Scanning Microscopy

Volume 1 | Number 3

Article 60

5-27-1987

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SEED COAT STRUCTURE IN SOME SPECIES OF ATYLOSIA (Phaseoleae - Cajaninae)

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(Received for publication December 08, 1986, and in revised form May 27, 1987)

Abstract

Scanning electron microscopy and light micros-copy were used to examine seed characters of ten species of the legume genus Atylosia. Seeds of all the species are arillate with a central hilar groove, a character useful for specific delineation of the genus. The testa of A.albicans, A.cajanifolia, A. lanceolata, A.scarabaeoides and A.volubilis is multi-reticulate. It is rugulate in the rest of the species. Differences in the size and arrangement of rugae can be utilized to separate them from one another. The shape of the hilum and micropyle is distinctive for some species. It was observed that the length of palisade cells and hour glass cells are not correlated with seed size. The tracheid bar is present and is of the common fabaceous type; tracheoids show variations in pitting and vesturing. Based on the character of the aril, spermoderm, tracheoids and pits, a key has been designed to identify species of Atylosia. It is hoped that this study will be helpful taxonomically.

KEY WORDS: <u>Atylosia</u>, Papilionaceae (Fabaceae), aril, spermoderm, hilum, tracheid bar, tracheoids.

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Introduction

Atylosia, a papilionoid legume genus of the subtribe Cajaninae, Benth. of the tribe Phaseoleae is represented by about 35 species distributed from Asia to Australia. Plants may be erect, trailing or twining herbs or shrubs with large arillate seeds. Some of its species are economically important e.g. A.goensis Dalz (=A.barbata Baker), which is said to be beneficial in rheumatism and fever (Chopra, 1933) while <u>A.goensis</u> and <u>A.scarabaeoides</u> are used as green manure (Burkill, 1935). <u>Atylosia</u> is considered one of the wild relatives of the pigeon pea. Therefore, many of its species are used in <u>Cajanus</u> breeding to acquire many of the desired features in cultivated pigeon pea such as higher pod index, disease and insect resistance, and early flowering. Biosystematic studies, encompassing morpho-cytological and electrophoretic analyses of <u>Cajanus</u> <u>cajan</u>, seven species of <u>Atylosia</u>, and one of <u>Rhynchosia</u>, revealed that A.cajanifolia is closest to Cajanus cajan followed by A.lineata, A.scarabaeoides, A.sericea, A.albicans, A.volubilis and A.platycarpa (Pundir and Singh, 1985). Further, Pundir and Singh, 1985 have suggested that the taxonomic status of Cajanus and Atylosia may be retained; based on the closeness as well as following rules of nomenclature - species like <u>A</u>. cajanifolia, A.lineata, A.sericea, A.scarabaeoides and <u>A.albicans</u> should be placed under the genus <u>Cajanus</u>. <u>A.platycarpa</u> and <u>A.volubilis</u> are quite distant from the pigeon pea, hence these two species should be retained under the genus Atylosia.

The spermoderm of some members of this subtribe (Cajanus cajan, Dunbaria rotundifolia, Eriosema floribundum, Rhynchosia minima and Flemingia floribundum) has been studied by Lersten (1981), who has surveyed 30 tribes of the Papilionoideae and reported levigate, rugulate, rugulate-pitted and papillose pattern in these species. Further, Lersten (1982) has reported nonvestured to vestured pits in the tracheoids of the tracheid bar of these species. Since no earlier report on Atylosia is available, in the present investigation ten species of this genus were taken into consideration. All the species have been compared for spermoderm, hilum, tracheid bar and pits which may help in their identification at the specific level.

Materials and Methods

Seeds were obtained from the Genetic Resources

Unit, ICRISAT, Patancheru (A.P.). A minimum of ten seeds per species were examined by scanning electron microscope and light microscope. Seeds were kept whole or sectioned longitudinally or transversely through the hilum and were examined under the light microscope to select good sections. To study the shape of the hilum the aril was removed with a fine blade. Seeds were mounted on brass stubs with double stick carpet tape and coated with gold-palladium in a sputter coater. Seed surfaces were examined at mid-seed and near the hilum. To maintain uniformity, portion immediately adjacent to the hilum was considered for spermoderm studies. Seeds were studied in a JEOL-JSM-35C SEM at an accelerating voltage of 20 kV and recorded on 120 mm ASA. The descriptions of the shape of seeds and hilum are based on the terminology used by Radford et al. (1974), the micropyle area was fixed as the apical point of reference. The testa topography terminology used here follows that of Lersten (1981).

