

Study on seed coat of some *Vigna* species following scanning electron microscopy (SEM)

D. Nath and T. Dasgupta

Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta

Abstract- Variation in spermoderm features along with macromorphological characters of eighteen wild and cultivated genotypes of *Vigna* were studied by scanning electron microscopy (SEM). On the basis of wax deposition, seed surface type is grouped in four groups. It is observed that though there were differences in seed coat colour and seed size in the wild progenitors of *Vigna* but the pattern of wax deposition on the seed coat is similar type.

Index Terms- Mungbean, Scanning electron microscopy (SEM), Seedcoat, Seedsurface, Spermoderm, *Vigna*, Wax deposition

Eighteen different genotypes of *Vigna* (Table-I) varying in seed size, colour and texture were used as experimental materials for SEM study. The genotypes were collected from different places of India. The seeds were treated with glycerol : acetone (2:1) in osmium tetroxide. The treated seeds were dehydrated in a series of ethanol and coated with gold. Seed surface observation were taken through Scanning Electron Microscopy (Make: Carl Zeiss, SBF-SEM SIGMA 3View) under different magnification at 20KV. Seed size, shape, colour, dry seed weight and seedcoat types were observed for the genus *Vigna* subgenus *Ceratotropis* separately for each genotypes.

I. INTRODUCTION

The subgenus *Ceratotropis* of the genus *Vigna* comprises of - five important Asiatic pulses; mungbean (*V. radiata* (L) Wilczek), blackgram (*V. mungo* (L) Hepper), mothbean (*V. aconitifolia* (Jacq) Marechal), ricebean (*V. umbellata* (Thunb) Ohwi and Ohashi) and adzukibean (*V. angularis*). Among these mungbean and blackgram are major beans from ancient time in Asia.

Vigna is native to India-Burma region of Asia and has been grown in India since ancient times. Principally it is protein rich crop and an important source of high calories next to cereal crops. At present mungbean is cultivated worldwide because of its easy digestibility than blackgram.

External seed morphology is one of the important taxonomic characters. Though illustrations on seed surface of the genus *Vigna* is represented before but little such work has been done through scanning electron microscopic study (SEM) of seed surface. Gonzales-Andres and Ortiz [1], Lopez *et al.* [2] worked on seedcoat surface of Leguminosae using SEM. Gunn [3] has summarized the major seed characters of Leguminosae. In *Vigna* seed coat is generally smooth and patterned type. Hilum plays an important role in seed morphology. Pithy pods are formed which completely obscures the central position of hilum [4] as dense mass of white tissue at hilum remains behind. SEM of spermoderm was found to be useful in establishing taxonomic and phylogenetic relationship in the Indian species of *Vigna*. In the present investigation an attempt has been made to characterize seed coat on different species of *Vigna* based on scanning electron microscopy (SEM).

II. MATERIALS AND METHODS

Spermoderm features, hilum structure and cellular structure of seed coat of wild and cultivated species of the genus *Vigna* were studied using scanning electron microscopy (SEM).

III. RESULTS AND DISCUSSION

The macromorphological variability of seeds including size, shape, colour and spermoderm features of different species of *Vigna* were studied and summarized in Table –II. Remarkable variation was found among these species. SEM study of seed coat was found to be very useful in establishing phylogenetic relationship among the species of *Vigna*.

Seed colour: The seeds of *Vigna radiata* var *sublobata* (2/2) were blackish green and *Vigna radiata* var *sublobata* (18A) were green in colour. The two other wild species of *Vigna* namely *Vigna radiata* var *setulosa* (2) and *Vigna radiata* var *setulosa* (4) showed black seedcoat colour. The colour of seedcoat of wild cowpea was black whereas that of cultivated species is yellow with smooth and shiny surface. The seed surface texture of accessions of *V. aconitifolia* were rough with green coloured seed coat. Seeds of *V. minima* blackish green in colour with shiny texture whereas in seeds of *V. umbellata* were yellow, bold, smooth and shiny.

