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Seed coat sculpture of subgenus *Ceratotropis* (Piper) verdc., genus *Vigna* Savi in India and its taxonomic implications

Suraj D. Umdale[a,b], Pravin D. Patil[a], Surendra K. Malik[a], M. Latha[c], Satyawada R. Rao[d], Shrirang R. Yadav[e], Nikhil B. Gaikwad[e] and Kangila V. Bhat[a]

[a]National Bureau of Plant Genetic Resources, Pusa campus, New Delhi, India; [b]Department of Botany, Yashvantrao Chavan Institute of Science, Satara, India; [c]National Bureau of Plant Genetic Resources Regional Station, KAU PO, Thrissur, India; [d]Department of Biotechnology & Bioinformatics, North-Eastern Hill University, Shillong, India; [e]Department of Botany, Shivaji University, Kolhapur, India

ABSTRACT
The seed coat morphology was studied using scanning electron microscopy in 21 species (of which two are both cultivated and wild) of subgenus *Ceratotropis* (Piper) Verdc. and one species each from subgenus *Plectotropis* and subgenus *Vigna* of genus *Vigna*. The macromorphological and micromorphological seed characters, which include seed shape, testa texture, hilum, aril, testa pattern and ornamentation of periclinal wall, exhibited well-defined variability among the studied taxa. The smallest seeds were recognized in *Vigna subramaniana* (1.97 × 1.91 mm) and the largest in *Vigna unguiculata* (7.02 × 4.45 mm). The smallest hilum was observed in *Vigna subramaniana* (0.50 ± 0.01 mm) and the largest in *Vigna umbellata* (3.57 ± 0.05 mm). Novel morphological features such as reticulate, reticulate-foveate, alveolate, colliculate-pusticulate, substriate, ruminate-reticulate and ruminate-channelled reticulations of periclinal wall of the testa cell and hilum structure were observed in addition to those reported in earlier studies. In the present investigation, we reported the seed micromorphology of wild endemic *Vigna* species, namely *Vigna sahyadriana*, *Vigna subramaniana*, *Vigna hainiana*, *Vigna indica*, *Vigna khandalensis* and *Vigna konkanensis* for the first time. Based on seed characters, the taxonomic identity and species complex in the subgenus *Ceratotropis* has been discussed. A key to the species has been developed based on seed characters.

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Introduction

The genus *Vigna* Savi (Leguminosae) is subdivided into five subgenera, namely *Ceratotropis* (Piper) Verdc., *Haydonia* (Wilczek) Verdc., *Lasiospron* (Benth.) Verdc., *Plectrotropis* (Schum.) Baker and *Vigna* Savi. It consists of over 104 species throughout the world (Thulin et al. 2004; Lewis et al. 2005; Delgado-Salinas et al. 2011; Takahashi et al. 2016). The subgenus *Ceratotropis* is known as the Asian *Vigna* (Tomooka, Vaughan et al. 2002). Tomooka, Vaughan et al. (2002) described 21 species within subgenus *Ceratotropis*, which was divided into three sections namely, section *Aconitifoliae* N. Tomooka & Maxted, section *Angulares* N. Tomooka & Maxted, and section *Ceratotropis* N. Tomooka & Maxted. The sectional delineation of the species within subgenus *Ceratotropis* was carried out by several authors on the basis of pollen morphology and molecular phylogenetic analyses (Maréchal, Mascherpa, and Stainier 1978; Doi et al. 2002; Saini and Jawali 2009; Javadi et al. 2011; Takahashi et al. 2016; Umdale et al. 2017). Babu, Sharma, and Johri (1985) revised the tribe *Phaseoleae* in India and listed 23 species of genus *Vigna* including wild and cultivated species. In addition to subgenus *Ceratotropis*, the occurrence of some species of the subgenus *Plectrotropis* and subgenus *Vigna* has also been reported from India (Verdcourt 1970; Tomooka, Vaughan et al. 2002; Yadav et al. 2014).

Recently, *Vigna trilobata* (L.) Verdc. var. *pusilla* Naik & Pokle has been raised to the rank of species as *Vigna indica* T.M. Dixit, K.V. Bhat & S.R. Yadav by Dixit et al. (2011). Aitawade et al. (2012) identified one new species, *Vigna sahyadriana* Aitawade, K.V. Bhat & S. R. Yadav and one new combination, *Vigna silvestris* Aitawade, K.V. Bhat & S.R. Yadav (= *Vigna mungo* var. *silvestris* Lukoki, Marechal & Otoul). A new species *Vigna konkanensis* Latha, K, K.V. Bhat, I.S. Bisht, Scariah, Joseph John and Krishnaraj has also been described from the west coast of India (Latha et al. 2014).

The potential taxonomic significance of ultrastructural patterns of seed coat morphology has been recognized in several taxa (Koul, Nagpal, and Raina 2000; de Queiroz, Gaulart de Azevedo Tozzi, and Lewis 2013; Patil et al. 2015). Seed morphological characters have provided reliable information in systematic studies of various Legume genera (Leese 1958; Trivedi, Bagchi, and

CONTACT Suraj D. Umdale milindumdale@gmail.com

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Bajpai 1979; Manning and van Staden 1987; Kirkbride, Gunn, and Weitzman 2003; de Queiroz, Gaulart de Azevedo Tozzi, and Lewis 2013).