Results

The size and shape of seeds vary a great deal (Table 1). The seed coats of all the species appear smooth, shining, chocolate brown to black or mottled with light brown patches under low magnifications of light microscope. Seeds are ovate in <u>A.lineata</u>, <u>A.mollis</u> and <u>A.sericea</u>, ovate to oblong in <u>A.albicans</u>, <u>A.cajanifolia</u> and <u>A.scarabaeoides</u>, ovate to reniform in <u>A.volubilis</u>, ovate to hemispherical in <u>A.lanceolata</u>, hemispherical in <u>A.platycarpa</u> and hemispherical to orbicular in <u>A.goensis</u>. The aril is present in all the species and is bilobed, horny and variable in colour from dull cream to grey (Table 2).

Differences have also been observed in the amount of the area of hilum covered over by the aril. Aril covers almost the entire hilum in <u>A.mollis</u> (Fig.1) or leaves behind the central region as in the rest of the species (Fig.2). In <u>A.goensis</u> and <u>A.volubilis</u> (Fig.3) aril is rim-like due to which the central furrow of the hilum is clearly visible. However, in the remaining species the central furrow becomes apparent only when the aril is removed (Figs.4-6). The Hilum and Micropyle

The shape of the hilum is narrowly elliptical in <u>A.cajanifolia</u> (Fig.4), <u>A.goensis</u>, <u>A.lanceolata</u>, <u>A.lineata</u> (Fig.5) and <u>A.volubilis</u>; and elliptical in <u>A.albicans</u>, <u>A.mollis</u>, <u>A.platycarpa</u>, <u>A.scarabaeoides</u> (Fig.6) and <u>A.sericea</u>. The micropyle is linear in <u>A.albicans</u>, <u>A.platycarpa</u>, <u>A.lanceolata</u> and <u>A.volubilis</u> (Fig.3) but is deltoid in the remaining species (Fig.6). In all the species a circular lens confluent to the hilum is also clearly visible (Fig.6).

The Testa

The testae of all the species exhibit a smooth surface at low magnifications of S.E.M. and under light microscope. However, at higher magnifications of S.E.M. the surface patterns become evident with uniform features at midseed region and throughout the rest of the seed. The seed surface is found to be multi-reticulate in A.albicans (Fig.7). A.cajanifolia (Fig.8), A.lanceolata, A.scarabaeoides (Figs.9,10) and A.volubilis and rugulate in the rest of the species (Figs.11-15). In A.albicans (Fig.7) and A.scarabaeoides (Figs.9,10) reticulations are with heavy ridges which are open at some places. In A.volubilis the reticulum is not as prominent as in other species. Clumping of some rugae is found at places in <u>A.cajanifolia</u> (Fig.8).

In <u>A.goensis</u> (Fig.11), <u>A.mollis</u> (Fig.12) and <u>A.platycarpa</u> (Fig.13) rugae are compact, irregularly arranged and separated by uniform furrows. Among these species minor differences have been observed in the size and arrangement of rugae. Rugae of <u>A.goensis</u> (Fig.11) are long and appearing to be arranged in rows; in <u>A.mollis</u> rugae are small and thick (Fig.12) while in <u>A.platycarpa</u> rugae are long and thin, giving the appearance of a multi-reticulate pattern.

In <u>A.lineata</u> and <u>A.sericea</u> rugae are of different sizes. Some rugae are short, irregular in shape and arrangement while others are longer forming clumps at several places. Rugae of <u>A.lineata</u> are small, thick and slightly beaded (Fig.14) in comparison to those of <u>A.sericea</u> (Fig.15) in which they are long, thin and sub-striate.