Seed size: The seed size ranges from 1mm to 3.5mm. The seed size of *Vigna radiata* var *setulosa* were small in size as compared to *Vigna radiata* var *sublobata*. The seed size of *V. aconitifolia* were very small around 0.3cm and non-hairy in nature. The hilum is large and exposed. The seed size of *Vigna mungo* var *silvestris*-10 (wild) is small with hardy seedcoat and prominent hilum compared to *Vigna mungo* (T9) which is cultivated type with large, shiny and varigated seedcoat. The two other genotypes namely *V. minima* and *V. umbellata* depicted similarity on seed coat sculpturing pattern i.e. maculo-reticulate type sculpturing with minutely longitudinal reticulate-striae with varied shapes and sizes of the meshes. Seeds of *V. minima* were minute in size. The seeds of *V. trilobata* were blackish green in colour with hard seed coat and prominent hilum

Seed shape: The cultivated species of *Vigna radiata* exhibited oval shaped seed along with their progenitor *Vigna radiata* var *sublobata*. The seeds of two accessions of *Vigna*

radiata var *setulosa*, *V. aconitifolia* and *Vigna hainiana* were found square and hardy in nature. Both wild and cultivated species of *Vigna unguiculata* showed reniform shaped seed which is unique among the studied materials.

Seed coat ornamentation: Through SEM study distinctive sculpturing pattern of seed coat due to various types of wax deposition over the seedcoat in different genotypes is observed. High diversity is found in sculpturing pattern on seedcoat among different species. The wild cultivars have thick deposition compared to cultivated ones. Based on the ornamentation pattern and structure of epidermal cells of the seedcoat, particularly the arrangement of the cells and the extent of elevation of the anticlinal cell walls, four morphological types could be distinguished, viz. reticulate, punctulate, rugose and captor-ridged. The captor-ridged type is unique, occurring in only one species. Punctulate type is also uncommon occurring only in two members. The other two types were common. Reticulate type could be further divided into subdivisions according to the arrangement of the epidermal cells of the seedcoat. Spermoderm features of these genotypes were shown in Table- II.

Vigna sublobata is a polymorphic taxon [5]. It had two distinct morphological groups *Vigna radiata* var *sublobata* and *Vigna radiata* var *setulosa*. These were wild progenitors of cultivated mungbean. During the study of sculpturing pattern of the seedcoat of the two accessions of these genotypes namely *Vigna radiata* var *sublobata* and *Vigna radiata* var *setulosa* it is found that despite of differences in seed coat colour and seed size, pattern of wax deposition on the seed coat is similar type i.e. maculo reticulate. Though the cultivated species of mungbean (*V. radiata*) also exhibited same type of seedcoat pattern but their wax deposition is much less or thin than that of the wild genotypes. The ornamentation pattern of seed coat of different genotypes of cultivated species of *Vigna radiata* is typically maculo reticulate type (Table-II).

Similarly the sculpturing pattern on seed coat surface of both wild and cultivated species of cowpea (*V. unguiculata*) is maculo-reticulate type.

The genotypes namely *V. minima*, *V. trichuriensis* and *V. umbellata* depicted similarity on seed coat sculpturing pattern. All of these have maculo-reticulate type sculpturing with minute longitudinal reticulate-striae with varied shapes and sizes of the meshes.

Among the two genotypes of *V. mungo* studied *Vigna mungo* var *silvestris*-10 is wild type and *Vigna mungo* (T9) is cultivated type. The interesting point is that though there were differences in the macromorphological characters of seeds, while comparing the pattern of wax deposition on seed coat no

difference is observed. The primary ornamentation of seed coat is punctulate type with differences in quantity of wax deposition.

Study of sculpturing pattern of two accessions of *V. aconitifolia* viz. *V. aconitifolia* -2 and *V. aconitifolia* -3 is found unique. Primary ornamentation is captor-ridged, with incomplete or complete ridges.

The other two genotypes namely *V. trilobata*, and *V. hainiana*-14 though showed similar sculpturing pattern but in *V. hainiana*-14 honeycomb-like supercellular pits is observed.

Macromorphology of seed, and cellular structure of seed coat are found characteristic to each species and appeared to be species-specific. Considerable morphological similarities were observed between cultivated species of *V. radiata* with its related wild species *V. radiata* var. *sublobata* and *Vigna radiata* var *setulosa* and *V. mungo* with that of *V. mungo* var. *silvestris*. Similar findings was also observed by Chandel *et. al.* [6]. So SEM study of seed coat structure could be a useful in establishing taxonomic and phylogenetic relationship among the Indian species of *Vigna*.