Rajendra, Mujeeb, and Bates (1979) evaluated the seed coat of *Vigna sinensis* (L.) Savi ex Hausskn. and *Vigna sequipedalis* (L.) Fruwirth hybrids and suggested that the seed coat pattern is genetically controlled in *Vigna* species. Significant seed surface polymorphism has also been reported in different populations of *Phaseolus sublobatus* Roxb. (Sharma, Babu, and Johri 1983) and *Vigna sublobata* (Roxb.) Babu & S.K.Sharma (Ignacimuthu and Babu 1985). Kumar and Rangaswamy (1984) observed species-specific seed coat in 12 wild and cultivated populations within the genus *Vigna*. The seed coat morphology of *Vigna mungo* (L.) Hepper, *Vigna radiata* (L.) R. Wilkzek and their wild relatives has been studied by Chandel (1981), Chandel, Lester, and Sterling (1984), and Trivedi and Gupta (1986). Due to the similarity in the reticulate surface pattern, it is often difficult to differentiate *V. silvestris* and *V. sublobata* (Trivedi and Gupta 1986). The detailed study of Asian *Vigna* species showed that the species-specific nature of the seed coat and hilum morphology can be potentially useful in taxonomic differentiation (Chandel, Malik, and Nayar 1991; Nath and Dasgupta 2015).

Probably due to incorrect identification, several authors wrongly described the seed coat of species belonging to subgenus *Ceratotropis* (Tomooka, Vaughan et al. 2002; Dixit et al. 2011). For instance, Sharma et al. (1977) and Sharma, Babu, and Johri (1983) wrongly designated *V. sublobata* as *V. silvestris*, whereas Babu, Sharma, and Johri (1985) described these two taxa as a single species. To date, the seed morphological characters have been insufficiently investigated in the subgenus *Ceratotropis*. Also, the seed coat characteristics have never been described in detail and have not been explored for taxonomic differentiation of species of the subgenus *Ceratotropis*.

The aim of the present study was (1) to assess the taxonomic significance of seed morphological and micromorphological characters using scanning electron microscopy with respect to the species delineation, and (2) to formulate a taxonomic key to the species of the subgenus *Ceratotropis*.

Materials and methods

Plant materials

A total of 109 accessions, which included 17 wild and six cultivated species, were collected from their natural habitats and cultivated fields from India during 2008 to 2013. All *Vigna* species were identified by Dr S.R. Yadav, Head, Department of Botany, Shivaji University, Kolhapur, Maharashtra, India. All accessions of *Vigna* species were authenticated by Dr E. Roshni Nayar, Principal Scientist, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Voucher specimen and seeds have been deposited at the National Herbarium of cultivated Plants (NHCP), NBPGR, New Delhi, India. The collection areas and the accession numbers of the representative taxa studied are given in Table 1. Ten seeds from each accession were used to examine morphological characters, which included seed size, seed shape, seed colour, hilum shape and hilum length–width. Each seed sample was thoroughly cleaned using 70% ethanol, air dried and then two or three seeds were mounted on brass stubs using double-adhesive carbon tape. Mounted stubs were uniformly coated with a thin layer of gold using ion sputter Jeol JFC-100. After coating, the specimens were examined using a scanning electron microscope (JEOL JSM-840A) with accelerating voltages at 10–15 kV. Seeds were uniformly scanned at the hilum, the surface surrounding both sides of the hilum, and the surface pattern was highlighted to observe the cellular and intercellular patterns. The terminology for describing seed coat patterns follows Barthlott (1981, 1990) and Stearn (1966).

Numerical analysis

A total of 16 characters were evaluated for each species, comprising 10 qualitative and six quantitative characters. The 23 species were clustered based on morphological and morphometric characters. A similarity index was calculated using the Euclidean distance coefficient and the dendrogram was developed using Ward's clustering. This analysis was executed by calculating the eigenvectors and eigenvalues from the Eigen program in PAST (Paleontological Statistics Software Package for Education and Data Analysis) software 3.01. The principal components analysis used a correlation matrix, plotting scores for the first principal component (PC1) against the scores for the second component (PC2). To concede the most intrinsic characters, factor loadings were applied, which enhanced the separation of the studied taxa.

