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TAXONOMIC SIGNIFICANCE OF SEED COAT STRUCTURE AND SPERMODERM PATTERNS IN FOUR SPECIES OF *VIGNA* (FABACEAE)

UJALA NYOLA and K.C. SHARMA
Department of Botany, University of Rajasthan, Jaipur – 302 004, India.
Email : Kailashcs@satyam.net.in

Seed coat development, mature structure and spermoderm patterns in four species of *Vigna* (*V. aconitifolia* (Linn.) Marechal, *V. mungo* (Linn.) Walp, *V. radiata* (Linn.) Hepper and *V. unguiculata* (Linn.) Wilczek) were studied. All the four species have bitegmic ovules and seed coat development takes place only from the outer integument. However, expansion of the hour-glass cells is different in *V. aconitifolia* than that in the other three species and the hilar region is specifically shaped in the four species.Spermoderm patterns are also species specific. Identification key to the species is proposed based on these characters. *V. mungo, V. radiata and V. unguiculata* are closely related to each other as compared to *V. aconitifolia* whereas *V. mungo* and *V. radiata* are nearer as compared to *V. unguiculata*.

Keywords : Hilum; Palisade; Seed coat ontogeny; Surface ornamentation.

Introduction

Significance of seed characters such as anatomical, morphological and spermoderm patterns etc., in taxonomic considerations have been emphasized upon time to time[1-6]. Papilionoideae have been studied by several investigators on these lines. Literature reveals conflicting views on Phaseolae based on seed characters[7-11] and development of seed coat of *Phaseolus lunatus*. Sterling[7] mentioned that uniformity of construction of the mature seed coat of member of Leguminosae has long been noted by many botanists. Deviations in details of seed coat structure are also characteristic and have served to permit generic and even specific description. Marechal[8] found that the somatic number in both *Vigna* and *Phaseolus* are same. Behl and Tyagi[11]described the seed coat and fruit development in *Vigna catjang* and *V. cylindrica* and discussed their taxonomical resemblance with *Phaseolus*. Seed coat structure and spermoderm patterns in some legumes have been undertaken and data on *Vigna aconitifolia, V. mungo, V. radiata* and *V. unguiculata* are presented here. The data could be instrumental in assessing bean maturity and provide basic information on developmental processes in an important leguminous seed[7].

