermis (Fig. 2 G, e), b) a mesocarp divided in two parts by the vascular bundles and the secretory ducts (Fig. 2 G, me, h, c, mi) a two-layered hypodermis and internal bistratified epidermis (Fig. 2 G, hip, i).

**Fruit development**

Free exposed flowers always presented pollen grains on the stigmata. In unbagged inflorescences, almost half the mature fruits did not produce a normal seed. Some empty fruits presented seeds with undeveloped embryos arrested at different stages, although the lack of an embryo was the most common condition. In the population of Santa Fe, fruits from freely pollinated flowers containing normal seeds were 24.58% and 56.66% in 2004 and 2005, respectively. In Corrientes city, 58.4% of the fruits were normal and the remainders were empty.

No pollen was observed on the stigmata of sampled flowers from the bagged inflorescences, confirming the efficacy of the bagging treatment. All of the bagged inflorescences produced some fruits. Within the bags it was possible to distinguish the following: a small proportion of flowers aborted at bud stage (8.5%); flowers aborted at anthesis ranged from 27.35 to 41.01%; around 9% of very small fruits of 2-5 mm were found, and around 40% of flowers produced seemingly normal fruits. However, the percentage of such fruits containing fully developed seeds was 2.5% in 2004 and 8.5% in 2005.

In both analyzed flowers -free exposed and bagged ones- the development of the pericarp was the same. After the petal abscission there was a rapid increase in the ovary size whilst styles and stigmata were still present. The apical portion of the ovary grew initiating the wing (Fig. 3 A). The uniseriate external epidermis formed the exocarp, the cells maintaining active anticlinal divisions and increased in size forming a palisade with cells of 20-25 µm high (Fig. 3 C, ex). As the pericarp developed, the exocarp cells acquired a lobulated outline. New stomata were formed and the surrounding cells divided anticlinally (Fig. 3 D).

The cells of the middle zone of the carpel continued increasing in number and size forming the mesocarp, the external zone of which remained composed of elliptic to spherical cells, filled with big starch granules with stellate hilum (Fig. 3 C, me). The cells of the internal mesocarp acquired an irregular shape with a lobulated outline, also filled with starch granules (Fig. 3 C, mi). Parenchyma cells close to the secretory ducts collapsed and became flattened.

The endocarp at this time consisted of four layers, which derived from the internal epidermis and the carpel hypodermis (Figs. 3 C, en; 4 F). The cells of the internal epidermis formed a palisade 20-25 µm high with very vacuolated cells, although the nuclei remained in a central position (Fig. 4 F). Since the contour of these cells changed gradually from polygonal to undulated, as seen from above (Figs. 3 E, 4 D), it is very difficult to identify them in a transversal section of the pericarp (Fig. 3 C, en). As the fruit continued developing, the cells of the internal epidermis of the endocarp became sclereids (Fig. 4 G). The remaining three layers of the endocarp presented elongated cells placed periclinaly (Figs. 3 C, en, 4 G): two intermediate layers formed by sclereids, and an inner layer (derived from the hypodermis and in contact with the mesocarp) formed by parenchymatic cells (Fig. 3 C).

The mature fruit wall (Fig. 3 B, F, G) presented the following anatomical features:

a. Exocarp: was composed of the external epidermis, with thickened and lignified cells walls. A layer of cuticle covered the whole surface (Fig. 3 G, ex). Seen from above, these exocarp cells had a markedly undulate outline (Fig. 4 E). The 2-4 sub-epidermal layers belonging to the external part of the mesocarp maintained their thin walls but they became suberized,
Fig. 3. Fruit development: A, general view of the young fruit; B, mature fruit; C, young pericarp in transverse section; D, epicarp and stomata; E, endocarp; F, diagram showing the arrangement of tissues in transverse section of fruit; G, mature pericarp in transverse section; H, detail of sclereid of mesocarp. Abbreviations: c, secretory ducts; cr, prismatic crystal; en, endocarp; ex, exocarp; h, vascular bundles; me, external mesocarp; mex, corky layer; mi, internal mesocarp; st, stomata; zt, transition zone. Scale bar: A, B = 0.5 cm; C-E, G, H = 10 µm; F = 0.15 mm.
forming a cory layer (Fig. 3 F, G, mex). Following fruit dispersal these suberized layers became dry and collapsed to form, with the external epidermis, the exocarp (*sensu lato*); stomata were somewhat sunken in relation to the epidermal cells.

b. Mesocarp: the external portion of the vascular bundles remained as a zone of irregular cells with thin walls forming intercellular spaces (Fig. 3 G, me). The cells of the internal mesocarp differentiated into sclereids (Fig. 3 G, mi; H). The limit between both layers of the mesocarp was due to the presence of vascular bundles always externally associated with secretory ducts and by a transition zone where the cells changed gradually from lobulated to isodiametric, while cell walls became thinner (Figs. 3 F, c, h; 3 G, zt, c, h). When the fruit was mature and dry, the whole internal mesocarp was formed by sclereids and constituted a third part of the body of the pericarp (Fig. 3 F, mi). The wing of the samara consisted of the external mesocarp, consisting of parenchymatic cells and vascularized by a net of tiny vascular bundles.

c. Endocarp: was formed by four layers: the cells of the internal epidermis formed a palisade of macrosclereids (Fig. 3, F, G, en). Both subepidermal layers also differentiated into sclereids of quadrangular section with branched pits (Figs. 3 G, en, 4 H). The cells of the inner layer of the hypodermis, with thin cellulose cell walls, each contained a conspicuous cuboid or prismatic crystal (Figs. 3 G, 4 C). This crystalliferous layer appeared as a natural breaking zone since the three layers of the endocarp remained attached to the seed when the fruit was opened manually. Empty fruits presented a seminal cavity delimited by the three layers of the endocarp which contained a vestigial ovule. A very long funiculus was present although it did not show any degree of mucellar development, suggesting the presence of an aborted embryo. There were no differences in the initial pericarp development between bagged and unbagged flowers nor did we detect anatomical or morphological differences between the pericarp of empty fruits and fruits containing seeds. Before maturity, both kinds of fruits were initially red, becoming brown when dry.

