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SEM studies on seed surface of wild and cultivated species of *Vigna* Savi

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Abstract. Scanning electron microphotographs of seed surface patterns of three wild and five cultivated species of *Vigna* are provided and described. The seed surface patterns are species-specific and showed no interpopulation variation. To promote the use of technical terms in SEM studies of seed surface and to serve as a ready reference a table explaining the 13 terms adopted by us is given. The importance of study of seed surface especially in taxonomy is discussed.

Keywords. Seed surface; *Vigna*; Leguminosae; wild populations; cultivated species; scanning electron microscopy

1. Introduction

Vigna Savi is one of the pulse-yielding genera. Of 100–150 species which the genus comprises, 20 are native to India. *V. angularis*, *V. radiata*, *V. sinensis*, *V. umbellata* and *V. unguiculata* are cultivated species; *V. aconitifolia* is found in both cultivated and wild states. The remaining 14 species occur wild.

Generally the wild relatives of crop plants are adapted to greater variations in climatic and edaphic conditions than are the crop plants. Therefore the wild relatives constitute an invaluable gene pool of natural variations (Boerma 1970; Zohary 1970). However, studies on the wild relatives of pulse crops are meagre; *Vigna* Savi is no exception to this.

In his studies of Papilionoideae Verdcourt (1970) has stated that ". . . the whole matter [of taxonomy of *Vigna*] is very confused and no sound decisions can [we] come to until a great deal of work has been done . . . ." Further, the exhaustive tabulation by Brisson and Peterson (1976, 1977) on scanning electron microscopy (SEM) of seeds shows that of over 700 angiosperms studied only one species is of *Vigna* (Nwanze *et al* 1975). Later studies on seed coats of some four species of *Vigna* (Rajendra *et al* 1979; Jain and Babu 1982), on those of *Phaseolus* spp. (Sharma *et al* 1977, 1982), on those of the tribe Vicieae (Trivedi *et al* 1978; Lersten 1979), and on those of some Mimosoideae and *Bauhinia* spp. (Trivedi *et al* 1979, 1980) reaffirm the value of SEM studies as a reliable tool in elucidating taxonomic and genetic relationships. Recognizing this, we undertook SEM studies on seed surface patterns of three wild and five cultivated species of *Vigna*.

2. Materials and methods

Seeds of 12 populations of *Vigna* species were studied (table 1). From each population, mature, naturally dried seeds collected at random from several individuals were

Table 1. Seed collection data on species of *Vigna* Savi.

Species*	Population code	Place of collection
Wild species		
V. aconitifolia (Jacq.) Marech.	AO I	Rocky habitat near Hirakud Dam, Sambalpur Distt, Orissa
	AO II	Railway embankments, Hirakud, Sambalpur Distt, Orissa
V. trilobata (L.) Verd.	TM I	Borai, Sitanadi Range, MP
	TM II	Bachi Gaon, Sitanadi Range, MP
V. vexillata (L.) A. Rich.	VB I	Ranchi, Bihar
	VM II	Chotta Donger, Bastar Distt, MP
Cultivated species		
V. aconitifolia (Jacq.) Marech. cv Moth PLMO-21	VA I	Univ. Delhi (seeds originally from Indian Agricultural Research Institute, New Delhi)
V. ricciardiana (Tenore) Babu*	RB I	Netrahat, Bihar
V. sinensis (L.) Hassk.	SSM I	Chotta Donger, Bastar Distt, MP
	SSM III	Gura Bera, Bastar Distt, MP
V. umbellata (Thunb.) Ohwi & Ohashi cv NBPGR-1	VUM I	Indian Agricultural Research Institute New Delhi
V. unguiculata (L.) Walp var. V-16	VUN I	

* The circumscriptions of all the species except *V. ricciardiana* are after Verdcourt (1970).

studied. The untreated seeds (figure 1A) were cleared ultrasonically for 10 min and mounted with a silver paste along the circumference of the specimen disc with the hilum laterally anticlockwise and toward the circumference of the disc (figure 1B). The mounted seeds were gold-coated (500 A) in an Edward's Vacuum Evaporation Unit and the free surface adjacent to the hilum (figure 1B) was scanned at a constant angle of 45° at an accelerating potential of 10 kV in a $S_4$10 Model SEM (Cambridge). The microphotographs were taken between 265× and 7400×.

To understand the broad chemical nature of the seed surface, mature dry seeds of only *V. aconitifolia* were treated with (i) 70% ethanolic solution of Sudan III, (ii) cold concentrated H_2SO_4 for 2 min, (iii) cold concentrated H_2SO_4 for 2 min followed by IKI reagent, (iv) Sudan Black B, (v) chlor-zinc iodide, (vi) cold chloroform, and (vii) boiling chloroform.