Material and Methods

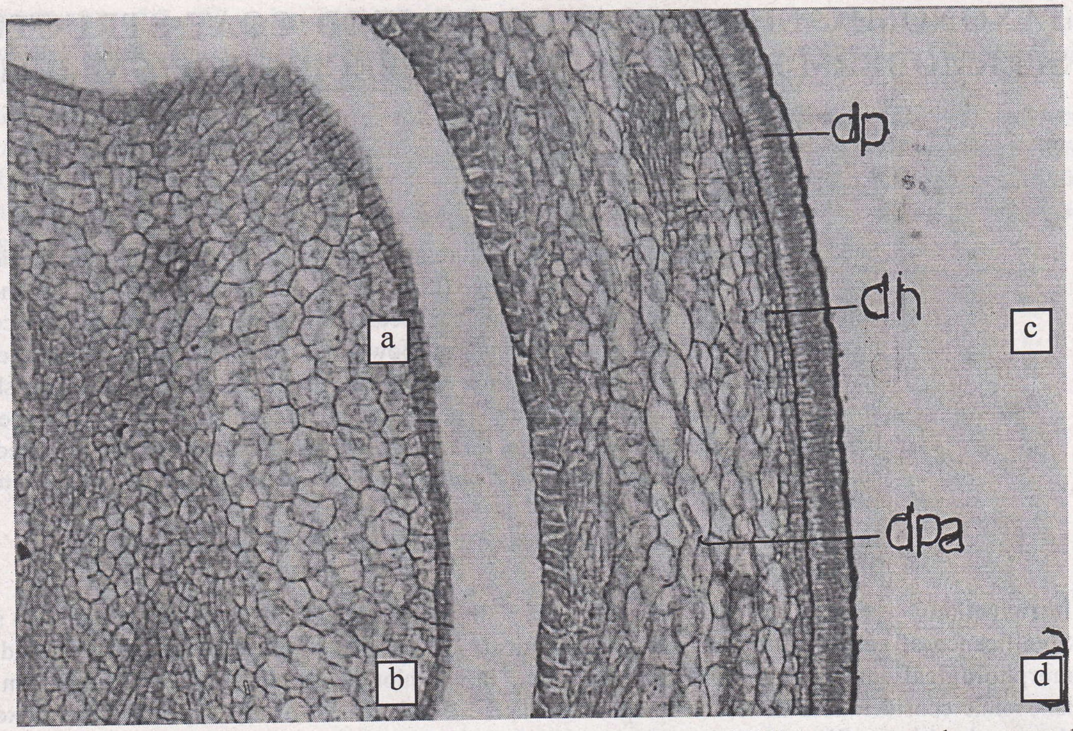
Ovaries and fruits at different developmental stages were collected from the plants of *Vigna aconitifolia* (Linn.) Marechal, *Vigna mungo* (Linn.) Walp, *Vigna radiata* (Linn.) Hepper and *Vigna unguiculata* (Linn.) Wilczek grown in University Botanic Gardens. The materials were fixed in FAA, dehydrated through TBA series, sectioned and stained with safranin, light green and tannic acid-iron chloride combinations. For spermoderm patterns mature dried seeds of these species were affixed on aluminum stub with the help of transparent adhesive, coated with gold and examined at a range of magnifications in a Leo 435 VP Scanning Electron Microscope.

Results and Discussion

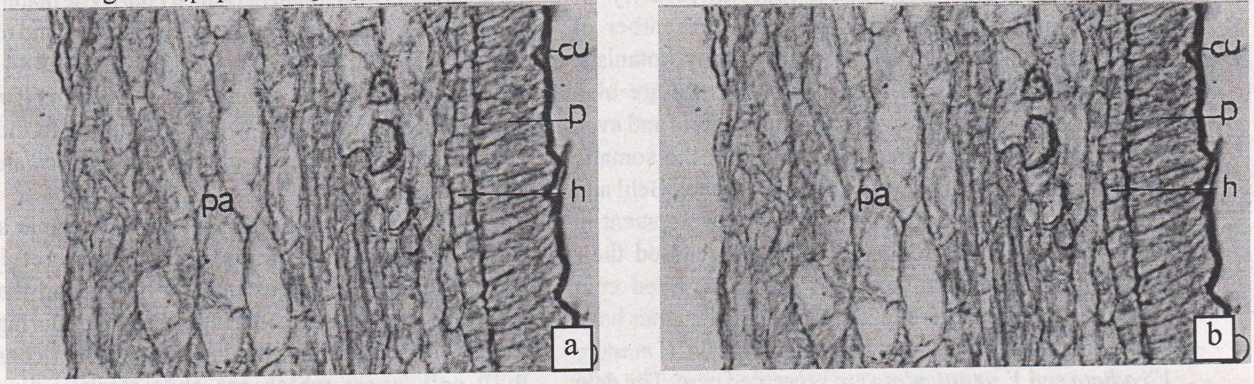
Structure and development of seed coat - The mature seeds are brown, black, green and creamish brown and measure about 4.5×3.0, 5.0×3.5, 4.0×3.0 and 7.0×4.5 mm respectively in *Vigna aconitifolia, V. mungo, V. radiata* and *V. unguiculata.* They are oblong in the former three species and slightly curved-oblong in *V. unguiculata* (Figs. 5a, 5c, 6a, 6c).

