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Comparative Seed Coat Anatomy of Some Indian Edible Pulses [1])

By

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With 44 Figures

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Summary

Seed coat anatomy of 13 edible pulses of India has been worked out. Macrosclereid, osteosclereid and hilum anatomy are comparatively studied, and an anatomical key for identification of 11 species is evolved. Species are divided into three groups — (1) seeds with hilum raised above the seed coat surface, (2) with hilum at or almost at the level of seed coat surface, and (3) with hilum situated in a pit below the seed coat surface. Similarly aril bearing species and species without aril, and species with persistant and species without funicle in dry seed hilum are recognized.

Zusammenfassung

Die Anatomie der Samenschalen von 13 in Indien angebauten Leguminosen wird untersucht. Es wird der Bau der Macro- und der Osteosclereiden und des Hilums studiert und ein Bestimmungsschlüssel für 11 Arten aufgestellt. Die untersuchten Arten werden in drei Gruppen geordnet: 1. Samen mit erhabenem Hilum, 2. Hilum etwa in der Höhe der Samenoberfläche liegend und 3. Hilum in einer Vertiefung liegend. Weiters baut der Bestimmungsschlüssel auf dem Vorkommen eines Arillus bzw. des Funiculus (am Hilum des trockenen Samens verbleibend oder fehlend) auf. (Editor).

1. Introduction

Pulses, members of *Papilionaceae*, are very widely used in India for their protein. They form the reachest source of protein and yet are cheaper

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than any other protein food. The dry seeds are the chief supply of the food material. However, certain species provide green podes as vegetables in different parts of India. Such an important group of plants was taken up for developmental and anatomical studies in a programme to investigate economic plants of India. Previous contributions deal with shoot apical (PATEL & VENKATESWARA RAO 1975), root apical (PATEL, SHAH & SUBBAYAMMA 1975) organization and comparative anatomy of leaf, node and internode (PATEL 1975) in Indian pulses. This is yet another contribution on the Indian pulses describing seed coat anatomy. Seed coat of various pulse species has been worked out mainly for its embryological and developmental aspects (ZIMMERMANN 1936, KONDO 1913, SINGH 1964, REEVE 1946a, b, CORNER 1951, HYDE 1954). Recently CHOWDHURY & BUTH (1970), published their observations on anatomy of Indian pulses. Nonetheless though their work is important, it describes mainly the external morphology and generalized anatomy of the pulse seed coat. In this situation, I took up the problem to investigate details of seed coat anatomy with special reference to hilum structure and find out new points, if any, of identification of Indian species.

2. Materials and Methods

The investigated species and the sources of their seeds are given in Table 1. The seeds were hydrated in hot water for 24 hrs, and soften in a glycerine-water mixture (50 : 50) for 3 to 8 days in various species. The seed coats were peeled off the seeds before softening. To avoid the fungal growth, a small amount of phenol was added. The soften hilum part was cut off the rest seed coat and dehydrated, processed in customary ways (SASS 1958). The transections, 8 to 12 μm thick, were stained with tannic acid-ferric chloride (FOSTER 1934), mordant safranin and fast green combinations. Other part of the seed coat was macerated using 10% chromic acid and 10% nitric acid (1 : 1) fluid. Macerated material was stained with PAS and mounted in 10% glycerine water. The edges of the coverslip were sealed with DPX mountant.

3. Observations

The seed coat of leguminous species shows characteristic and interesting structure. Its general anatomy has already been described in earlier literature (see CHOWDHURY & BUTH 1970). Hence, that part of the investigation is not described here. However, the nature of sclereids, which form a hard part of the seed coat, in different pulse species has been described. Next to the outer cuticular and waxy layer is a layer of long sclereids known as macrosclereids which is followed by a layer of hour-glass cells or osteosclereids. Number of macrosclereid and osteosclereid layers may differ in some species, but usually they are single layered.

