Botanical Journal of the Linnean Society, 2007, **153**, 169–179. With 37 figures

Development of megagametophyte, embryo, and seed in *Senna corymbosa* (Lam.) H.S. Irwin & Barneby (Leguminosae – Caesalpinioideae)

MARTIN RODRIGUEZ-PONTES*

Universidad de la República, Facultad de Agronomía, Unidad Asociada Biología de Leguminosas Nativas, Eduardo Victor Haedo 1995/101, 11200 Montevideo, Uruguay

Received March 2006; accepted for publication August 2006

The main aspects of seed ontogeny in *Senna corymbosa* were studied by standard anatomical microtechniques for light microscope observations. The results revealed an ana-campylotropous, bitegmic, and crassinucelate mature ovule. A single archesporocyte developed by an archesporial cell enlargement from the subhypodermal multicellular archesporium. Meiosis originated linear or T-shaped megasporic tetrads. The functional megaspore was the chalazal one. Megagametophytic development conformed to the *Polygonum* type. Fertilization was porogamic. Endosperm development was free nuclear and conformed to a chalazal haustorium. Cellular endosperm was initiated from the micropylar end during the globular embryo stage. Embryogeny derived from a linear proembryonal tetrad. The mature embryo showed an oblique axis. The testa derived from the outer ovular integument. Nucellar and endosperm remnants, and the micropylar region of the inner ovular integument, persisted at embryo maturity. The absence of a pleurogram would be adaptative to wetland habitats. The taxonomic use of the mature embryo axis in the Cassieae and the phylogenetic employment of megasporic arrangements in Leguminosae needs some reinterpretation.

ADDITIONAL KEYWORDS: Cassieae – embryogenesis – endosperm – Fabaceae – fertilization – megasporogenesis – ontogeny.

INTRODUCTION

The reproductive and embryological knowledge of economically important plants, such as many Leguminosae, provides useful data in relation to the fields of cell biology, reproductive ecology, and taxonomy and for purposes related to seed production and crossbreeding. According to embryological data compilations (Prakash, 1987; Johri, Ambegaokar & Srivastava, 1992), there is a considerable amount of information on this family, with emphasis on the Papilionoideae. Most reports for the subfamily Caesalpinioideae concern Euro-Asiatic species and diverse North American representatives. However, some Brazilian species of this subfamily have been investigated embryologically more recently (Oliveira, 1999), including the seed coat (Melo-Pinna, Martins-Neiva & Alencar Barboza, 1999) and the relationship between seed behaviour and anatomy (De Pádua Teixeira, Carmello-Guerreiro & Rodriguez, 2004). A recent report by Delachiave & Zambello de Pinho (2003) about seed germination of the medicinal species, *Senna occidentalis* (L.) Link, also shows that knowledge of seed anatomy provides useful complementary data for the interpretation of physiological results about legume seed biology.

Senna corymbosa (Lam.) H.S.Irwin & Barneby (= *Cassia corymbosa* Lam.) is a riparian shrub native to the Paraná–Uruguay–Río de la Plata fluvial system (Irwin & Barneby, 1982). It is widely employed in diverse Latin American zones as an ornamental and medicinal species (Burkart, 1952). It has similar uses to the 'sen' (*Senna alexandrina* Mill.), having diverse active principles, such as anthrachinones, naphtochinones, alkaloids, anthocyanidins, flavonoids, triterpenoids, phenolic compounds and amines (Bandoni,

---

*E-mail: martinangel@adinet.com.uy

1976). Apart from a short morphological description of its suspensor made by Guignard in 1881 (Lersten, 1983), no more embryological data are available concerning this medicinal legume. The objective of our study of *Senna corymbosa* was to describe megasporogenesis, megagametophyte development, and the pattern of development of the zygotic embryo. The ontogeny of endosperm and seed coat were also included.

MATERIAL AND METHODS

Flowers and buds in diverse developmental stages of three individuals of *Senna corymbosa* were collected in a riverside wetland in the Department of Colonia (south-western Uruguay). They were fixed in formalin–acetic–alcohol. Voucher specimens were deposited in the Bernardo Rosengurtt Herbarium (Universidad de la República, Uruguay). Dissected gynoecia and seeds were dehydrated in ethanol series with ulterior toluene infiltration and embedding in paraffin wax. Erythrosin in 70% ethanol was used as a general stain. Sections were cut at 8–12 μm with a Minot American optical rotary microtome. The slides were stained in safranin–fast green and mounted in Canada balsam for light microscopy observation (Johansen, 1940). Digital photomicrographs were taken using an Olympus Vanox photomicroscope. Drawings were made with the aid of some of these photographs, which served as a pattern. A Leitz polarized light microscope was used for starch recognition.

RESULTS AND DISCUSSION

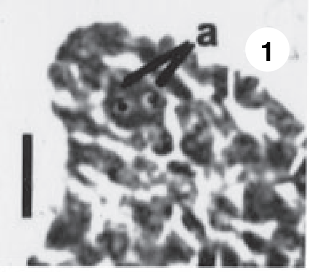
MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

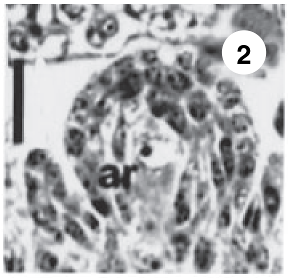
The mature ovule is ana-campylotropous to anatropous, bitegmic, and crassinucelate with a zig-zag micropyle (Fig. 10), as is usual in Caesalpinioideae (Kopooshian & Isely, 1966; Prakash, 1987). The subhypodermal archesporium consists of two to four archesporial cells (Fig. 1). A single archesporocyte (Fig. 2) is differentiated by the direct enlargement of an archesporial cell. Whereas a single or bicellular archesporium with direct archesporocyte development was observed in *Cercis canadensis* L., a unicellular hypodermic archesporium with a parietal cell produced before the differentiation of the archesporocyte was reported for *Chamaecrista nictitans* (L.) Moench (= *Cassia nictitans* L.), *Cassia abbreviata* Oliver, and *Gleditsia triacanthos* L. (Rembert, 1969a). However, this latter seems to be the usual archesporial condition in Caesalpinioideae (Rembert, 1972), and archesporocyte differentiation from direct enlargement of an archesporial cell only occurs when a subhypodermal archesporium is present (Prakash, 1987). Dnyansagar (1951) mentioned deep-seated archesporial cells for *Cassia tora* L. and *Cassia tomentosa* L. The presence of two archesporocytes in the same ovule was reported for *Cassia glauca* Lam. and *Senna siamea* (Lam.) H.S. Irwin & Barneby (= *Cassia siamea* Lam.) (Prakash, 1987) and both may have simultaneous megasporogenic viability in *Gleditsia triacanthos* (Rembert, 1969a) resulting in a pair of tetrads.