Ovules in all the species are bitegmic and the outer integument, shows several distinct layers, transforming themselves into the testa, and the inner integument disappears during ontogeny. During early stages of development, the inner integument consists of 9-10 cell layers which are characteristically lesser cytoplasmic, penta-or hexagonal shaped and have larger vacuoles as compared to the cells in the outer integument (Fig. 1a).

Cells in the outermost layer of the outer integument expand radially with a few cells showing anticlinal divisions during development (Figs. 1b, 2a-b, 3a-c). These cells elongate about three folds and become thick walled. Tannin deposition also takes place and these form the epidermis of the mature seed coat, the outer face



Figs.1. *Vigna aconitifolia*.L.S.of developing seed coat. A. At globularstageX100.b.At mature embryo stage showing different zonesX100.c.Enlarged view of mature seed coatX400.d.L.S.of mature seed through hilumX100. (ar – aerenchyma, cp – counter palisade, cu – cuticle, dh – differentiating hour-glass cell, dii – degenerating inner integument, dp–differentiating palisade, dpa–differentiating parenchyma, h–hour-glass cell, ii–inner integument, oi–outer integument, p–palisade, pa–parenchyma, tb–trachied bar)



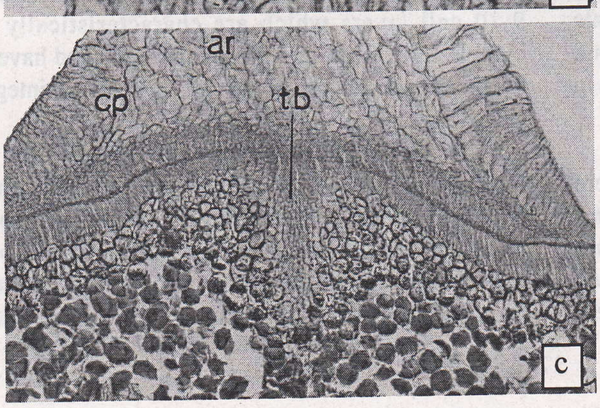
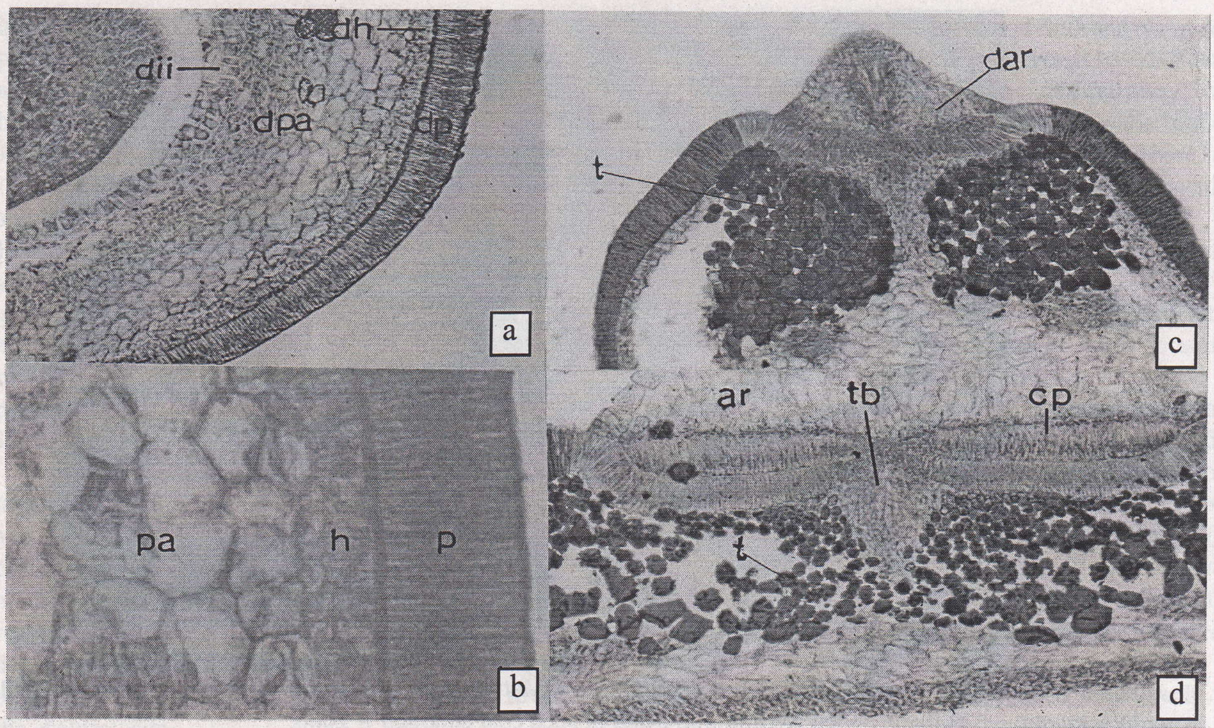
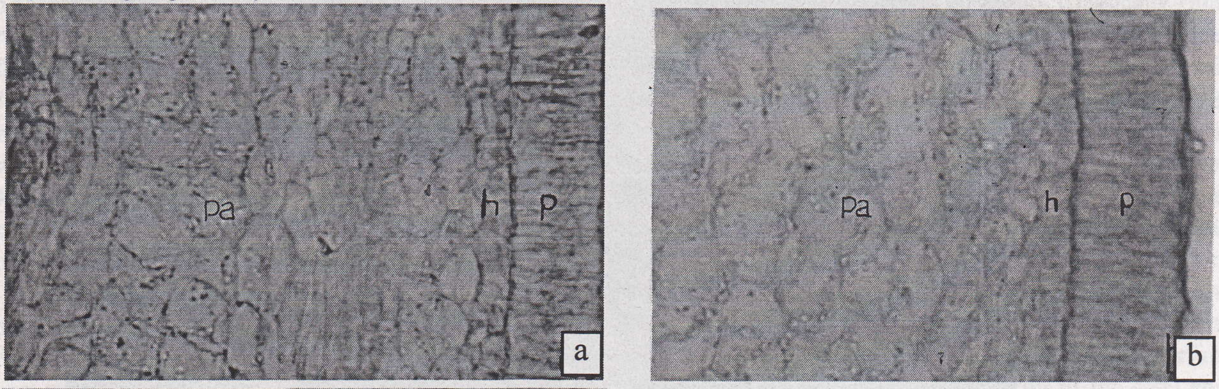