Critical examination of the testa in transection in all the species has revealed differences in the sizes of the cells of the outermost layer, i.e., palisade cells (Table 3). These cells are longest near the hilum, thick walled and elongated (P. Fig.16). Below the palisade layer is a layer of hour glass cells (H) with prominent intercellular spaces. This layer has longer cells near the hilum, becoming shorter towards the mid-seed area (Fig.16). Below the sclereid layer, a poorly defined zone of partially or completely crushed cells-remnant layer is visible, which may consist of remnants of endosperm cells (Fig.16). The Tracheid Bar and Tracheoids

The tracheid bar (TB) when observed in transection is present below the hilar groove (HG, Fig.16) and at the upper region is attached to the counter palisade layer (CP, Fig.17). It is embedded in a mass of spongy parenchyma and is generally elliptical in shape but varies in size and shape from narrow to broad (Table 3). Its length is not correlated with the size (length and breadth) of the respective seed. Tracheoids (small tracheids) are oriented vertically; these merge with the ovular bundle opposite the micropyle as can be seen in a longitudinal section of the seed (Figs.18,19). The tracheoids in all the species are thick walled with prominent end walls (Figs.19,20). Tracheoids of A.albicans, A.cajanifolia, A.goensis (Figs.17,21) and A.lineata are broad whereas those of the remaining species are narrow. In A. albicans, A.goensis and A.scarabaeoides (Fig. 19) tracheoids vary from pitted to scalariform whereas in <u>A.cajanifolia</u>, <u>A.lineata</u>, <u>A.mollis</u>, <u>A.platycarpa</u> and <u>A.volubilis</u> (Fig.20) they are simply pitted. However, in A.lanceolata and A.sericea they generally have scalariform pittings. The most common condition of pitting noticed in all the species is vestured (Figs.22-27) but variations have been recorded in the degree of vesturing. However, in A.cajanifolia and A.goensis some pits tended to be warty (Fig.24). All the three types of pits, i.e., nonvestured, vestured and warty are seen in A.mollis and A.platycarpa, but in A.platycarpa warty pits are as common as vestured ones (Fig.27).

In A.goensis (Fig.21), A.albicans (Figs. 22,23), A.cajanifolia (Fig.24), A.lineata (Fig.26), A.scarabaeoides vestures are short to long, unbranched to branched and sparsely arranged while some vestures probably join to form bars across the pits; a small number of pits of A.cajanifolia (Fig.24) and A.goensis

Atylosia Seed Coats



- Figure 1. Figure 2.
- Seed of <u>Atylosia mollis</u> showing aril. Bar = 1000/um. Seed of <u>A.scarabaeoides</u> showing bilobed aril and hilum. Bar = 1000/um. Seed of <u>A.volubilis</u> showing rim-like aril, a linear micropyle (M) and a central hilar groove. Figure 3. Bar = 1000 jum.
- Seed of A.cajanifolia after removal of aril to show narrowly elliptical hilum. Bar = 1000 µm. Figure 4.
- Seed of <u>A.scarabaeoides</u> after removal of aril showing narrowly elliptical hilum. Bar = $1000 \,\mu\text{m}$. Seed of <u>A.scarabaeoides</u> after removal of aril showing a deltoid micropyle (M), lens (L) and a central hilar groove. Bar = $1000 \,\mu\text{m}$. Figure 5. Figure 6.

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Figure 7. <u>A.albicans</u> - Surface showing multi-reticulate pattern. Bar = $10 \,\mu\text{m}$. Figure 8. <u>A.cajanifolia</u> - Surface showing multi-reticulate pattern with clumping of some rugae. Bar = $10 \,\mu\text{m}$. Figures 9-10 <u>A.scarabaeoides</u> - Surface showing multi-reticulate pattern. Bar = $10 \,\mu\text{m}$.

- Figure 9-10 <u>A.scarabaeoides</u> Surface showing multi-reticulate pattern. Bar = 10 μ m. Figure 11. <u>A.goensis</u> Surface showing rugulate pattern. Bar = 10 μ m. Figure 12. <u>A.mollis</u> Surface showing rugulate pattern. Bar = 10 μ m. Figure 13. <u>A.platycarpa</u> Surface showing rugulate pattern with long rugae. Bar = 10 μ m. Figure 14. <u>A.lineata</u> Surface showing rugulate pattern with rugae forming clumps at places. Bar = 10 μ m. Figure 15. <u>A.sericea</u> Surface showing rugulate pattern with substriate rugae. Bar = 10 μ m.

