

SEED STRUCTURE IN SOME *RHYNCOSIA* SPECIES (PAPILIONOIDEAE)

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Abstract

Seed development has been studied in *Rhyncosia minima* DC., and general morphology and anatomy of mature seeds have been investigated in *R. albiflora* (Sims.) Alston, *R. bracteata* Benth., *R. minima* and *R. rothii* Benth. The ovules are bitegmic, crassinucellate and campylotropous. The outer integument alone contributes to the formation of seed coat. The seed coat comprises the outer epidermis of macrosclereids, a single-layered hypodermis of osteosclereids and a few layers of collapsed parenchymatous cells. The hilar region comprises the characteristic 'counter-palisade' layer and the 'tracheid bar'. The osteosclereids are absent in this region. Mature seeds are exalbuminous, almost reniform and smooth. They are arillate in *R. bracteata* but exarillate in others. Seed colour varies in different species. The hilum is nearly oblong to oval in shape. The mature embryo is elongated-oval with a notch on radicular side in all the species. The hypocotyl-root-axis is very small and curved. The two cotyledons are thick and fleshy.

Introduction

The genus *Rhyncosia* Lour. is placed in the subtribe Cajaninae, tribe Phaseoleae of the subfamily Papilionoideae and family Leguminosae (Lackey, 1981). Netolitzky (1926), Corner (1951, 1976), Singh (1964) and more recently Gunn (1981) have reviewed the work on seed structure in this family. Various aspects of seed development in the members of Phaseoleae have been worked out by Brown (1917), Anantaswamy Rau (1951, 1953), Sterling (1954, 1955), Takao (1962), Deshpande and Bhasin (1974), Prakash and Chan (1976), Berg (1979) and Rao *et al.* (1979). Anantaswamy Rau (1953) made some observations on the nature of endosperm. Ambegaokar (1976) investigated only the mature seed coat anatomy together with other leguminous taxa. Behl and Tiagi (1977) described the development of seed in *Rhyncosia*. Perusal of existing literature thus reveals that only meagre information is available on seed structure in *Rhyncosia* species. With this background, an effort has been made to study the development of seed in *Rhyncosia minima*, and gross morphology and anatomy of seed in *R. albiflora* (Sims.) Alston, *R. bracteata* (Benth.), *R. minima* DC. and *R. rothii* (Benth.).

Material and method

For the purpose of developmental study, the floral buds, flowers and fruits of *R. minima* at different stages of growth were fixed in formalin-acetic acid-alcohol (F.A.A.). Mature and dry seeds of different species collected from various sources were softened by an overnight treatment in 30 per cent ethanol at 50°C. Customary methods of dehydration in tertiary-butyl-alcohol (TBA) series and embedding in paraffin wax were followed. Serial microtome sections cut between 6 to 9 μ m thickness were stained in safranin-fast green combination. The measurements of macrosclereids and osteosclereids were taken from the sections of mature seed coat with the help of ocular micrometer in the middle

of seed. Mature and dry seeds were measured with a scale. Colours of the seeds were matched with those given in the colour dictionary (Macrez & Paul, 1950).

Observations

Ovule—Ovules—generally two, rarely one in number per ovary—are produced on the marginal placenta. They are bitegmic, crassinu-cellate and campylotropous (Text-fig. 1A). The outer integument is thicker than the inner and completely overgrows the latter during the course of ovule development. The micropyle is somewhat zigzag in outline and is mainly formed by the outer integument.