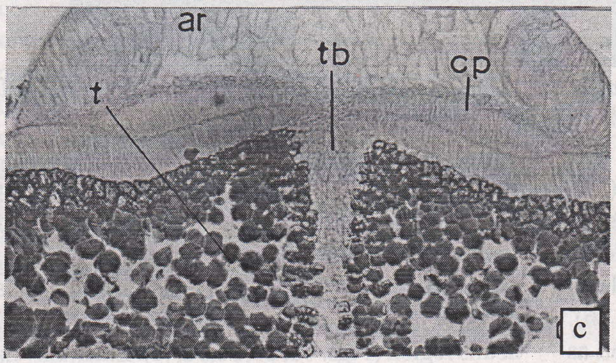
Fig.2. *Vigna mungo*. L.S.of developing seed coat.a. At torpedostageX100.b. At mature stageX400.c. L.S.ofmature seed through hilum X100. (ar–aerenchyma, cp–counterpalisade, cu–cuticle, dh–differentiating hour-glass cell, dp – differentiating palisade, dpa–differentiating parenchyma, h–hour-glass cell, h – hour-glass cell, p – palisade, pa – parenchyma, tb–trachiedbar)



Figs. 3. *Vigna radiata.* L.S. of developing seed coat. a. At torpedo stage X 400. b. At mature stage X 400. c. L.S. of developing seed at torpedo stage through hilum X 100. d. L.S. of mature seed through hilum X 100.