Atylosia Seed Coats



Figure 16. A.albicans-Transection of hilum showing aril (A), hilar groove (HG), tracheid bar, palisade cells (P), hourglass cells (H), and cotyledons (COT). Bar = 100 µm.

Figure 17. <u>A.goensis-Transection of hilum showing elliptical tracheid bar (TB) with pitted tracheoids</u>, counter palisade layer (CP) and palisade layer (P). Bar = $100 \,\mu$ m.

Figure 18. <u>A.goensis</u>-Sagittal view of the tracheid bar showing ovular bundle at left (arrow). Bar = 100 μm.
Figure 19. <u>A.scarabaeoides</u>-Sagittal section of tracheid bar with generally thin and long scalariform tracheoids. Arrow indicates ovular bundle and its confluence with tracheid bar. Bar = 10 μm.
Figure 20. <u>A.volubilis</u>-Longisection of tracheid bar to show pitting of tracheoids. Bar = 10 μm.
Figure 21. <u>A.goensis</u>-Tracheoids showing pits. Bar = 10 μm.



Figures22-23 <u>A.albicans</u>-Tracheoids showing vestured pits with long and branched vestures. Bar = 10 μm.
 Figure 24. <u>A.cajanifolia</u>-Pits with short to long, unbranched to branched vestures, some pits are slit like while others have one or two warts. Arrow indicates the pit membrane occurring on the pit. Bar = 10 μm.
 Figure 25. <u>A.volubilis</u>-Pits with few short and unbranched vestures. Remnants of pit membrane is seen in

some pits. Bar = 10 µm. Figure 26. <u>A.lineata</u>-Pits with extensive long and branched vestures. Arrow indicates remnants of pit membrane. Figure 27. <u>A.platycarpa</u>-Pits showing warts and vestures. Bar = $10 \,\mu\text{m}$.

Atylosia Seed Coats

Spe	ecies	Pedigree	Origin	Shape	Colour	Spermoderm	Size [*] LxBxT in mm
1.	A. albicans Benth.	PR-4816	Tamil Nadu	Compressed ovate or oblong	Chocolate brown to black mot- tled with light brown patches	Multi-reticulate	3.26x 3.83x 2.14
2.	A. cajanifolia Haines.	PR-4876	Orissa	-do-	-do-	-do-	3.27x3.90x2.16
3.	<u>A. goensis</u> Dalz. syn <u>A</u> . <u>barbata</u> Baker.	JM-3501	Kerala	Hemispherical or orbicular	-do-	Rugulate	4.06x3.21x2.40
4.	A. lanceolata W.V. Fitzq.	EC-137220	Australia	Compressed ovate- hemi- spherical	-do-	Multi-reticulate	4.25x3.44x1.93
5.	A. <u>lineata</u> Wight & Arn.	JM-3366	Tamil Nadu	Compressed ovate	Chocolate brown mot- tled with light brown patches	Rugulate	3.35x3.58x2.06
6.	A. mollis Benth.	JM-4311	Uttar Pradesh	-do-	Black mot- tled with light brown patches	-do-	2.70x 3.20x 1.95
7.	<u>A. platycarpa</u> Benth.	PR-4557	M a harashtra	Compressed hemispherical	Chocolate brown to black mot- tled with light brown patches	-do-	4.25x5.26x2.34
8.	A scarabaeo- ides Benth.	JM-2958	Himachal Pradesh	Compressed ovate or oblong	-do-	Multi-reticulate	2.78x3.31x1.96
9.	<u>A. sericea</u> Benth.	EC-121208	Australia	Compressed ovate	-do-	Rugulate	2.83x3.22x2.16
10.	<u>A. volubilis</u> Gamble.	NKR-73	Karnataka	Ovate to reniform	-do-	Multi-reticulate	4.44x4.10x3.00

Table 1. Seed characters in species of Atylosia

* Mean of 10 readings., Abbreviations : L = length (Length is measured parallel to hilum); B = Breadth; T = Thickness.

are slit-like. In <u>A. lanoeolata</u>, <u>A. mollis</u>, <u>A. volubilis</u> (Fig.25), <u>A.platycarpa</u> (Fig.27) and <u>A. sericea</u> vestures are short, unbranched sparsely arranged and less in number.