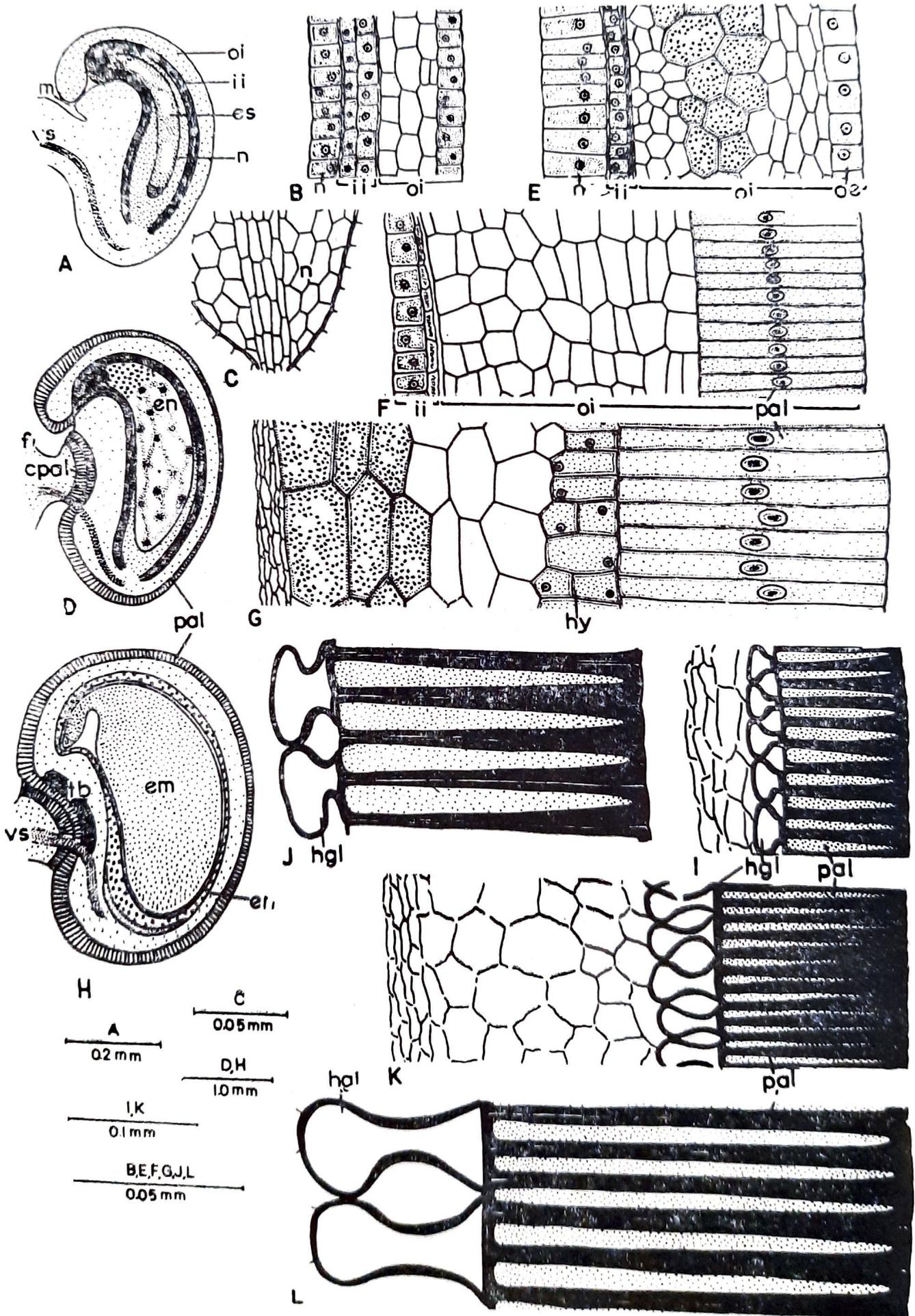
The outer integument at the organized female gametophyte stage is four-layered (Text-fig. 1 B), but at the swollen micropylar end it is 9 to 10 cell layers thick. The outer epidermal cells are full of tanniferous substance and are slightly elongated in radial direction. The cells of remaining layers are lightly stained and are elongated more in tangential direction. Some of these cells divide periclinally. The cells of the epidermis, on the other hand, divide in anticlinal plane except at the micropylar and where periclinial divisions are also seen.

The inner integument is uniformly two-layered on both, raphe and antiraphe side, but it is 3 to 4-layered at the swollen micropylar end. The nucellus is quite massive on the chalazal side while it is 4 to 5-layered on the lateral sides of the embryo sac. On the contrary, only a few cell layers are seen above the embryo sac. The nucellar cells, in general, are thin-walled, polygonal and very light-stained but those of the nucellar epidermis are radially elongated and densely cytoplasmic. Some of the nucellar cells lying directly below the embryo sac are elongated and simulate conducting tissue, probably they assist in the conduction of nutrients to the growing embryo sac (Text-fig. 1C). The vascular supply passes through the funicle and terminates at the chalazal end of the ovule.