(ar – aerenchyma, dar - differentiating aerenchyma cp – counter palisade, dh – differentiating hour-glass cell, dii – degenerating inner integument, dp – differentiating palisade, dpa – differentiating parenchyma, h – hour-glass cell, p – palisade, pa – parenchyma, t – tannin, tb – trachied bar)





Figs. 4. *V. unguiculata.*L.S. of developing seed coat. a. At torpedo stage X 100. B. At mature stage X 400. c. L.S. of mature seed through hilum X 100.

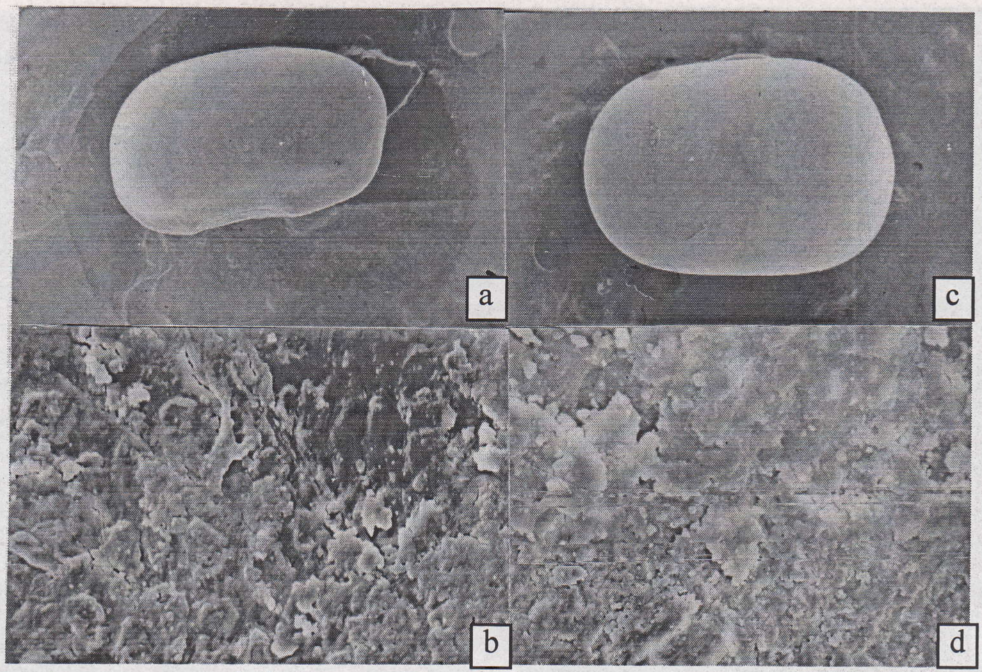


Fig.5. Scanning electron micrographs in *V. aconitifolia*. a. Mature seed X 12. b. A portion of seed coat enlarged X 2000; - in *V. mungo* – c. A mature seed X 12. d. A portion of seed coat enlarged X 2000.