Most of the earlier SEM studies on seed surface have described the seed surface patterns in non-technical terms or in comparison to animal or vegetable specimens such as octopus, sea anemone, and cauliflower. We have, however, adopted the technical terms used by Murley (1951, see Stearn 1966) for describing the seed surface patterns revealed through optical microscopy (table 2).

3. Observations

3.1 *Wild species*

3.1a *Vigna aconitifolia*: The seed surface is scrobiculate in both the wild populations studied (figure 1C–F). In whole seeds treated with an alcoholic solution of Sudan III

the surface stained red. From water-soaked seeds a discrete covering could easily be removed; it presents a compartmentalized pattern. The surface covering disintegrates in cold concentrated sulphuric acid. All the other chemical tests with whole seeds as well as with the removed covering showed the latter to be largely waxy. Optical sections of both sulphuric acid-treated seeds and seeds from which the surface covering had been mechanically removed showed exposed palisade layer.

3.1b *Vigna trilobata*: In both the populations studied the seed surface is reticulate-foveate to reticulate (figure 1G, H, J). The reticulum is presumably waxy and the wax deposit is rather thick at the junction points. In the interspaces of the reticulum a network of polygonal areas which in turn are made of a finer network are seen in relief (figure 1H, J). This structure in relief is more discrete in the population TM I than in TM II; in all other aspects the seed surface pattern in both the populations is identical.

3.1c *Vigna vexillata*: The seed surface is only apparently reticulate; a closer examination shows a scalariform pattern (figure 1K, L). However, owing to compression of the spaces between the crossbands, the overall pattern can be best considered intermediate to reticulate and scalariform. The surface deposition was more in the population VM II than in VB I; nevertheless, no interpopulation variation could be observed.

Thus in each of the three wild species studied the microsculpture of seed surface is distinct.

3.2 *Cultivated species*

3.2a *Vigna aconitifolia*: In contradistinction to the seed of wild population, that of the cultivated population shows a pusticulate surface (figure 2A). The arrangement of the pustules is only apparently reticulate. Underlying the pusticulate surface and seen in relief is a cord-like and at places a granular matrix. The similar appearances of the surface and the matrix in electron microphotographs (figure 2B–D) permit the assumption that the two are chemically identical. Except for some micro-variation in the outline of the pustules, all seeds examined showed a pusticulate surface (figure 2B–D).

Also, in contrast to the seed surface of the wild population, that of the cultivated population gave only a mild reaction for waxes and fats. Thirdly, upon water-soaking, the seeds of the cultivated population did not yield any detachable covering.

3.2b *Vigna ricciardiana*: All the seeds scanned showed a glebulate-ruminate surface (figure 2E, F).

3.2c *Vigna sinensis*: The seed surface is favulariate and shows some microvariations (figure 2G–J).

3.2d *Vigna umbellata*: The seeds scanned showed a ruminate surface (figure 2K–M), and seldom a falsifoveate to foveolate surface (figure 2N, P).

3.2e *Vigna unguiculata*: All seeds studied showed a glebulate surface (figure 2Q, R).

In all the species studied the seed surface pattern is species-specific. No intrapopulation variation (except in *V. umbellata*), nor any interpopulation variation could be discovered; however, the wild and the cultivated populations of *V. aconitifolia* showed differences in their seed coat surface patterns.