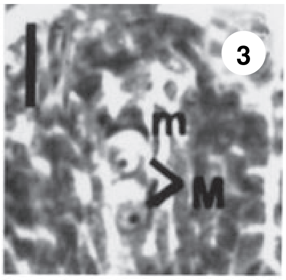
The megasporogenic meiosis cuts off both linear and T-shaped tetrads of megaspores (Figs 3–6). A single tetrad is observed in each ovule. Some tetrads show the persistence of both the chalazal and subchalazal megaspores (Figs 3, 5). In some cases, the persistence of the chalazal megaspore and the submicropylar one was observed (Fig. 4). However, mitotic activity that originates the megagametophyte was only observed in the chalazal megaspore. All these megasporic pattern variations could be observed in ovules of the same ovary. Maldonado (1982) also observed diverse megaspore tetrad patterns in the same ovary in some *Astragalus* (Papilionoideae) species. Rembert (1972) proposed a phylogenetic hypothesis for legume taxa

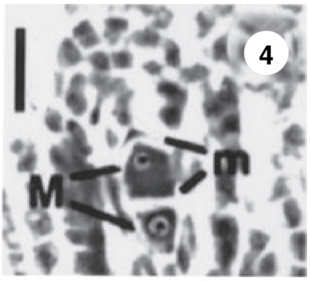
→

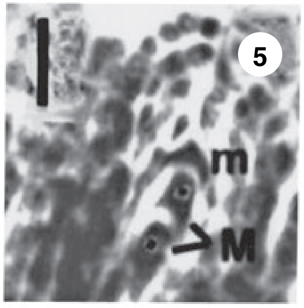
Figures 1–15. Megasporogenesis and development of the embryo sac and the proembryo. Fig. 1. A young ovule with a bicellular archesporium (*a*). Fig. 2. An archesporocyte (*ar*). Fig. 3. A linear megasporic tetrad with two chalazal persistent megaspores (*M*) and the two degenerating micropylars (*m*). Fig. 4. A tetrad with submicropylar and chalazal persistent megaspores (*M*) and the two degenerating ones (*m*). Fig. 5. A T-shaped tetrad with two persistent chalazal megaspores (*M*) and the micropylar degenerating ones (*m*). Fig. 6. A T-shaped tetrad with the chalazal persistent megaspore (*M*) and the three micropylar degenerating ones (*m*). Fig. 7. A binucleate coenocytic embryo sac (*2es*). Fig. 8. A four-nucleate coenocytic embryo sac (*4es*). Fig. 9. A mature embryo sac showing the synergids (*sy*), the egg cell (*ec*), polar nuclei (*pn*), and the antipodals (*an*). The hypostase (*hy*) and the nucellus (*nu*) are also observed. Fig. 10. A mature ovule showing the embryo sac (*es*), the nucellus (*nu*), the inner (*ii*) and the outer integument (*oi*), the micropyle (*mi*), and the funicle (*fu*). Fig. 11. Fertilization: zygote (*z*), functional synergid (*sy*), pollen tube remnants (*pt*), and dividing primary endosperm nucleus (*en*). Fig. 12. A resting zygote (*z*) with initial nuclear endosperm (*ne*) and remnants of pollen tubes (*pt*). Fig. 13. A bicelled proembryo with the apical (*ca)* and the basal cell (*cb*), and the nuclear endosperm (*ne*). Fig. 14. A proembryo with the superior (*cc*), inferior (*cd*), and the basal cell (*cb*). Nuclear endosperm (*ne*). Fig. 15. A proembryonal tetrad with the superior (*cc*), inferior (*cd*), and the two basal cells (*m, ci*). Enlarging nuclear endosperm (*ne*). Scale bars: Figs 1–8 = 20 μm; Figs 9, 11–15 = 15 μm; Fig. 10 = 50 μm.