3. 1. Osteosclereids

The height of the osteosclereids differs at different loci on the seed coat. They are longest near to the hilum region and gradually decrease in height as they are traced away from the hilum region. Figure 29 shows a very small osteosclereid, and Figs. 30—33 show different sizes of osteosclereids. Usually, the shortest osteosclereids are dumb-bell shaped with two large

Table 1

Species investigated and their sources

Species	Local name	Source
Cajanus cajan L.	Tur or Tuver	BACA
Cicer arietinum L.	Chanā	IARI
Cyamopsis tetragonoloba DC.	Guvār	BACA
Glycine max L.	Soyābean	BACA
Lablab purpureus (L.) SW (*Dolichos lablab* L.)	Pāpadi	BACA
Lathyrus sativus L.	Lāng	IARI
Lens culinare Medic. (*Lens esculenta* MOENCH.)	Masūr	IARI
Phaseolus vulgaris L.	Fansi	BACA
Pisum sativum L.	Vatānā	IARI
Vigna aconitifolia (JACQ.) MARECHAL	Math	ACJ
V. angularis (WILLS.) OHWI & CHASHI (*Phaseolus mungo* L.)	Urid	UPIA
V. radiata (L.) WILCZEK (*P. aureus* ROXB.)	Moong	IARI
V. unguiculata (L.) WALP. (*V. catjang* L., WALP.)	Cholā or Lobiā	IARI

Sources: ACJ = Agricultural College, Junagarh, Gujarat (India); BACA = Bansilal Amritlal College of Agriculture, Anand, Gujarat, India; IARI = Indian Agricultural Research Institute, Delhi; UPIA = Utter Pradesh Institute of Agricultural Sciences, Kanpur (India).

heads and a narrow middle part (Figs. 29—31, 34). Very long osteosclereids may be humerus shaped (Fig. 33) or sometimes, they are narrow at one end and very broad at the other end (Fig. 32). However, in all the cases the end parts of the sclereids are broad and form structure like a humerus bone end (Figs. 29—33). The adjoining osteosclereids are in contect with each other only at their broad ends leaving a very large inter-sclereid space (Figs. 31, 34). Such inter-sclereid spaces, when seen in longisectional view, appear like an hour-glass. Hence, they are very frequently refered as hour-glass cells. The lateral walls of middle part of the osteosclereid are very thick and,

usually, uniform in thickness (Figs. 29—32, 34). But sometimes as in soybean very long osteosclereids may show uneven wall thickening (Fig. 33). The thickness of the lateral wall tapers at the broad ends. The transverse walls of the broad ends of the sclereids are thinner than that of the lateral ones. The osteosclereid layer is not continuous beneath the hilum region in most of the species.

3. 2. Macrosclereids

Unlike the osteosclereids the macrosclereids are compactly arranged leaving no inter-sclereid spaces. Their length at different loci of the seed coat does not differ sharply. Average height of the macrosclereids in different species has been given in Table 2.

Table 2

Height, wall thickening and other characters of the Macrosclereids in various pulse species of India

Species	Height (μm)	Outer wall surface	Shape of the base	Surface of the wall adjacent to lumen	Other characters (if any)
C. arietinum	175±25	UE	B	W	End curved
C. cajan	75±5	UE	—	W (S)	Lumen very narrow
C. tetragonoloba	72±5	UE	—	S	One end narrow and notched
G. max	35±2	UE	—	S	Dumb-bell or oval base
L. purpureus	92±3	UE	B (sm)	S (W)	.....
L. sativus	76±19	UE	—	S—W	The thick wall not tapering gradually
L. culinare	35±5	UE	—(B)	S	.....
P. vulgaris	49±5	UE	—(B)	S	Lumen narrow or broad
P. sativum	70±10	UE	B	W	.....
V. aconitifolia	37±2	UE	—	S	Hour-glass shaped lumen
V. angularis	48±8	UE	—	S	Notches at thinner end
V. radiata	46±4	UE	—	S	.....
V. unguiculata	36±6 70±5	UE	—(B)	S	Cup shaped lumen

Abbreviations: UE = Uneven thickening; B = Bulbous base of the lumen; W = Wavy surface; S = Smooth surface; sm = small; — = Shape of the basal part of the lumen not certain; ..... = No particular character is present.