Results

The seed morphological characters of the studied taxa observed under light microscopy and scanning electron microscopy are presented in Tables 2, 3 and 4 and Figures 1–5. The seeds of the genus *Vigna* varied in their shape, colour, size, hilum structure, seed ornamentation and testa cell characteristics. The rough testa was observed in *V. mungo* (Figure 1A), *V. silvestris* (Figure 1D), *V. sahyadriana* (Figure 1G), *V. sublobata* (Figure 1M), *V. konkanensis* (Figure 2A), *Vigna subramaniana* (Babu ex Raizada) M. Sharma (Figure 2D), *Vigna hainiana* Babu, Gopin. & S. K. Sharma (Figure 2G), *V. indica* (Figure 3G), *Vigna khandalensis* (Santapau) Raghavan & Wadhwa (Figure 3J), *Vigna trinervia* var. *trinervia* (Heyne ex Wall) Tateishi & Maxted (Figure 4M) and *V. trinervia* var. *bourneae* (Gamble) Tateishi & Maxted

Table 1. List of selected *Vigna* species studied with Number of Accessions, collection number, status and Voucher information.

Subgenus	Section	Taxon	Status	No. of Accessions	Voucher information and location
Ceratotropis	*Ceratotropis*	*V. mungo* (L.) Hepper	Cultivated	05	Tamilnadu; 77°54'N, 08°79'E; TMV-1 (VMUN)
		V. silvestris (Lukoki, Marechal & Otoul) Aitawade, K.V. Bhat & S.R. Yadav	Wild	05	Raigarh fort, Raigarh, Maharashtra; 73°22'N, 18°50'E; BB-2666 (VSILV)
		V. sahyadriana Aitawade, K.V. Bhat & S.R. Yadav	Wild	04	Pasarani Ghat, Satara, Maharashtra; 73°21'N, 18°46'E; SUK-156 (VSAH)
		V. radiata (L.) R. Wilkzek	Cultivated	05	Kanpur, Uttar Pradesh; PDM-11; 26°29'N, 80°16'E (VRAD)
		V. sublobata (Roxb.) Babu & S.K. Sharma	Wild	05	Sirohi, Rajasthan; 73°52'N, 25°70'E; BB-2726 (VSBL)
		V. konkanensis Latha, K, K.V. Bhat, I.S. Bisht, Scariah, Joseph John & Krishnaraj	Wild	03	Ratnagiri, Maharashtra; 73°69'N, 16°59'E; BBL-64–2000 (VKON)
		V. subramaniana (Babu ex Raizada) M. Sharma	Wild	04	Thrissur, Kerala; 76°16'N, 10°32'E; SUK-170 (VSUR)
		V. hainiana Babu, Gopin. & S. K. Sharma	Wild	05	Chittorgarh, Rajasthan; 74°63'N, 24°57'E; BBYD-2709 (VHAI)
	Aconitifoliae	*V. aconitifolia* (Jacq.) Marechal	Cultivated	05	Rajasthan; 73°739'N, 24°39'E; RMO-257 (VACN)
		V. aconitifolia (Jacq.) Marechal	Wild	05	Udaipur, Rajasthan; 73°73'N, 24°39'E; BBYD-2718 (VACW)
		V. stipulacea (Lam.) Kuntz	Wild	05	IGAU campus, Raipur, Chattisgarh; 82°17'N, 21°07'E; BBD-20–01B (VTRI)
		V. trilobata (L.) Verdc	Wild	04	Kanyakumari, Tamil Nadu; 77.36'N, 08°30'E; TCR-303 (VSTI)
		V. indica T.M. Dixit, K.V. Bhat & S.R. Yadav	Wild	06	Sumerpur, Pali, Rajasthan; 73°52'N, 25°70'E; BB-2618 (VIND)
		V. khandalensis (Santapau) Raghavan & Wadhwa	Wild	04	Panchagani, Satara, Maharashtra; 74°17'N, 17°66'E; BB-2678 (VKHA)
	Angulares	*V. angularis* (Willd.) Ohwi & Ohashi	Cultivated	06	Shimla, Himachal Pradesh; 77°05'N, 30°50'E; HPU-51 (VANG)
		V. dalzelliana (Kuntz.) Verdc	Wild	05	Udupi, Karnataka; 74°68'N, 13°34'E; TCR-387 (VDAL)
		V. umbellata (Thunb.) Ohwi & Ohashi	Cultivated	05	Meghalaya; 91°55'N, 25°35'E; TCR-95 (VUME)
		V. umbellata (Thunb.) Ohwi & Ohashi	Wild	05	Meghalaya; 91°54'N, 25°36'E; SKM-09 (VUMW)
		V. glabrescens Marechal, Mascherpa & Stainer	Wild	02	Harighat, Madhya pradesh, 78°12'N, 22°12'E; IC-251372 (VGLA)
		V. trinervia var. *trinervia* (Heyne ex Wall) Tateishi & Maxted	Wild	06	Koraput, Odisha; 82°72'N, 18°81'E; BBD-09–01B (VTRN)
		V. trinervia var. *bourneae* (Gamble) Tateishi & Maxted	Wild	05	Thrissur, Kerala; 76°31'N, 10°47'E; TCR-345 (VBOR)
Vigna	*Catiang*	*V. unguiculata* (L.) Walp	Cultivated	05	Gujarat; 72°28'N, 24°63'E; CG-4 (VUNG)
Plectotropis	*Plectotropis*	*V. vexillata* (L.) A. Rich	Wild	05	Pune, Maharashtra; 74°06'N, 18°57'E; TCR-4 (VVEX)

