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Fruit and seed ontogeny related to the seed behaviour of two tropical species of *Caesalpinia* (Leguminosae)

SIMONE DE PÁDUA TEIXEIRA[1]*, SANDRA MARIA CARMELLO-GUERREIRO[2] and SÍLVIA RODRIGUES MACHADO[3]

[1]*Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (USP), Ribeirão Preto, SP, 14040-903 Brazil*
[2]*Departamento de Botânica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), CP 6109, Campinas, SP, 13083-970 Brazil*
[3]*Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Botucatu, SP, 18618-000 Brazil*

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Caesalpinia echinata and *C. ferrea* var. *ferrea* have different seed behaviours and seed and fruit types. Comparison of the seed ontogeny and anatomy partly explained the differences in seed behaviour between these two species of Brazilian legumes; some differences were also related to fruit development. The seed coat in *C. ferrea* consisted of two layers of osteosclereids, as well as macrosclereids and fibres, to form a typical legume seed coat, whereas *C. echinata* had only macrosclereids and fibres. In *C. echinata*, the developing seed coat had paracytic stomata, a feature rarely found in legume seeds. These seed coat features may account for the low longevity of *C. echinata* seeds. The embryogeny was similar in both species, with no differences in the relationship between embryo growth and seed growth. The seeds of both species behaved as typical endospermic seeds, despite their different morphological classification (exendospermic orthodox seeds were described for *C. echinata* and endospermic orthodox seeds for *C. ferrea*). Embryo growth in *C. ferrea* accelerated when the sclerenchyma of the pericarp was developing, whereas embryonic growth in *C. echinata* was associated with the conclusion of spine and secretory reservoir development in the pericarp. Other features observed included an endothelial layer that secreted mucilage in both species, a nucellar summit, which grew up into the micropyle, and a placental obturator that connected the ovarian tissue to the ovule in *C. ferrea*.

ADDITIONAL KEYWORDS: Brazilwood – *Caesalpinia echinata* – *C. ferrea* – embryogeny – endosperm – stomata.

INTRODUCTION

Caesalpinia echinata Lam. (Brazilwood) and *C. ferrea* Mart. ex Tul. var. *ferrea* (Jucá) are two Brazilian legume species that occur preferentially in the north-eastern Atlantic rainforest (Polhill & Vidal, 1981). *Caesalpinia echinata* has been almost exterminated as a result of its use as a source of red dye for fabrics and ink, and the current total size of natural stands of this species is low (Cardoso *et al*., 1998). *Caesalpinia ferrea*, a species used in Brazilian folk medicine, has been investigated for its pharmacological properties. The active constituents of *C. ferrea* have anticancer (Nakamura *et al*., 2002a, b), as well as anti-inflammatory and analgesic (Carvalho *et al*., 1996) properties.

The fruit and seed morphology of *Caesalpinia* species is highly variable. *Caesalpinia echinata* and *C. ferrea*, which belong to the subgenera *Gillandina* and *Mezoneuron*, informal group Libidibia, respectively (Lersten & Curtis, 1994), have different seed behaviour, seed and fruit types: *C. echinata* has exendospermic orthodox seeds and spiny folicular fruits, whereas *C. ferrea* has endospermic orthodox seeds and smooth bacoid legumes (Lewis, 1987; Barroso *et al*., 1999; Barbedo, Bilia & Figueiredo-Ribeiro, 2002).

Legume seeds have been classified as exendospermic (exalbuminous) and endospermic (albuminous)

*Corresponding author. E-mail: spadua@fcfrp.usp.br

based on the absence or presence of a discernible endosperm in the mature seed (Boelcke, 1946; Gunn, 1981; Barroso *et al*., 1999). Bentham (1841) and Burkart (1934) were among the first to consider the absence/presence of an endosperm in seeds as a character of potential taxonomic value, especially at the tribal level. In Boelcke's (1946) seed key of Argentinian caesalpinoids, the first couplet divides into species 'with albumen' and 'without albumen'. This morphological classification does not consider seed ontogeny, and seeds with a similar development may be included in different categories.

There is considerable discussion concerning the seed behaviour of tropical species, with the current classification distinguishing between orthodox and recalcitrant seeds, based on features such as tolerance to desiccation, longevity during storage, seed dormancy, the main reserve materials and the species natural habitat (Werker, 1997; Barbedo & Bilia, 1998). The seeds of *C. echinata* have a low longevity of approximately three months (Barbedo *et al*., 2002), with immediate germination after shedding. In contrast, the seeds of *C. ferrea* can remain on the soil for more than eight months in natural conditions and germinate only after scarification (Lorenzi, 1998).

In this study, we examined the ontogeny of the seeds and fruits of *C. echinata* and *C. ferrea* in order to: (i) elucidate the origin of the morphological variations related to seed behaviour (ii) determine the distinctive seed and fruit characters of potential taxonomic value, and (iii) obtain information about the seeds and fruits of potential in the conservation of these two important tropical species.

MATERIAL AND METHODS

Plant material

Material of *Caesalpinia echinata* were collected during the spring of 2001 from plants cultivated in Campinas and on the Fazenda Campininha, Mogi-Guaçu, São Paulo State, Brazil. The *C. ferrea* samples were from plants cultivated in Campinas and Botucatu, São Paulo State, Brazil, and were collected during the summer and autumn of 2002. Voucher specimens were deposited in the herbarium of the Universidade Estadual de Campinas (UEC), São Paulo State, Brazil, under the accession numbers 26368 and 51731 for *C. echinata* and 60661 and 66698 for *C. ferrea*.

Anatomy and ontogeny

Fruits and seeds in several stages of development were fixed in Karnovsky solution for 24 h (Karnovsky, 1965), followed by gradual dehydration in an alcohol series and embedding in historesin (Gerrits, 1991). Sections 2–6 μm thick were stained with 0.05% toluidine blue (O'Brien, Feder & McCully, 1964) and permanent slides were mounted in Permount resin (Gerlach, 1969). Ovules and seeds of early developmental stages were also cleared in Herr's fluid (Herr, 1971) and examined using Nomarski differential interference contrast microscopy in order to study the embryogeny.

In order to detect phenolic compounds, fruits and seeds were fixed in formalin mixed with iron sulphide and then embedded in paraffin, sectioned (8–10 μm), and mounted in synthetic resin (Jensen, 1962). Control material was obtained by squashing the paraffin-embedded sections in methanol to extract phenolic compounds.

Photomicrographs were taken using a Leica model DMR microscope.

For ultrastructural studies, ovules and seeds at several stages of development were fixed with Karnovsky's solution (0.075 M in phosphate buffer, pH 7.2–7.4, for 4 h) (Karnovsky, 1965), postfixed with osmium tetroxide (1% in the same buffer for 1 h), dehydrated in an acetate series and embedded in Araldite. Ultrathin sections were stained with 2% uranyl acetate for 15 min (Watson, 1958) and with lead citrate for 15 min (Reynolds, 1963) and then examined using a Philips EM 301 electron microscope.

