

Scanning Electron Microscope Studies of Spermoderm Patterns of the Three Species of *Vigna* and their Cultivars

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ABSTRACT

Spermoderm patterns studies using SEM of three species of *Vigna* i.e., *Vigna radiata*, *Vigna aconitifolia*, *Vigna mungo* and their 24 cultivars were studied. Each species had 8 cultivars. The general pattern of all the species showed many similarities and there are many similarities are seen between some cultivars but the variability among cultivars are considerable and may be of taxonomic and evolutionary significance.

INTRODUCTION

The importance of spermoderm patterns of seeds by using SEM technique has been widely used as taxonomic features for identification of plants and differentiation between different taxa. The fabaceae or leguminosae, commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. Seed morphology and structure in the Leguminosae has been the base of numerous studies by Corner and Gunn^[1]. The importance of ultrastructural pattern analysis of the seed coat observed under the SEM has been well recognised as a reliable approach for assessing phenetic relationship and identification of species or taxa^[2]. A substantial amount of this variability is adaptive and of genetic origin^[3]. Recent SEM studies in seed coats reveal substantial diversity in topography^[4]. SEM studies have already provided a considerable amount of taxonomically useful information in many genera and families^[5].

MATERIALS AND METHODS

Seeds of the 3 species of *Vigna* i.e., *Vigna radiata* linn, *Vigna mungo* linn, *Vigna aconitifolia* linn and their 24 cultivars were collected from NBPGR, New delhi. The mature dried seeds were affixed on aluminium stab with transparent adhesive, coated with gold particles and then examined at a range of magnification in a Zeiss EVO 18 Scanning Electron Microscope at UOR, Jaipur.

Eight Cultivars of *Vigna radiata*

GM-3

The seed is small, dark green, oblong shaped with maximum length and breadth of 3.987 and 2.852mm respectively (**Figure 1.1a**). Cell wall is irregular. Surface of seed shows reticulate pattern (**Figure 1.1b**). Anticlinal walls are raised and at some places form low rugae-like structures. The surface have wax flakes depositions. The hilum contains heavy globular waxy depositions of various sizes and rugose structures are clearly seen. The hilum is centrally placed. It is oval with defined and raised boarder and with broad posterior and narrow anterior end (**Figure 1.1c**). There is a very narrow longitudinal slit which joins the micropyle with the anterior end of the seed (**Figure 1.1e**).

Kcg-89

The seed is small having length and breadth of 3.941 and 2.955 mm respectively (**Figure 1.2a**). Its colour varies from brown to dark green. The surface contains furrow like longitudinal cells with raised thicker anticlinal walls and depressed periclinal walls forming a network almost in a striated form structure with light waxy depositions (**Figure 1.2b**). The hilum is sub apical and contains globular shaped depositions (**Figures 1.2a and 1.2d**). The surface pattern is of striated reticulate type (**Figure 1.2b**). The

anticlinal walls remain intact while periclinal walls are degenerated at some places forming perforations. Long slit is seen between anterior end of the seed and micropyle. The hilum boundary (rim) is clearly defined (**Figure 1.2c**).

ML-131

The seed is small, light green in colour. Its length and breadth are 4.457 and 3.037 mm respectively (**Figure 1.3a**). The periclinal walls are depressed and anticlinal walls are raised and form a reticulate pattern (**Figure 1.3b**). The surface exhibits at some places that the anticlinal walls are condensed and form cell mounds with light waxy depositions. The hilum is sub-apical placed having undulated structure with network of thick anticlinal walls (**Figure 1.3c**). Periclinal walls degenerate to form perforations. The longitudinal slit is seen in between micropyle and anterior end of the seed (**Figure 1.3e**). The hilum rim is well defined (**Figure 1.3d**).

K-851

The seed is small, dark green in colour with length and breadth of 3.941 and 2.955 mm respectively (**Figure 1.4a**). The surface shows structure like that seen in ML131. The surface contains heavy waxy deposition (**Figure 1.4b**). The hilum is oval and sub- apical with broad posterior end and narrower anterior end (**Figure 1.4c**) the surface pattern in the hilum becomes condensed and have perforations formed due to degeneration of periclinal walls (**Figure 1.4d**). The anticlinal walls form undulated structure with waxy depositions. The longitudinal slit is also present. Hilum rim is well defined.