There are three height groups of the macrosclereids, in the investigated varieties of pulse species, (i) 35—50 μm as in *Vigna radiata*, *V. unguiculata*, *V. aconitifolia*, *V. angularis*, *Lens culinare*, *Glycine max*, *Phaseolus vulgaris*; (ii) 70—100 μm as in *Vigna unguiculata*, *Pisum sativum*, *Lathyrus sativus*, *Lablab purpureus*, *Cyamopsis tetragonoloba*, *Cajanus cajan*, and (iii) more than 150 μm as in *Cicer arietinum*. From this it is very clear that though the pulse species investigated here differ sharply in shape and size of their seeds, majority of them fall in the first height group, i. e. 35—50 μm. In *V. unguiculata* two types of macrosclereids (in height) are found (Table 2). Size and shape of macrosclereids in various species have been shown in Figs. 1—28, and the details of wall thickening and eye-catching characters, if any, are given in Table 2. Usually, the upper end wall of the sclereid is thicker than the lower one (e. g., *V. radiata*, *V. unguiculata* etc.), but in *V. angularis*, sometimes, both the end walls are thick and show pits (Fig. 19). Lateral walls are thickest towards the upper end and gradually taper towards the lower end except in *C. arietinum* (Figs. 1, 2) where at the basal part they show more and uneven, wavy thickening; *V. angularis* (sometimes) where they are uniform (Fig. 19); some cases of *G. max* in which they are thickest in the middle and taper towards both the end walls (Fig. 20). The wall thickening in *P. sativum* (Figs. 7—9), *C. cajan* (Fig. 28) and *L. sativus* (Figs. 12—14) is tapering at the base but the inner surface of the wall is wavy throughout the length or at the base. The formation of a bulbous lumen at the base of the sclereid in *C. arietinum*, *L. purpureus* (small), *L. culinare*, *P. sativum*, *V. unguiculata* and *P. vulgaris* is very interesting. The shape of the bulbous base lumen in these species varies (see Figs. 1—28). This can be used for the identification of the species. The outer wall surface is smooth in most of the cases, but in *C. arietinum* (at arrow, Figs. 1, 2) and *P. sativum* (Figs. 8, 9), sometimes, it shows wavy surface at lower end. Whereas in *L. culinare*, *V. angularis*, *C. tetragonoloba* and *P. vulgaris* (Fig. 25), sometimes, small projections are found on the lateral wall at lower end (Figs. 10, 17, 24, 25, darts).

C. arietinum stands out from other species by the presence of very long sclereids with their outer ends curved (Figs. 1, 2). *V. aconitifolia* has macrosclereids with a slightly swollen part in the middle (Figs. 15, 16). Other characters are given in Table 2. All these wall and lumen characters along with general shape form important identification keys for the species of pulses investigated here.

3. 3. Hilum

The structure, both morphological and anatomical, of the hilum region of the seed coat in pulse species is of much importance as it shows a range of variations in characters (Figs. 35—44). The important parts of hilum are: the white corky outgrowths around the hilum also known as aril, funicle,

macrosclereid layers and tracheid bar. The size, width and lenght of the hilum varies greatly in various species but that character has not been taken into consideration here for the reason that it may vary even in various varieties of the same species. Moreover rough idea of this character is available in the work of CHOWDHURY & BUTH (1970). Hilum is, in most of the species, at or almost at the level of surface of seed coat except *C. arietinum* in which it is situated in a deep cup-like structure below the level of seed coat (Fig. 40), and *P. sativum* (Fig. 43) and *L. culinare* (Fig. 44) in which it is raised above the surface of the seed coat forming a pout-like structure.