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AUTHORS

First Author – Dr. Disharee Nath, Ph.D., Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta, ndisharee@gmail.com

Second Author – Prof. T. Dasgupta, Ph.D., Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta, tapashdg@rediffmail.com

Table-I : List of Materials

Name	Status
<i>V. radiata</i> var <i>radiata</i> (B1)	Cultivated
<i>V. radiata</i> var <i>radiata</i> (4441D1)	Cultivated
<i>V. radiata</i> var <i>sublobata</i> -2/2	Wild
<i>V. radiata</i> var <i>sublobata</i> -18A	Wild
<i>V. radiata</i> var <i>setulosa</i> -2	Wild
<i>V. radiata</i> var <i>setulosa</i> -4	Wild
<i>V. mungo</i> (T9)	Cultivated
<i>V. mungo</i> var <i>silvestris</i> -10	Cultivated
<i>V. unguiculata</i>	Cultivated
<i>V. unguiculata</i>	Wild
<i>V. umbellata</i>	Cultivated
<i>V. trichuriensis</i>	Wild
<i>V. trilobata</i>	Wild
<i>V. hainiana</i> -14	Wild
<i>Allo sp</i> 6/3	Allopolyploid
<i>V. aconitifolia</i> -2	Cultivated
<i>V. aconitifolia</i> -3	Wild
<i>V. minima</i>	Wild

Table-II : Macro-morphological and spermoderm features of different genotypes of mungbean

Plant material	Seed shape	Seed colour	Mean dimension of seeds		100 seed-weight (gm)	Seed surface type
			Length (± 0.02) (cm)	Breadth (± 0.01) (cm)		
<i>Vigna radiata</i> var <i>sublobata</i> -2/2	Oval	Blackish-green	0.3	0.2	3.73	Maculo reticulate
<i>Vigna radiata</i> var <i>sublobata</i> -18A	Roundish square	Green	0.4	0.6	3.08	Maculo reticulate
<i>Vigna radiata</i> var <i>setulosa</i> -2	Square	Black	0.6	0.3	1.92	Maculo reticulate
<i>Vigna radiata</i> var <i>setulosa</i> -4	Square	Black	0.6	0.3	2.12	Maculo reticulate
<i>Vigna radiata</i> var <i>radiata</i> (4441D1)	Oval	Green	0.5	0.3	2.38	Maculo reticulate
<i>Vigna radiata</i> var <i>radiata</i> (B1)	Oval	Yellow	0.5	0.4	3.35	Maculo reticulate
<i>Vigna unguiculata</i> (wild)	Remiform	Black	0.4	0.3	13.9	Maculo reticulate
<i>Vigna unguiculata</i>	Remiform	Cream	0.7	0.5	14.2	Maculo reticulate
<i>Vigna hainiana</i> -14	Square	Brown	0.3	0.2	3.66	Rugose
<i>Vigna umbellata</i>	Cylindrical	Cream	0.9	0.6	6.94	Maculo reticulate
<i>Vigna trichuriensis</i>	Cylindrical	Black	0.2	0.3	3.53	Maculo reticulate
<i>Vigna trilobata</i>	Cylindrical- round	Blackish green	0.3	0.3	1.85	Rugose
<i>Vigna mungo</i> (T9)	Cylindrical -round	Black	0.4	1.2	2.72	Puncticulatae
<i>Vigna mungo</i> var <i>silvestris</i> -10	Square	Black	0.3	0.5	2.87	Puncticulatae
<i>Allo sp.</i> - 6/3	Cylindrical	Black	0.4	0.3	2.23	Maculo reticulate
<i>Vigna aconitifolia</i> -3	Roundish square	Green	0.3	0.2	1.24	Captor ridged
<i>Vigna aconitifolia</i> -2	Square	Green	0.3	0.2	1.12	Captor ridged
<i>Vigna minima</i>	Cylindrical	Blackish Green	0.2	0.1	1.36	Maculo reticulate

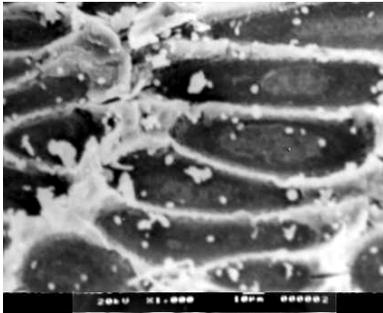


Fig:I. V. radiata var radiata (B1)