Quantitative studies

Two-phase regressions models (Hinkley, 1971) were tested to determine whether they could explain the relationship between fruit and seed lengths during seed development in *C. echinata* and *C. ferrea*. These analyses were performed using ODDJOB v.6.5 software (Dallal, 1989). Sixty-six pods of *C. echinata* and 67 of *C. ferrea* were analysed. The lengths of the seeds and pods of both species in early embryogeny were also compared graphically. Forty-one pods of *C. echinata* and 45 of *C. ferrea* were analysed. In all of these analyses, five individuals of each species were used.

RESULTS

Gross morphology

The gross morphological features of the seeds and fruits of *C. echinata* and *C. ferrea* showed considerable differences. The fruits of *C. echinata* were spiny whereas those of *C. ferrea* were smooth. The testa was chartaceous and exfoliate in *C. echinata* and bony and nonexfoliate in *C. ferrea*. The seeds in both species were round and asymmetrical, with raphes and antiraphes of different lengths.

Fruit

Ovary wall anatomy: There were three ovules per ovary in *C. echinata* and 7–9 in *C. ferrea*. The ovary outer epidermis was one-layered and its cells were elongated (Fig. 1), and stained positively for phenolic compounds (Fig. 1). The stomata were paracytic. The cuticle was thicker in *C. ferrea*. The mesophyll of the ovary wall consisted of an outer and an inner zone. The outer zone had 5–7 layers of parenchymatous cells with a parietal nucleus, and most of these cells had a flocculate phenolic content (Fig. 1). The inner zone consisted of nine layers of shorter cells, with a dense cytoplasm and very prominent nucleus (Fig. 1). The inner epidermis of the ovary had a simple layer of rectangular cells with a dense cytoplasm and highly stained nuclei. The placental cells themselves were papillose in *C. echinata* (Fig. 1) and trichomatous in *C. ferrea* (Fig. 10). These trichomes were formed by one basal cell and a stipe of two or three cells.

There were two vascular bundles in the sutural region and a larger one in the dorsal region of *C. ferrea* ovaries. In *C. echinata*, two vascular bundles occurred in the sutural and dorsal regions. Thick-walled parenchymatous cells occurred externally to the vascular bundles and scattered cells with phenolic compounds occurred amongst the vascular elements.

Sharp pointed structures formed by the outer epidermis of the ovary and the subepidermal layers occurred in all pericarpial extensions of *C. echinata*. Secretory reservoirs (Fig. 1) occurred in the outer zone of the median layers of *C. echinata* ovaries.

Pericarp anatomy: The exocarp had a thin cuticle in *C. echinata* (Fig. 2). The outer layers of the mesocarp had thick-walled cells and lignified vascular fibres were observed above the collateral vascular bundles. The inner layers of the mesocarp and the endocarp layers together formed about 20 layers of sclerenchymatous tissue, of which the outer layer consisted of brachysclereids and the underlying inner layers of fibres. The fibres ran parallel to the longitudinal axis of the pod. In the sutural and dorsal zones, a separation tissue of short parenchymatous cells was observed between the two carpellary vascular bundles. No secretory reservoirs were observed in the mesocarp of the mature fruit.

In *C. ferrea*, the exocarp was two-layered and had a thick cuticle (Fig. 8). The mesocarp had well-developed macrosclereids (Fig. 9) above the collateral vascular bundles. The longitudinal axis of these macrosclereids ran transverse to the longitudinal axis of the pod (Figs 8, 9). The endocarp tissue was formed by brachysclereids, and no separation tissue was observed in the sutural and dorsal zones.

Pericarp ontogeny: The exocarp (fruit epidermis) was derived from cell divisions of the outer epidermis of the ovary wall. In *C. echinata*, the divisions occurred mainly in the anticlinal plane whereas in *C. ferrea*, the divisions occurred in the periclinal and anticlinal planes, thereby adding another epidermal layer to the exocarp (Fig. 8).

In *C. echinata*, the brachysclereids of the mesocarp were derived from cells of the outer zone of the ovary (Fig. 7). The mesocarp and endocarp fibres resulted from cells of the inner zone and inner epidermis of the ovary. These cells divided several times in the anticlinal plane (Fig. 7) and subsequently became wall-thickened and lignified.

In *C. ferrea*, the macrosclereids of the mesocarp were derived from cells accompanying the vascular bundles that underwent wall thickening and lignification (Fig. 8). The brachysclereids of the endocarp resulted from sclerification of the inner epidermis, and occurred later than the formation of macrosclereids.