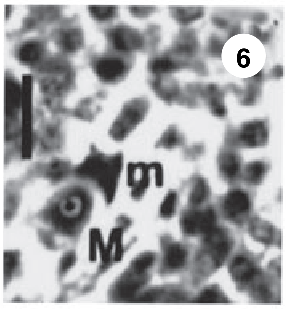

1
a

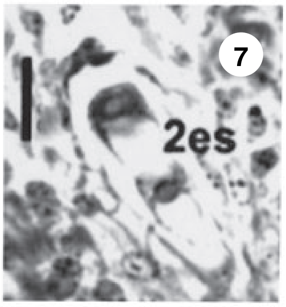

2
ar

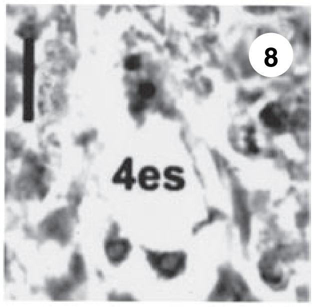

3
m
M

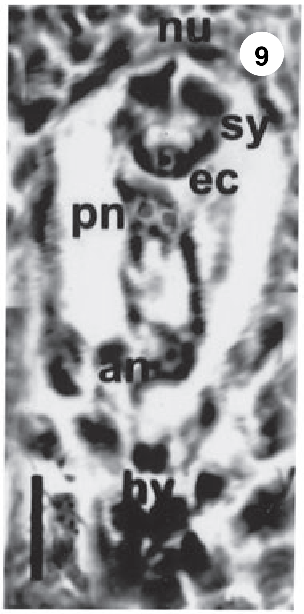

4
m
M


5
m
M


6
m
M

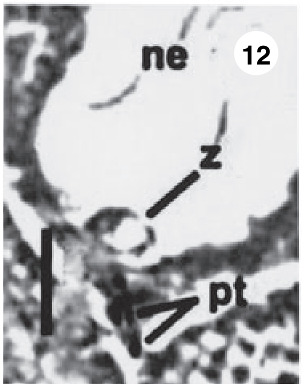

7
2es

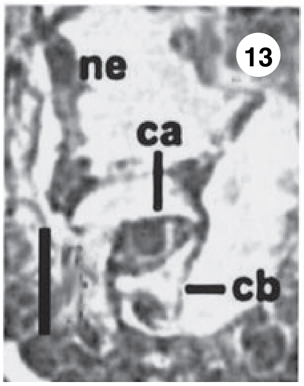

8
4es

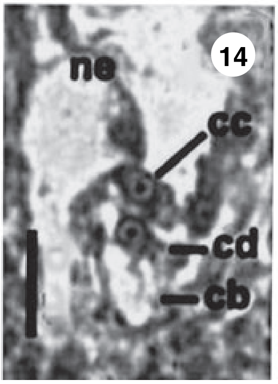

9
nu
sy
ec
pn
an
hy

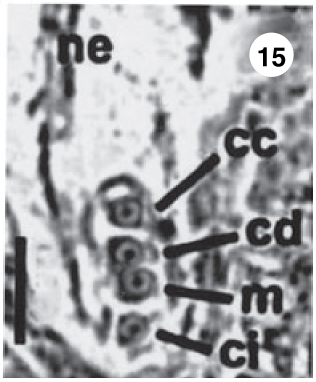

ov
10
hy
es
nu
ii
oi
mi
fu


pt
11
z
sy
en


ne
12
z
pt


ne
13
ca
cb


ne
14
cc
cd
cb


ne
15
cc
cd
m
ci

based on their numerous types of megasporic tetrad pattern. This phylogenetic model considers linear tetrads as the primitive condition from which the rest of the tetrad patterns are derived. The occurrence of diverse types of tetrad pattern in the same genus was mentioned by this author for *Medicago*, *Lathyrus*, *Melilotus* and *Desmodium* (Papilionoideae), *Acacia* and *Leucaena* (Mimosoideae), and *Cassia* and *Tamarindus* (Caesalpinioideae); or even in the same species, as in *Cassia abbreviata* (Caesalpinioideae) (Rembert, 1969a) and *Pueraria lobata* (Willd.) Ohwi (Papilionoideae) (Rembert, 1969b). However, his phylogenetic hypothesis does not mention the simultaneous occurrence of both a primitive (linear tetrad) and a derivative (T-shaped) condition of the megasporic arrangements in the same gynoecium, and subsequently in the same individual. This is the case in *Senna corymbosa*, and not anywhere else in the Caesalpinioideae or even the rest of Leguminosae. Some reinterpretation considering these tetrad pattern variations between ovules of the same plant would provide a quite significant contribution for this phylogenetic model based on the megasporic arrangements of the Leguminosae.

Dnyansagar (1951) mentioned the chalazal or distal (micropylar) megasporic functionality for some representatives of this subfamily. Prakash (1987) reported some tendency to the viability of the subchalazal megaspore in the Caesalpinioideae.

The embryo sac development is monomegasporic and follows three mitotic free nuclear divisions (Figs 7, 8) corresponding to the *Polygonum* type of octonucleate bipolar megagametophytic ontogeny. The straight mature embryo sac (Figs 9, 10, 16) shows an egg apparatus with hooked synergids, and the paired polar nuclei that fuse during anthesis. The three antipodals are ephemeral. The *Polygonum*-type development of the embryo sac is a general character of Leguminosae (Prakash, 1987), despite some secondary variations in this megagametophytic ontogeny reported for some Australian representatives of the Papilionoideae (Cameron & Prakash, 1994).

A barrier tissue or hypostase differentiates at the chalazal region close to the embryo sac (Figs 9, 10, 30). The possible role of this structure in the ovule is to transfer nutritional factors to the megagametophyte (Masand & Kapil, 1966).