Remnants of pit membranes have been seen occasionally in pits of <u>A.albicans</u>, <u>A.goensis</u>, <u>A.</u> <u>lanceolata</u> and <u>A.sericea</u>; while majority of pits in <u>A.cajanifolia</u> (Fig.24), <u>A.lineata</u> (Fig.26), <u>A.mollis</u>, <u>A.platycarpa</u>, <u>A.scarabaeoides</u> and <u>A.volubilis</u> have remnant membrane.

Discussion

Seeds of all the species of <u>Atylosia</u> vary in shape, size and colour. Seeds of <u>A.lanceolata</u>, <u>A.</u> <u>platycarpa</u> and <u>A.volubilis</u> are largest among all the species. Seeds of <u>A.goensis</u> and <u>A.platycarpa</u> can be distinguished from the rest of the species due to hemispherical or orbicular shape.

Variations noticed in the shape and size of the hilum can be utilized for taxonomic purposes. The hilum is narrowly elliptical in <u>A.cajanifolia</u>, <u>A.goensis</u>, <u>A.lanceolata</u> <u>A.lineata</u> and <u>A.volubilis</u>. The significance of the hilum as an important taxonomic

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Species		Shape	Colour	Size [*] Length x Breadth (mm)	Aril colour	
1.	A. albicans	Elliptical	Dark Brown	1.60x0.90	Grey	
2.	A. cajanifolia	Narrowly elliptical	-do-	1.89x0.44	-do-	
3.	A. goensis	-do-	-do-	1.60x0.60	Cream	
4.	A. lanceolata	-do-	-do-	1.16x0.40	Grey	
5.	<u>A. lineata</u>	-do-	-do-	1.52x0.37	Cream	
6.	A. mollis	Elliptical	-do-	0.87x0.47	-do-	
7.	A. platycarpa	do-	-do-	1.18x0.55	-do-	
8.	A. scarabaeo- ides	do-	Brown	1.15x0.45	Steel grey	
9.	A. sericea	-do-	-do-	1.15x0.57	Cream	
10.	<u>A.</u> volubilis	Narrowly elliptical	Dark Brown	2.10x0.75	Cream	

Table 2. Hilum and aril character in species of Atylosia

* Mean of 10 readings.

Table 3. Seed Unaracters in ir

Spec	cies	Palisade Length (Cell Hor (µm)* Ler	urglass Cell ngth (μ m)*	Tracheid bar Shape Length x breadth (μm)		Tracheoid pit pattern
1.	A. albicans	111 (10	6-117) 35	(29-40)	W.E.	200 x 133	V
2.	A. cajanifolia	100 (9	9-101) 23	(17-33)	W.E.	253 x 167	V(W)
3.	A. goensis	122 (11	7-133) 25	(17-33)	W.E.	240 x 167	V(W)
4.	A. lanceolata	67 (5	7-101) 25	(25)	W.E.	253 x 150	V
5.	A. lineata	91 (8	1-99) 28	(22-44)	Е.	272 x 130	V
6.	A. mollis	118 (11	5-131) 23	(22-26)	Е.	144 x 60	V(N,W)
7.	A. platycarpa	115 (11	4-159) 63	(28-74)	Е.	386 x 193	V, W(N)
8.	A. scarabaeoides	80 (6	7-83) 17	(17)	W.E.	180 x 100	v
9.	A. sericea	82 (7	7-83) 11	(11)	N.E.	264 x 99	V
10.	A. volubilis	98 (9	2-117 22	(15-48)	Е.	300 x 177	v

* Mean of 10 readings. Figure in parenthesis indicates range. Abbreviations: E. - Elliptical; N.E. - Narrowly elliptical; W.E. - Widely elliptical; N - Nonvestured; W - Warty; V - Vestured; () - Variation from common condition.

character was emphasized by Bridges and Bragg (1983) and Bragg (1983). The present study has supported their contention.