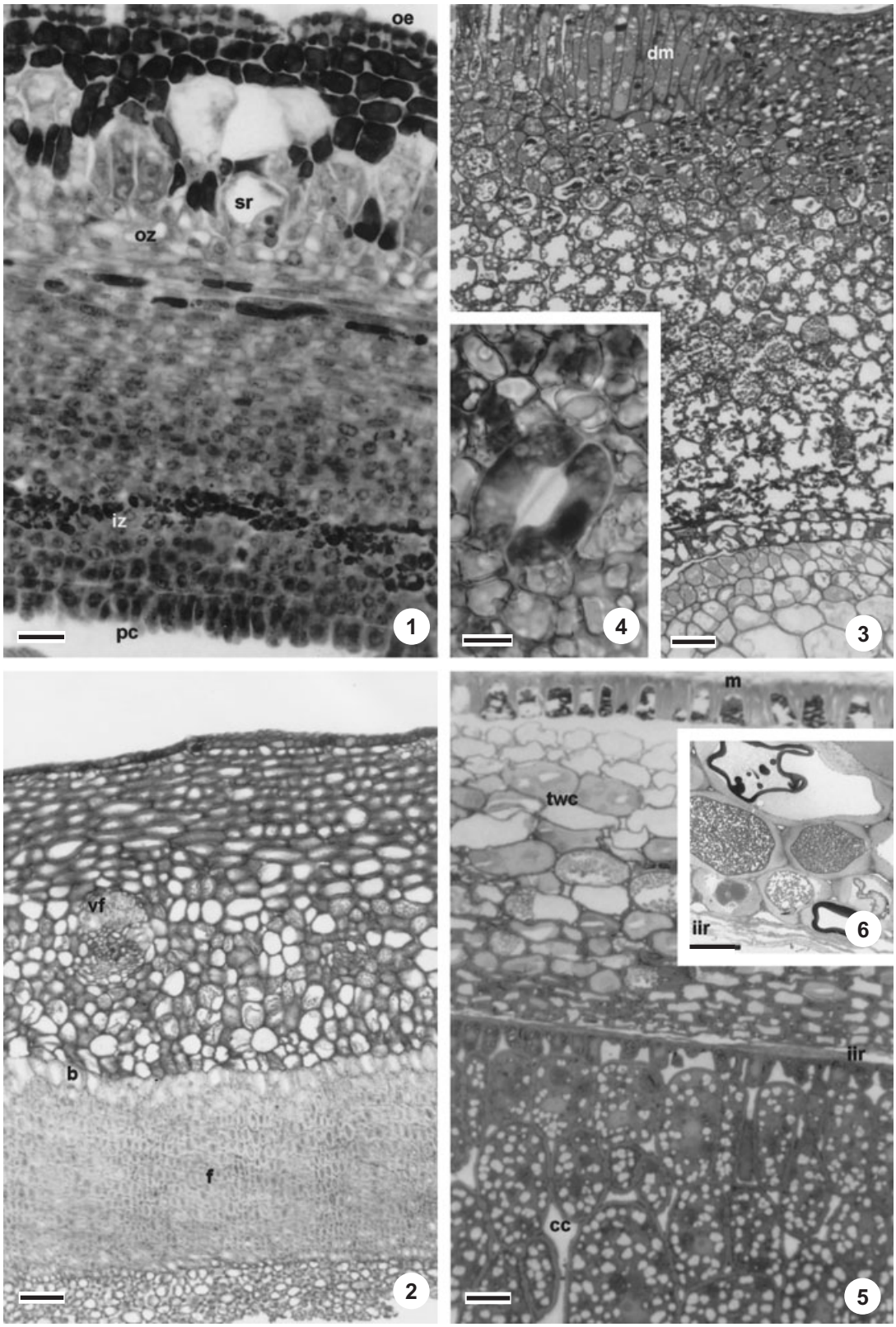
Seed

Ovule anatomy: The ovules were anatropous, bitegmic and crassinucellar, with a zig-zag micropyle (Figs 10, 14). In *C. echinata*, the funicle was short (Fig. 14), but it was long in *C. ferrea* (Fig. 10).

The outer integument was generally ten-layered in *C. echinata* (Fig. 14) and eight-layered in *C. ferrea* (Fig. 10), except in the micropylar zone where it became thinner. In the funicular zone, the outer integument formed a lateral projection through an increase in cell number (Fig. 14). The outer epidermis of the outer integument had elongated cells and reacted positively to phenolic compound staining (Fig. 14). The remaining outer integument consisted of vacuolated, thin-walled cells (Figs 10,14). A collateral vascular bundle ran through the entire length of the outer integument.

The inner integument was four-layered in the chalazal and micropylar zones (Fig. 19), with the inner epidermis being endothelial (Figs 15, 20). The endothelial cell wall was thin and numerous plasmodesmata connected neighbouring cells. The cytoplasm was dense and contained small vacuoles and dictyosomes with many associated vesicles (Fig. 20). The nucleus was highly stained (Fig. 15). The remaining inner integument cells showed sinuous walls, a dense cytoplasm with vacuoles, extensive rough endoplasmic reticulum, mitochondria and chloroplasts (Fig. 19). The nucleus had a conspicuous nucleolus.

Most integumentary cells in the micropylar zone stained positively for phenolic compounds (Fig. 14). A viscous substance was observed in the micropylar zone (Fig. 15), in the embryo sac cavity, and in the inner integument layers. This substance stained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pink with toluidine blue, indicating a mucilaginous composition.

The nucellar cells contained highly stained, central nuclei and showed intensive division, especially in the micropylar zone (Fig. 15). The ovule of *C. ferrea* had a nucellar protrusion into the micropyle that contacted a trichomatous obturator (Fig. 10) of placental origin. The protrusion cells were round and thin-walled, with highly stained central nuclei (Fig. 11).

Seed anatomy. The mature embryos of *C. echinata* had two fleshy cotyledons of equal size and required about 70 days to develop from the zygote. The epidermal cells of the cotyledons were smaller and round while those of the mesophyll were elongated (Fig. 5). Starch grains were observed in all cotyledon cells. In *C. ferrea*, the mature embryo was chlorophyllous, with two leaf-like cotyledons of equal size and required about 180 days to develop from the zygote. The epidermal cells in the cotyledons were smaller and round. The mesophyll of the cotyledons was divided into two strata, spongy and palisadic. The cotyledons contained several starch grains.

The endosperm of *C. echinata* was completely consumed by the developing embryo, but this was not the case with *C. ferrea*. Positive staining of the endosperm with toluidine blue indicated the presence of mucilage.

The seed coat of *C. echinata* had a layer of macrosclereids with a thick cuticle and nearly 20 layers of lignified fibres (Figs 5, 13). In addition to macrosclereids and fibres, the seed coat in *C. ferrea* had two layers of osteosclereids, one above (Fig. 13) and the other below the fibres. The macrosclereids and most other seed coat cells contained phenolic compounds (Fig. 12), which were homogeneously dispersed or flocculated in the vacuole (Fig. 22). Sieve tubes and companion cells were observed in the seed coat of *C. ferrea*, but not of *C. echinata*.

Seed ontogeny. The first division of the zygote was transverse. The number of anticlinal and periclinal divisions increased in the globular stage of the proembryo (Fig. 16), to become very intense in the heart-shaped stage of the proembryo. In these stages, a short suspensor was observed (Fig. 17).

The endosperm was of the nuclear type and formed an aggressive haustorium in the chalazal zone, which invaded the adjacent tissues (Figs 10, 14). Cell formation (Fig. 18) started in the heart-shaped stage of the proembryo.

The outer epidermis cells of the outer integument became elongated and thick-walled (Figs 3, 12), and formed the macrosclereids of the seed coat (Figs 5, 13). In the hilar zone, the cells of the outer epidermis underwent greater elongation to produce macrosclereids that were more elongated than in other zones. The formation of macrosclereids occurred in the heart-shaped stage of the proembryo in *C. echinata* and in the globular stage of the proembryo in *C. ferrea*.

The outer epidermis of the seed coat in *C. echinata* contained paracytic stomata (Fig. 4) with a large stomatal chamber. The stomata persisted until the heart-shaped stage of the proembryo. No stomata were observed in *C. ferrea*.

In *C. ferrea*, the layer beneath the outer epidermis of the outer integument was formed by cells with sinuous walls, parietal and rounded nuclei, prominent nucleoli, dispersed chromatin and a large, central vacuole (Fig. 21). In later stages of seed development, these cells formed a single layer of osteosclereids (Fig. 13). At even later stages, the inner epidermis of the outer integument also formed a layer of osteosclereids.

In both species, the median layers of the outer integument consisted of regularly shaped cells with thin walls (Figs 3, 12) and rounded central nuclei. In the cotyledon stage of the embryo, these cells underwent wall thickening (Figs 5, 6) and lignification to produce fibres (Fig. 13). Cells of the inner integument became vacuolated at the first zygote division and collapsed in the cotyledon stage of embryonic development.

QUANTITATIVE STUDIES

The seeds and embryos were larger in *C. echinata* than in *C. ferrea*, although the fruit length was similar in both species (Table 1, Fig. 23). The relationship between fruit and seed lengths in both species was best explained by two regression lines with significantly different slopes (*C. echinata*: $U = 10.500$;

Figures 1–6. Light micrographs of *Caesalpinia echinata* fruits and seeds. Fig. 1. TS ovary showing the outer epidermis (oe), the median layers divided into outer (oz) and inner (iz) zones and the papillose placental cells (pc). Darkly stained cells contain phenolic compounds. Developing secretory reservoirs (sr) occur in the outer zone of the median layers. Scale bar = 22 μm. Fig. 2. TS fruit in the late stage of development. Note the vascular fibres (vf) and the inner sclerenchymatous layers formed by brachysclereids (b) and fibres (f). Thick-walled parenchymatous cells occur in the outer layers of the mesocarp. Scale bar = 22 μm. Fig. 3. LS seed in early stages of development. Note the developing macrosclereids (dm) in the seed coat. Scale bar = 22 μm. Fig. 4. Paracytic stomata in the seed coat. Scale bar = 9.5 μm. Fig. 5. LS seed in the late stage of development. Note the layer of macrosclereids (m), the thick-walled parenchymatous cells (twc), the inner integumentary remnants and the cotyledon cells (cc). Scale bar = 22 μm. Fig. 6. Detail of the thick-walled parenchymatous cells and the inner integumentary remnants (iir). Scale bar = 5.7 μm.