(Figure 5A), whereas in *V. radiata* (Figure 1J), *Vigna aconitifolia* (Jacq.) Marechal (cult.) (Figure 2J), *V. aconitifolia* (wild) (Figure 2M), *Vigna stipulacea* (Lam.) Kuntz (Figure 3A), *V. trilobata* (L.) Verdc (Figure 3D), *Vigna angularis* (Willd.) Ohwi & Ohashi (Figure 3M), *Vigna dalzelliana* (Kuntz.) Verdc (Figure 4A), *Vigna umbellata* (cult.) (Thunb.) Ohwi & Ohashi (Figure 4D), *V. umbellata* (wild) (Figure 4G), *Vigna glabrescens* Marechal, Mascherpa & Stainer (Figure 4J), *Vigna unguiculata* (Figure 5D) and *Vigna vexillata* (Figure 5G) seed coat surface was found to be smooth.

Seed shape, size and colour

Seed shape was rectangular in *V. sahyadriana* (Figure 1G), *V. radiata* (Figure 1J), *V. sublobata* (Figure 1M), *V. konkanensis* (Figure 2A), *V. subramaniana* (Figure 2D), *V. hainiana* (Figure 2G), *V. stipulacea* (Figure 3A), *V. trilobata* (Figure 3D), *V. indica* (Figure 3G), *V. khandalensis* (Figure 3G), *V. trinervia* var. *trinervia* (Figure 4M) and *V. trinervia* var. *bourneae* (Figure 5A). However, other shape types were observed, namely round or ovoid in *V. silvestris* (Figure 1D) and *V. konkanensis* (Figure 2A), globular in *V. aconitifolia* (cult.) (Figure 2J), *V. aconitifolia* (wild) (Figure 2M), *V. umbellata* (cult.) (Figure 4D) and *V. umbellata* (wild) (Figure 4G), oblong in *V. mungo* (Figure 1A), *V. angularis* (Figure 3M) and *V. glabrescens* (Figure 4J), and oblong to reniform in *V. unguiculata* (Figure 5D) and *V. vexillata* (Figure 5G). Diverse seed colours were observed varying from black to blackish brown, greyish brown, yellowish brown, green and rarely white. The two species *V. trilobata* and *V. umbellata* showed variegated seed testa. The seed length in the species studied ranged from 1.97 to 7.02 mm and seed width from 1.57 to 4.45 mm (Table 2). In most of the species, seed length ranged from 2 to 4 mm; the longest seeds were observed in *V. mungo*, *V. radiata*, *V. angularis*, *V. glabrescens* and *V. unguiculata* (Table 2). The smallest width was recorded in *V. subramaniana* (Figure 2D), whereas the largest was recorded in *V. unguiculata* (Figure 5D). The seeds with minimum length : width ratio of 1.03 were observed in *V. subramaniana* whereas the highest ratio, 1.8, was recorded for cultivated *V. umbellata* (Table 2).

Hilum

In most of the examined seeds, the hilum was located in a central position except in *V. indica* (Figure 3G), *V. umbellata* (Figures 4D, G), *V. dalzelliana* (Figure 4A), *V. unguiculata* (Figure 5D) and *V. vexillata* (Figure 5G), where the hilum was subcentral. The hilum was oblong in most of the species (Figures 1J, 1M, 2G, 2J, 2M, 3A, 3J, 4D, 4G, 4M), but linear in *V. angularis* (Figure 3M), *V. dalzelliana* (Figure 4A) and *V. trinervia* var. *bourneae* (Figure 5A), elliptic to linear in *V. mungo* (Figure 1A), *V. silvestris* (Figure 1D) and *V. sahyadriana* (Figure 1G),

Table 2. Seed morphometric characteristics of species of subgenus *Ceratotropis*.