Morphology and colour of the aril can also be an important character at different taxonomic levels. Hutchinson (1967) used it at a tribal level while Grear and Dengler (1976) and Polhill (1976) has used this at the generic level. Polhill and Raven (1981) have remarked that seeds of Atylosia have large arils. All ten species examined in this study have an aril with variations in its morphology and extent of hilum coverage. In A.mollis it almost completely covers the hilum while in A.goensis and A.volubilis it is in the form of a rim. However, in the rest of the species it is a bilobed structure. Thus, from these observations it can be assumed that the shape of the hilum and aril can be used for identification at the specific level. Lersten (1981) did not examine the spermoderm of any species of <u>Atylosia</u> but examined other members of sub-tribe Cajaninae and observed a papillose pattern in <u>Dunbaria</u> rotundifolia; levigate in <u>Eriosema</u> floribundum and <u>Flemingia</u> floribundum; rugulate in <u>Rhynchosia</u> minima; and rugulate-pitted in <u>Cajanus</u> <u>cajan</u>. Among the ten species of this genus the pattern is multi-reticulate in <u>A.albicans</u>, <u>A.cajanifolia</u>, <u>A.lanceolata</u>, <u>A.scarabaeoides</u> and <u>A. volubilis</u> and rugulate in the remaining five species. Minor differences found in the size, thickness and arrangement of rugae are useful in separating them. Rugae of <u>A.lineata</u> and <u>A.sericea</u> form clumps at several places while those of <u>A.mollis</u> are small and thick. However, the rugae of <u>A.goensis</u> are long **and appear to be arranged in rows while those of** <u>A.platycarpa</u> are long and thin, giving the appearance of a multi-reticulate pattern. Thus testa patterns in this genus appear to be species specific with a low range of variations.

Baker (1879) divided Atylosia into two sub-genera based on the character of petals falling before or after the pod develops. In sub genera-I. Atylia with marcescent petals (remaining till the pod develops) Baker recognizes two categories based on an erect or twiner habit of the plant. He has retained A.lineata and A.sericea under an erect habit and A.mollis under a twiner habit.

In the present study, the sub-genus Atylia is found to have rugulate testa pattern, although in species with an erect habit, rugae form clumps at several places and in species with twiner habit, rugae are small and thick.

The second sub-genus, viz., <u>Cantherospermum</u> (characterised by petals falling off before pod develops) shows multi-reticulate and rugulate testa pat-A-albicans and A-scarabaeoides with sub-digitate leaflets is found to have multi-reticulate testa patterns, thus differing completely from the sub-genus Atylia. But the second category of this sub-genus comprising species <u>A.goensis</u> and <u>A.platycarpa</u> resembles the sub-genus Atylia in having rugulate patterns but differ in having longer rugae in comparison to the rugae present in sub-genus <u>Atylia</u>. Thus, from the observations made in the present study the division of this genus based on petal and leaf characters can be partially supported on the basis of patterns of the spermoderm.

Further, all species of this genus resemble <u>Cajanus cajan</u> and <u>Rhynchosia</u> <u>minima</u> in having ru-gulate pattern. Lersten (1981) remarked that the reticulate pattern is of epidermal origin while rugulate pattern is of cuticular origin.

In the present study no correlation has been found between the seed size and the length of palisade and hour-glass cells. In all the species the shape of the tracheid bar and its relation to the ovular bundle is uniform which supports Lersten's (1982) observations of the remarkable homogeneity of the tracheid bar in the diversified Papilionoideae and of the closely "inter-locked" relationship between the tracheid bar and ovular bundle. Furthermore, no correlation was found between the tracheid bar length and seed size (length & width).

All the species resemble each other in having thick walled and pitted tracheoids except A.albicans, A.goensis and A.scarabaeoides in which scalariform pitting occurs. In all the species, pits are vestured with variations in the degree of vesturing. In A.mollis and A.platycarpa nonvestured, warty and vestured pits were seen in the same tracheid bar. In A.platycarpa warty pits were as common as vestured ones and in A.mollis warts and vestures were seen in the same pit. In A.cajanifolia and A.goensis some pits are slightly warty. Pits although variable in species of <u>Atylosia</u> are being reported here for the first time. This provides additional information for this genus.

The degree of vesturing of the pits is also useful for the identification of some species. In A.lanceolata, A.mollis, A.platycarpa, A.sericea and A.volubilis vestures are short, unbranched and few in number whereas in the remaining species they vary from short to long and unbranched to branched.