Seed Development—During development of the seed various changes are brought about in the ovule.

Endosperm—The primary endosperm nucleus divides earlier than the zygote. The endosperm development is of the Nuclear type and considerable number of free nuclei are formed prior to the division of the zygote. The free endosperm nuclei aggregate densely in the close vicinity of the developing embryo. Wall formation in the free nuclear endosperm initiates at the micropylar end near the growing proembryo extending further towards the chalazal side (Text-fig. 1 D). The free nuclear endosperm at the chalazal end persist till the heart-shaped stage of the embryo. The whole endosperm finally becomes cellular by the time dicot embryo stage is reached. The endosperm during further seed development is much consumed by the developing embryo and in a nearly mature seed a few cell layers are seen around the embryo (Text-fig. 1 H). As the seed matures, the endosperm is fully utilized and no trace of this tissue is seen in a fully mature seed (Text-fig. 2 K).

Nucellus—Soon after fertilisation, the rapidly enlarging embryo sac crushes the nucellar cells above its micropylar end and it also penetrates deeply in the nucellar tissue on the chalazal side. All the nucellar cells except the epidermal ones on the lateral sides become highly vacuolated. The developing endosperm consumes these cells starting from the micropylar end and gradually proceeding towards chalaza, and by the time late globular proembryo stage is reached only a few cell layers are seen at the chalazal end. Concomitantly, the epidermal cells, which show elongation in radial direction even prior to fertilisation, elongate more radially up to globular embryo stage and simulate endothelium (Text-fig. 1E). These cells are longest at the chalazal end. These radially elongated



Text-fig. 1

nucellar epidermal cells show signs of degeneration at the heart-shaped stage of embryo, starting from the extreme micropylar end and gradually proceeding towards chalaza. By the time dicot stage of embryo is reached, nearly the entire of nucellar tissue degenerates except at the extreme chalazal end where a few layers persist till the embryo is well differentiated, but finally these are also consumed during seed maturation.