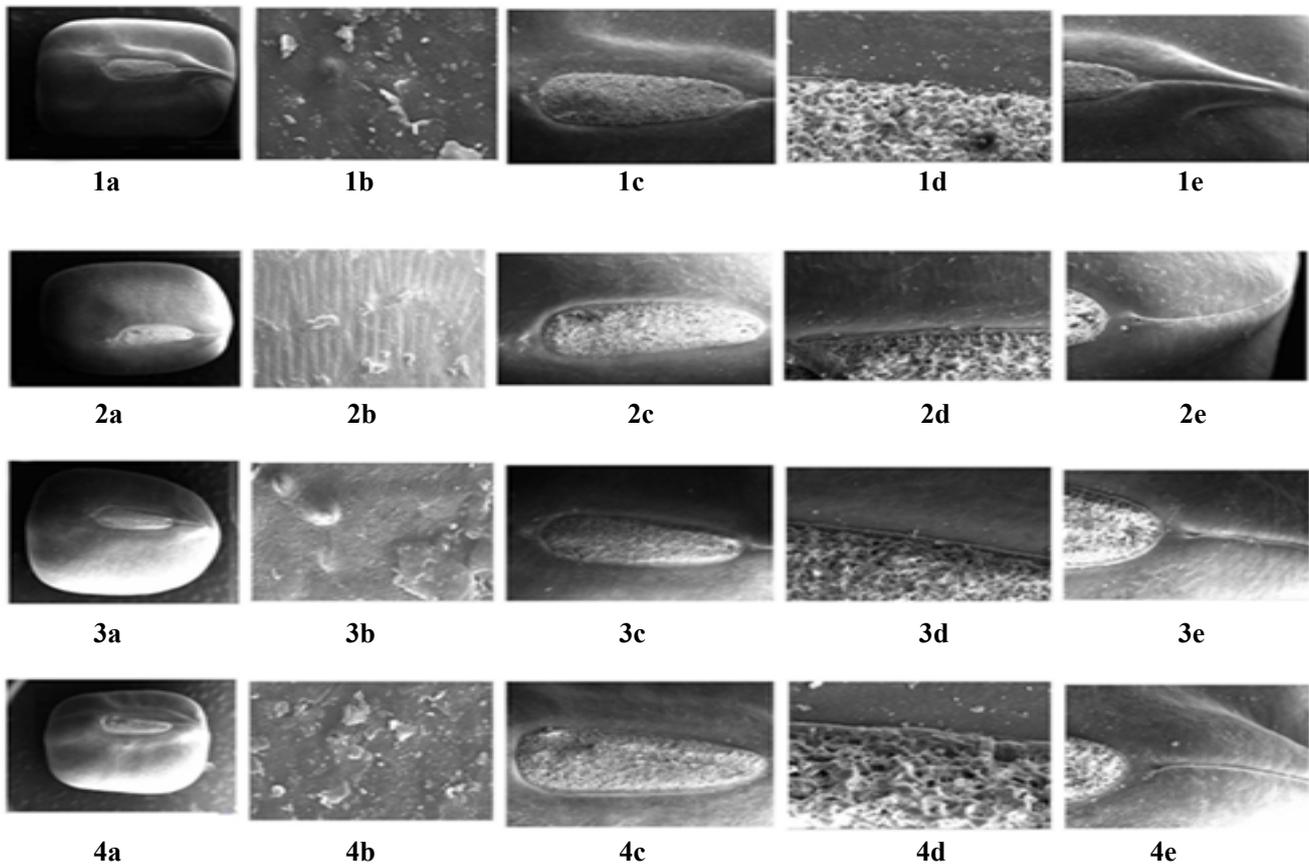


Figure 1. Scanning electron micrographs of cultivars of *Vigna radiate*; 1. GM3 (a) Whole seed(148X) (b) Seed surface enlarge view (20KX) (c) hilum(396X) (d) rim aril(3.5KX) (e) micropylar end(904X); 2. kcg-89 (a) whole seed (148X) (b) Seed surface enlarge view (20KX) (c) hilum(540X) (d) rim aril(1.4KX) (e) micropylar end(923X); 3. ML-131 (a) Whole seed(140X) (b) Seed surface enlarge view (20KX) (c) hilum(439X) (d) rim aril(1.73KX) (e) micropylar end(1kX); 4. K-851 (a) Whole seed (148X) (b) Seed surface enlarge view (20KX) (c) hilum(481X) (d) rim aril(3.5KX) (e) micropylar end(904X).

IC-11312

The seed is small, brownish to light green in colour with maximum length and breadth of 4.415 and 2.957 mm respectively (**Figure 2.5a**). The surface having net like structure with clear and raised anticlinal wall and deeply depressed periclinal walls (**Figure 2.5b**). The hilum is sub apical and oval shaped. It contains outwardly raised transverse anticlinal ends of radial walls forming globular shaped structures (**Figure 2.5c**). Periclinal walls degenerate to form perforations. The longitudinal slit is present and raised above the seed surface (**Figure 2.5c**). It is also covered with condensed anticlinal walls.

TM-9937

The seed is small and dark green in colour having length and breadth of 4.064 and 3.428 mm respectively (**Figure 2.6a**).

The surface contains reticulum of anticlinal walls covered with some waxy flakes (Figure 2.6b). The hilum is centrally placed with broad anterior end and narrow posterior end (Figure 2.6c). The reticulate pattern is clearly defined due to deeply depressed or periclinal walls (Figure 2.6b). The longitudinal slit is clearly seen which joins micropyle with anterior end of hilum and rose above the seed surface like that in Moong IC11312 (Figure 2.6e).

Wgg-37

The seed is small, dark green in colour with length and breadth of 4.212 and 3.389 mm respectively (Figure 2.7a). The hilum is sub-apical, oval with broader posterior end and narrower anterior end (Figure 2.7c). Its surface is reticulate with light waxy flakes (Figure 2.7b). A longitudinal slit which joins anterior end of seed with the micropyle is present (Figure 2.7e). The boundary of hilum is well defined and seen like interwoven strings. The transverse ends of the radial walls are slightly raised in the form of small globules. Wax flakes are seen in the hilum region (Figure 2.7c).