Taxon	Seed Length (mm)		Seed Width (mm)		Length/width ratio	Hilum Length (mm)		Hilum Width (mm)		Length/width ratio
	Range	$\bar{x} \pm s\bar{x}$	Range	$\bar{x} \pm s\bar{x}$		Range	$\bar{x} \pm s\bar{x}$	Range	$\bar{x} \pm s\bar{x}$	
V. mungo (cult.)	2.65–5.28	4.18±0.41	2.35–3.38	3.22±0.23	1.3	1.78–2.09	1.93±0.06	0.90–1.15	1.07±0.05	1.8
V. silvestris	2.41–3.13	2.71±0.56	2.10–2.84	2.44±0.50	1.11	1.97–2.24	2.05±0.06	1.08–1.20	1.13±0.02	1.81
V. sahyadriana	2.80–3.08	3.75±0.24	2.15–2.77	2.47±0.15	1.52	1.73–1.84	1.80±0.03	0.98–0.99	0.95±0.03	1.9
V. radiata (cult.)	4.15–4.36	4.10±0.14	2.50–3.57	2.86±0.15	1.43	1.25–1.42	1.34±0.03	0.49–0.52	0.51±0.01	2.63
V. sublobata	2.26–3.39	2.77±0.19	1.92–2.88	2.25±0.15	1.23	0.74–1.14	0.90±0.07	0.35–0.45	0.41±0.02	2.19
V. konkanensis	2.29–2.35	2.33±0.02	1.43–1.78	1.63±0.06	1.43	0.70–1.03	0.86±0.07	0.34–0.43	0.38±0.02	2.26
V. subramaniana	1.96–1.98	1.97±0.01	1.89–1.93	1.91±0.01	1.03	0.49–0.52	0.50±0.01	0.28–0.31	0.30±0.01	1.67
V. hainiana	2.03–2.78	2.49±0.12	1.68–1.85	1.86±0.08	1.34	0.92–1.15	1.04±0.06	0.31–0.36	0.34±0.01	3.56
V. aconitifolia (Cult.)	3.45–4.25	3.76±0.14	2.22–2.39	2.30±0.03	1.63	1.13–1.37	1.22±0.04	0.32–0.39	0.39±0.02	3.13
V. aconitifolia (wild)	2.18–4.43	3.46±0.33	1.68–2.60	2.18±0.14	1.59	1.08–1.28	1.20±0.04	0.32–0.42	0.36±0.02	3.33
V. stipulacea	2.48–2.86	2.70±0.07	1.98–2.35	2.09±0.08	1.29	1.25–1.51	1.34±0.05	0.58–0.72	0.63±0.03	2.13
V. trilobata	1.93–3.09	2.67±0.20	1.42–1.85	1.71±0.07	1.56	0.72–1.13	0.93±0.10	0.71–0.99	0.85±0.06	1.09
V. indica	2.49–3.24	2.81±0.14	1.41–2.24	1.80±0.12	1.56	0.44–0.59	0.49±0.03	0.23–0.27	0.25±0.01	1.96
V. khandalensis	3.40–4.16	3.81±0.15	2.85–3.29	3.06±0.08	1.24	1.27–1.62	1.44±0.06	0.50–0.62	0.56±0.03	2.57
V. angularis (cult.)	4.26–6.23	5.23±0.46	2.78–4.58	3.83±0.44	1.36	2.39–3.42	2.73±0.18	0.51–0.78	0.61±0.04	4.47
V. dalzelliana	2.45–2.85	2.67±0.08	1.11–1.89	1.57±0.15	1.7	1.30–1.73	1.51±0.07	0.43–0.56	0.49±0.02	3.08
V. umbellata (Cult.)	5.68–7.25	6.63±0.36	2.93–3.41	3.16±0.09	2.11	3.45–3.71	3.57±0.05	1.11–1.21	1.16±0.02	3.08
V. umbellata (wild)	2.79–6.74	4.78±0.70	1.79–4.02	2.66±0.36	1.8	1.64–4.00	2.98±0.37	0.46–1.49	1.03±0.14	2.89
V. glabrescens	3.96–4.49	4.20±0.12	2.87–3.17	3.00±0.07	1.4	1.71–2.10	1.93±0.08	0.59–0.69	0.64±0.02	3.01
V. trinervia var. *trinervia*	2.55–2.84	2.73±0.07	2.46–2.53	2.45±0.02	1.11	1.52–2.06	1.72±0.08	0.45–0.70	0.49±0.05	3.51
V. trinervia var. *bourneae*	2.87–3.30	3.12±0.08	2.67–3.09	2.80±0.08	1.11	1.41–1.72	1.54±0.08	0.45–0.47	0.46±0.01	3.35
V. unguiculata (cult.)	6.60–7.86	7.02±0.20	4.12–4.62	4.45±0.08	1.57	1.69–2.51	1.97±0.22	0.63–1.39	0.90±0.20	2.19
V. vexillata	3.13–4.42	3.92±0.23	2.13–2.61	2.33±0.07	1.62	1.37–1.78	1.51±0.07	0.45–0.84	0.69±0.07	2.19

Arithmetic mean $\bar{x}$; standard deviation $s\bar{x}$

elliptic in *V. glabrescens* (Figure 4J) and *V. vexillata* (Figure 5G), obovate in *V. konkanensis* (Figure 2A), broadly obovate or orbicular in *V. trilobata* (Figure 3D), truncate in *V. unguiculata* (Figure 5D), and rectangular in *V. subramaniana* (Figure 2D) and *V. indica* (Figures 3G; Table 3). The minimum hilum length was found in *V. subramaniana* (0.50 mm) and *V. indica* (0.59 mm), and the maximum was recorded in *V. umbellata* (4.0 mm). Minimum hilum width was found in *V. indica* (0.23–0.27 mm) and the maximum in *V. mungo* (1.08–1.2 mm). The seeds with the lowest hilum length : width ratio (1.09) were observed in *V. trilobata* and the highest (4.47) in *V. angularis* (Table 2). An aril was well developed in *V. mungo* (Figure 1A), *V. silvestris* (Figure 1D), *V. sahyadriana* (Figure 1G), *V. trilobata* (Figure 3D), *V. dalzelliana* (Figure 4A), *V. umbellata* (Figure 4D, G), *V. unguiculata* (Figure 5D) and *V. vexillata* (Figure 5G).