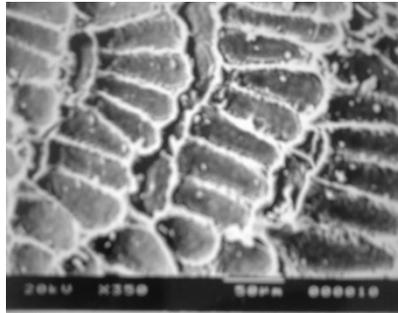


Fig:II. V. radiata var radiata (4441D1)

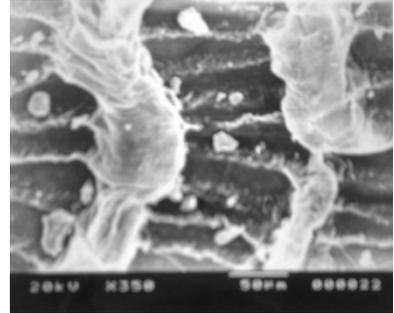


Fig:III. V. radiata var sublobata-2/2

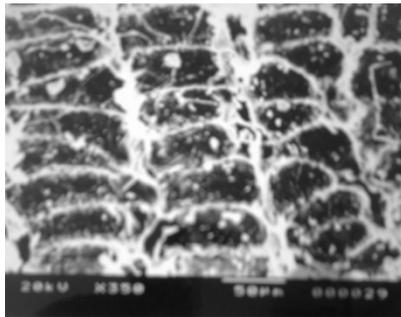


Fig:IV. V. radiata var sublobata-18A

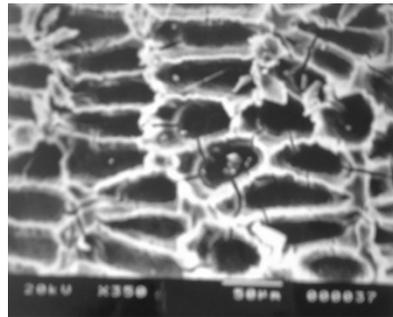


Fig:V. V. radiata var setulosa-2

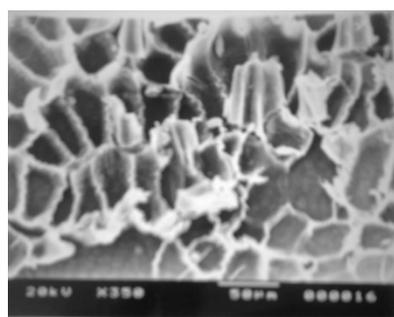


Fig:VI. V. radiata var setulosa-4

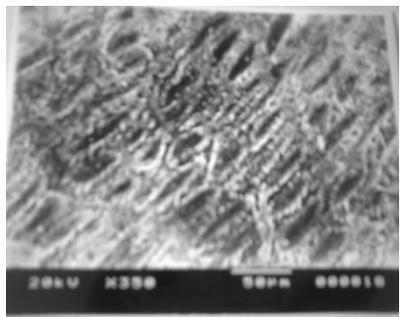


Fig:VII. V. mungo (T9)

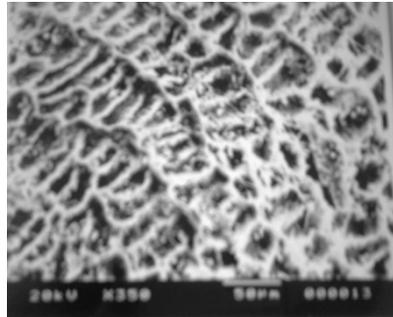


Fig:VIII. V. mungo var silvestris-10

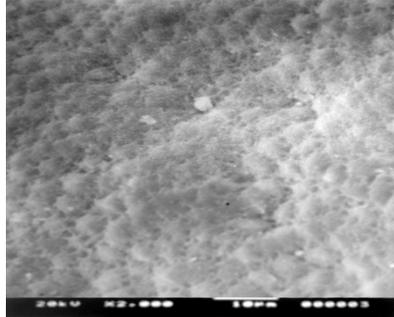


Fig:IX. V. unguiculata (Cultivated)

Fig I-IX. Scanning electron micrographs of seed coat patterns of the of the *Vigna* sp.

