Ontogeny and Structure of the Pericarp of Schinus terebinthifolius Raddi (Anacardiaceae)

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ABSTRACT

The fruit of Schinus terebinthifolius Raddi is a globose red drupe with friable exocarp when ripe and composed of two lignified layers: the epidermis and hypodermis. The mesocarp is parenchymatous with large secretory ducts associated with vascular bundles. In the mesocarp two regions are observed: an outer region composed of only parenchymatous cells and an inner region, bounded by one or more layers of druse-like crystals of calcium oxalate, composed of parenchymatous cells, secretory ducts and vascular bundles. The mesocarp detaches itself from the exocarp due to degeneration of the cellular layers in contact with the hypodermis. The lignified endocarp is composed of four layers: the outermost layer of polyhedral cells with prismatic crystals of calcium oxalate, and the three innermost layers of sclereids in palisade.

Key words: Anacardiaceae; Schinus terebinthifolius; pericarp; anatomy; pericarpo; anatomia

INTRODUCTION

Schinus terebinthifolius Raddi, also known as the Brazilian Pepper Tree, belongs to the tribe Rhoideae (Rhoeae) of the Anacardiaceae family. This species is limited in its natural distribution to South America. The trees are used ornamentally and their fruits are used in cooking. Based on the studies produced so far, Netolitzky (1924) and Corner (1976) considered that the protective function of testa or the tegmen in Anacardiaceae seems to have been transferred to the endocarp, and thus, the seed integuments, which are not so specialized, do not provide criteria for its classification. The lack of specialization of the seed integuments of Anacardiaceae gives to the pericarp structure a considerable taxonomic significance particularly at a generic level. However, further ontogenic studies of the Anacardiaceae family are necessary to compare the homologous structures in the various taxa (Von Teichman, 1989). In most cases, generic limits in Anacardiaceae are mainly established by considering the type of fruit, placentation and the embryo characteristics (Santin and Leitão-Filho, 1991).

The vast majority of the taxonomic treatments of Anacardiaceae have described the fruit as drupaceous. Descriptions of individual genera are frequently superficial and provide little information on the anatomy and morphology of the fruit. There are few descriptions of the fruits of individual species, even fewer of the groups of species (Wannan and Quinn, 1990).

Wannan and Quinn (1990) conducted a broad
research on the structure of the pericarp in 29 genera of Anacardiaceae and observed little uniformity in the structures of the exocarp and mesocarp, as well as two distinct types of endocarp which were designated *Anacardium*-type and *Spondias*-type.

This work aimed to study the ontogeny and the structure of the pericarp of *Schinus terebinthifolius* to contribute to the characterization of this species, as well as to establish the generic limits within Anacardiaceae.

**MATERIALS AND METHODS**

Fruits of *Schinus terebinthifolius* at different stages of development were used in this study. These were collected from specimens grown in the Botanical Garden of the Instituto de Biociências, Universidade Estadual Paulista, Campus de Botucatu-SP, Brazil. The morphological and anatomical studies were carried out using fresh material and material fixed in FAA 50 (Johansen, 1940). The morphological characteristics of the fruits were described and illustrated using 100 individual fruits sampled from at least five different trees. The nomenclature used in this study were taken from the following: Radford *et al.* (1974) for the form of the fruits; Spjut (1994) for the type of the fruit; and Roth (1977) for the pericarp layers.

Permanent slides were made with the fixed samples and embedded in glycol metacrylate, using the technique described by Gerrits (1991). The embedded material was glued to blocks of wood with epoxi-glue. Sections were cut on a rotary microtome with steel knives type C. These sections were stained using 0.05% toluidine blue in acetate buffer with pH 4.7 (O’Brien *et al.*, 1964), and mounted in synthetic resin.

For the histochemical tests, the sections of fresh material were submitted to specific dye and/or reagents such as: aqueous solution of FeCl₃ for detection of phenolic compounds, phloroglucinol-HCL for lignified walls (Sass, 1951), Sudan IV for lipids, chloridric and sulfuric acids for crystals (Johansen, 1940).