Seed coat ornamentation

Seed coat surface ornamentation was found to be reticulate in most of the cases (Figures 1E, 1H, 1K, 1N, 2B, 2E, 2H, 3E, 3K, 4N, 5B, 5E, 5H); occasionally, other patterns were also observed: reticulate to alveolate in *V. mungo* (Figure 1B); foveate to reticulate in *V. stipulacea* (Figure 3B); reticulate-pusticulate in *V. glabrescens* (Figure 4K); reticulate to scalariform in *V. vexillata* (Figure 5H); ruminate in *V. angularis* (Figure 3N), *V. dalzelliana* (Figure 4B), *V. umbellata* (cult.) (Figure 4E) and *V. umbellata* (wild) (Figure 4H); substriate in *V. indica* (Figure 3H) and colliculate-pusticulate in *V. aconitifolia* (cult.) (Figure 2K) and *V. aconitifolia* (wild) (Figure 2N) (Table 4).

Testa cell structure

Testa cells were hexagonal to polygonal in shape while channelled testa cells were observed in *V. dalzelliana* (Figure 4C). The anticlinal walls were granulated, irregularly thick in *V. mungo* (Figure 1C), *V. silvestris* (Figure 1F), *V. sahyadriana* (Figure 1I) and *V. hainiana* (Figure 2I); raised undulated in *V. sublobata* (Figure 1O), *V. subramaniana* (Figure 2F), *V. aconitifolia* (cult.) (Figure 2L), *V. angularis* (Figure 3O), *V. umbellata* (cult) (Figure 4F), *V. umbellata* (wild) (Figure 4I), *V. glabrescens* (Figure 4L) and *V. vexillata* (Figure 5I); raised papillate in *V. trinervia* var. *trinervia* (Figure 4O) and *V. trinervia* var. *bourneae* (Figure 5C); raised sharp in *V. radiata* (Figure 1L), *V. stipulacea* (Figure 3C) and *V. vexillata* (Figure 5F); whereas in *V. trilobata* this seems to have disappeared at the centre of the testa cell (Figure 3F). Periclinal walls were flat to concave; granulated in *V. mungo* (Figure 1C), *V. silvestris* (Figure 1F), *V. sahyadriana* (Figure 1I), *V. hainiana* (Figure 2I), *V. aconitifolia* (cult.) (Figure 2L), *V. aconitifolia* (wild) (Figure 2O) and *V. angularis* (Figure 3O); compact-reticulate in *V. radiata* (Figure 1L), *V. sublobata* (Figure 1O), *V. trilobata* (Figure 3F), *V. khandalensis* (Figure 3L), *V. trinervia* var. *trinervia* (Figure 4O) and *V. trinervia* var. *bourneae* (Figure 5C); simple-reticulate in *V. konkanensis* (Figure 2C), *V. subramaniana* (Figure 2F), *V. hainiana* (Figure 3H), *V. stipulacea* (Figure 3C) and *V. glabrescens* (Figure 4L); ruminate in *V. umbellata* (cult.) (Figure 4F), *V. umbellata* (wild) (Figure 4I) and *V. vexillata* (Figure 5I); psilate in *V. dalzelliana* (Figure 4C) and *V. unguiculata* (Figure 5F) and pusticulate in *V. glabrescens* (Figure 4L) (Table 4).

Table 3. Macromorphological seed characters to distinguish the studied species of subgenus *Ceratotropis*.

Taxon	Form	Colour	Testa texture	Hilum position	Hilum shape
V. mungo (cult.)	Round, oblongoid	Black	Rough	Central	Elliptic to linear, protruding
V. silvestris	Ovoid	Dark brown, black	Highly rough,	Central	Elliptic to linear, protruding
V. sahyadriana	Rectangular with roundish ends	Dark brown	Rough	Central	Elliptic to linear, protruding
V. radiata (cult.)	Rectangular to oblongoid	Shiny, green or brown	Smooth, shiny	Central	Oblong
V. sublobata	Rectangular	Blackish brown	Highly rough	Central	Oblong
V. konkanensis	Ovoid	Blackish brown	Rough	Central	Obovate
V. subramaniana	Oblong or rectangular	Dark brown	Rough	Central	Rectangular
V. hainiana	Rectangular with roundish ends	Blackish brown	Rough	Central	Oblong
V. aconitifolia (cult.)	Globular to elongated with roundish ends	Pale Brown	Smooth, shiny	Central	Oblong
V. aconitifolia (Wild)	Globular to elongated with roundish ends	Pale brown, yellowish brown	Smooth, shiny	Central	Oblong
V. stipulacea	Rectangular with roundish ends	Brownish	Smooth	Central	Ellipsoidal, oblong, protruding
V. trilobata	Rectangular to globular with roundish ends	Brown, yellowish brown with black spots	Smooth	Central	Broadly ovate or orbicularprotruding,
V. indica	Rectangular with truncate ends or hourglass shaped	Brown to maroon	Rough	Subcentral	Rounded rectangular
V. khandalensis	Rectangular to oblong	Light to dark Black	Rough	Central	Oblong
V. angularis (cult.)	Oblong, rounded at ends	Greyish brown, Blackish brown	Smooth, shiny	Central	Linear
V. dalzelliana	Subcylindrical, truncate	Dark brown	Smooth	Subcentral	Broadly linear, less protruding
V. umbellata (cult.)	Globular elongated with roundish ends	Blackish brown, black, variegated	Smooth, shiny	Subcentral	Oblong
V. umbellata (Wild)	Globular elongated with roundish ends	Blackish brown to black, spotted	Smooth, shiny	Subcentral	Oblong
V. glabrescens	Oblong	Brownish Black	Smooth	Central	Narrowly elliptic
V. trinervia var. trinervia	Rectangular	Blackish brown to black	Rough	Central	Oblong
V. trinervia var. bourneae	Rectangular with roundish ends	Blackish Brown	Rough	Central	Linear
V. unguiculata (cult.)	Oblong to reniform	Reddish Brown to black or white	Smooth	Subcentral	Obovate
V. vexillata	Oblong to reniform	Reddish Brown to black	Smooth	Subcentral	Broadly elliptic