3. 3. 1. Aril

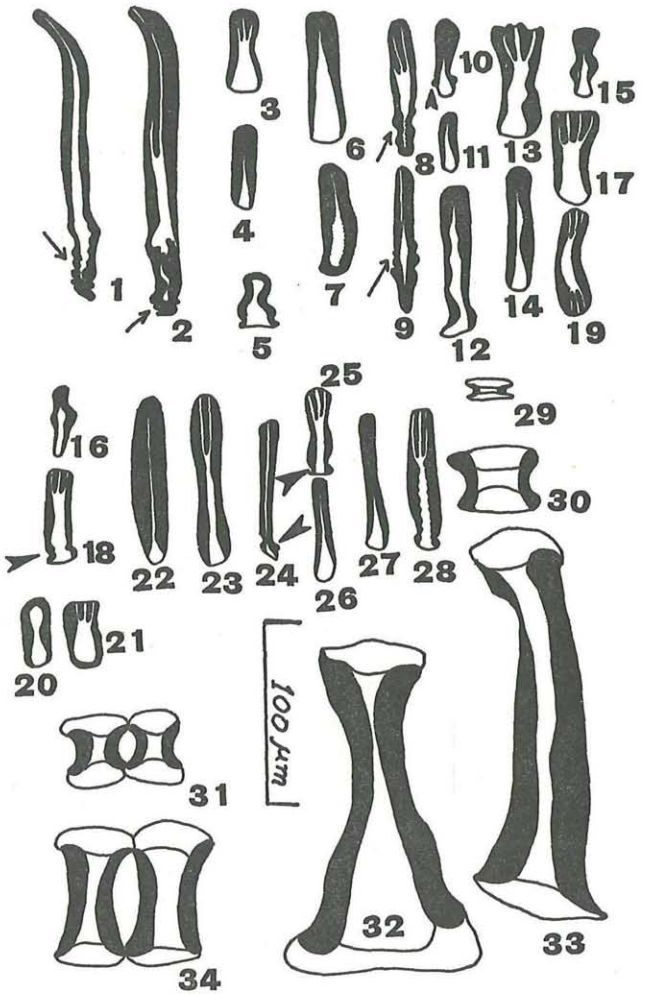
The studied 11 species can be divided into two major groups based on the presence or absence of aril. (i) With aril — *V. radiata* (Fig. 35), *V. aconitifolia* (Fig. 36), *V. angularis* (Fig. 37), *V. unguiculata*, *P. vulgaris* (Fig. 38), *L. purpureus* (Fig. 39), *C. cajan* (Fig. 41) and *G. max* (Fig. 42); (ii) without aril — *C. arietinum* (Fig. 40), *P. sativum* (Fig. 43), *L. culinare* (Fig. 44).

Aril is quite prominent, massive and leave a very narrow slit in *C. cajan* (Fig. 41). In transection it appears as club shaped projection on either flanks of the hilum and is multiseriate. *V. angularis*, *V. unguiculata* (Fig. 37), *P. vulgaris* (Fig. 38) and *L. purpureus* (Fig. 39) have prominent aril but it is not as large as that in *C. cajan*. Whereas, in *V. radiata* (Fig. 35) and *V. aconitifolia* (Fig. 36) the aril is scanty on the periphery of the hilum. It has a very short vertical rows of cells, sometimes of only 1—2 cells. In these two species aril does not project above the seed coat level unlike in other species. In *G. max* (Fig. 42) aril is formed of only 2—3 vertical rows of cells as seen in transectional view.

The aril in general is formed of long cells arranged in vertical rows. Usually, these vertical rows arch over the central slit of the hilum. But in *V. radiata* and *V. aconitifolia* they arch towards the periphery of the hilum (Figs. 35, 36).

3. 3. 2. Funicle

In dry seeds funicle is not persistant and could not be found in any of the microscopic preparations in *C. arietinum* (Fig. 40), *C. cajan* (Fig. 41), *G. max* (Fig. 42), *P. sativum* (Fig. 43) and *L. culinare* (Fig. 44). It is persistant in other species (Figs. 35—39). The dry tissue, formed of irregular parenchymatous cells, of funicle usually does not project out of the aril except in *L. purpureus* (Fig. 39) where funicle is seen outside the aril even with naked eye. The cells of funicle show inter cellular spaces. Funicle when present is surrounded by aril. When aril is absent and the hilum is raised above the surface of the seed coat, funicle is absent. In *C. cajan* where aril is too massive funicle is absent.