**Figures 1-3 - Schinus terebinthifolius.** 1. General aspect of the mature fruit. 2. Longitudinal section of the mature fruit. 3. Transversal section of the mature fruit. (ex=exocarp; ms=mesocarp; sc=secretory cavity; en=endocarp; se=seed; em=embryo; cl=crystal layer).
RESULTS

At the time of anthesis the ovary wall of *S. terebinthifolius* showed the outer epiderm cells radially elongated, the parenchymatous zone between outer and inner epidermis with secretory cavities and vascular vessels and the inner epidermis, which comprised the initials of the future endocarp.

The fruit was a globose (Fig. 1) red drupe 5.5 mm in diameter, uniloculate, unisperm with friable exocarp when ripe. The mesocarp was parenchymatous with large secretory cavities (Figs. 2, 3) which produced a substance that gave the mesocarp an amber color. The endocarp was lignified.

The longitudinal and transversal sections of the immature fruits showed similar anatomical characteristics at different stages of development, as could be seen from the following:

a) The uniseriate outer epidermis (Fig. 4, 7-10) was covered by a cuticle with non-glandular and glandular trichomes and stomata. The cells were radially elongated with evident nuclei (Figs. 7-9).

b) The hypodermis consisted of thin-walled cells, these cells were larger than those of the epidermis (Figs. 8-10).

c) The central parenchymatous zone was characterized by secretory cavities associated with vascular bundles in which two regions could be observed: an outer region composed only of parenchymatous cells, and an inner region composed of parenchymatous cells, secretory cavities and vascular bundles bound by one or more layers of druse-like crystals of calcium oxalate (Figs. 2-7).

*Figure 4-7 –* 4) Transversal section of the mature fruit. Arrow indicates endocarp. (scale bar=100µm). 5) Detail showing secretory cavities (asterisk) associated with vascular bundles. (scale bar=250µm). 6) Secretory cavities and vascular bundles bounded by layers of druse-like crystals of calcium oxalate (arrows). (scale bar=500µm). 7) Detail showing crystal-layer (arrows). (scale bar=50µm). (em=embryo; ex=exocarp; ph=phloem; ms=mesocarp; star=xylem).
d) The multiseriate inner epidermis was composed of four layers of cells: the three outermost layers were of polyhedral cells, and the innermost layer, in contact with the locule, was radially elongated (Figs. 4, 12-14) with a cuticle.

Differentiation during the development towards the mature pericarp affected mainly the outer epidermis (future exocarp) and the multiseriate inner epidermis (future endocarp) (Fig. 11). The exocarp (*sensu lato*) was composed of two layers: the outer epidermis of the ovary and the hypodermis which lignified in the mature fruit, turning the exocarp friable (Fig. 10). The mesocarp was characterized by a parenchymatous zone with secretory cavities associated with vascular bundles with a layer of idioblasts containing druses of calcium oxalate, forming a sheath close to the secretory cavities (Figs. 2, 3, 6, 7). In the mature fruit, the mesocarp was secretory, yellowish, and detaches itself from the exocarp due to the degeneration of the outer cellular layers of the mesocarp which were in contact with the hypodermis (Figs. 9, 10).

The endocarp (*sensu stricto*) presented four layers that derived from the inner epidermis of the ovary wall (Figs. 11, 12). In the mature fruit, the endocarp was composed of a layer of crystalliferous cells (prismatic crystals), and three layers of sclereids in palisade (Figs. 13, 14). Between two sclereids layers, there was one layer in which the cells in palisade were smaller (Figs. 13, 14). The walls of the four layers became thickened and lignified (Fig. 14).

**DISCUSSION**

The morphological and anatomical analysis of the fruits of *S. terebinthifolius* showed that this species had well-delimited exo-, meso- and endocarp. The endocarp was sclerified and the fruits could be typified as drupes. This typification of the species agrees with that of Barkley (1942, 1957), Spjut (1994) and Barroso *et al.* (1999).