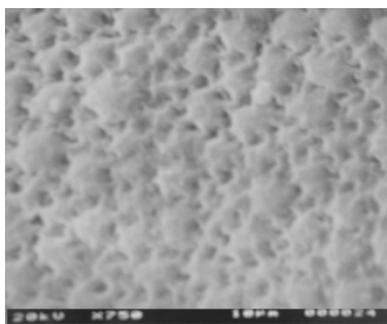


Fig:X. V. unguiculata (Wild)

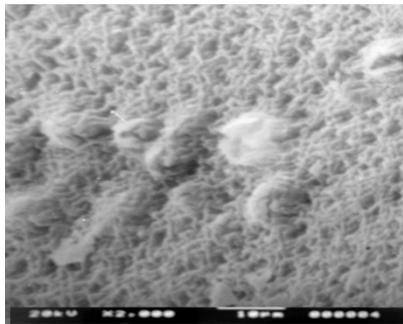


Fig:XI. V. umbellata

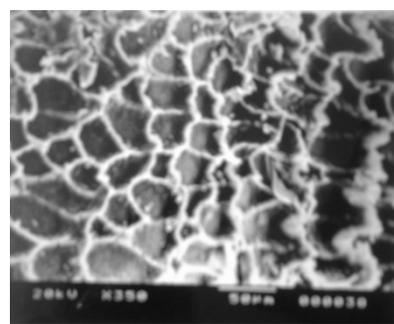


Fig:XII. V. trichuriensis

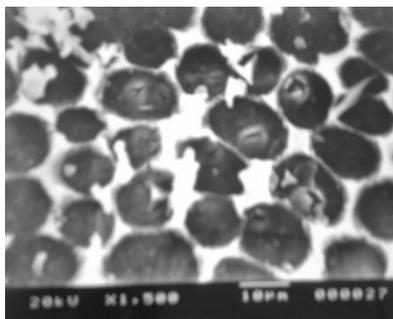


Fig:XIII. V. trilobata

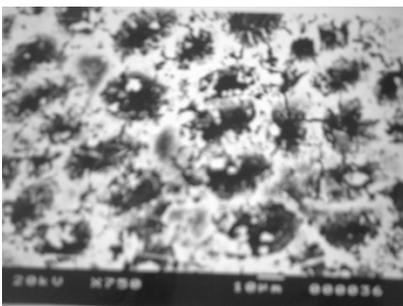


Fig:XIV. V. hainiana-14

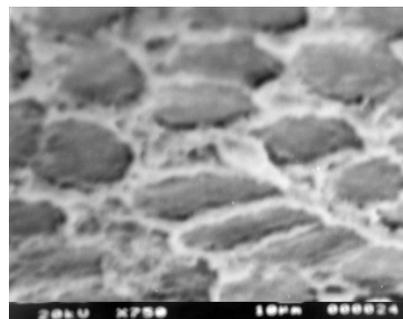


Fig:XV. Allo sp 6/3

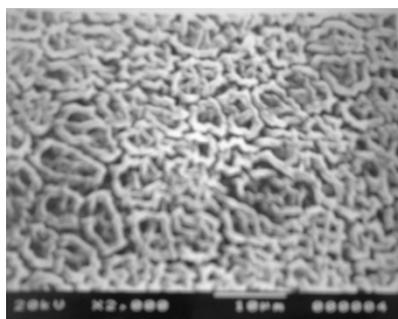


Fig:XVI. V. aconitifolia-2

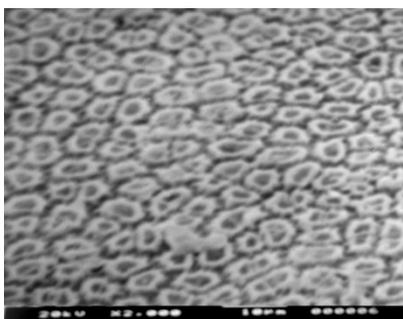


Fig:XVII. V. aconitifolia-3

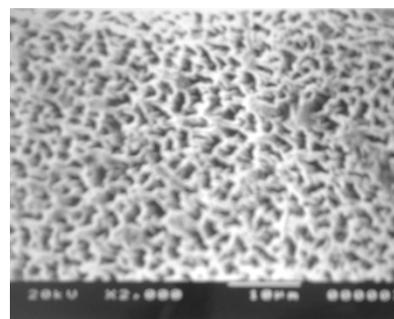


Fig:XVIII. V. minima

Fig X-XVIII. Scanning electron micrographs of seed coat patterns of the *Vigna* sp.