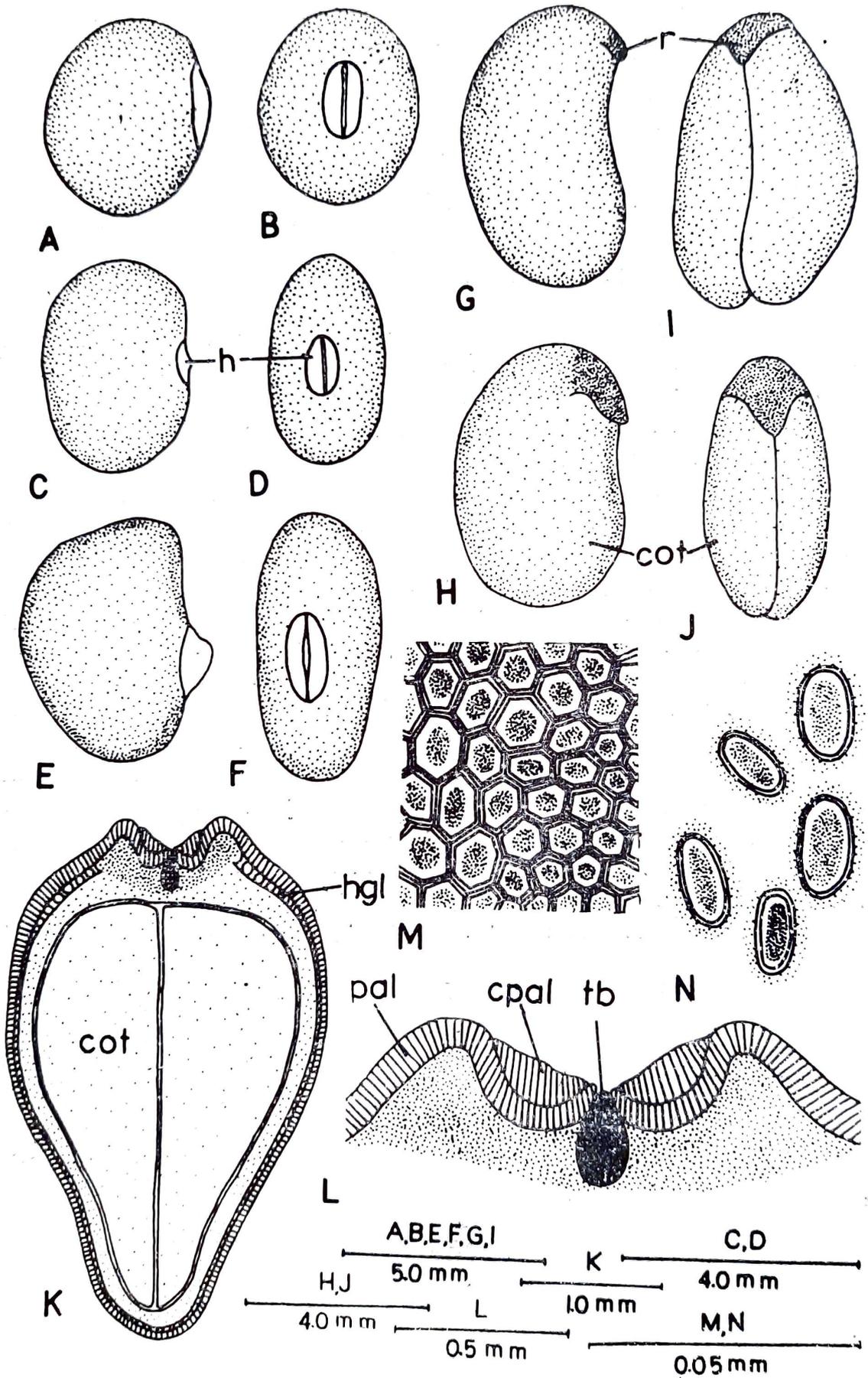
Inner integument—The two-layered inner integument remains healthy up to fertilisation. Following fertilisation, the cells of the outer epidermis get depleted in their contents and become tangentially stretched (Text-fig. 1 E, F) and by the time late globular embryo stage is reached, these cells totally degenerate except at the micropylar end where they persist till heart-shaped stage. The cells of the inner epidermis persist for a longer period than the outer one but finally they also degenerate on lines similar to the cells of the outer epidermis. The inner integument, thus does not participate in the formation of the seed coat.

Outer integument—The cells of the outer integument undergo a series of changes during seed development. The cells of all the layers except of the outer epidermis divide rapidly in all planes resulting in 9 to 10-layered outer integument on the raphe and antiraphe sides by the time globular proembryo stage is reached (Text-fig. 1 E). At this stage the outer integument can be distinguished into four zones below the embryo level. The outer most zone consists of the epidermal cells. The next zone is composed of about 4 layers of cells which are tangentially elongated and light-stained. The third zone consists of large cells with tanniniferous depositions. The inner most zone is made up to 4 to 5 layers of smaller cells with no tanniniferous deposits (Text-fig. 1 E). The secretory cells, as reported by Anantawamy Rau (1953) in the outer integument could not be observed during the present study. The cells of the outer integument at the micropylar end behave in a different manner. All the cells below the epidermis get more regularly arranged resembling the cambial cells to a great extent (Text-fig. 1 F). They divide actively up to globular stage of proembryo adding more layers at the extreme micropylar end. After this stage, the cells do not divide anymore, rather they increase in size and become highly vacuolated. As the embryo develops further, the cells of the outer integument are slowly consumed beginning from the inner zone and progressing towards the outer (Text-fig. 1 G).

The outer epidermal cells elongate more radially at the micropylar and chalazal ends and elongation also extends at other places. By the time embryo is well differentiated, these cells acquire maximum length becoming palisade-like. The palisade cells are

Text-fig. 1—A-L *Rhynchosia* species; A-J. *R. minima*; K, L. *R. albiflora*. **A**, Longi-section of ovule at the organised female gametophyte stage; **B**, Longi-section of part of ovule at the organised female gametophyte stage showing integuments and nucellar epidermis; **C**, Longi-section of part of nucellus at the chalazal side showing narrowly elongated cells resembling the 'conducting cells'; **D**, Longi-section of developing seed at the globular stage of embryo; **E**, Longi-section of part of developing seed at the globular stage of embryo showing radially elongated nucellar epidermis and thick outer integument in the middle region; **F**, Same at micropylar side showing cambium-like arrangement of subepidermal cells and radial elongation of outer epidermal cells of the outer integument; **G**, Longi-section of part of developing seed at the dicot stage of embryo showing radially elongated palisade-like cells and differentiation of hypodermis outer integument; **H**, Longi-section of young seed at well organised embryo stage; **I**, **K**, Longi-section of part of seed coat of *R. minima* and *R. albiflora* respectively; **J**, **L**, Magnified view of macrosclereids and hour-glass' cells of *R. minima* and *R. albiflora* respectively in longi-section.