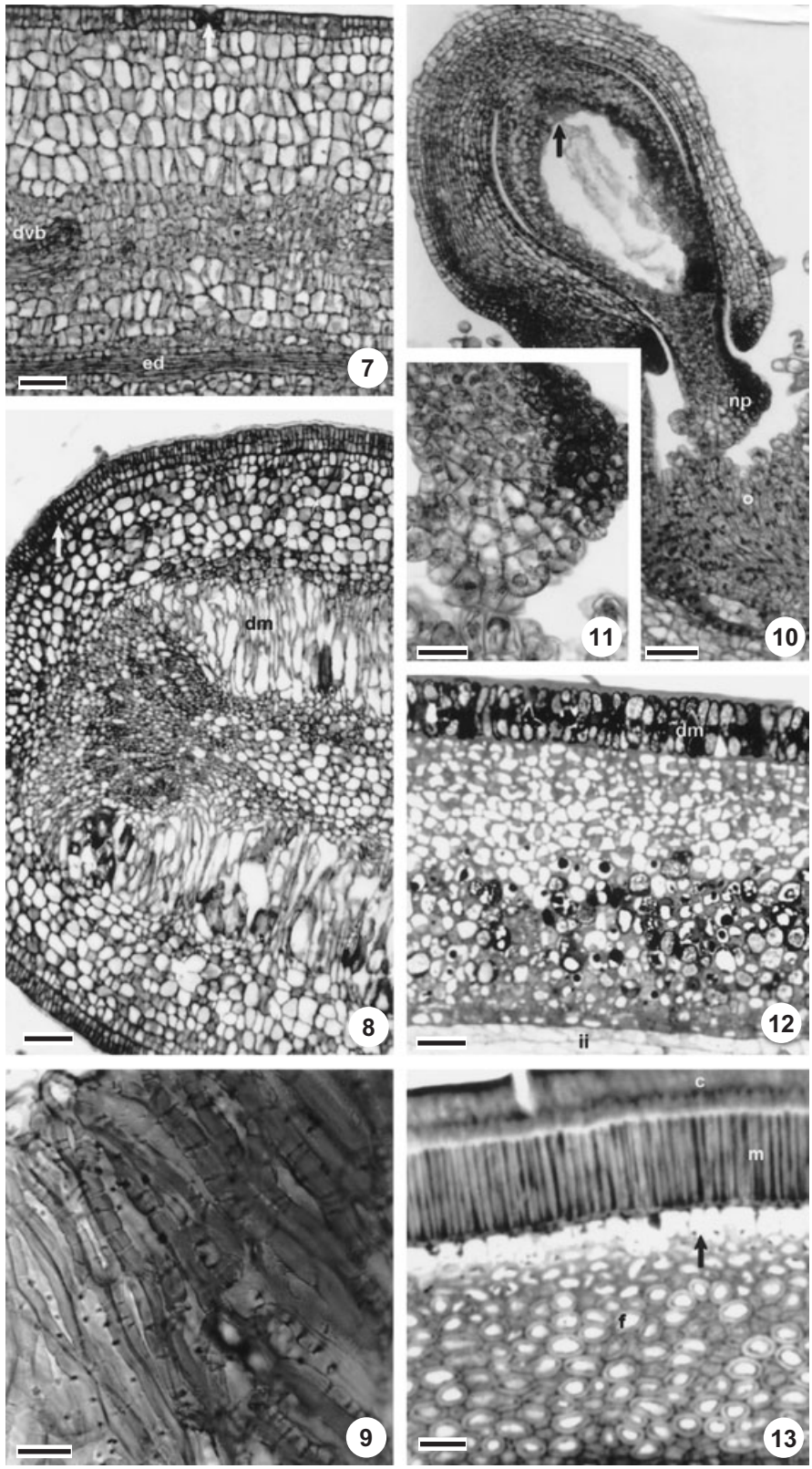
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Table 1. Fruit, seed and embryo characteristics at several stages of development in *Caesalpinia echinata* and *C. ferrea*

Embryo shape	Species	Length			Fruit anatomy
		Fruit (mm)	Seed (μm)	Embryo (μm)	
Proembryo (Fig. 14)	*C. echinata*	40	1500	38	Most cells of the outer mesocarp layers contain phenolic compounds and the others remain nucleate
	C. ferrea	37	1200	24.4	Beginning of sclereid development in the mesocarp (Fig. 8)
Globular (Fig. 15)	*C. echinata*	42	2500	42.75	Intensive divisions in mesocarp and endocarp cells
	C. ferrea	–	–	–	Stage not found
Cordiform	*C. echinata*	55	3000	400	Endocarp cells undergo vacuolation and wall thickening
	C. ferrea	50	3900	200	Sclereids in the mesocarp are fully lignified (Fig. 9)
Cotyledons	*C. echinata*	66.5	5200	1100	Fibres in the endocarp are completely lignified and vascular fibres in the mesocarp begin to lignify
	C. ferrea	55	4200	600	Sclereids in the endocarp begin to lignify
Well-developed cotyledons	*C. echinata*	80	12 500	1700	Vascular fibres in the mesocarp are completely lignified (Fig. 2)
	C. ferrea	75	9500	850	Sclereids in the mesocarp and endocarp are completely lignified

$P < 10^{-4}$ and *C. ferrea*: $U = 8.196$; $P = 0.0001$) (Fig. 23). The slopes before the switch point (*C. echinata*: 0.0601 ± 0.0562; *C. ferrea*: 0.0190 ± 0.0608) were shallower than those after this point (*C. echinata*: 0.3420 ± 0.0665; *C. ferrea*: 0.1850 ± 0.0211). The switch point was 395.3 mm for *C. echinata* and 193.4 mm for *C. ferrea* (Fig. 23).