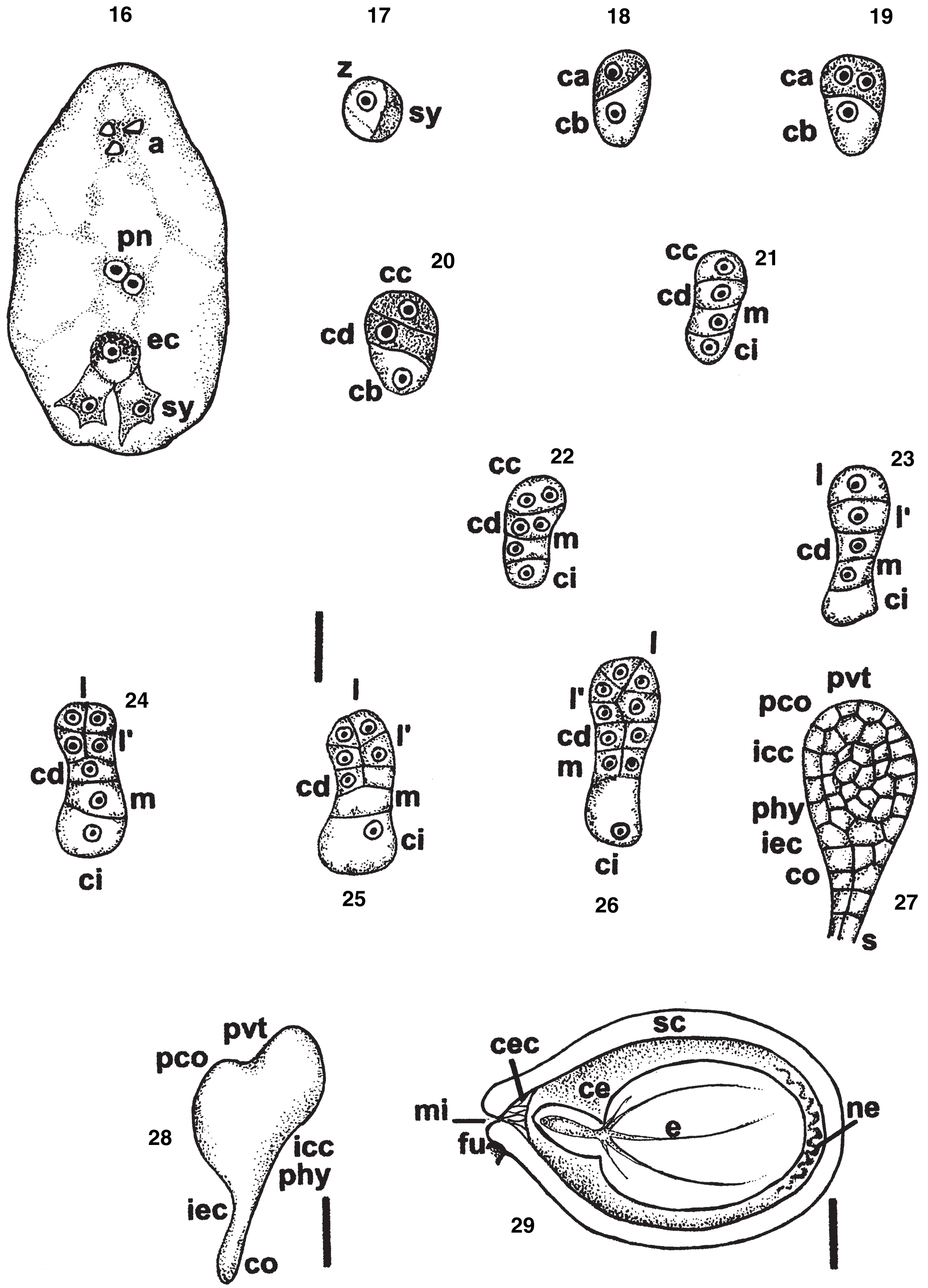
FERTILIZATION AND EMBRYOGENESIS

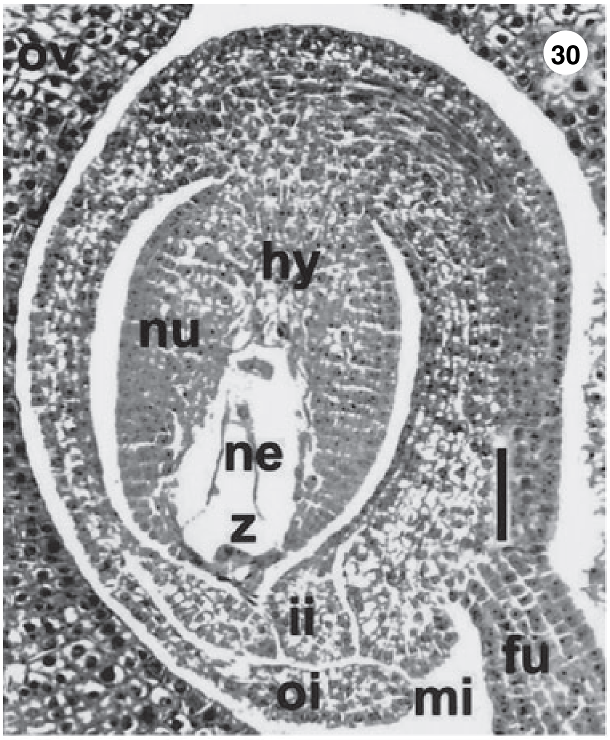
Fertilization is porogamous. The functional synergid is dark staining and the other synergid degenerates following the pollen tube discharge (Figs 11, 17). Many degenerating pollen tubes were observed in the micropylar region (Figs 11, 12). Suri & Deshpande (1981) observed degenerating synergids, intact antipodals, and starchy deposition close to the primary endosperm nucleus in *Chamaecrista absus* (L.) H.S. Irwin & Barneby (= *Cassia absus* L.) and *Cassia auriculata* L. However, this presence of megagametophytic starch is not a common feature in the Caesalpinioideae (Prakash, 1987).

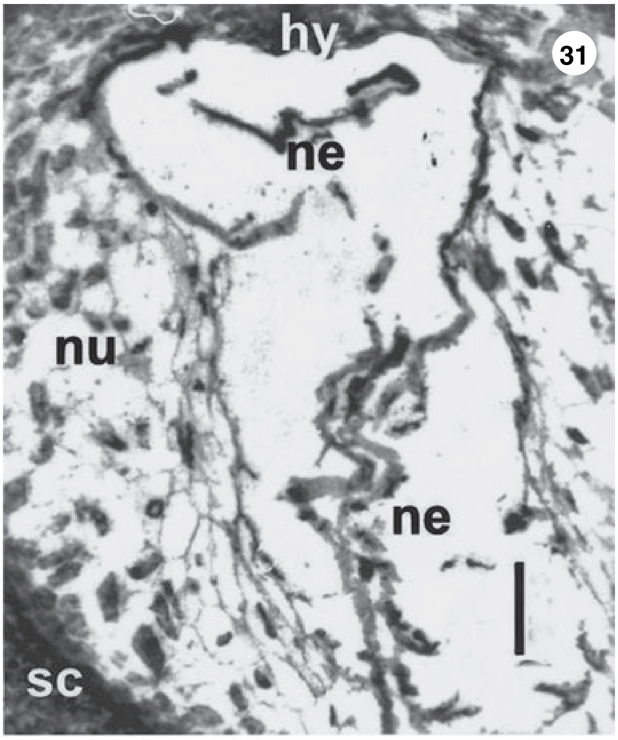
After an undetermined period of time after fertilization (Fig. 12), the zygote divides transversely to produce a bicelled proembryo provided with a dense apical cell (*ca*) *and* a hyaline basal cell (*cb*) (Figs 13, 18, 19). Only *ca* will contribute to the embryo proper. Both *ca and cb* divide transversely. In one case, a binucleate cell *ca* was observed as a consequence of a nuclear division prior to cell wall formation (Fig. 19). An apical cell *cc* and an inferior intermediate cell *cd* are derived from *ca* (Figs 14, 20), whereas an intermediate cell *m* and the basal terminal vesicular cell *ci* are derived from *cb* (Figs 15, 21, 22). Thus, embryogeny is derived from a linear proembryonal tetrad (Natesh & Rau, 1984). A transverse division of *cc* originates a pair of tiers *l* and *l′* (Fig. 23). Longitudinal divisions will take part from the apical tier *l* (Figs 24–26).

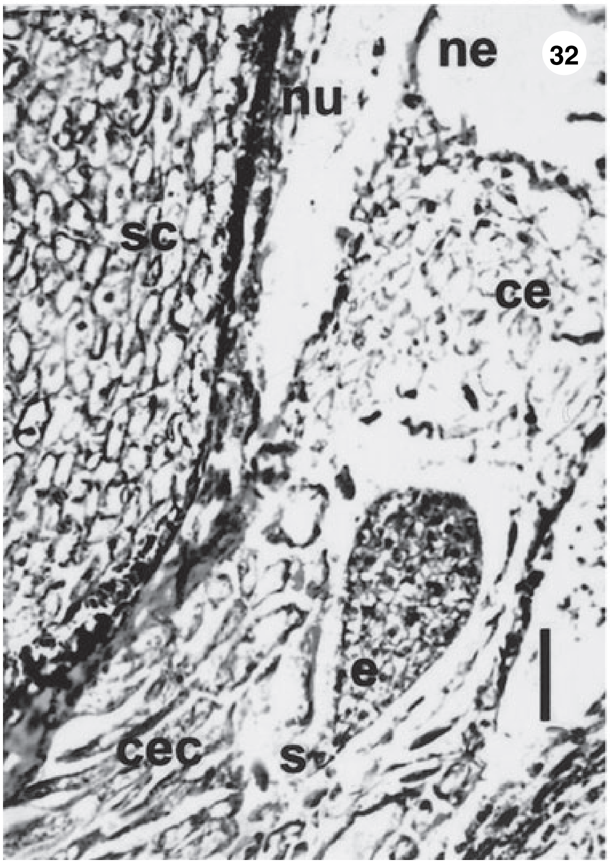
A poorly developed suspensor is observed during the globular embryo stage (Figs 27, 32). Its morphology is distally filamentous with a gradual transition to the embryo proper. It is crushed during radicle and cotyledonary development (Fig. 28). The suspensors may be reduced or absent in Caesalpinioideae (Lersten,