The pericarp, in drupe-like fruits, showed well-delimited exo-, meso- and endocarp. The exocarp or epicarp generally functioned as a protective layer for the fruit. The mesocarp was generally parenchymatous and lied between the exocarp and the endocarp. This latter was hard and has sclerified layers that protect the seed (Roth, 1977). The exo- and endocarp could be considered either *sensu stricto* or *sensu lato*. These were considered *sensu stricto* when they developed from the cells of the outer and inner epidermis of the ovary wall, or from their derivatives; or *sensu lato* when they developed from the epidermal layers and adjacent subepidermal layers, or regions of the ovary wall (Roth, 1977).

The exocarp of *S. terebinthifolius* was composed by outer epiderm and a hypoderm, then considered an exocarp *sensu lato*. The endocarp was considered *sensu stricto* because it developed from the inner epidermis. According to Roth (1977), the pericarp has several functions; its main function is to protect the embryo from damage. Roth also reported that a certain relationship was observed between the pericarp development and the seed coat structure. When the pericarp is strongly developed taking over seed protection, the integuments remain parenchymatous or may even be reduced and partly disintegrated or disappear.
during development. Another important function of the pericarp is the dispersal of seeds, which in berries and drupes, seems to be attributed to the fleshy fruit part, usually the mesocarp.

In a comparative study of the pericarp within the Anacardiaceae family, Wannan and Quinn (1990) reported that in general the fruits were drupes, but there were marked differences in the nature and origin of sclerified and parenchymatous functional layers. However, all pericarps contained a central zone that included vascular bundles and secretory ducts and often fiber bundles or zones of sclerenchyma. The regions outside and inside this zone varied considerably in thickness and structure, but in all cases lacked the secretory ducts and vascular tissue. Therefore, the region containing parenchymatous cells, secretory ducts and vascular tissues was defined as mesocarp, and the regions outside and inside of it were defined as exocarp and endocarp, respectively.

The exocarp of *S. terebinthifolius* is composed of two cellular layers, the epidermis and the hypodermis, both consisting of lignified polygonal cells. A lignified epidermis has been previously reported in *Astronium, Cotinus, Euroschinus, Laurophyllus, Loxostylis, Rhus, Schinopsis* and *Schinus areira*. A hypodermis with lignified parenchyma is reported only in *Laurophyllus* and *Schinus areira* (Wannan and Quinn, 1990).

**Figures 11-14** - Transversal section of the mesocarp and endocarp. 11. Dividing inner epidermis of the ovary (arrows) (Scale bar: 25µm). 12. Radially elongated inner epidermis of the ovary (star). Arrows indicate dividing cells. (Scale bar: 50µm). 13. Mature fruit showing the composition of the endocarp (*sensu stricto*): crystalliferous layer (straight arrow) and 3 layers of not much lignified sclereids in palisade. The curved arrow indicates a smaller layer of sclereids. (Scale bar: 125µm). 14. Detail of the lignified endocarp. Arrow indicates crystalliferous layer. (Scale bar: 50µm) (ex=exocarp; ms=mesocarp).
The mesocarp is mainly composed of parenchymatous cells interspersed with secretory ducts which are associated with vascular bundles, and commonly surrounded by groups of sclereids or crystalliferous cells (Wannan and Quinn, 1990). The presence of sclereids around the secretory ducts seems to be characteristic of some species, for instance, *Pistacia* (Grandwag, 1976). In some genera, the mesocarp becomes partially or totally lignified, as observed in *Schinus*, *Rhodosphaera*, and *Ozoroa* (Wannan and Quinn, 1990), and in *Rhus lancea* (Von Teichman and Robbertse, 1986). In some species of *Mauria*, *Parishia*, *Astronium* and *Lithraea*, the secretory ducts form a compact arrangement one beside the other (Wannan and Quinn, 1990; Carmello-Guerreiro, 1996). Secretory ducts, randomly arranged close to the endocarp and surrounded by druse-like crystals of calcium oxalate, were observed in the mesocarp of *Schinus terebinthifolius*.