Figs. 1—34: Sclereids in the seed coat of various pulses. Macrosclereids: 1, 2 = *Cicer arietinum*; 3, 4 = *Vigna radiata*; 5, 6 = *Vigna unguiculata*; 7—9 = *Pisum sativum*; 10, 11 = *Lens culinare*; 12—14 = *Lathyrus sativus*; 15, 16 = *Vigna aconitifolia*; 17—19 = *Vigna angularis*; 20, 21 = *Glycine max*; 22, 23 = *Lablab purpureus*; 24 = *Cyamopsis tetragonoloba*; 25, 26 = *Phaseolus vulgaris*; 27, 28 = *Cajanus cajan*. Osteosclereids: 29 = *Vigna radiata*; 30 = *Pisum sativum*; 31—34 = *Glycine max*

3. 3. 3. Macrosclereid layers

In hilum region there are two layers of macrosclereids, the outer one known as counter macrosclereid layer (Figs. 35—44). The cells of the counter macrosclereid layer taper at both the ends, central and peripheral. The counter macrosclereid layer is found only up to the base of the aril, if present. And its limit is up to the rim of the hilum in the species where aril is absent. In *C. arietinum* where hilum is found at the base of a cup-shaped pit the counter macrosclereid layer covers only the bottom of the pit (Fig. 40). The inner macrosclereid layer is in continuation with the outermost layer of seed coat. Its cells at the central region of the hilum are very short and gradually increase in height as traced towards the periphery and finally uniform height of sclereids is established. However, in *P. vulgaris* the macrosclereids of inner layer in the hilum region are very long as compared to those at other loci on the seed coat (Fig. 38). The osteosclereids are not present within the hilum region but their shortest cells appear just at the rim region of the hilum where the cells of counter macrosclereid layer taper.

3. 3. 4. Vascular bar

Vascular bar is the source of vascularization of entire seed when it is in the pode. It gets connection with the pode through funicle. In dry seeds a small group of tracheary elements is found at the slit point in the hilum (Figs. 35—44). It has been named as "tracheid bar" because the earlier authors thought that all the constituents of this structure are tracheids (Corner 1951, Ott & Ball 1943, Cutter 1971, Chowdhury & Buth 1970). However, maceration of hilum region shows that the vessel members are also present in the so-called tracheid bar. I have shown this in *P. vulgaris* (Patel 1973). In all the species except in *C. tetragonoloba* and *C. arietinum* the identification of vessel elements was confirmed. The vessel elements in various species show variations. (i) Vessel elements may show two or three perforation plates. (ii) Blind vessel elements with only one perforation plate are very common. (iii) Vessel elements are very short. (iv) The shape may be cylindrical, triangular, branched, club-shaped or very irregular. (v) The pits on the walls are simple.

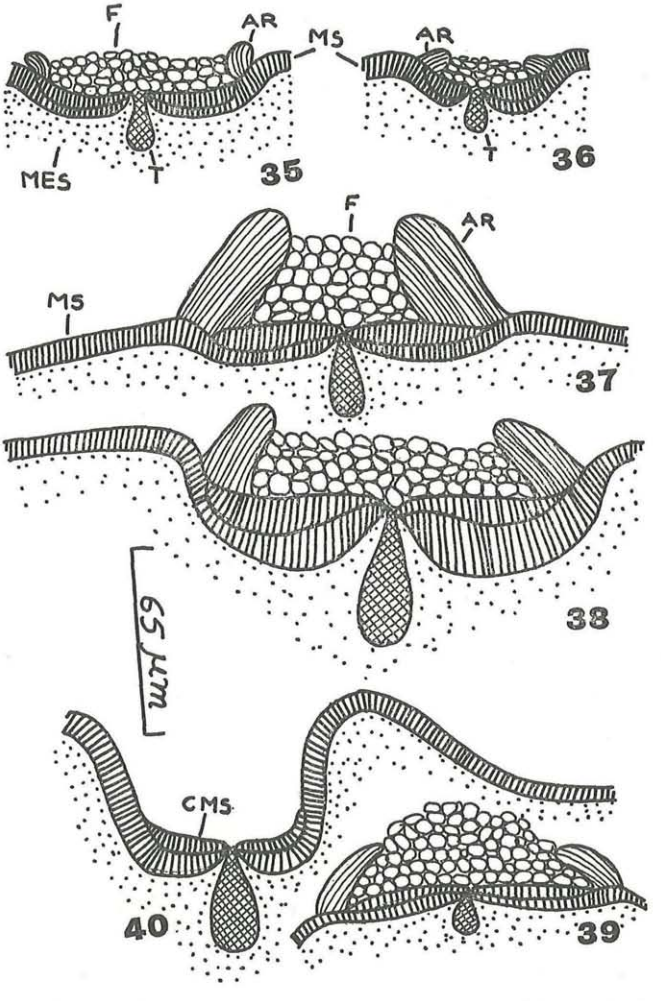
Alongwith vessel elements short, irregularly shaped tracheids and xylem parenchyma cells are also observed. The cuticule and the mesophyll are described by Chowdhury & Buth (1970) hence not given here.