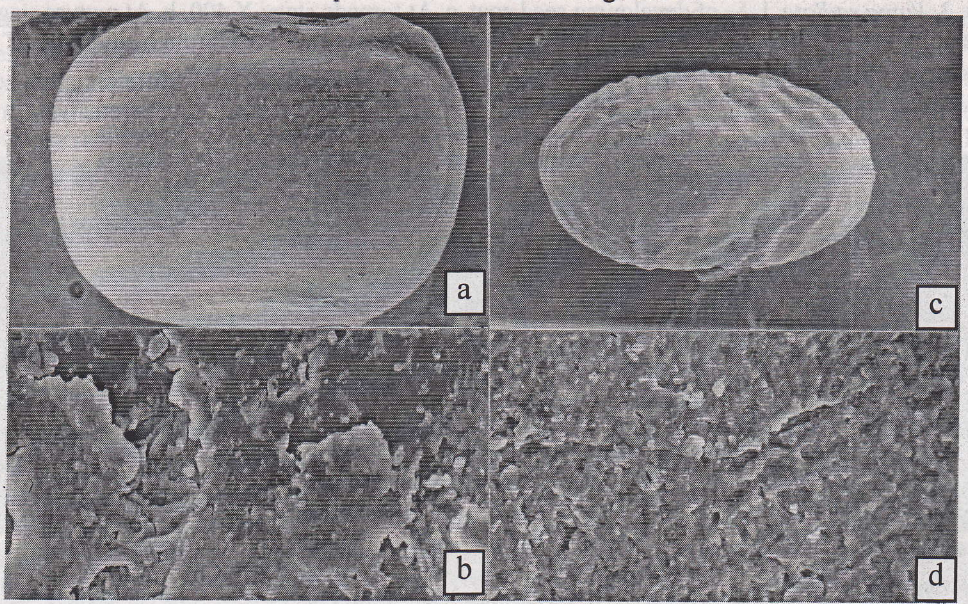


Fig. 6. Scanning electron micrographs in *Vigna radiata*. a. Mature seed X 12. b. A portion of seed coat enlarged X 2000; - in *V. unguiculata* – c. A mature seed X 12. d. A portion of seed coat enlarged X 2000.

of which shows deposition of thick cuticular layer (Fig. 1d). Due to their shape and arrangement, these cells are termed as palisade cells. In *V. unguiculata* the nuclei in all the palisade cells are arranged at almost same level giving a characteristic appearance to this layer (Fig. 4b).

Characteristically shaped is the hilar region as seen in L.S. It is 'w' shaped in *V. aconitifolia,* a low dome in *V. mungo,* an arcuate structure in *V. radiata* and almost flat to low dome in *V. unguiculata.* The hilum on either sides (in L.S.) shows slightly larger sized cells in the palisade layer. The palisade also becomes double layered in this region (Figs. 1d, 2c, 3c,3d,4c). Externally the hilum is covered by a conical strophiolar outgrowth of large parenchymatous cells. Inner to the hilar groove is present a pear shaped trachied bar in *V. aconitifolia.* The trachied bar is inverted pear shaped in *V. radiata* and elongated in *V. mungo* and *V. unguiculata.*

The hypodermal layer of the outer integument shows thickening of cell walls and enlargement of cells. However, they remain smaller than the outer cells and have wider intercellular spaces. These cells constitute the hour-glass cells layer. Specific pattern of wall thickenings in this layer results in cell expansion in the basal region alone in *V. aconitifolia.* This gives them the characteristic pyriform appearance. These cells are lighter stained in *V. unguiculata.*

The remaining subhypodermal cells of the outer integument become enlarged, thicker walled (at maturity) and have larger vacuoles. It forms 10-15 layered parenchymatous zone of the mature seed coat and have black contents deposited particularly in the micropylar region. In *V. mungo* and *V. radiata* these contents start depositing in the micropylar region at very early stages of development and this activity spreads laterally towards the chalazal side.

The hilum is also formed by the outer integument only. It is reniform to ovate. The palisade is double layered in this region. The subhilar tissue is aerenchymatous having stellate cells filled with dark contents.

Spermoderm patterns - The spermoderm pattern is reticulate type in all the four species. In *Vigna aconitifolia* the surface pattern showed distinct cell boundaries and in *V.mungo* the surface pattern showed indistinct cell boundaries. Both species had heavy wax depositions. The walls are unevenly thickened in *V.mungo*(Figs.5b,5d).In *V.unguiculata* the surface is covered with low and irregular projections(Fig. 6d).Where as in *V.radiata* the waxy coatings are thinner than the other species(Fig.6b).

Ovules in all the four species of *Vigna* studied here are bitegmic and the outer integument shows several distinct cell layers and transforms finally into the seed coat (testa) while the inner integument disappears during ontogeny. Corner[12], while studying development of seed coat in leguminous species, found that the inner integument during post-fertilization ultimately obliterates and the seed coat develops from the outer integument only. Dnyansagar[13], however, reported involvement of both the integuments in the formation of seed coat in *Leucaena.* But the present reports, as also previous ones[14-18], are in agreement with Corner[12].