Based on the endocarp structure of the tribe Rhoeae, Wannan and Quinn (1990) divided this tribe into groups A, B and C. Groups A and B present an *Anacardium*-type endocarp, and group C a *Spondias*-type endocarp. Group A with endocarps composed of four-cell layers. The three inner layers are formed by palisade-like sclereids, whereas the outer layer is formed by small and sometimes unlignified crystalliferous cells. The three layers in palisade have been previously described for *Schinus molle* (Copeland, 1959), *Rhus diversiloba* (Copeland and Doyel, 1940), *Euroschinus* (Wannan and Quinn, 1990). Although Copeland and Doyel (1940) illustrated the crystalliferous layer in *T. diversiloba*, only in *R. lancea* (Von Teichman and Robbertse, 1986), it was observed that this layer derived from the inner epidermis by periclinal divisions, taking part in the endocarp formation.

Group B in which the endocarp appears to derive from group A. The endocarp is composed of two or three parenchymatous cellular layers. This characteristic results from the absence of one or two layers in palisade, since the crystalliferous layer is always present (Wannan and Quinn, 1990). Group C has an endocarp composed of irregularly oriented sclereids, but there is no visible arrangement into four layers or into palisade-like sclereids. In *Pentaspadon*, the inner epidermis is parenchymatous, and in *Camposperma* the inner epidermis is lignified. The presence of an endocarp composed of irregularly oriented sclereids suggests that these genera are related to Spondiaceae (Wannan and Quinn, 1990). The fruits of *S. terebinthifolius* possess endocarps with the characteristics presented by Wannan and Quinn (1990) for the *Anacardium*-type endocarp of the tribe Rhoeae-group A, which are composed of four sclerified layers. The three innermost layers are composed of palisade-like sclereids of different sizes: the innermost layer is larger, and the outermost layer is sclerified and crystalliferous. Wannan and Quinn (1990) also reported that the outer lignified epidermis observed here is restricted to the taxa with *Anacardium*-type endocarp.

When the endocarp derives entirely from the inner epidermis of the ovary wall or from its direct derivatives, it is called *sensu stricto*, as previously mentioned. This capacity of dividing itself periclinaly exhibited by the inner epidermis seems to be a characteristic of the Anacardiaceae (Roth, 1977). In, *Anacardium occidentale*, *Pistacia chinensis*, *Schinus molle*, *Rhus lancea*, *Rhus diversiloba* (Wannan and Quinn, 1990) *Rhus problematodes* (Von Teichman and Van Wyk, 1991), *Ozoroa paniculosa*, *Protorhus namaquensis* (Von Teichman and Van Wyk, 1993; 1994), and *Protorhus longifolia* (Von Teichman, 1991). *Lithraea molleoides* and *Myracrodruon urundeuva* (Carmello-Guerreiro, 1996) all of the tribe Rhoeae, the four layers of the endocarp derive from the inner epidermis of the ovary wall. *Schinus* follows the same pattern of endocarp development described for the other species of the tribe Rhoeae.

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**RESUMO**

O fruto de *Schinus terebinthifolius* Raddi é uma drupa vermelha, globosa, com exocarpo friável quando maduro e composto de duas camadas lignificadas: a epiderme e a hipoderme. O mesocarpo é parenquimatício com grandes canais secretores associados aos feixes vasculares. No mesocarpo distinguem-se duas regiões uma externa composta apenas de células parenquimáticas e uma interna, delimitada por uma ou mais camadas de células contendo cristais.
de oxalato de cálcio do tipo drusa, composta de células parenquímaticas, canais secretores e feixes vasculares. O mesocarpo desaparece devido à degeneração das camadas celulares em contato com a hipoderme. O endocarpo é lignificado e composto de quatro camadas: uma externa de células poliédricas com cristais prismáticos de oxalato de cálcio e três camadas internas de esclereídeos em paliçada.

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