Pdm-11

The seed is small, almost quadrangular shaped (Figure 2.8a). The colour varies from dark brown to dark green with length and breadth of 4.452 and 3.147 mm respectively. Hilum is oval and centrally placed (Figure 2.8c). The surface of seed contains very light waxy flakes here and there and has elevated periclinal walls and depressed anticlinal walls forming almost pentagonal to hexagonal structures (Figure 2.8b). A longitudinal slit is present which joins anterior end of seed to the hilum (Figure 2.8e).

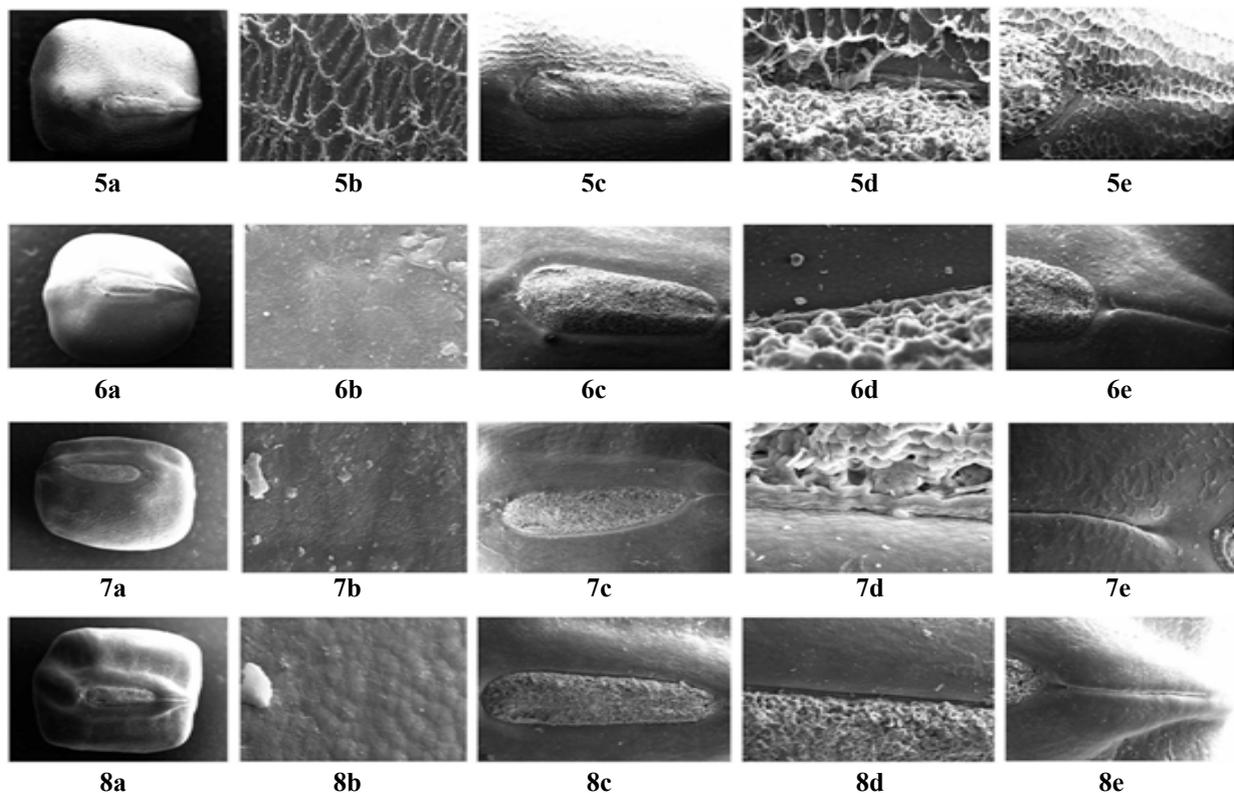


Figure 2. Scanning electron micrograph of cultivars of *Vigna radiate*; 5. 1C-11312 (a) Whole seed(148X) (b) Seed surface enlarge view(20KX) (c) hilum(439X) (d) rim aril(2.7KX) (e) micropylar end(1.5X); 6. TM-9937(a) whole seed (180X) (b) Seed surface enlarge view(20KX) (c) hilum(540X) (d) rim aril(5.43KX) (e) micropylar end(2.0X); 7. Wgg-37 (a) Whole seed(140X) (b) Seed surface enlarge view(20KX) (c) hilum(380X) (d) rim aril(4.23KX) (e) micropylar end(2.1kX); 8. Pdm-11 (a) Whole seed(165X) (b) Seed surface enlarge view(20KX) (c) hilum(488X) (d) rim aril(1.2KX) (e) micropylar end(7704X).

Eight Cultivars of *Vigna aconitifolia*

Jwala

The seed is small, very light yellow and oblong shaped with maximum length and breadth of 4.339 and 2.705 mm respectively. Hilum is sub-apical (Figure 3.1a). Surface of seed have heavily reticulate patterns of rugue with waxy drops here and there (Figure 3.1b). The boundary of hilum has net of interwoven string like structure (Figure 3.1d). The hilum is oval with broader posterior and narrower anterior end (Figure 3.1c). The boundary of hilum is clearly defined and slightly protruded out. There is a rod like very narrow slit which joins the micropyle with the anterior end of the seed (Figure 3.1e).