The data support the earlier observations that beans and peas have a highly specialised epidermis of seed-coat[19]. Choudhary and Buth[9] and Buth and Narayan[20] categorised palisades into type I and type II based on wall thickenings. The palisade cells with end of cell away from cuticle becoming bulbous and the radial walls having uniform thickening in all the four species studied here fall under the type II. However, the presence of a linea-lucida, considered to be the characteristic feature of Papilionaceous seeds[2, 21], was not observed in the species investigated here.

Gopinathan and Babu[22] while studying structural diversity and adaptive significance of seeds in some species of *Vigna* suggested that this region is the zone of weakness and permeability. Buth and Narayan[20] stated that the hilar region is a characteristic feature in all the papilionaceous seeds with a very specialised organisation. The species studied at present showed a 'w' shaped (*Vigna aconitifolia*), low dome (*V. mungo*), arcuate structure (*V. radiata*) and almost flat to low dome (*V. unguiculata*) shaped hilum. External to this is present a strophiolar outgrowth. Inner to the hilar groove is present a trachied bar which is an "upright pear" shaped structure in *V. aconitifolia,* an "inverted pear" shaped structure in *V. radiata* and an elongated "spindle-like" structure in *V. mungo* and *V. unguiculata.* These features are important taxonomically.

Behl and Tyagi[11] mentioned that in phaseoleae, there is a greater variation in the vasculature of the seed as compared to other tribes of the family. Classically *Vigna* is differentiated from *Phaseolus* on the basis of the degree to which the beak of the keel is incurved[23]. But the somatic number in both *Vigna and Phaseoslus* is the same[8] *i.e.* $2n = 22$. They described the seed coat and fruit development in *Vigna catjang* and *V. cylindrica* and discussed their taxonomical resemblances with *Phaseolus.* The structure of seed coat and type of spermoderm patterns in the four species of *Vigna* revealed a similar basic plan of development (seed coat developing from outer integument alone and inner integument degenerating during development) and the final pattern of the surface ornamentation (reticulate) at maturity of the seed coat within the genus. Still all the four species showed some distinguishing characters.

The spermoderm ornamentation revealed a reticulate pattern with indistinct boundaries in *Vigna aconitofolia,* distinct cell boundaries in *V. mungo,* irregular cell boundaries in *V. radiata* and a smooth reticulum in *V. unguiculata.* Waxy depositions are also present on the surface. Earlier Sharma[16] in some *Tephrosia* spp. and Vyas[17] in some legumes reported this type of ornamentation with distinct differences depending upon the species as also in the present report. The data suggest that though all the four species studied here showed broadly a similar pattern, still differences based on type of cell boundaries and thickness of waxy depositions etc. may be used for a comparative analysis of all the species.

On the basis of developmental and mature structure and surface patterns of seed coat the four species studied here can be arranged in the following order:

1a Hour-glass cells expand in their basal region; Seeds brown at maturity; Hilum is 'W' shaped; Spermoderm with distinct boundaries and heavy waxy depositions; .... *V. aconitifolia*

1b Hour-glass cells expand uniformly throughout their length.

2a Black contents are deposited during later stage of development; Nuclei of palisade cells acquire almost same position in all the cells; Hilum is flat to low dome; Spermoderm with irregular boundaries and covered with irregular projections; Seeds are creamish at maturity; .... *V. unguiculata*

2b Deposition of black contents in the micropylar region starts at an early stage.

3a Hilum is arcuate; Spermoderm is with thin waxy coating and smooth reticulate type; Seeds are green at maturity. .... *V. radiata*

3b Palisade layer is uniform; Hilum is a low dome; Spermoderm is with heavy waxy disposition; Seeds black at maturity. ....*V. mungo*

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