Table 4. Micromorphological seed characters to distinguish the studied species of subgenus *Ceratotropis*.

Taxon	Aril	Testa pattern	Testa cell	Anticlinal wall	Periclinal wall
V. mungo (cult.)	Well developed	Reticulate, alveolate	Elongated polygonal	Granulate, waxy mounds giving papillate appearance	Flat to slightly concave, granulate
V. silvestris	Well developed	Reticulate	Broad polygonal	Granulate, waxy mounds with indistinct thickness	Flat to slightly concave, granulate
V. sahyadriana	Well developed	Reticulate	Elongated polygonal	Raised, undulate	Concave granulate
V. radiata (cult.)	Absent	Reticulate	Elongated polygonal arranged in tires	Raised, sharp and thin	Concave, smooth
V. sublobata	Absent	Reticulate	Elongated hexagonal arranged in tires	Raised, undulate with thick longitudinal thin transverse wall	Concave, compact reticulate
V. konkanensis	Absent	Reticulate	Broad hexagonal, irregularly arranged	Undulate with thin longitudinal and transverse wall	Concave, loose reticulation
V. subramaniana	Absent	Reticulate	Broad polygonal arranged in tires	Raised, undulate with thick longitudinal and thin sharp transverse wall	Flat to concave, reticulate
V. hainiana	Absent	Reticulate	Broad polygonal	Granulate, waxy mounds with indistinct thickness	Flat to slightly Concave, granulate
V. aconitifolia (cult.)	Absent	Colliculate-pusticulate	Broad polygonal	Not much Raised, Wavy with indistinct thickness	Unevenly reticulate
V. aconitifolia (Wild)	Absent	Colliculate-pusticulate	Broad polygonal	Raised, undulate	Unevenly reticulate
V. stipulacea	Less developed	Reticulate-foveate	Polygonal	Raised, sharp, thick wax deposition	Flat, reticulate
V. trilobata	Well developed	Reticulate	Broad polygonal, arranged in tires	Thick longitudinal wall and transverse wall thin, sharp disappear at centre,	Concave, compact reticulate
V. indica	Absent	Substriate	Broad polygonal arranged in tires	Raised thin	Flat to concave, reticulate
V. khandalensis	Absent	Reticulate	Broad polygonal arranged in wavy tires	Irregular thickness	Flat to concave, compact reticulate
V. angularis (cult.)	Absent	Ruminate	Polygonal	Raised, undulate	Granulate
V. dalzelliana	Less developed	Ruminate-channelled	Channelled	Smooth, channelled	Psilate
V. umbellata (cult.)	Well developed	Ruminate-reticulate	Polygonal with loosely arranged	Raised, smooth and wavy	Reticulate
V. umbellata (Wild)	Well developed	Ruminate-reticulate	Polygonal with tightly arranged	Raised, smooth and wavy	Reticulate
V. glabrescens	Absent	Reticulate pusticulate	Polygonal	Raised, undulate	Reticulate
V. trinervia var. *trinervia*	Absent	Reticulate	Broad hexagonal	Raised, papillate, irregular thickness	Flat to concave, compact reticulate
V. trinervia var. *bourneae*	Absent	Reticulate	Broad hexagonal arranged in wavy tires	Raised, papillate, thick longitudinal and thin, sharp transverse wall	Flat to concave, compact reticulate
V. unguiculata (cult.)	Well developed	Reticulate	Hexagonal	Raised, smooth and uniform thickness	Psilate
V. vexillata	Well developed	Reticulate to Scalariform	Polygonal	Raised, smooth and undulate	Granulate