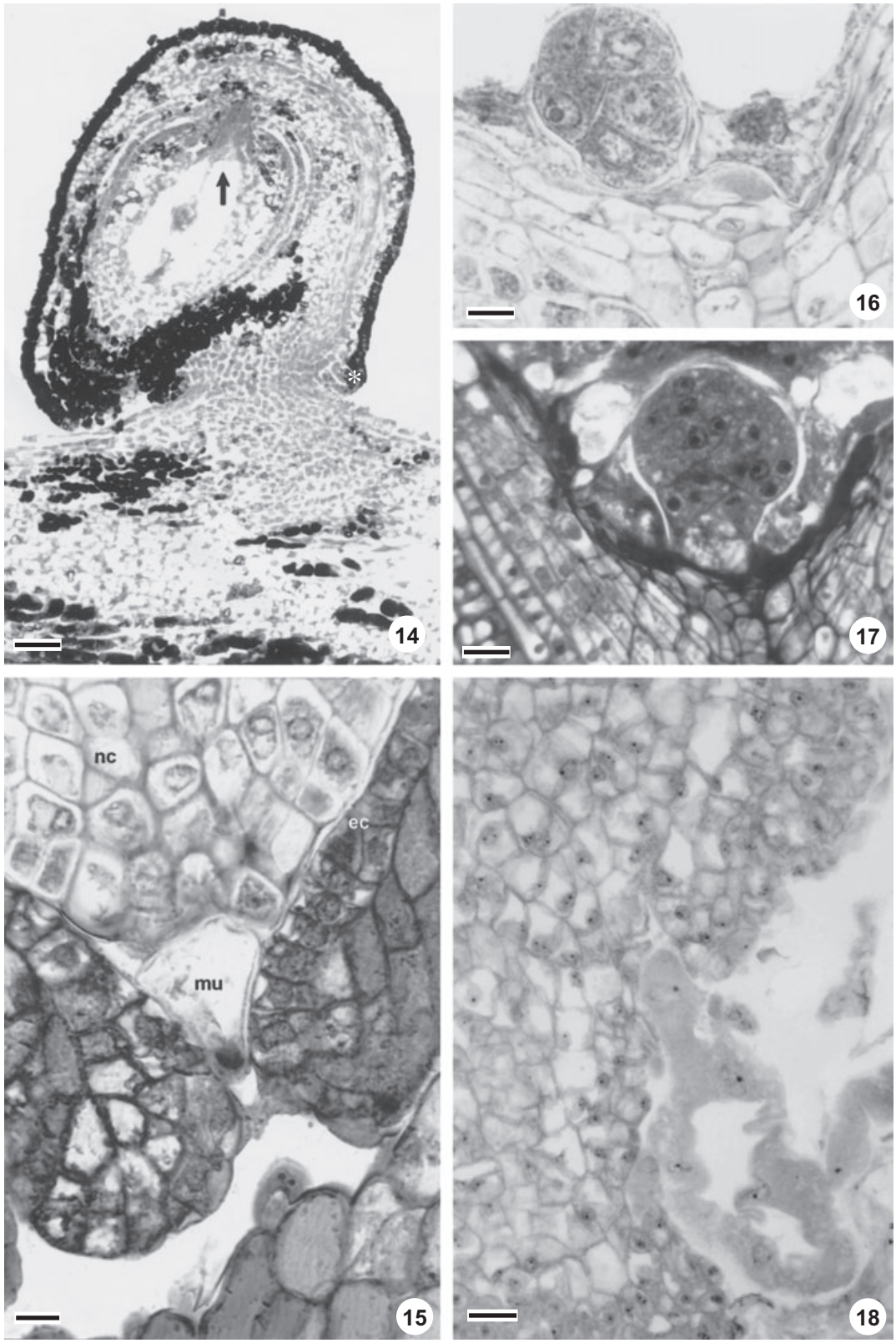
When compared with fruit growth, the seed growth was not accentuated in the early developmental stages (Fig. 24). After the initial growth, the seeds of *C. echinata* and *C. ferrea* stopped growing when about 1.5 mm and 0.5 mm long, whereas the fruits grew 25 mm and 20 mm, respectively (Fig. 24). Seed growth accompanied the beginning of sclereid development in the mesocarp of *C. ferrea*, when the fruits and seeds were about 30 mm and 1 mm long, respectively (Table 1, Fig. 24). The seeds and fruits of *C. echinata* grew simultaneously from a fruit length of 55 mm until maturity (Fig. 24), when the endocarp cells underwent wall thickening (Table 1). When the fruits of *C. echinata* were nearly 65 mm during late seed development, the seeds were of variable sizes (Fig. 23). Similarly, in *C. ferrea* seed size differed for the same values of the fruit size (Fig. 23), with the seeds collected in the last months being larger.

DISCUSSION

FRUIT

A relationship between embryogeny and development of the pericarpial sclerenchyma similar to that described for *Acacia paniculata* (Souza, 1993) also appears to occur in *C. ferrea*, i.e. the embryo grows slowly until the pericarp develops fibres and sclereids. Embryonic and seed growth in *C. ferrea* are associated with complete lignification of the mesocarpial sclereids. In addition to sclereids, the fruit at this stage also has a rigid dorsal region consisting of several fused vascular bundles. The relationship between pericarp sclerification and fruit size was less obvious in *C. echinata*. Sharp pointed structures and secretory reservoirs seen in the pericarp as soon as fertilization has occurred could protect the seeds of *C. echinata* against herbivory (Krzyzanowski, 1998).

Figures 7–13. Light micrographs of *Caesalpinia ferrea* fruits and seeds. Fig. 7. TS fruit in early stage of development showing exocarp and a stomate (arrow), the dividing mesocarp cells, the developing vascular bundles (dvb) and the endocarp layers (ed). Scale bar = 60 μm. Fig. 8. TS sutural region of a fruit in late stage of development. Note the single hypodermal layer (arrow) and the developing macrosclereids (dm) in the mesocarp. Scale bar = 60 μm. Fig. 9. Detail of macrosclereids in the mesocarp. Scale bar = 30 μm. Fig. 10. LS anatropous ovule showing the endosperm haustorium (arrow) and the nucellar protrusion (np) in contact with the trichomatous obturator (o). Scale bar = 61.5 μm. Fig. 11. Detail of the nucellar protrusion. Note the rounded cells with stained central nuclei. Scale bar = 14 μm. Fig. 12. LS seed in the early stage of development. Note the developing macrosclereid layer (dm) and the inner integumentary layers (ii). Darkly stained cells contain phenolic compounds. Scale bar = 28 μm. Fig. 13. LS seed in the late stage of development. Note the thick cuticle (c), the macrosclereids (m), the osteosclereids (arrow) and several layers of fibres (f). Scale bar = 54 μm.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In both species, fruits of the same size contained seeds of different sizes during late fruit development as a result of accelerated seed growth during late embryo development. A similar finding has been described for *Glycine max* (Laszlo, 1994). This growth occurred when the storage reserves were transferred to the embryo and characterized fruit ripeness.

Anatomical comparison of the indehiscent fruit of *C. ferrea* and the dehiscent fruit of *C. echinata* helped in identifying the tissues associated with mechanisms of seed release in these species. Various arrangements of sclereids have been observed in the pericarp of indehiscent fruits, including those of *C. ferrea* studied here, *Arachis hypogaea* (Halliburton, Glasser & Byrne, 1975) and *Senna spectabilis* (Souza, 1988b). Fibres have been found in the endocarp, among parenchymatous cells of the mesocarp, and among vascular bundles in dehiscent fruits (Fahn & Zohary, 1955; Izaguirre, Mérola & Beyhaut, 1994) of *C. echinata* (present study), *Senna multijuga*, *S. occidentalis* and *S. macranthera* var. *micans* (Souza, 1988b). However, *Dalbergia nigra* (Paoli, 1992), *Peltophorum dubium* (Santiago & Paoli, 1999) and *Lonchocarpus muehlbergianus* (Souza, 1988a), which have indehiscent fruits, also have fibres in the endocarp. Thus, fibre and sclereid arrangement in the pericarp, rather than the type of sclerenchymatous elements, appears to be more important for the mechanism of dehiscence.