3. 4. Key

Based on my observations on seed coat anatomy of 11 species and sclereid morphology of 13 Indian edible species I have given their identification key below. Chowdhury & Buth (1970) have given a key but it is based on morphological characters and structure of macrosclereids only

Whereas my key shows hilum anatomy and morphology of macrosclereids. This might be more useful to those who work on archeological plant remains (as suggested by CHOWDHURY & BUTH 1970).

1. Hilum raised above the seed coat level.
   (i) Macrosclereids 70 ± 10 µm in height ..... *P. sativum*
   (ii) Macrosclereids 35 ± 5 µm in height ..... *L. culinare*

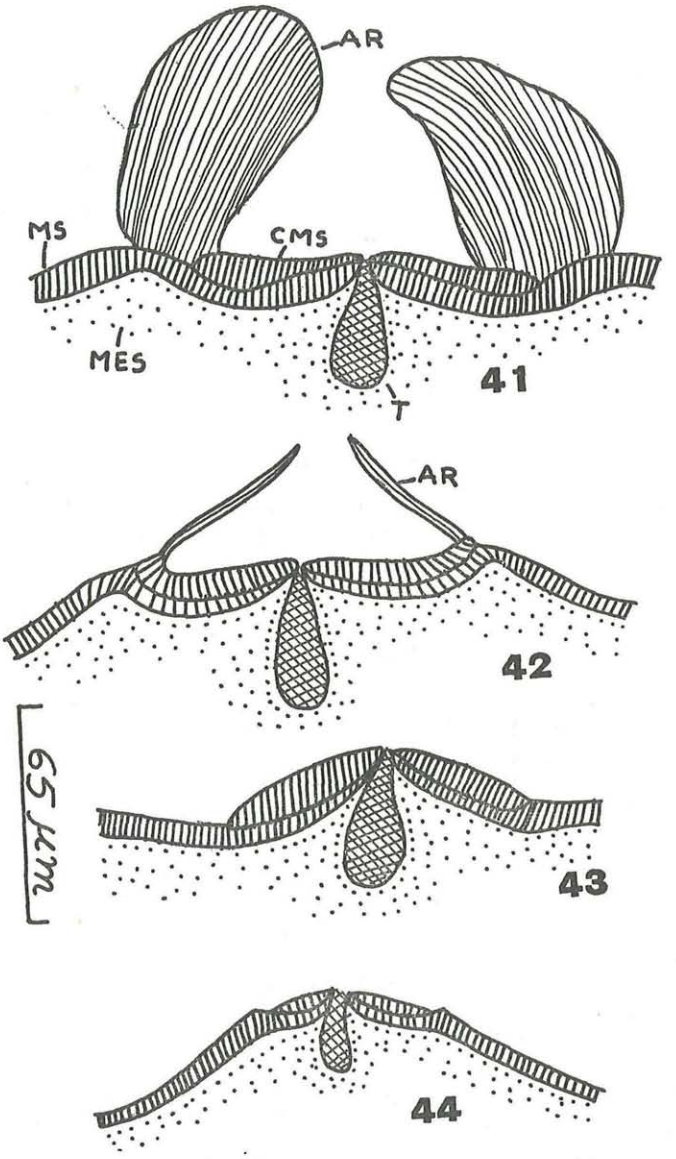


Figs. 35—40: Transections through the hilum region of the seed coat. 35 = *Vigna radiata*; 36 = *Vigna aconitifolia*; 37 = *Vigna angularis*; 38 = *Phaseolus vulgaris*; 39 = *Lablab purpureus*; 40 = *Cicer arietinum*. AR = aril, CMS = counter macrosclereid layer, F = funicle, MES = mesophyll, MS = macrosclereid layer, T = vascular bar