IC-120986

The seed is small, light green, oblong shaped with maximum length and breadth of 4.710 and 2.943 mm respectively (Figure 3.1a). Hilum is placed almost in the middle of the seed. Surface of seed shows thickly interwoven net of string like structure of

anticlinal walls with very light waxy droplets here and there. Periclinal walls are deeply depressed or disintegrated (**Figure 3.2b**). The hilum contains reticulate pattern of broader anticlinal walls looking like interwoven net of strings (**Figures 3.2c and 3.2d**). Periclinal walls are completely disintegrated. There is a longitudinal very narrow slit which joins the micropyle and anterior end of the seed. Waxy drops are seen in micropyle region also (**Figure 3.2e**).

RMO-40

The seed is small, oblong shaped with maximum length and breadth of 4.080 and 2.581 mm respectively (**Figure 3.3a**). The colour of seed is brown. The seed surface resembles that of M-IC-120986 and made up of irregular net of anticlinal walls which are highly raised and form an interwoven net of string like structure (**Figure 3.3b**). Periclinal walls are almost degenerated with very light waxy depositions. The hilum is almost centrally placed and the narrow longitudinal slit is seen in between micropyle and anterior end of the seed (**Figures 3.3c and 3.3e**). Waxy deposition is heavier than that seen in M-IC-120986.

IC-36011

The seed is relatively small with max. Length and breadth of seed is 3.872 and 2.655 mm respectively. The colour of seed varies from yellow to brownish. Hilum is placed in the sub-middle region and slightly towards the anterior end of the seed (**Figure 3.4a**). A longitudinal slit is also present (**Figure 3.4e**). The surface shows reticulum of raised anticlinal walls whereas periclinal walls are deeply depressed with light waxy depositions (**Figure 3.4b**). The boundary of hilum protrudes out slightly (**Figure 3.4c**). The surface pattern in the hilum region becomes condensed in the hilum region (**Figure 3.4d**). Most of the periclinal walls degenerate here.

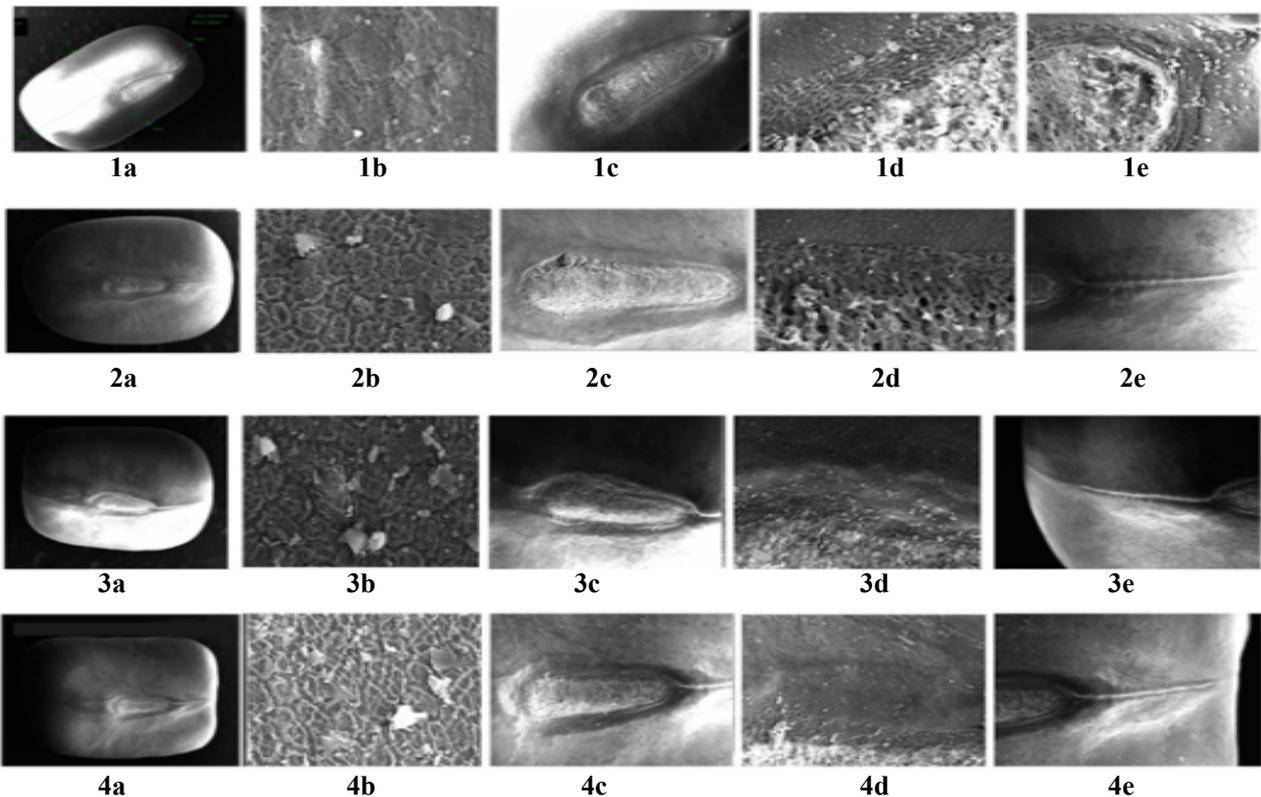


Figure 3. Scanning electron micrograph of cultivars of *Vigna aconitifolia*; 1. Jwala (a) Whole seed(151X) (b) Seed surface enlarge view(15KX) (c) hilum(519X) (d) rim aril(4.2KX) (e) micropylar end(3.4X); 2. IC-120986 (a) whole seed(164X) (b) Seed surface enlarge view(20KX) (c) hilum(764X) (d) rim aril(5KX) (e) micropylar end(830X); 3. RM-040 (a) Whole seed(198X) (b) Seed surface enlarge view(20KX) (c) hilum(515X) (d) rim aril(1.7KX) (e) micropylar end(549KX); 4. IC-36011 (a) Whole seed(159X) (b) Seed surface enlarge view(20KX) (c) hilum(712X) (d) rim aril(5KX) (e) micropylar end(952X).