Fruit extracts of *C. ferrea* var. *ferrea* have been investigated (Carvalho *et al*., 1996), and the antitumour activity of two constituents, gallic acid and methyl gallate, has been confirmed (Nakamura *et al*., 2002a,b). In the present study, phenolic compounds were found in the exocarp and mesocarp and also in the integument of *C. echinata* and *C. ferrea* seeds. Pharmacological studies should be expanded to other *Caesalpinia* species, including *C. echinata*, and should include other plant structures, such as seeds.

SEED

Analysis of the seed coat in *C. ferrea* revealed why these seeds have a greater longevity and are more tolerant to desiccation than those of *C. echinata*. In *C. ferrea*, the seed coat was bony with several layers of sclerenchyma (macrosclereids, osteosclereids and fibres) and no stomata, while that of *C. echinata* was chartaceous with no osteosclereid layers but with stomata. In this case, the stomata may be involved in water uptake in the dry seed prior to germination, as suggested by Werker (1997). The seed coat of *Inga fagifolia* also lacks the osteosclereid and other sclerenchyma layers, and is considered undifferentiated (Oliveira & Beltrati, 1994). According to Barbedo & Bilia (1998), the seeds of *Inga* species cannot tolerate desiccation. In contrast, soybean seeds are resistant to desiccation and this resistance is directly related to the lignin content of the seed coat (Alvarez *et al*., 1997).

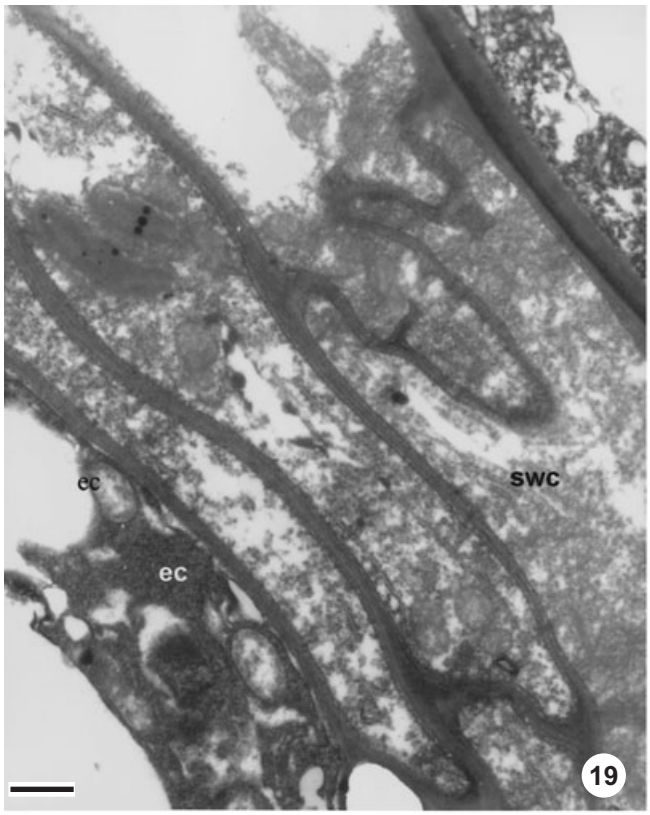
The seed coat and pod wall (pericarp) characteristics may be related to the different mechanisms of seed release in *C. echinata* and *C. ferrea*. Abrupt opening of the fruit and consequent expulsion disperse the seeds of *C. echinata* some distance from the mother tree. Germination occurs after soaking for two or three days. In contrast, the dispersal of *C. ferrea* seeds requires the pod wall (pericarp) to be crushed. Hence, the seeds of this species can remain dormant in the soil for more than eight months (Lorenzi, 1998).

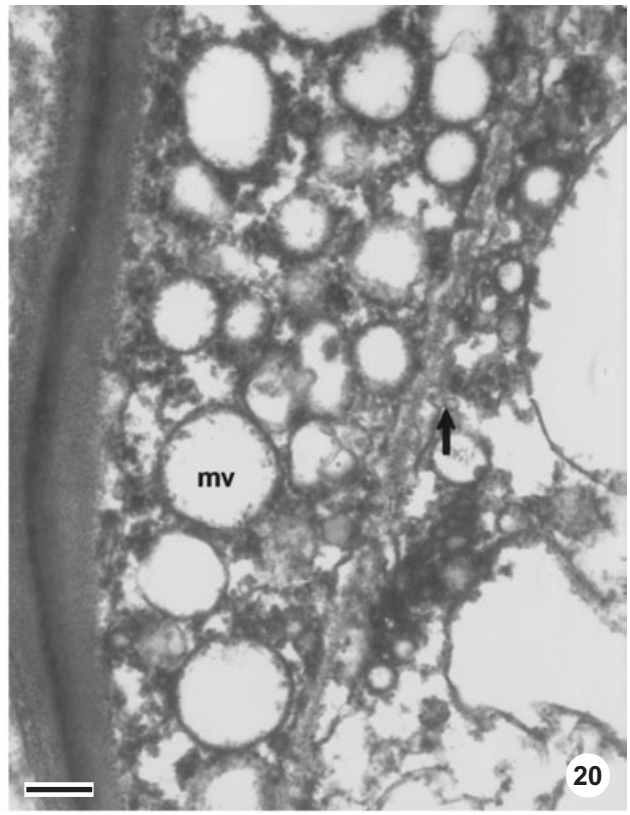
The two osteosclereid layers in the seed coat of *C. ferrea* may be related to seed aeration, as suggested by Corner (1951), especially considering the lack of stomata in this species. The occurrence of stomata and osteosclereids in the seed coat are distinguishing characteristics of the species studied. Thus, osteosclereids were not observed in *C. echinata*, but occurred in *C. ferrea*, and stomata occurred in *C. echinata* but not in *C. ferrea,* or other caesalpinoid species, such as *Senna micranthera* (Áqüila, 1995). The genus of *Bauhinia*, in a similar way to *Caesalpinia*, also contains some representatives with seed stomata, such as *B. variegata* (Werker, 1997), and others without, such as *B. forficata* (Beltrati & Paoli, 1998). Seed stomata have been reported for papilionoid species in particular, e.g. *Andira humilis*, *Inocarpus edulis* and *Olneya tesota*, but, in general, they are considered rare in Leguminosae (Werker, 1997).