(**cpal**—Counter palisade layer, **em**—embryo, **en**—endosperm, **es**—embryo sac, **f**—funicle, **hgl**—'hour-glass' cells, **hy**—hypodermis, **ii**—inner integument, **m**—micropyle, **oe**—outer epidermis, **oi**—outer integument, **pal**—palisade layer, **tb**—tracheid bar, **vs**—vascular supply).



Text-fig. 2

densely cytoplasmic containing centrally located nucleus (Text-fig. 1 F, G). During maturation of seed, the radial walls of all the palisade-like cells become thickened due to lignification. These cells form the protective macroscleried layer. The cells of hypodermal layer show signs of differentiation nearly at the mature embryo stage in the major part of the seed (Text-fig. 1 G). However, at the micropylar and chalazal ends their differentiation starts much earlier. All the hypodermal cells ultimately assume nearly a flask-shaped appearance (Text fig. 1 I). As the seed matures, these cells become thick-walled osteosclereids, designated also as 'Hour-glass' cells.

Hilum—Initially, the cells in the hilar region are thin-walled. At the globular stage of proembryo the cells of two layers elongate radially and become palisade-like (Text-fig. 1 D). The inner palisade layer forms the continuation of the general palisade layer of seed, whereas the outer palisade layer forms the counter-palisade layer and is restricted to the hilar region only. As the embryo matures, a few cells on the inner side of palisade layer are transformed into tracheid-like structure forming the characteristic 'tracheid-bar' which extends to quite an extent in the hilar region (Text-fig. 1 H). Remaining hilar cells, which usually become stellate, contain tanniferous deposits.

Mature seed—The seeds are nearly oval in *R. albiflora* (Text-fig. 2 A), but they are somewhat reniform in *R. bracteata*, *R. minima* and *R. rothii* (Text-fig. 2 C, E). The lateral sides of the seeds in all the species are convex but the convexity is maximum in *R. albiflora*.

The colour of the seeds is very distinct in all the species. The seeds of *R. albiflora* are uniformly shining dark blue (admiral) coloured. They are greenish-grey (dusty green) in *R. bracteata*, dull creamish to light grey (sheepskin moth) in *R. minima* and creamish (malmaison) in *R. rothii*.

The seed surface is almost smooth and dull in appearance in *R. minima* and *R. rothii*. In *R. albiflora* and *R. bracteata* the surface is shiny. The seeds of *R. bracteata* are arillate to some extent (Text-fig. 2 E) whereas in others they are devoid of this structure. The aril is greenish-white, small, horny and bilobed (Text-fig. 2 F). The shape of the hilum is nearly oval in outline in *R. minima* (Text-fig. 2 D) and somewhat elongated oval in other species (Text-fig. 2 B). The colour of the hilum is dull white in *R. albiflora* and *R. bracteata*, dull grey-white surrounded by dark brown coloured area in *R. minima* and cream colour surrounded by brown coloured area in *R. rothii*. The seed sizes of different species are recorded in Table 1 and thus it is evident that the largest seeds are of *R. bracteata* and smallest of *R. minima*.