IC-36161

The seed is small, brown in colour with maximum length and breadth of 4.331 and 2.684 mm respectively (**Figure 4.5a**). The seed surface shows reticulate pattern with raised string like anticlinal walls and deeply depressed periclinal walls (**Figure 4.5b**). The hilum is oval with broader posterior and narrower anterior end and is joined with the end of the seed through a narrow longitudinal slit (**Figures 4.5c and 4.5e**). The hilum region shows heavily waxy deposition with denser reticulate patterns (**Figure 4.5d**).

Jadia

The seed is small with maximum length and breadth of 4.844 and 3.020 mm respectively (**Figure 4.6a**). The colour of the seed is dark yellow. The surface showed reticulate pattern with waxy droplets here and there

(Figure 4.6b). The hilum boundary is distinct (Figure 4.6d.). Periclinal walls in this region are almost degenerated and the thicker anticlinal walls are condensed and overlapped and contain heavy waxy deposition. The longitudinal slit connecting hilum with anterior end of the seed is present (Figure 4.6e).

IC-39626

The seed is small very light yellow coloured with maximum length and breadth of 4.396 and 2.993 mm respectively (Figure 4.7a). The seed surface shows reticulate pattern with raised anticlinal and depressed periclinal walls (Figure 4.7b). Waxy drops are seen here and there. Hilum contains heavy waxy deposition and anticlinal walls are very much condensed (Figure 4.7d). It is placed in the sub-middle region of the seed and showed a longitudinal slit upto the anterior end of the seed (Figure 4.7e).

Maru

The seed is relatively larger with maximum length and breadth of 4.317 and 2.914 mm respectively (Figure 4.8a). The colour of the seed is yellow. The seed surface shows very heavy waxy deposition which overshadows the network of anticlinal walls (Figure 4.8b). The reticulum is very much condensed in the hilar region (Figure 4.8d). However, it is more visible than the remaining surface of the seed due to depression and disintegration of periclinal walls. Hilum is oval and placed in the sub-middle region and showed the longitudinal anterior slit (Figures 4.8c and 4.8e).