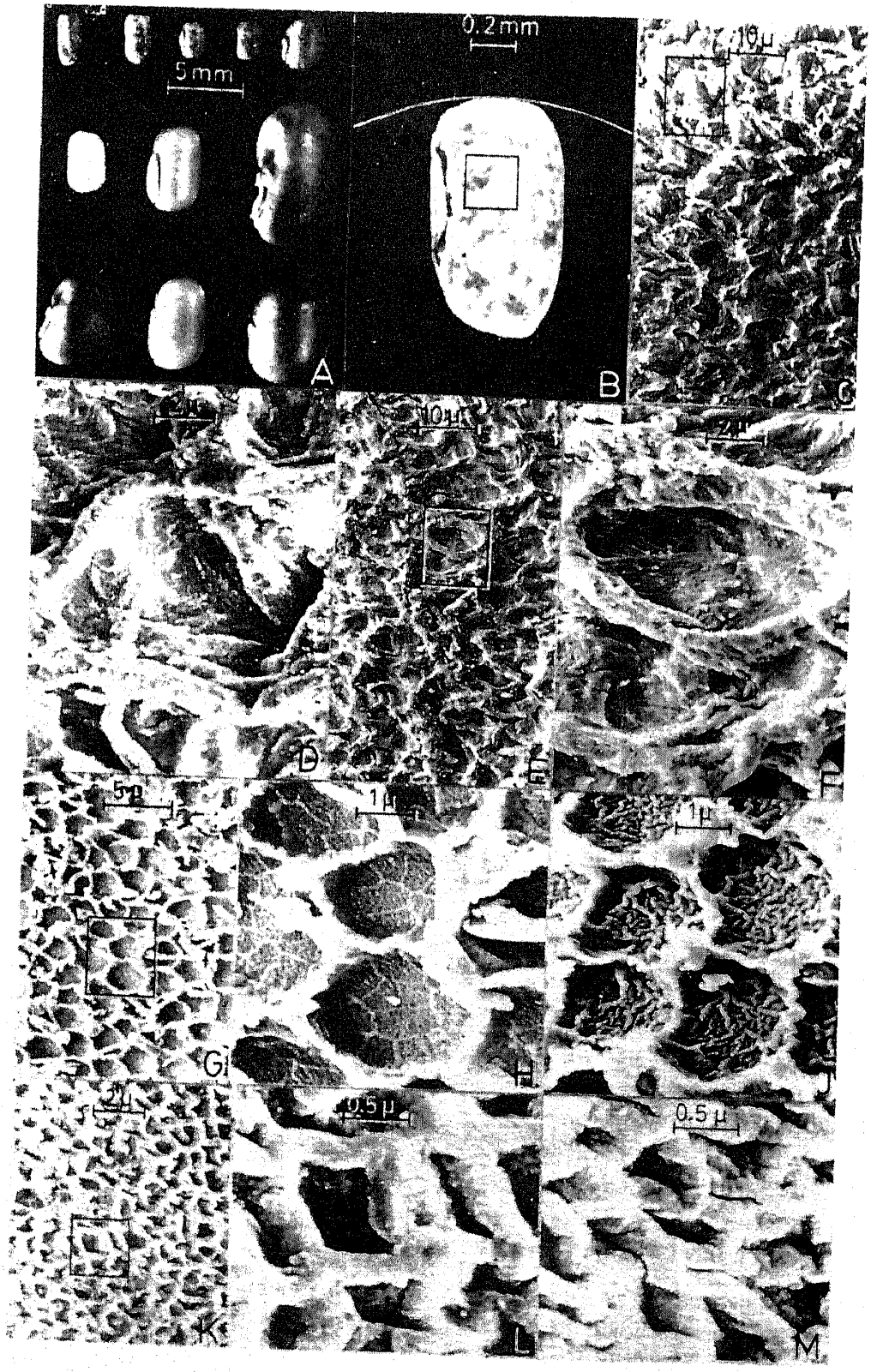
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Table 2. Technical terms* adopted to describe seed surface patterns.