Seed coat—The seed coat in the major part of the seed consists of 8 to 12 layers of cells of which the two outermost layers are very characteristic (Text-fig. 1 I, K). The outermost epidermal layer shows uniformity of structure in all the species, and is composed of highly lignified macrosclereids (Text-fig. 1 I, J, K, L). Thickening on the cell walls is more towards the outer side and less on the inner side resulting in broader lumen towards the base of cells and narrower towards the outer side (Text-fig. 1 J, L). However, Ambegaokar (1976) has described

Text-fig. 2—A-N, *Rhynchosia* species; A, B, *R. albiflora*; E, F, *R. bracteata*; C, D, H, J-N, *R. minima*. A, C, E. Seeds in lateral view; B, D, F. Seeds showing hilum in front view; G, H. Lateral view of mature embryos; I, J. Front view of mature embryos showing two fleshy cotyledons; K, Cross-section of mature seed through hilar region; L, Part of cross-section of mature seed through hilar region showing palisade, counter-palisade and tracheid bar; M, Cross-section of macrosclereids; N, Cross-section of 'hour-glass' cells.

(cot-cotyledon, cpal-'counter-palisade' layer, h-hilum, hgl-'hour-glass' cells, pal-palisade layer, r-radicle, tb-tracheid-bar.).

Table 1—Seed morphology in *Rhynchosia* spp.

Species	Seed			Hilum		Arid	Seed size L×B×T (mm)
	Shape	Colour	Surface	Shape	Colour		
<i>R. albiflora</i>	Reniform	Navy blue (Admiral)	Smooth	Elongated oval	Dull white	Absent	4.5×2.5×2
<i>R. bracteata</i>	Nearly oval	Greyish green (Dusty green)	Slightly rough	Narrow elliptical	Dull white	Small greenish white coloured	6×4.5×1.5
<i>R. minima</i>	Reniform	Peach (Sheeps kin moth)	Smooth	Nearly oval	Dull grey white surrounded by dark brown area	Absent	3.5×2×1
<i>R. rothii</i>	Reniform	Creamish or mustard (Malmaison)	Smooth	Elongated oval	Cream coloured surrounded by brown	Absent	4.5×3×2

uniform thickening in the macrosclereids and the lower part becoming bulbous, a description which does not tally with his figure 19. In surface view, as well as, in cross-sections, the macrosclereids are polygonal, thick-walled and filled with brown contents (Text-fig. 2 M), a feature also reported by Ambegaokar (1976). The length and breadth are quite variable in different species and the data are given in Table 2. It is evident from the table that the length of the macrosclereids in the middle of the lateral sides of seeds in *R. bracteata* is minimum, i.e. 72 microns and that in *R. albiflora* it is maximum, i.e. 120 microns. The breadth of macrosclereids does not show that much variation and ranges between 11 to 16 microns. In all the species the macrosclereids near the hilar region are longer. The macrosclereid layer is followed by a hypodermal layer of osteosclereids which are flask-shaped, thick-walled with large air gaps in between (Text-fig. 1 I, J, K, L). The

Table 2—Length and breadth of macrosclereids and Osteosclereids in *Rhynchosia* spp.

Species	Macrosclereids		Osteosclereids		
	Length in (microns)	Breadth in (microns)	Length in (microns)	Breadth in (microns)	
				'BASE'	'NECK'
<i>R. albiflora</i>	120	16	40	21	15
<i>R. bracteata</i>	72	15	24	35	15
<i>R. minima</i>	86	11	16	18	11
<i>R. rothii</i>	96	11	24	34	11

differentiation of these hour-glass cells is not uniform and sometimes they are totally lacking in many parts of the seed coat. Besides, all similarities, the 'neck' part of the osteosclereid is very long in *R. albiflora* compared to other three species (Text-fig. 1 K, L). Table 2 gives an idea of the length and breadth of osteosclereids. In *R. minima* they are 16 microns while in *R. albiflora* they are 40 microns in length. The breadth of the basal part varies from 35 microns (*R. bracteata*) to 18 microns (*R. minima*), however, the breadth of the 'neck' region is 15 microns in *R. albiflora* and *R. bracteata* while it is 11 microns in other two species. The osteosclereids near the hilum region are more conspicuous and longer. According to Behl and Tiagi (1977), the hour-glass cells near the hilum lack air-spaces and remain unlignified. No osteosclereids are differentiated in hilar region in any of the four species studied presently (Text-fig. 2 K). As seen in cross-sections, the osteosclereids are thick-walled and oval-elliptical in outline (Text-fig. 2 N). These cells are filled with brown contents and are surrounded by a mucilaginous layer. Remaining part of the seed coat is composed of 6 to 10 layers of degenerating parenchymatous cells (Text-fig. 1 I, K) with no lysigenous cavities or starch grains as reported by Behl and Tiagi (1977).