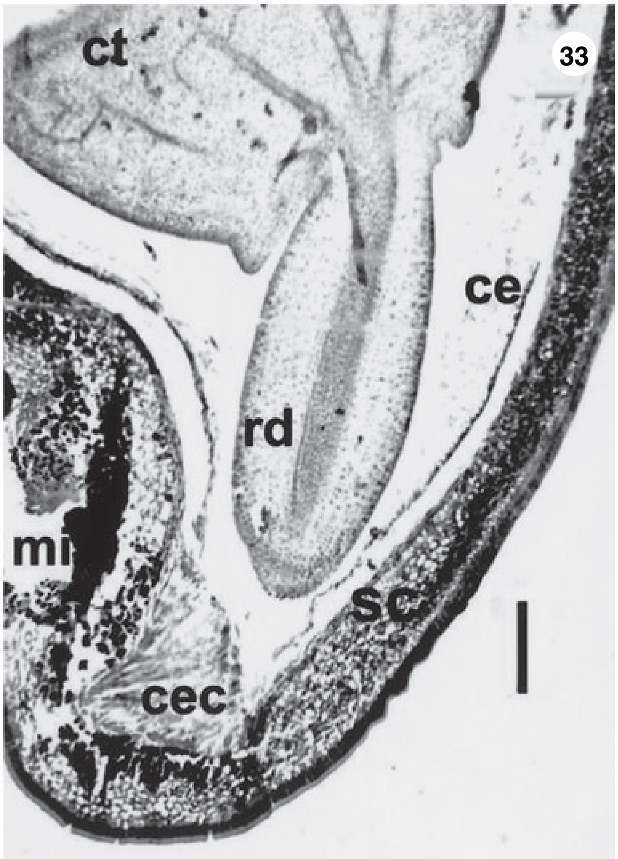
Figures 16–29. Mature embryo sac and embryogenesis. Fig. 16. A mature embryo sac with antipodals (*a*), polar nuclei (*pn*), egg cell (*ec*), and synergids (*sy*). Fig. 17. A zygote (*z*) and a functional synergid (*sy*). Figs 18, 19. Bicellular proembryos with apical (*ca)* and basal cells (*cb*). Fig. 20. A three-celled proembryo with superior (*cc*), inferior (*cd*), and basal cells (*cb*). Figs 21, 22. Proembryonal tetrads showing the superior (*cc*), inferior (*cd*), and the two basal cells (*m, ci*). Fig. 23. A proembryo showing the resultant apical cells (*l, l′*) from the division of *cc*. Figs 24 – 26. Advanced proembryos showing the longitudinal divisions from the cell *l* of the apical pole. Fig. 27. A late globular embryo showing the hypothetic future cotyledonary region (*pco*), stem tip (*pvt*), hypocotyledonary region (*phy*, *icc*), root cortex (*iec*), root cap (*co*), and the suspensor (*s*). Fig. 28. An initial chordiform embryo showing the hypothetic organogenical regions and the suspensorial reabsortion. Fig. 29. A diagram of the mature seed showing the seed coat (*sc*), micropyle (*mi*), funicle (*fu*), cellular endosperm cap (*cec*), cellular endosperm (*ce*), nuclear endosperm (*ne*), and embryo (*e*). The few peripheral nucellar remnants are not visible at this scale. Scale bars: Figs 16–27 = 15 μm; Fig. 28 = 100 μm; Fig. 29 = 1000 μm.


16
a
pn
ec
sy
17
z
sy
18
ca
cb
19
ca
cb
20
cc
cd
cb
21
cc
cd
m
ci
22
cc
cd
m
ci
23
l
l'
cd
m
ci
24
l
l'
cd
m
ci
25
l
l'
cd
m
ci
26
l
l'
cd
m
ci
27
pvt
pco
icc
phy
iec
co
s
28
pvt
pco
icc
phy
iec
co
29
sc
cec
ce
mi
fu
e
ne


ov
30
hy
nu
ne
z
ii
fu
oi
mi


hy
31
ne
nu
ne
sc


ne
32
nu
sc
ce
e
cec
s


ct
33
ce
rd
mi
sc
cec

Figures 30–33. Longitudinal sections of seeds in diverse stages of development. Fig. 30. Initial seed development from the end of fertilization: resting zygote (*z*), initial nuclear endosperm (*ne*), nucellus (*nu*), hypostase (*hy*), outer (*oi*) and inner (*ii*) ovular integuments, micropyle (*mi*), and funicle (*fu*). Fig. 31. A nuclear endosperm haustorium (*ne*) during the globular embryo stage in contact with the hypostase (*hy*) and the nucellus (*nu*). A developing seed coat (*sc*). Fig. 32. A young embryo (*e*) with a rudimentary suspensor (*s*). A cellular endosperm cap (*cec*), cellular (*ce*) and nuclear endosperm (*ne*). Nucellar remnants (*nu*) and a developing seed coat (*sc*). Fig. 33. The micropylar end of a mature seed showing the oblique insertion between the hypocotyle–radicle axis (*rd*) and the cotyledonary region (*ct*) of the embryo. Micropyle (*mi*). Scale bars: Figs 30–32 = 50 μm; Fig. 33 = 100 μm.

1983). Lersten mentioned a short description made by Guignard in 1881 related to a small- and large-celled suspensor for *Senna corymbosa*. However, the suspensors observed by Suri & Deshpande (1981) in *Chamaecrista absus* and *Cassia auriculata* had the same small-celled filamentous morphology as the suspensors of *Senna corymbosa* observed in our study (Figs 27, 32).

The mature embryo axis is oblique (Figs 29, 33). A straight radicle arises from the hypocotyl. A conspicuous procambial cylinder is observed (Fig. 33). The cotyledons are elliptic and basally subchordate. Although the three central procambial cotyledonary veins are obvious, the two marginal ones are less evident (Fig. 33). Both the plumule and the epicotyl are poorly differentiated. According to Kirkbride, Gunn & Richtie (1994), the poorly differentiated plumules of Cassieae are typical of the Caesalpinioideae. Recent anatomical observations from this subfamily (Oliveira, 1999) suggest that when the epicotyl is well developed, a conspicuous plumule is present, such as in *Caesalpinia leiostachya* (Benth.) Ducke. Less differentiated epicotyls and plumules are present in *Peltophorum dubium* (Spreng.) Taub. and *Cassia ferruginea* Schrad. ex DC., and both the epicotyl and the plumule are rudimentary in *Senna multijuga* (Rich.) H.S. Irwin & Barneby and *Dimorphandra mollis* Benth. Subsequently, there would be a direct correlation between the plumule and the epicotyl concerning their degree of differentiation in this legume subfamily.

The morphology of the oblique mature embryo in *Senna corymbosa* agrees with the general descriptions of this topic for Caesalpinioideae (Van Staden, Manning & Kelly, 1989). However, the mature embryo morphology of this subfamily is usually straight with a thick and short radicle (Kopooshian & Isely, 1966). A more recent report showed that a straight embryonic axis is predominant in Caesalpinioideae and Mimosoideae, whereas it is mainly incurved in Papilionoideae (Oliveira, 1999). The presence of an oblique embryonic axis is indicated for the genus *Chamaecrista* and its straight condition is mentioned for the related genus *Senna* (Gunn, 1991). Thus, a reinvestigation of the taxonomic delimitation of this character for *Senna corymbosa* and Cassieae is necessary.