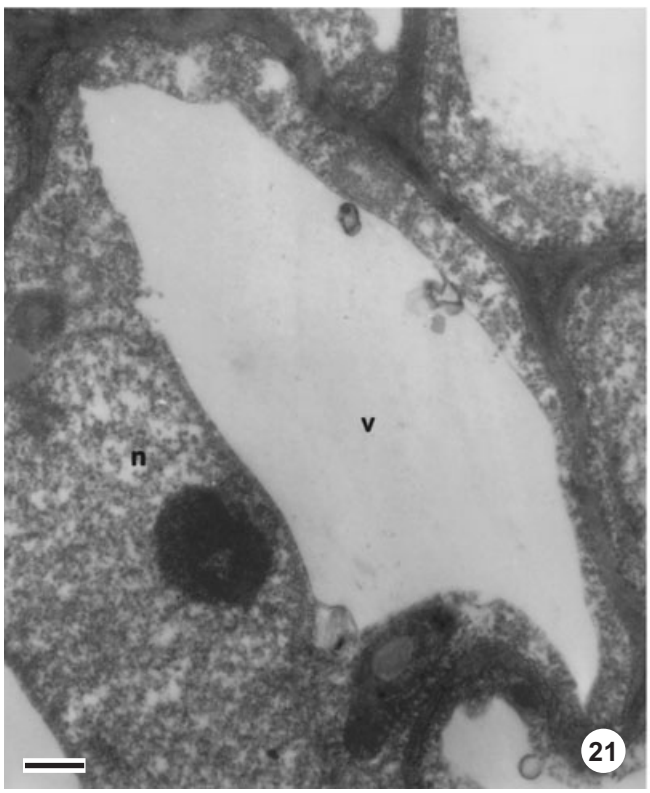
The seeds of *C. ferrea* can be considered as typical legume seeds (Corner, 1951), in contrast to those of *C. echinata*. Other characters found in both species, such as the anatropous ovule, the haustorial endosperm, the single vascular bundle in the seed coat and lack of a tegmen in mature seeds, are common in the Caesalpinioideae (Corner, 1951) and in the Leguminosae as a whole (Prakash, 1987).

The inner epidermis of the inner integument was cytologically distinct from other inner integument layers. The ultrastructural features of the endothelial

Figures 14–18. Light micrographs of *Caesalpinia echinata* seed tissue. Fig. 14. LS fertilized ovule showing the endosperm haustorium (arrow) and a lateral projection (*) formed by the outer integumentary cells. Darkly stained cells contain phenolic compounds. Scale bar = 43 μm. Fig. 15. Detail of the micropylar zone showing nucellar cells (nc), endothelial cells (ec) and a drop of mucilaginous secretion (mu). Scale bar = 9.5 μm. Fig. 16. Proembryo at the beginning of cell division. Scale bar = 9.5 μm. Fig. 17. Globular proembryo with a short suspensor. Scale bar = 9.5 μm. Fig. 18. Endosperm cellarization. Scale bar = 43 μm.


ec
ec
swc
19


mv
20


v
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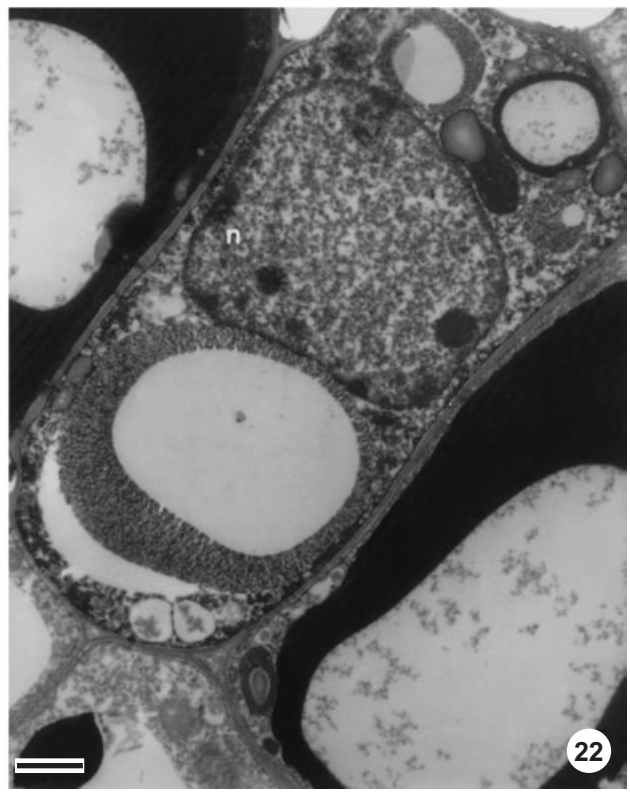
21


n
22

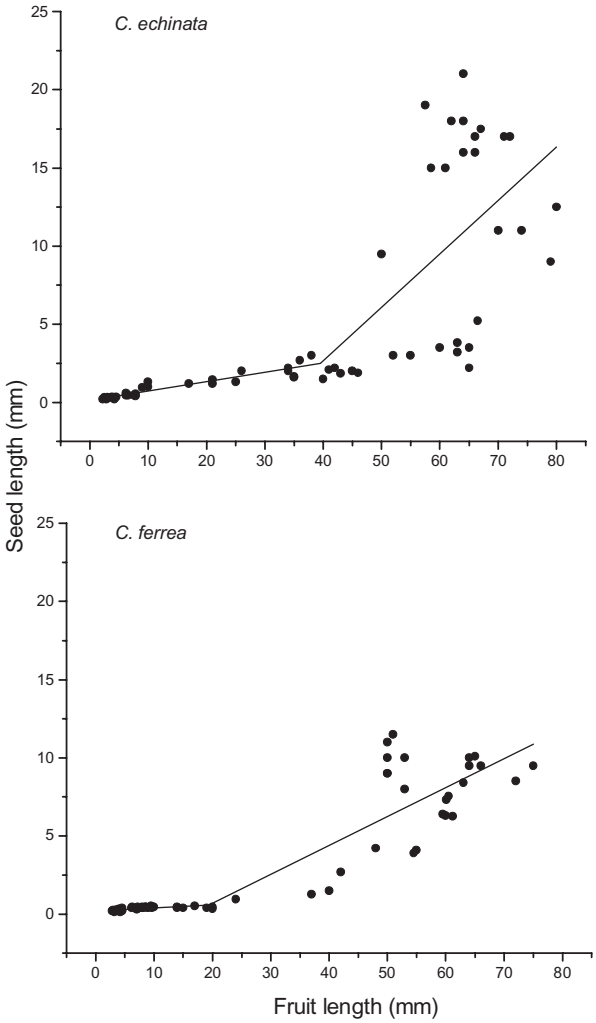


Figure 23. Relationship between fruit length and apical seed length in *Caesalpinia echinata* ($N = 66$ fruits) and *C. ferrea* ($N = 67$ fruits).

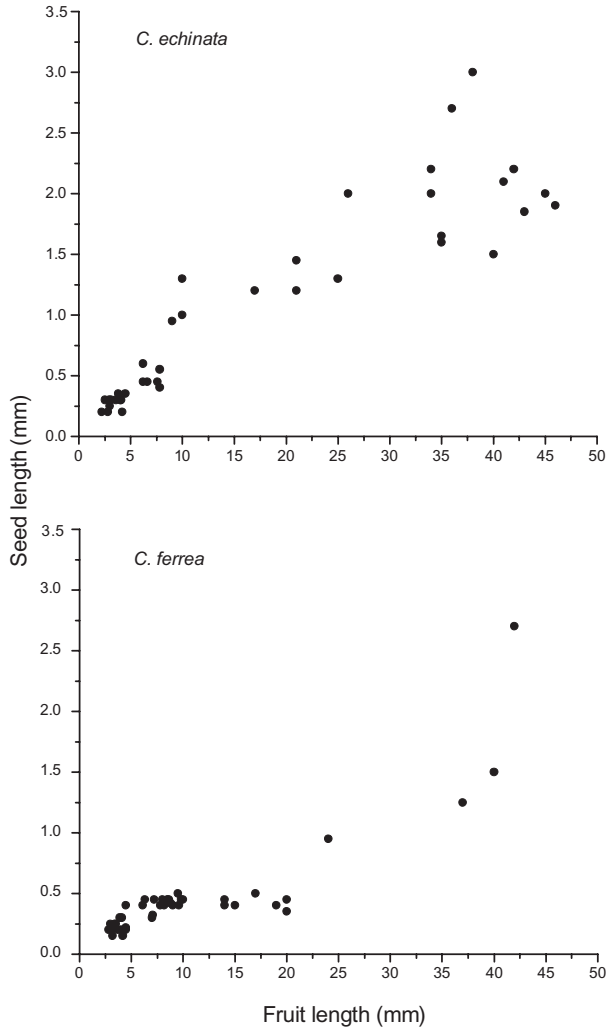


Figure 24. Relationship between fruit length and apical seed length in *Caesalpinia echinata* ($N = 41$ fruits) and *C. ferrea* ($N = 45$ fruits) for fruits less than 40 mm long.