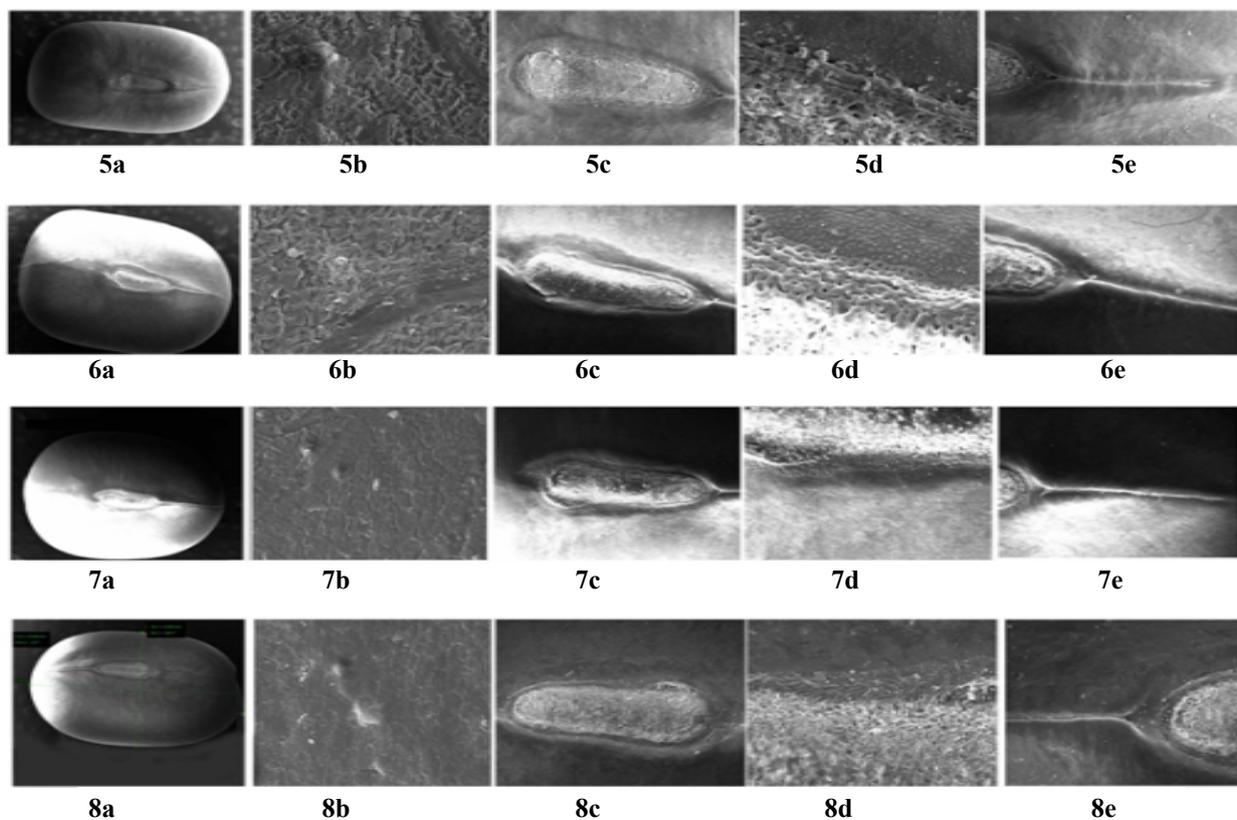


Figure 4. Scanning electron micrograph of cultivars of *Vigna aconitifolia* 5.1C-36161; (a) Whole seed(159X) (b) Seed surface enlarge view(20KX) (c) hilum(712X) (d) rim aril(5KX) (e) micropylar end(952X); 6. Jadia (a) whole seed(176X) (b) Seed surface enlarge view(158KX) (c) hilum(688X) (d) rim aril(3.2KX) (e) micropylar end(1.50X); 7. IC-39626 (a) Whole seed(190X) (b) Seed surface enlarge view(20KX) (c) hilum(551X) (d) rim aril(1.38KX) (e) micropylar end(885kX); 8. Maru (a) Whole seed(183X) (b) Seed surface enlarge view(10KX) (c) hilum(723X) (d) rim aril(2.20KX) (e) micropylar end(1.34KX).

Eight Cultivars of *Vigna mungo*

PBG-1

The seed is comparatively larger with length and breadth of 5.067 and 3.518 mm respectively (Figure 5.1a). The colour of the seed is dark black. The hilum is centrally placed with protruded border forming rim which is covered with aril (Figure 5.1c). The anticlinal walls form square to pentagonal shaped structure and rose while periclinal wall are depressed. The surface pattern is reticulate (Figure 5.1b). The hilum contains condensed surface pattern (Figure 5.1c). The periclinal walls are degenerated forming perforations. A longitudinal slit is present which joins the micropyle with the anterior end (Figure 5.1e).

LBG-22

The seed is small and faded black coloured with green patches. Its length and breadth are 4.425 and 3.141 mm respectively (Figure 5.2a). The surface of seed contains heavy wax deposition covering the entire reticulate surface of the testa (Figure 5.2b).

The hilum is centrally placed with raised border forming rim having aril and condensed reticulated cells (Figures 5.2c and 5.2d). A longitudinal slit is also present on the anterior side. Granulated mounds are present in the aril region (Figure 5.2e).

MBG-207

The seed is small black coloured with green patches having 4.998 mm length and 3.769 mm breadth (Figure 5.3a). The reticulate surface contains light waxy flakes (Figure 5.3b). The hilum is centrally placed with reticulate perforate pattern (Figure 5.3c). The rim aril is formed of condensed mounds of clubbed granules (Figure 5.3d). Anterior longitudinal slit is present (Figure 5.3e).

LBG-17

The seed is small, having 4.962 mm length and 3.637 mm breadth (Figure 5.4a). The seed colour is black with green patches on its surface. The surface having reticulate pattern with slightly raised anticlinal walls and having light waxy flakes here and there (Figure 5.4b). The hilum is sub-apical and with raised boundary forming rim aril (Figures 5.4e and 5.4d). The rim aril is granulated. A longitudinal slit is also present to join the micropyle with the anterior end (Figure 5.4e).