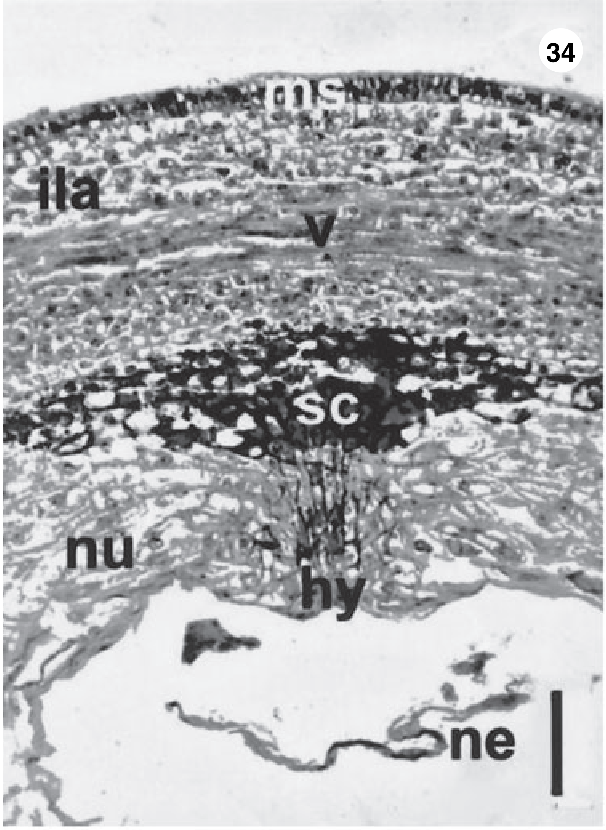
ENDOSPERM

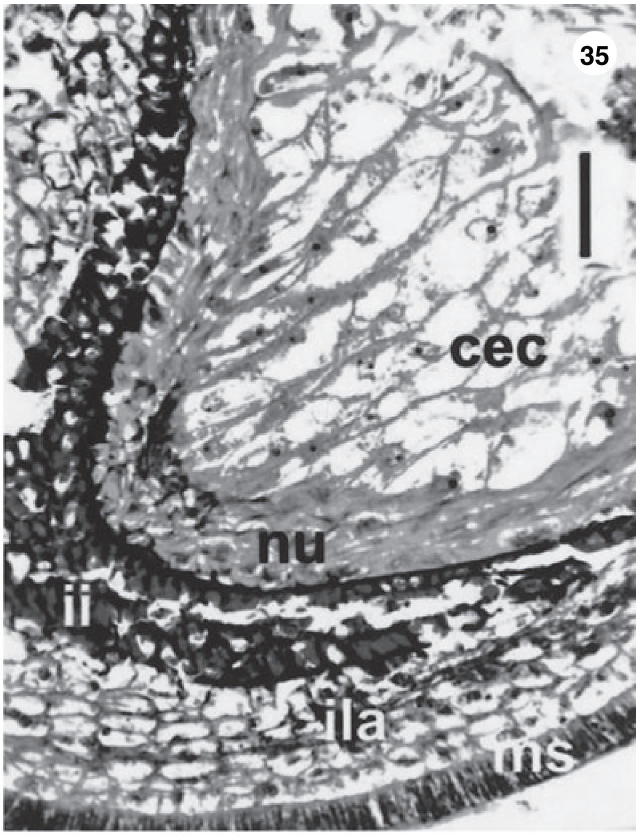
The primary endosperm nucleus divides by mitosis just after fertilization (Fig. 11) and the initial free nuclei are peripherally distributed surrounded by a cytoplasm mantle (Figs 12–15, 30). After the fourth mitotic cycle from the primary endosperm nucleus is reached, the distal nuclear endosperm enlarges towards the chalazal end to produce a sac-like haustorium (Fig. 31). This endosperm haustorium differs morphologically from the tubular haustorium reported for *Cassia tora* (Anantaswamy Rau, 1950) and it also lacks the sac-like and distally tubular morphology described for the haustorium of *Cassia sophera* L. (Johri & Garg, 1959). The main similarity we found is to the haustorial morphology of *Chamaecrista absus* (Johri & Garg, 1959; Suri & Deshpande, 1981).

During the globular embryo stage, the free nuclear endosperm becomes cellular, developing from micropyle to chalaza (Fig. 32), as is usual in Caesalpinioideae (Davis, 1966).

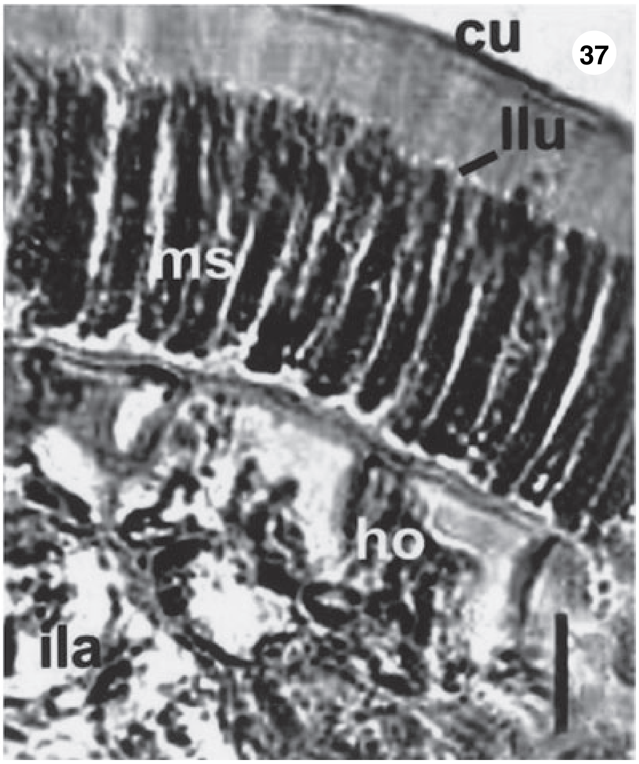
The endosperm cells located at the micropylar region enlarge axially, forming a cap-like structure or cellular endosperm cap (Figs 29, 32, 33, 35). This was also observed in *Chamaecrista absus*, but not in *Cassia auriculata*, by Suri & Deshpande (1981). Despite both longitudinal and transverse sections revealing the endospermic origin of this micropylar cap in *Senna corymbosa*, the nature of this cap-like structure was reported as suspensorial by Johri *et al.* (1992) for other Cassieae representatives.