**Discussion**

According to the endocarp organization, Wannan & Quinn (1990), proposed a classification of the different types of pericarp in the family Anacardiaceae. The genus *Schinopsis* was classified as *Anacardium*-type, based only in a brief description of *S. haenkeana*, the endocarp was regularly guided and consists of four layers of cells: an internal crystalliferous layer and three external layers formed by sclereids in palisade. The presence of a lignified outer epidermis is restricted to this type of endocarp. The exocarp of *S. haenkeana* was formed by 6 layers: the outer lignified epidermis plus 5 underlying parenchyma layers, the mesocarp was almost completely lignified. The ontogeny of the fruit of *S. haenkeana* was not reported by Wannan & Quinn (1990).

Following the Wannan & Quinn’s classification, the endocarp of *S. balansae* presented an *Anacardium*-type. The characteristic of their pericarp agrees with the description of *S. haenkeana*.

According to Roth (1977) the endocarp or exocarp could be formed by the epidermis of the ovary and its immediate derivates, in which case it was referred to as “*sensu stricto*”; if the endocarp/exocarp also includes layers of the mesocarp then it was referred to as “*sensu lato*”. Our ontogenetic study confirm that the exocarp and the endocarp in *S. balansae* were formed *sensu lato*, i.e. the exocarp was made up of the external epidermis of the carpel, which contains cells with thickened walls, and suberized layers originated from the underlying parenchyma. The endocarp was formed by four layers derived from the divisions of the internal epidermis and carpel hypodermis, the latter not derived from the protoderm.

A feature of seeds contained within a lignified pericarp is that the function of the seed cover is transferred to the pericarp, and the episperm is relatively undeveloped (Boesewinkel & Bouman, 1984). This characteristic also appeared in the samaras of other species i.e., *Tipuana tipu* (Benth.) Kuntze (Martins & Oliveira, 2001) and *Pterodon emarginatus* Vogel, Leguminosae (Oliveira & Paiva, 2005). In *S. balansae* the protective functions of the seed coat are transferred to the endocarp, indeed the columnar cells of the endocarp resemble the cells commonly encountered in seed testa. This transfer of functions has already been described as a generalized condition for the family (Corner, 1976). The irregular arrangement of the exocarp cells, with their lobulated shape and a densely interlocked pattern, and their highly lignified cell walls, explain the indehiscence of the *S. balansae* fruit.

*Schinopsis balansae* is a parthenocarpic species: it produces a great amount of fruits, many of them lacking seeds. Bagging treatment confirmed that the absence of pollen do not prevent fruiting. Self pollination is not possible because anthers of pistillated flowers -apparently normal- do not produce sporogenous tissue and there is no pollen formation. The low percentage of seeded fruits produced under pollen exclu
tion should be checked for eventual maternal progeny production.

Seed abortion at different development stages did not lead to fruit abscission in this species. This is a common condition in the family. Parthenocarpy in Anacardiaceae is due to several causes: a) pre fertilization including funicle or embryo sac degeneration, embryo sac absence, vascular transport to ovules

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**Fig. 4.** Optical microscope photographs: **A,** longitudinal section of pistillate flower at anthesis; **B,** longitudinal section of ovary at floral anthesis; **C,** polarized light photograph of crystalliferous layer of endocarp; **D,** superficial view of endocarp; **E,** superficial view of exocarp; **F-H,** transverse section of endocarp in three different stages of development, showing the formation of sclereids; **F,** endocarp in young fruit, phase of cell division; **G,** endocarp in immature fruit; **H,** endocarp in mature fruit. Abbreviations: e, external epidermis; hip, hypodermis; i, internal epidermis. Scale bar: **A** = 0.5 cm; **B** = 20 µm; **C-H** = 10 µm.
blocked at the placenta or in the funicle (Polito, 1999); b) lack of fertilization and of pollen tube penetration in the embryo sac (Shuraki & Sedgley, 1994) and c) post fertilization failure of embryo development or cellularisation of the endosperm (Grundwag & Fahn, 1969; Ram & al., 1976; Shuraki & Sedgley 1994).

In the current study we found normal fruits containing ovules, aborted embryos at different stages of development and seeds. Further studies -ecological and ontogenetic- will be necessary to figure out the several steps and causes of seed abortion.

Parthenocarpy seems to be a plesiomorphic trait within the Anacardiaceae. In other reported cases, in species with fleshy, bird-dispersed fruits, it has been suggested that empty fruit retention could be advantageous presumably in helping to attract the seed dispersers, and this may be the case in genera with endozoocorous drupe or drupaceous fruits (Traveset, 1993; Verdú & García-Fayos, 1998; 2002).

Stenospermocarpy is a term describing a small seedless fruit caused by embryo abortion after fertilization. This characteristic was fully described in mango (Mangifera indica L.), and related to environment conditions, specially the temperatures during pollination or early fruit set (Lakshminarayana & Aguilar, 1975; Davenport & Núñez-Elisea, 1983; Soule, 1985; Whiley & al., 1988).

The occurrence of partenocarpic samara seems rather difficult to explain. However, like Schinopsis, species of the genera Astronium Jacq. and Loxopterygium Hook. fil. (Anacardiaceae) have wind dispersed seeds, and the presence of parthenocarpy in these genera should be investigated. Whether parthenocarpy has an adaptive value or otherwise an example of phylogenetic baggage remains unclear in Schinopsis.

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