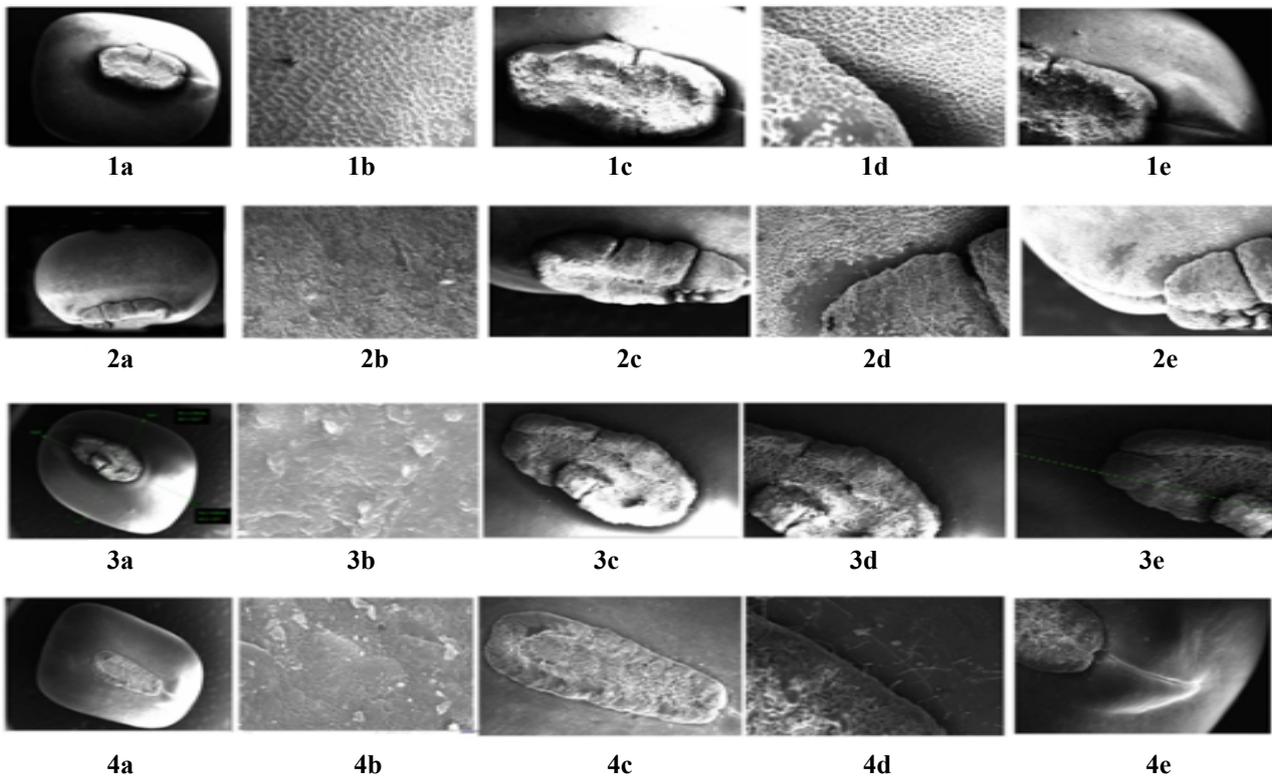


Figure 5. Scanning electron micrograph of cultivars of *Vigna mungo*; 1. PBG-1 (a) Whole seed(132X) (b) Seed surface enlarge view(2KX) (c) hilum(364X) (d) rim aril(1.96KX) (e) micropylar end(421X); 2. LBG-22 (a) whole seed(186X) (b) Seed surface enlarge view(2KX) (c) hilum(369X) (d) rim aril(1.13KX) (e) micropylar end(4696X); 3. MBG-207 (a) Whole seed(140X) (b) Seed surface enlarge view(20KX) (c) hilum(342X) (d) rim aril(448KX) (e) micropylar end(420kX); 4. LBG-17 (a) Whole seed(140X) (b) Seed surface enlarge view(33.17KX) (c) hilum(420X) (d) rim aril(1.59KX) (e) micropylar end(488KX).

LBG- 709

The seed is black, small with length and breadth of 4.815 mm and 3.371 mm respectively (Figure 6.5a). The surface reticulum is covered with heavy waxy deposition (Figure 6.5b). The hilum is sub-apical with raised border and condensed. The rim aril is composed of highly condensed mounds of small granules (Figure 6.5d). A longitudinal slit is present at anterior end which joins with the micropyle end (Figure 6.5e).

LBG- 623

The seed is small, oblong shaped with length and breadth of 4.994 and 3.287 mm respectively (Figure 6.6a). The reticulate seed surface contains waxy flakes. The surface shows cracks with undulated structure (Figure 6.6b). The hilum is centrally placed with raised border forming rim aril which consist o granular mounds (Figure 6.6c). A longitudinal slit is present which joins anterior end with the micropyle end (Figure 6.6e). The hilum region shows raised anticlinal walls and periclinal walls disintegrated to form perforations (Figure 6.6d).

LBG- 645

The seed is small, black and oval shaped with length and breadth of 4.905 and 3.652 mm respectively (Figure 6.7a). The

surface of the seed is relatively smooth with light waxy flakes and reticulate pattern (**Figure 6.7b**) the anticlinal walls are slightly raised. The hilum is white coloured sub middle with protruded border and condensed cells forming rim aril composed of (**Figures 6.7c and 6.7d**). A longitudinal slit is clearly seen (**Figure 6.7e**).

LBG -685

The seed is small, oval shaped, measuring 4.923 mm and 3.590 mm length and breadth respectively (**Figure 6.8a**). The surface of the seed contain heavy waxy deposition with reticulate pattern (**Figure 6.8b**). The hilum is sub middle with raised border forming rim aril composed of large granular mounds (**Figures 6.8c and 6.8d**). An anterior longitudinal slit is present (**Figure 6.8e**).