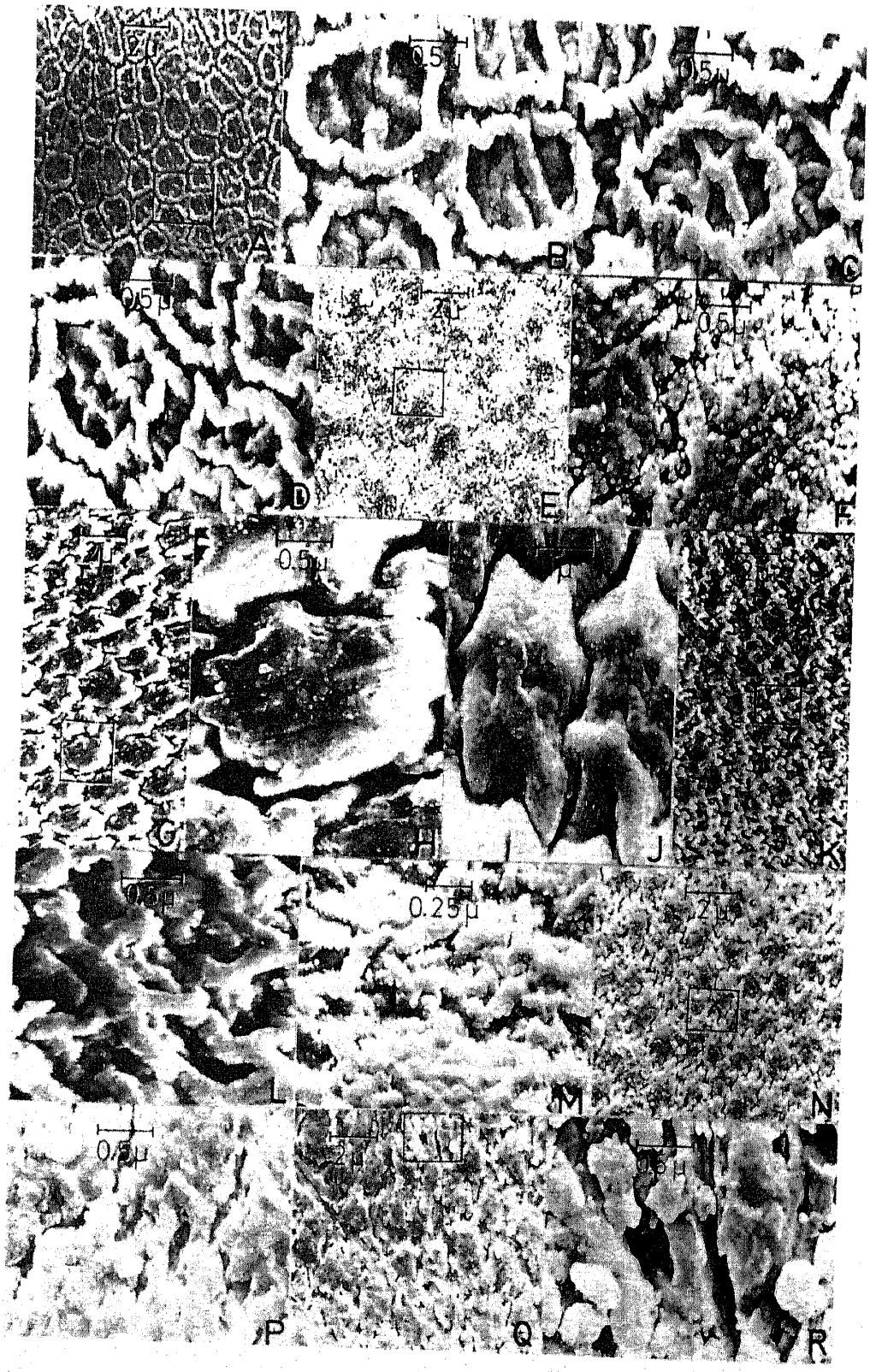
Term	Explanation
Colliculate	Having rounded broad elevations closely spaced covering the seed coat
Falsifoveate	Having pits that do not have uniform depth, little depressions made laterally
Favulariate	Finely ribbed, the ribs separated by zig-zag furrows
Foveate	Pitted or having depressions
Foveolate	Marked with little pits
Glebulate	Having small clumps of randomly placed granules
Pusticulate	Having small broad elevations not so high or abundant as on colliculate surface and not having as abrupt elevations as a minutely tuberculate surface
Reticulate	Having a raised net work of narrow and sharply angled lines frequently presenting a geometric appearance, each area outlined by reticulum being an interspace
Reticulate-foveate	A type in between reticulate and foveate types
Ruminate	Penetrated by irregular channels giving an eroded appearance and running in different directions
Scalariform	Having small fairly regular cross bands suggestive of the rungs of a ladder
Scrobiculate	Having elongated shallow depressions or pits
Tuberculate	Having small smooth rounded projections or knobs

* From Stearn (1966).

4. Discussion

None of the seed surface patterns observed by us resembles that of any other four species or of the two interspecific hybrids of *Vigna* illustrated so far (Rajendra *et al* 1979; Jain and Babu 1982). The seed surface pattern of *V. ricciardiana* needs a special mention. According to Verdcourt (1970) *Phaseolus ricciardianus* Tenore is a synonym of *V. umbellata* (Thunb.) Ohwi and Ohashi. However, the unpublished observations of