Some disorganized cellular endosperm layers, the micropylar cap-like structure, and the crushed chalazal nuclear haustorium remnants persist at embryo maturity (Figs 29, 33, 36). The persistence of endosperm remnants surrounding the mature embryo does not occur in the main part of Leguminosae, whereas it was reported for many representatives of Caesalpinioideae belonging to the tribes Caesalpinieae, Sclerobeae, Cassieae, and Dimorphondreae (Dnyansagar, 1951) and also Cercideae (Gunn, 1991). Corner (1951) mentioned this occurrence for diverse genera such as *Caesalpinia*, *Peltophorum*, *Gleditsia*, *Cercidium*, *Parkinsonia*, *Bauhinia*, and *Erythrophloeum*. A recent report (De Pádua Teixeira *et al.*, 2004) also revealed endospermic seeds in *Caesalpinia ferrea*


34
ms
ila
v
sc
nu
hy
ne


35
cec
nu
ii
ila
ms


36
ila
nu
ce


cu
37
llu
ms
ho
ila

Figures 34–37. Longitudinal sections of seeds in advanced and mature stages. Fig. 34. The chalazal region of a seed in advanced development. Macrosclereids (*ms*), intermediate parechymatic layers (*ila*), vascular bundle (*v*), nucellus (*nu*), chalazal sclereid cap (*sc*) in contact with the hypostase (*hy*) and the haustorial nuclear endosperm (*ne*). Fig. 35. The micropylar region of a mature seed. Macrosclereid layer (*ms*), intermediate tegumentary parenchyma (*ila*), sclerified remnant of the ovular inner integument (*ii*), nucellar remnants (*nu*), and the cellular endosperm cap (*cec*). Fig. 36. A dorsal longisection of a mature seed showing the intermediate parenchymatic layers (*ila*), the nucellar remnants (*nu*), and the remaining cellular endosperm (*ce*). Fig. 37. A longisection of the testa in a mature seed. Linear external macrosclereids (*ms*) showing cuticle (*cu*) and 'linea lucida' (*llu*). Hourglass layer (*ho*) and intermediate parenchyma (*ila*). Scale bars: Figs 34, 35 = 50 μm; Fig. 36 = 20 μm; Fig. 37 = 15 μm.

Mart. Thus, the developed seeds of *Senna corymbosa* and other diverse representatives of Caesalpinioideae cannot be considered as completely exalbuminous, despite the seemingly disorganized remnants of this tissue.

SEED COAT

The mature testa is derived from the outer ovular integument. The epidermal palisade layer (Fig. 37) is composed of radially elongated linear macrosclereids. These sclereids show a larger size around the hilar zone. A conspicuous 'linea lucida' (Fig. 37) is observed as a consequence of the macrosclereids distal wall differential thickness. The outer periclinal walls of the palisade cells become gelatinous in *Erythrophloeum guineense* G. Don. (Caesalpinioideae) (Corner, 1951). The rest of the anatomy of this layer in *Senna corymbosa* conforms to other descriptions for this legume subfamily (Corner, 1951; Gunn, 1981; Suri & Deshpande, 1981; Prakash, 1987). However, recent studies of the seed coat anatomy in *Caesalpinia ferrea*, *Caesalpinia pyramidalis* Tul., *Senna martiana* (Benth.) H.S. Irwin & Barneby, and *Senna spectabilis* (DC.) H.S. Irwin & Barneby show an interspecific variation in the degree of compression of the macrosclereids (Melo-Pinna *et al*., 1999).

A conspicuous cuticle is present (Fig. 37). Thick cuticles are more common in seeds of Mimosoideae and Caesalpinioideae than in Papilionoideae (Gunn, 1981). This agrees with recent experimental data that suggest that the low seed coat permeability of the Leguminosae provides the hypoxic conditions required for embryo development (Rolletscheck *et al*., 2002). The absence of a pleurogram in *Senna corymbosa* would be accorded with its wetland habitat, whereas xerophytic Caesalpinioideae show a conspicuous pleurogram, such as in *Caesalpinia pyramidalis* (Melo-Pinna *et al*., 1999). A few representatives of Caesalpinioideae, such as *Caesalpinia echinata* Lam. (De Pádua Teixeira *et al*., 2004), present paracytic stomata in their developing seed coat.

A rudimentary hypodermal hourglass or osteosclereid layer was observed (Fig. 37). The absence of this layer is an uncommon feature in Leguminosae (Van Staden *et al*., 1989). Although mostly conspicuous in the Papilionoideae, the hourglass layer is rudimentary or absent in diverse Caesalpinioideae such as *Bauhinia* and *Caesalpinia,* although it is well developed in many *Cassia* species (Corner, 1951), with some exceptions, such as *Cassia auriculata* (Suri & Deshpande, 1981). However, two layers of osteosclereids were observed in *Caesalpinia echinata* and *Caesalpinia ferrea* (De Pádua Teixeira *et al*., 2004).

Various parenchymatous intermediate layers (Figs 34–37) remain during embryo maturation of *Senna corymbosa*. Polarized light analysis of these parenchymatous layers revealed the presence of starch grains inside their cells. In diverse Leguminosae, these intermediate layers become a chlorophyllous aerenchyma, whereas in some Caesalpinioideae, such as *Erythrophloeum*, *Bauhinia*, and *Cassia*, they remain parenchymatous (Corner, 1951). These layers become sclerified by cellulose in *Caesalpinia ferrea*, *Caesalpinia pyramidalis*, *Senna martiana*, and *Senna spectabilis* with an inner osteosclereid layer (Melo-Pinna *et al*., 1999). This latter layer is absent in *Senna corymbosa*.

During seed development, the nucellar tissue mainly disappears. A few nucellar layers were observed between the cellular endosperm and the testa, both in mature seeds of *Senna corymbosa* (Fig. 36) and in many other legumes (Corner, 1951; Suri & Deshpande, 1981).

The micropylar region of the inner ovular integument persists as short-armed astrosclereids (Fig. 35). Suri & Deshpande (1981) defined them as cells with dark contents in both *Chamaecrista absus* and *Cassia auriculata*. In most Leguminosae, the inner ovular integument is completely destroyed during seed development and does not take part in seed coat formation (Prakash, 1987). However, an endothelial layer or integumentary tapetum develops from this integument in *Caesalpinia echinata* and *Caesalpinia ferrea* (De Pádua Teixeira *et al*., 2004), which was not observed in *Senna corymbosa*.

During advanced embryogeny, another short-armed astrosclereid cap located between the hypostase and the conspicuous vascular bundle was observed at the chalaza (Fig. 34) as a suspected barrier against the endosperm haustorium activity. According to Bouman (1984), such barrier action is also made by the hypostase in developing seeds.