cells indicated that they were secretory, as confirmed by the presence of mucilage in this region during fertilization. Mucilage facilitates entry of the pollen tube into the ovule (Teixeira, Prakash & Ranga, 2001). Most plant families with an endothelium are characterized by a unitegmic and tenuinucellate ovule (Kapil & Tiwari, 1978). For many years, the only report of this feature in legumes (which generally have a bitegmic and crassinucellate ovule) was that of a false endothelium in *Vigna* (Kapil & Tiwari, 1978). More recently, however, Prakash (1987) has shown that various species in the tribes Abreae and Phaseoleae have seeds with an endothelial layer.

Several morphological structures that connect ovarian tissue to the ovule have been identified. These include an obturator in Euphorbiaceae and Liliaceae, a funicular protrusion in Anacardiaceae, and a placental extension in Myrsinaceae and Lentibulariaceae (Endress, 1998). An obturator of placental origin has also been described for other legumes (Johri, Ambe-

Figures 19–22. Electron micrographs of *Caesalpinia ferrea* seed coats (longitudinal sections). Fig. 19. Inner integument features: three layers of sinuous-walled cells (swc) and one layer of endothelial cells (ec). Scale bar = 2.5 μm. Fig. 20. Endothelial cell. Note the plasmodesmata in the cell wall, the microvacuoles (mv) and the dictyosomes accompanied by vesicles (arrow). Scale bar = 0.4 μm. Fig. 21. Developing osteosclereid cell. Note the large central vacuole (v) and the peripheral nucleus (n). Scale bar = 2.5 μm. Fig. 22. Outer integument cells. Note the central nucleus (n) and the phenolic compounds. Scale bar = 2.5 μm.

gaokar & Srivastava, 1992), and a nucellus protrusion reaching from the ovarian tissue to the ovule was found in *C. ferrea*, as described above.

The seed ontogeny of the species studied here differs from the seed classification based on endosperm consumption during embryogeny (Boelcke, 1946; Gunn, 1991; Barroso *et al*., 1999). The early embryonic development of both species did not occur simultaneously with the development of the other seed tissues. After reaching the proembryonic stage, the zygote stopped dividing until other tissues differentiated. Similarly, seed growth was slower than fruit growth, and may even cease for some time. As hypothesized by Kozlowski & Gunn (1972), and stressed by Boesewinkel & Bouman (1984), Johri *et al*. (1992) and Richards (1997), a seed that totally consumes the endosperm during embryogeny (exendospermic, represented here by *C. echinata*) shows simultaneous development of the embryo and endosperm, whereas a seed that does not consume the endosperm during embryogeny (endospermic, represented by *C. ferrea*) shows delayed growth of the embryo in relation to development of the endosperm and other seed tissues. However, the seeds of *C. echinata* and *C. ferrea* behaved similarly, like typical endospermic seeds. The chalazal haustorial endosperm was very aggressive and invasive, indicating that during early embryogeny in *C. echinata* and *C. ferrea* the endosperm accumulated reserves which came from the antipodals and the nucellus. The fleshy cotyledon morphology of *C. echinata* and the leaf-like morphology of *C. ferrea* correspond to exendospermic and endospermic seeds, respectively, as defined by Kozlowski & Gunn (1972). Cotyledon thickness is generally considered to be inversely proportional to the amount of endosperm present (Gunn, 1981).

The two species studied showed quite distinctive embryo characteristics based on Smith's (1981) cotyledon types: the cotyledons of *C. echinata* were type four and those of *C. ferrea* type one. These cotyledon types differ in mesophyll differentiation (undifferentiated in type four), in life-span (much shorter in type four), and in the contribution of photosynthesis to seedling growth (minimal in type four) and expansion during germination (no expansion in type four). In the tribe Caesalpinieae, including the genus *Caesalpinia*, species with cotyledons of all types were described by Smith (1981). The considerable differences observed here between *C. echinata* and *C. ferrea* suggest that the monophyly of this genus should be re-analysed, as also proposed by Gunn (1991). In this regard, Table 2 lists a suite of characters that may be useful in the taxonomy of this complex genus. Seed characters support the concept of a single family in the Leguminosae, as well as its infrafamilial division; De Candolle (1825) first divided the family into Curviembrieae and Rectembrieae based on embryonic characters (Boelcke, 1946). Seed shape, external morphology, structure of the testa, cotyledon type and general morphology of the embryo provide information that can be used in seed identification and in establishing phylogenetic relationships (Gunn, 1981; Smith, 1981).

An understanding of the seed coat and pericarpial properties may help in defining conservation strategies for tropical species. Successful cultivation, for example, requires the availability of high-quality seeds for planting (Krzyzanowski, 1998). In the case of *C. ferrea*, the seed coat's resistance to desiccation and to pathogenic microorganisms, the small seed size, and the low pod and cell wall permeability enhance the quality of the seeds. According to Werker (1997), this thick and sclerified seed coat serves in mechanical

Table 2. Distinctive features of the fruits and seeds of *Caesalpinia echinata* and *C. ferrea* (Leguminosae, Caesalpinioideae)

Features	*C. echinata*	*C. ferrea*
Fruit		
Type	Spiny dehiscent	Smooth indehiscent
Non-glandular trichomes on the exocarp	Present	Absent
Secretory reservoirs in the mesocarp	Present	Absent
Principal sclerenchyma component	Fibre	Sclereid
Placenta	Papillose	Trichomatous
Seed		
Funiculus	Short	Long
Macrosclereid cuticle	Thin	Thickened
Stomata	Present	Absent
Osteoesclereids	Absent	Present
Cotyledon	Fleshy	Leaf-like
Tissue bridge between the micropyle and the ovary	Absent	Present

protection against physical, chemical and biological damage. In contrast, the seeds of *C. echinata*, an endangered tropical species, are less viable.

Embryo culture *in vitro* could provide an alternative approach for the conservation of species such as *C. echinata*. The patterns of embryo growth *in vivo* provide a standard for assessing growth and developmental morphology in culture (Raghavan & Srivastava, 1982; Dodeman, Ducreux & Kreis, 1997). The results presented here may be useful in the culture of zygotic embryos, from determining the stage at which the embryo becomes sufficiently independent and autotrophic for it to be cultured *in vitro*, and the total period of embryo development, to constraining the seed size associated with each embryonic phase.

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