The exact function of tracheid bar is not known. Lersten (1982) has speculated that since the pits of tracheoids always lose their pit membrane by maturity, this enhances its efficiency in gas exchanges thus

supporting the conclusion of Hyde (1954) that "hilum is a hygroscopically activated valve in the impermeable epidermis of the testa". Vestures have been hypothesized by Zweypfenning (1978) to support the pit membrane when it is subjected to unequal water pressure in adjacent tracheary elements, simplification of tracheoid pits among papilionoid tribes may reflect an evolutionary shift away from water conduction in the tracheid bar.

In the present study elaborately branched vestured pits with pit membranes have been found in A.cajanifolia and A.lineata. Perhaps, it is by chance that the membrane is visible even in the mature pits and it may be functioning as a vestigial organ.

Based on the characters of the aril, spermoderm, tracheoids and pits, the following tentative key has been prepared.

Key for the identification of species of Atvlosia

- 1. Aril almost entirely covering the hilum (hilum concealed) A.mollis.
- 2. Aril covers the hilum like a rim (hilar groove visible) A.goensis, A.volubilis.
 - A. Seeds hemispherical to orbicular with rugulate surface A.goensis.
 - B. Seeds ovate to reniform with multi-reticulate surface A.volubilis.
- 3. Aril bilobed, partially covering the hilum A.albicans, A.cajanifolia, A.lanceolate, A.lineata, A.platycarpa, A.sericea, A.scarabaeoides.
 - A. Surface multi-reticulate A.albicans, A.cajanifolia, A.lanceolata, A.scarabaeoides.
 - i. Tracheoids scalariform to pitted with long vestured pits A.albicans, A.scarabaeoides. ii. Pits alternate with short to long, branched
 - vestures and pit membranes A.cajanifolia.
 - iii.
 - B. Surface rugulate A.lineata, A.platycarpa, A.sericea.
 - i. Pits with extensive branched vestures. A.lineata.
 - ii. Pits vestured and warty A.platycarpa.
 - iii. Pits with short, unbranched vestures A.sericea.

Acknowledgements

The authors thank Dr. D.G. Faris, Principal Pigeon pea breeder (ICRISAT) for providing identified seeds, to Director N.B.R.I. for providing Scanning Electron Microscope facilities and to V.K. Lal for technical assistance. One of us, M.G., is grateful to the University Grants Commission for awarding a Research Associateship.

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Discussion with Reviewers

W.J. Wolf: How were the arils removed from the seeds shown in Figures 4 and 5? The text indicates that it was also removed in Figure 6 but it appears to have been removed very cleanly or was not present to begin with.

Authors : In the figures 4, 5 and 6 aril was removed with a sharp blade and the seed was examined under light microscope to select good specimens. In figure 6 it was removed entirely thus giving the impression as if it was not present originally.

Reviewer II: Why was the portion of the spermoderm immediately below the hilum used for characterization of the seed coat pattern rather than the midseed portion? Did the spermoderm pattern at midseed and below the hilum differ within the individual species? If so, how did they differ in their patterns? Authors : The portion of the spermoderm immediately below the hilum was used because Lersten (1981) from the study made on 340 species from thirty of the thirty two tribes found that the testa patterns were most distinctive around the hilum for most of the seeds examined, but approximately ten percent of seeds had uniform surface patterns over the entire seed. Lersten and Gunn (1982) found differences in testa patterns at mid seed and near the hilum in species of Medicago, Ononis and Melilotus as they

were papillose near the hilum and non papillose at the mid seed region.

Reviewer II: Based on your experience of examining spermoderm patterns, do you agree with Lersten's (1981) assessment that reticulate patterns are of epidermal origin, whereas rugulate ones are of cuticular origin? Do your present observations substantiate Lersten's (1981) assessment?

Authors: To assess Lersten's view that reticulate patterns are of epidermal origin, whereas rugulate ones are of cuticular origin, seeds of <u>A.goensis</u> (rugulate type) and <u>A.scarabaeoides</u> (multi-reticulate type) were sectioned transversely but we could not find a clear cut demarcation between the sections of these two species. Since no clear distinction could be established it is not possible to comment on Lersten's assessment. We hope to comment on Lersten's views after we have obtained positive results. Figures of both patterns are provided below (Figs.28,29,30).



Fig. 28. A.goensis (rugulate) T.S. Spermoderm. Bar = 10 μm.



Figs. 29,30. A.scarabaeoides (multi-reticulate) T.S. Spermoderm. Bars = $10 \mu m$.