Figures 1A–M. **A.** One representative seed each of 11 of the 12 populations of *Vigna* species studied. Left to right, **top row**: *V. aconitifolia* wild populations AO I and AO II, *V. trilobata* populations TM I and TM II, and *V. vexillata* population VM II; **middle row**: *V. aconitifolia* cultivated population VA I, *V. ricciardiana* population RB I, and *V. sinensis* population SSM I; **bottom row**: *V. sinensis* population SSM III, *V. umbellata* population VUM I, and *V. unguiculata* population VUN I. **B.** Seed showing its orientation on specimen stub and the region of its surface that was scanned. White line in continuity with seed margin represents an arc of the specimen stub and the enblocked area the seed surface scanned. **C–F.** SEM graphs of surface of untreated seeds of wild populations AO I (**C, D**) & AO II (**E, F**) of *V. aconitifolia*. The seed surface is scrobiculate showing shallow depressions. **D, F** are magnified views of regions shown enblocked in **C** and **E** respectively. Contrast with **2A–D. G–J.** *V. trilobata* populations TM I (**G, H**) and TM II (**J**). Seed surface shows a reticulate-foveate to reticulate pattern. Reticulation is distinct at places; at other regions (arrows in **G**) it shows little pits (foveolate). **H.** Magnified view of region enblocked in **G. K–M.** *V. vexillata*; the underlying scalariform pattern is distinct in **L** which is the magnified view of region enblocked in **K. K, L** are of population VM II from MP and **M** is of population VB I from Bihar.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Dr C R Babu (University of Delhi) show that the pod characters of *P. ricciardianus* are so distinct that it deserves to be treated as a species of *Vigna* (following Verdcourt's circumscription of the two genera *Vigna* and *Phaseolus*), namely *V. ricciardiana* (Tenore) Babu (*cf.* footnote in table 1). Interestingly our SEM studies have shown that the glebulate-ruminate seed surface of *V. ricciardiana* bears some resemblance to the ruminate seed surface of *V. umbellata*. However, *V. umbellata* itself has exhibited intrapopulation variation. Therefore, the taxonomic identity of *P. ricciardianus vis-a-vis V. umbellata* and *V. ricciardiana* is still an open area for research. Likewise, the intrapopulation variation in *V. umbellata* also calls for further delimitation of the taxon into infraspecific categories.

Lersten (1979) observed the seed surface patterns in Vicieae to be distinctive from those of nearly 200 other genera of Papilionoideae. Like the work on eight species of Viciae (Trivedi *et al* 1978) our work has also shown the seed surface to be species-specific. The work of Nwanze *et al* (1975) has demonstrated that the seed surface patterns help distinguish varieties as well. SEM studies on *Vigna sinensis* and *V. sesquipedalis* and their hybrids have shown the seed surface patterns to be genetically controlled (Rajendra *et al* 1979). All these investigations including ours clearly prove that seed surface pattern is a conservative trait and can therefore be of great value in taxonomic delimitations and in hybridization as a genetic marker.

In our study the presence of a mechanically detachable thick covering on seeds of the wild populations of *V. aconitifolia* in contradistinction to those of a cultivated population of the same species is of interest. In *Glycine* of Phaseoleae (to which *Vigna* also belongs) remnants of the inner pod wall are known to be adherent to the seed. Ontogenetic studies are needed to establish whether or not the thick surface covering on seeds of the wild *V. aconitifolia* is *pro parte* the pericarp. Chemical tests have proved the waxy nature of this covering. The absence of such a waxy covering in the cultivated population of *V. aconitifolia* may indicate an adaptation to domestication in that it increases the permeability of seed coat to water enabling uniform germination under human care.

In *V. unguiculata* (cow pea) bruchid larvae penetrate the smooth seed coat varieties in greater numbers than they do the rough seed coat varieties (Nwanze and Horder 1976); in bruchid-resistant varieties of *Cicer arietinum* (chick pea) the seeds have a

Figures 2A–R. All are SEM graphs of surface of untreated seeds of *Vigna* species. **A–D** are of cultivated population of *V. aconitifolia*. Surface is pusticulate; closer examination falsifies the reticulate pattern. Contrast with surface of seeds of wild populations of *V. aconitifolia* (figure **1C–F**). **B.** Magnified view of region enblocked in **A. B** and **C** show granular region of matrix in relief. **B, C, D** are from different individuals. **E, F.** *V. ricciardiana*; the seed surface is glebulate-ruminate. **F.** Magnified view of region enblocked in **E**; compare with the patterns in **P** and **R. G–J.** *V. sinensis*; **G** shows general view of the favulariate pattern of seed surface, and **H** a magnified view of region enblocked in **G**. Both are from population SSM I from MP. **J** is magnified view of surface of seed from population SSM III, also from MP. Except for a microvariation in the contour of ribs and furrows the overall pattern is identical with that in **H. K–P.** *V. umbellata*; **K–M** show ruminate pattern. **L** is magnified view of region enblocked in **K, M** is of another seed. Pattern in **L** superficially resembles that in **1 L, M** (*V. vexillata*). **N, P.** Falsifoveolate-foveolate pattern. **P** is magnified view of region enblocked in **N. Q, R.** *V. unguiculata*; general view and enlarged view of seed surface respectively. Note the glebulate pattern.

rough surface which acts as a deterrent to oviposition by bruchids (Schalk *et al* 1973). These observations further point to the applied value of SEM studies on seed surface.

Indeed, Heywood's (1971) remark that SEM studies of seed coat will become a routine in studies of seed biology aptly concludes this discussion.

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