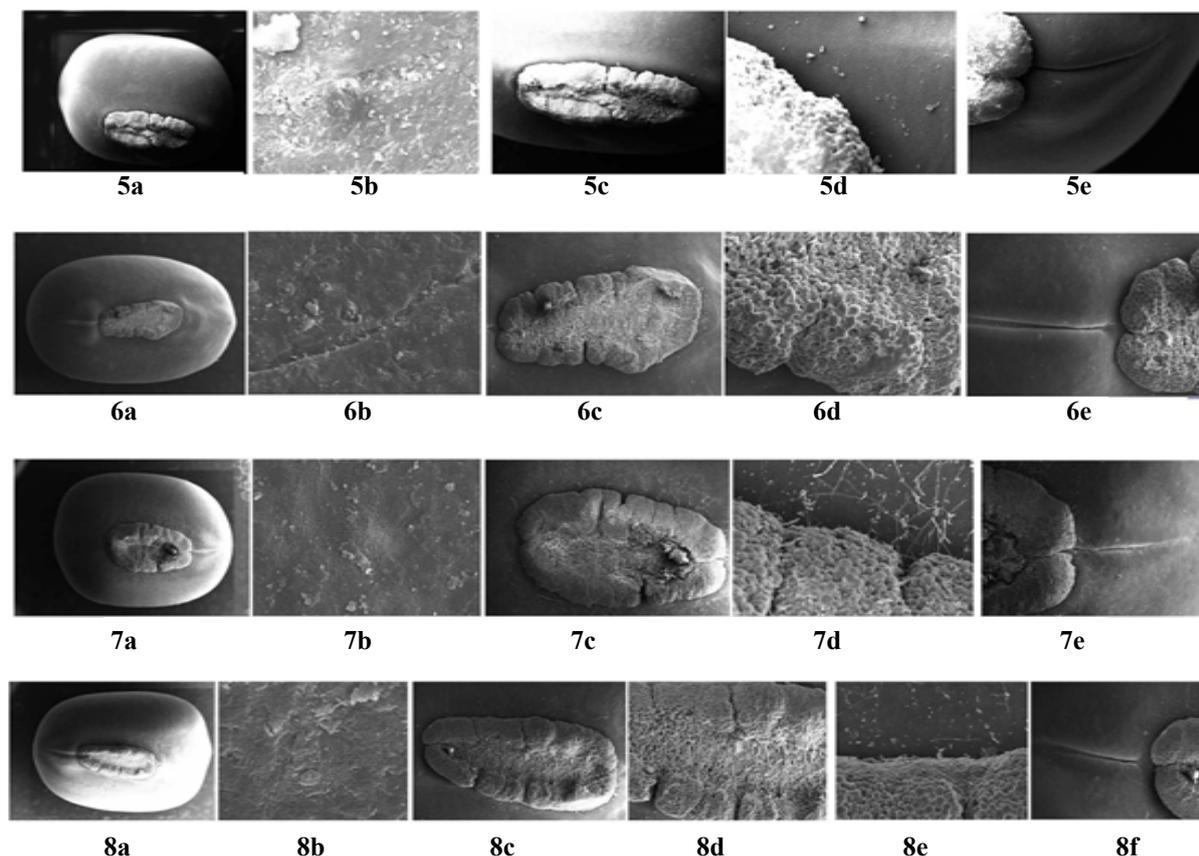


Figure 6. Scanning electron micrograph of cultivars of *Vigna mungo*; 5. LBG-709 (a) Whole seed(171X) (b) Seed surface enlarge view(20KX) (c) hilum(171X) (d) rim aril(2.18KX) (e) micropylar end(709X); 6. LBG-623 (a) whole seed(163X) (b) Seed surface enlarge view(10KX) (c) hilum(413X) (d) rim aril(163KX) (e) micropylar end(803X); 7. LBG-645 (a) Whole seed(132X) (b) Seed surface enlarge view(20KX) (c) hilum(358X) (d) rim aril(2.33KX) (e) micropylar end(566KX); 8.LBG-685 (a) Whole seed(140X) (b) Seed surface enlarge view (20KX) (c) hilum(413X) (d) rim aril(2.20KX) (e) micropylar end(739KX).

DISCUSSION

The seed coat patterns are species specific in the three species of *Vigna*. The surface pattern in all the three species is more or less reticulate covered with some waxy flakes here and there. The well-marked hilum boundary is protruded out in *Vigna mungo* while in other two species it is not raised or slightly depressed. The surface of *Vigna radiata* is smoother than the other two species. From the observation it is found that there are some variations in the seed coat of cultivars of same species like structure of anticlinal and periclinal walls, deposition of waxy flakes, hilum structure, position of hilum, structure of rim etc., but there are no significant differences in the cultivars of same species. The colour, size and shape of the seeds of three species are different.

In *Vigna aconitifolia* cell boundaries are distinct on seed surface and resembled with those observed by Nyola and Sharma [6]. In *Vigna mungo* the cell boundaries are indistinct. In *Vigna mungo* the waxy flakes are thick while in *Vigna radiata* waxy flakes are thinner than the other two species. The surface ornamentation showed reticulate pattern with irregular cell boundaries in *Vigna radiata*. Waxy flakes are present in all the three species with some variations. Observations were also made in species in some Tephrosia species and other legumes. This type of surface ornamentation with some differences depending upon the species was observed these workers [7,8]. In SEM studies of many papaveraceae species seeds have been reported to be reticulate super cellular pattern and this type of ornamentation has been considered as a family trait [9]. The seed coat patterns based on SEM studies revealed genetic diversity at many levels of taxonomic hierarchy and this variability was considered to be of adaptive and genetic origin [10]. It has been suggested that there are many uses of seed coat pattern to solve classification problems,

to determine evolutionary relationship, to act as genetic marker for the identification of genotypes in the hybrid progenies^[11]. Differentiation was made between different black berry cultivars through seed coat morphology^[12]. The variations in epicuticular wax morphology and size of the wax granules were suggested to be useful characters for plant taxonomy^[13]. The data gathered in the present report are in agreement to this surface pattern of seed coat of 12 natural populations of *Phaseolus sublobatus* were observed by SEM. As also SEM based seed coat patterns of leguminous taxa have been used for the classification purpose at or above species level^[14]. Study of seed coat microtopography in eight natural populations of *Vigna radiata* with SEM was made by Ignacimuthu and Babu which are in accord with present report^[15].

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