The hilar region is very prominent consisting of 'counter-palisade layer' which is restricted to the hilar region only in all the species. Both the palisade layers of individual species are uniform in structure and equal in size in the central region of the hilum except in *R. minima* where the cells of counter-palisade layer are longer. A groove slightly separating the palisade cells of hilum, almost in the centre of the hilum, is also discernible. The 'tracheid-bar' is present under this groove and extends to quite an extent along the hilar region. In cross sections the 'tracheid-bar' is pyriform (Text-fig. 2 K, L). Rest of the hilar cells consist of thick-walled polygonal cells with abundant tanniferous depositions.

Aril—On the outer side of counter palisade layer is the arillar zone in *R. bracteata*. The arillar tissue is parenchymatous and all the cells are full of brown contents.

Embryo—The shape of fully grown embryo is nearly elongated-oval with a notch on the radicular side in all the species (Text-fig. 2 G, H). The hypocotyl-root-axis is very short and curved. The two cotyledons are thick and fleshy (Text-fig. 2 J, K).

Discussion

The ovule is bitegmic, crassinucellate and campylotropous at the time of fertilisation as is found in majority of the leguminous taxa (Davis, 1966). The behaviour of the nucellar epidermis is of special interest for its cells elongate radially and simulate an endothelium. Nucellar epidermis simulating an endothelium has been described in *Phaseolus aconitifolius* a member of tribe Phaseoleae (Deshpande & Bhasin, 1974). Anantaswamy Rau (1953), as well as Behl and Tiagi (1977) could not observe this feature in *R. minima*, and they reported formation of *endothelium* by the inner layer of the inner integument.

The endosperm development is of the Nuclear type, a feature common to all the Papilionaceous taxa (Davis, 1966). The endosperm on the chalazal side remains free nuclear till late stages of seed development and probably functions as a haustorium. Similar observations have also been recorded earlier in many taxa (Anantaswamy Rau, 1951, 1953; Sterling, 1955; Takao, 1962; Deshpande & Bhasin, 1974; Behl & Tiagi, 1977; Berg, 1979).

The inner integument completely disintegrates during the late stages of seed development and does not play any role in the formation of mature seed coat as is reported for Papilionaceous seeds in general (Gunn, 1981).

The seeds are exarillate but in *R. bracteata* the seeds have small arils comparable to 'rim-aril' of Corner (1951). The seed coat is formed by the outer integument alone and its outermost layer consists of lignified macrosclereids, a feature common in leguminous taxa (Gunn, 1981). The differential lignification on the radial walls of these macrosclereids leads to the formation of a broader lumen towards the base of cells and narrower towards the outer side. However, Ambegaokar (1976) has described uniform thickening in the macrosclereids and the lower part becoming bulbous, a description which does not tally with his figure 19. The present observations regarding thickening pattern do not support Ambegaokar's findings. Linea lucida, considered to be a characteristic feature of Papilionoideae (Corner, 1951, 1976), could not be observed in any of the four species of *Rhyncosia*.

The osteosclereids or 'hour-glass' cells are flask-shaped, thick-walled with large air gaps in between. The 'hour-glass' cells of different shapes but with air gaps in between are most characteristic features of Papilionoideae (Netolitzky, 1926 ; Berg, 1979 ; Gunn, 1981). Ambegaokar (1976) has reported somewhat elongated and dumbbell shaped hour-glass cells while Behl and Tiagi (1977) observed the osteosclereids to have stellate ends with broad base and narrow upper end. However, according to the present investigation, neither Ambegaokar's nor Behl and Tiagi's observations regarding the shape of hour-glass cells are supported.

Hilar region comprises the characteristic 'counter-palisade,' palisade layer and a 'tracheid-bar' (Corner, 1951, 1976 ; Gunn, 1981). The aril of *Rhyncosia bracteata* is similar in position and structure to the earlier reported arils of papilionaceous seeds, which develop about the hilum as a swollen compact structure (Corner, 1976 ; Berg, 1979). The arils have also been observed in *Atylosia platycarpa* (Ambegaokar, 1976) and *Kennedia* and *Hardenbergia* (Berg, 1979).

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