The number of embryological studies in Neotropical Caesalpinioideae has increased in recent times, although these reports mainly concern individual topics such as the ontogeny of the seed coat (Melo-Pinna *et al*., 1999) or the anatomy of the mature embryo of many species (Oliveira, 1999). Furthermore, many embryological reports about Euro-Asiatic Caesalpinioideae also concern individual items (Prakash, 1987). On the other hand, the main embryological characters of *Senna corymbosa* are described in our report from early ovular stages until mature seed formation. These data concerning *Senna corymbosa* should be taken into account for further studies about the seed biology of this medicinal plant. It must be noticed that recent studies of the seeds of Caesalpinioideae (Rolletscheck *et al*., 2002; Delachiave & Zambello de Pinho, 2003; De Pádua Teixeira *et al*., 2004) have been carried out joining both physiological and anatomical aspects.

Our results also shed light on the legume phylogenetic megasporic model of Rembert (1972) and on the taxonomic employment of the mature embryo axis in Cassieae (Gunn, 1991). Both topics should be considered as the most significant contributions of our report for legume systematics. Some future complementary research guidelines concerning reproductive aspects of *Senna corymbosa* should be the ontogeny of the anthers and pollen, the floral biology, and the seed ecology of this species.

ACKNOWLEDGEMENTS

Financial support was provided by the Swedish Agency for Research Cooperation (SAREC). Primavera Izaguirre (UDELAR, Faculty of Agronomy) made this paper possible. Dr Alfredo Cocucci gave useful suggestions about the interpretation of the megasporogenesis. Mario Piaggio (UDELAR, Faculty of Sciences) helped in taking the photographs.

REFERENCES

Anantaswamy Rau M. 1950. Endosperm in *Cassia tora*. *Nature* **165:** 157.

Bandoni AL. 1976. Survey of Argentine medicinal plants. Folklore and phytochemical screening II. *Economic Botany* **30** (2)**:** 161–170.

Bouman F. 1984. The ovule. In: Johri BM, ed. *Embryology of angiosperms*. Berlin: Springer, 123–157.

Burkart A. 1952. *Las Leguminosas Argentinas Silvestres y Cultivadas*, 29 ed. Buenos Aires: Acme.

Cameron BG, Prakash N. 1994. Variations of the megagametophyte in the Papilionoideae. In: Ferguson IK, Tucker SC, eds. *Advances in legume systematics*, Vol. 6. Kew: Royal Botanic Gardens, 97–115.

Corner EJH. 1951. The leguminous seed. *Phytomorphology* **1:** 117–150.

Davis GL. 1966. *Systematic embryology of the angiosperms*. New York: Wiley.

De Pádua Teixeira S, Carmello-Guerreiro SM, Rodriguez Machado S. 2004. Fruit and seed ontogeny related to the seed behaviour of two tropical species of *Caesalpinia* (Leguminosae). *Botanical Journal of the Linnean Society* **146:** 57–70.

Delachiave MEA, Zambello de Pinho S. 2003. Germination of *Senna occidentalis* Link: seed at different osmotic levels. *Brazilian Archives of Biology and Technology* **46:** 163–166.

Dnyansagar VR. 1951. Embryological studies in the Leguminosae. XII. Status of the Leguminosae. *Saugar University Journal* **1:** 39–55.

Gunn CR. 1981. Seeds of Leguminosae. In: Polhill CH, Raven PH, eds. *Advances in legume systematics*, Vol. 2. Kew: Royal Botanic Gardens, 913–925.

Gunn CR. 1991. *Fruits and seeds of genera in the subfamily Caesalpinioideae (Fabaceae)*. Port Royal – Springfield: US Department of Agriculture.

Irwin HS, Barneby RC. 1982. The American Cassiinae. *Memoirs of the New York Botanical Garden* **35:** 1–454.

Johansen DA. 1940. *Plant microtechnique*. New York: McGraw-Hill.

Johri BM, Ambegaokar KB, Srivastava PS. 1992. *Comparative embryology of angiosperms*. Berlin: Springer.

Johri BM, Garg S. 1959. Development of endosperm haustoria in some Leguminosae. *Phytomorphology* **9:** 34–46.

Kirkbride JH Jr, Gunn CR, Richtie CA. 1994. Seed and fruit phylogenies of Caesalpinioideae and Mimosoideae (Fabaceae) and their tribes. In: Ferguson IK, Tucker SC, eds. *Advances in legume systematics*, Vol. 6. Kew: Royal Botanic Gardens, 117–140.

Kopooshian H, Isely D. 1966. Seed character relationships in the Leguminosae. *Bulletin of the Iowa Academy of Science* **73:** 59–66.

Lersten NR. 1983. Suspensors in Leguminosae. *Botanical Review* **49:** 233–251.

Maldonado S. 1982. Estudios embriológicos y palinológicos en *Astragalus* (Leguminosae). *Boletín de la Sociedad Argentina de Botánica* **20:** 201–225.

Masand P, Kapil RN. 1966. Nutrition of the embryo sac and embryo – a morphological approach. *Phytomorphology* **16:** 158–175.

Melo-Pinna GFA, Martins Neiva MS, Alencar Barboza DC. 1999. Estrutura do tegumento seminal de quatro espécies de Leguminosae (Caesalpinoideae), ocurrentes numa área de caatinga (PE – Brasil). *Revista Brasileira de Botânica* **22** (3)**:** 375–379.

Natesh S, Rau MA. 1984. The embryo. In: Johri BM, ed. *Embryology of angiosperms*. Berlin: Springer, 377–443.

Oliveira DMT. 1999. Morfo-anatomia do embrião de leguminosas arbóreas nativas. *Revista Brasileira de Botânica* **22** (3)**:** 413–427.

Prakash N. 1987. Embryology of the Leguminosae. In: Stirton CH, ed. *Advances in legume systematics*, Vol. 3. Kew: Royal Botanic Gardens, 241–266.

Rembert DH. 1969a. Comparative megasporogenesis in Caesalpiniaceae. *Botanical Gazette* **130:** 47–52.

Rembert DH. 1969b. Comparative megasporogenesis in Papilionaceae. *American Journal of Botany* **56** (6)**:** 584–591.

Rembert DH. 1972. Phylogenetic significance of megaspore tetrad patterns in Leguminales. *Phytomorphology* **21:** 1–9.

Rolletscheck H, Borisjuk L, Koschorreck M, Wobus U, Weber H. 2002. Legume embryos develop in a hypoxic environment. *Journal of Experimental Botany* **53** (371)**:** 1099–1107.

Suri RK, Deshpande PK. 1981. Development of seed in *Cassia absus* and *Cassia auriculata*. *Phytomorphology* **31:** 40–47.

Van Staden J, Manning JC, Kelly KM. 1989. Legume seeds – the structure: function equation. In: Stirton CH, Zaruchi JL, eds. *Advances in legume biology*. Monographs of Systematic Botany 29. Missouri: Missouri Botanical Garden, 417–450.