1. Hilum seated below the level of seed coat in a pit.
   (i) Macrosclereid 175±25 μm in height ..... *C. arietinum*
1. Hilum at (or almost at) the level of seed coat.
   2. Aril present.
      3. Funicle not found in dry seed.
         (i) Massive aril leaving very narrow slit ..... *C. cajan*
         (ii) Very narrow, long strip of aril ..... *G. max*
      3. Funicle present.
         4. Aril massive.
            5. Narrow hilum with small tracheid bar.
               (i) Macrosclereids 48±8 μm in height ..... *V. angularis*
               (ii) Macrosclereids 36±6 and 70±5 μm in height .... *V. unguiculata*
            5. Broad hilum with large tracheid bar.
               (i) Very narrow macrosclereids ..... *P. vulgaris*
         4. Aril small.
            (i) Funicle raised above the height of the aril ... *L. purpureus*
         4. Aril very short.
            (i) Macrosclereids cylindrical with conical lumen .... *V. radiata*
            (ii) Macrosclereids with bulbous middle part ..... *V. aconitifolia*

4. Discussion

The only detailed document on seed coat anatomy of Indian pulses is provided in the contribution by CHOWDHURY & BUTH (1970). They have described external morphology in details. But detailed seed coat structure is not dealt with. Three types of macrosclereids (palisade cells), type I with uniform wall thickness (*C. tetragonolobus*, *Dolichos lablab*); type II the end of the cell away from the cuticle is bulbous (*C. cajan*, *Dolichos biflorus*, *Lens culinaris*, *Phaseolus aconitifolius*, *P. aureus*, *P. mungo*, *Vigna catjung* and *V. sinensis*); type III in which inner surface of the cell is corrugated (*C. arietinum*, *Lathyrus sativus*, *P. sativum*, *Vicia faba*), have been recoganized by CHOWDHURY & BUTH (1970). In the present investigation *C. cajan* showed, in some cases, corrugated inner wall surface of the macrosclereids; *V. aconitifolia* has a bulbous central part of the sclereid and the inner wall surface of the wall of macrosclereids in *C. arietinum* is corrugated only at the basal part differing from the observations of CHOWDHURY & BUTH (1970). Over and above these I found certain peculiarities of macrosclereids in some of the species which help their easy identification, e. g., curved outer end in *C. arietinum*; notched ends in *C. tetragonoloba* and *V. angularis*; cup shaped lumen in *V. unguiculata* and an hour-glass shaped lumen in *V. aconitifolia*. The keys such as size and shape of the seeds and size of hilum in pulses, used by CHOWDHURY & BUTH (1970), may not be very useful in all the cases because these characters vary in different varieties



Figs. 41—44: Transection through the hilum region of the seed coat. 41 = *Cajanus cajan*; 42 = *Glycine max*; 43 = *Pisum sativum*; 44 = *Lens culinare*. Abbreviations see Figs. 41—44

of the same species, e. g. in *C. cajan*, *L. purpureus*, *C. arietinum*, *P. sativum* and *V. unguiculata*. Instead, the use of hilum, aril, funicle and sclereid characters are used to develop a key of identification.

The presence of "tracheid bar" (CORNER 1951, CHOWDHURY & BUTH 1970, CUTTER 1971) or "vascular isle" (HYDE 1954) is an interesting character of the seeds in the subhilum region in papilionaceous species. CORNER (1951) and HYDE (1954) reported the presence of short tracheids with large, reticulate pitting in tracheid bar. However, in *Phaseolus vulgaris* (PATEL 1973) and other species investigated in the present work show the presence of vessel elements with reticulate thickening and simple pits. Hence, I have prefered to designate the so-called "tracheid bar" as "vascular bar" as it represents the vascular connection between the seed and the pod wall, and consisted of both the xylem and phloem elements when in green pod.

5. Acknoledgements

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