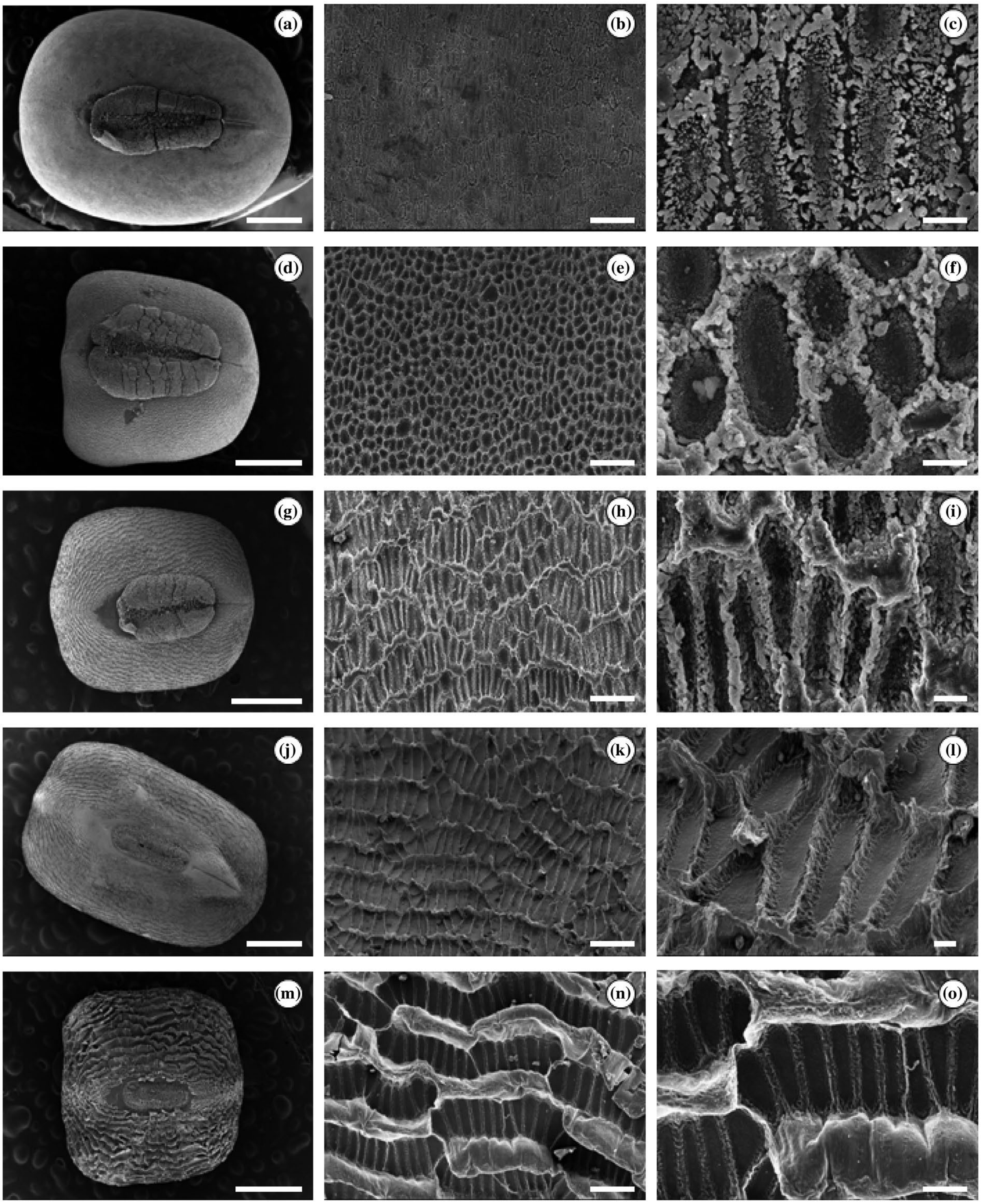

Figure 1. Scanning electron micrographs of seeds. **(A–C)** *Vigna mungo*; **(D–F)** *Vigna silvestris*; **(G–I)** *Vigna sahyadriana*; **(J–L)** *Vigna radiata*; **(M–O)** *Vigna sublobata*. Scale bars: A, D, G, J, M, 1 mm; B, E, H, K, N, 100 μm; C, F, I, L, O, 10 μm.

Principal components analysis

In the principal components analysis, the first five principal components (PC1 = 33.57%, PC2 = 17.57%, PC3 = 11.36%, PC4 = 9.26% and PC5 = 7.91%) accounted for a total variance of 93.25% differentiating the sixteen characters (Table 5). PC1 was most highly influenced by hilum position, hilum shape, aril, periclinal wall, seed length, seed width, seed length : width ratio, hilum length and hilum length : width ratio. For PC2, the characters presenting the major contributions to the total variance were testa texture, testa cell, seed length, seed width, hilum length and hilum width. In PC3, characters such as colour, testa pattern, anticlinal wall and seed length : width ratio showed comparatively minor contributions (Figure 6).

Cluster analysis

In the Ward's cluster analysis all taxa were divided into two major clusters, namely, cluster I and cluster II (Figure 7). Cluster I included all species of

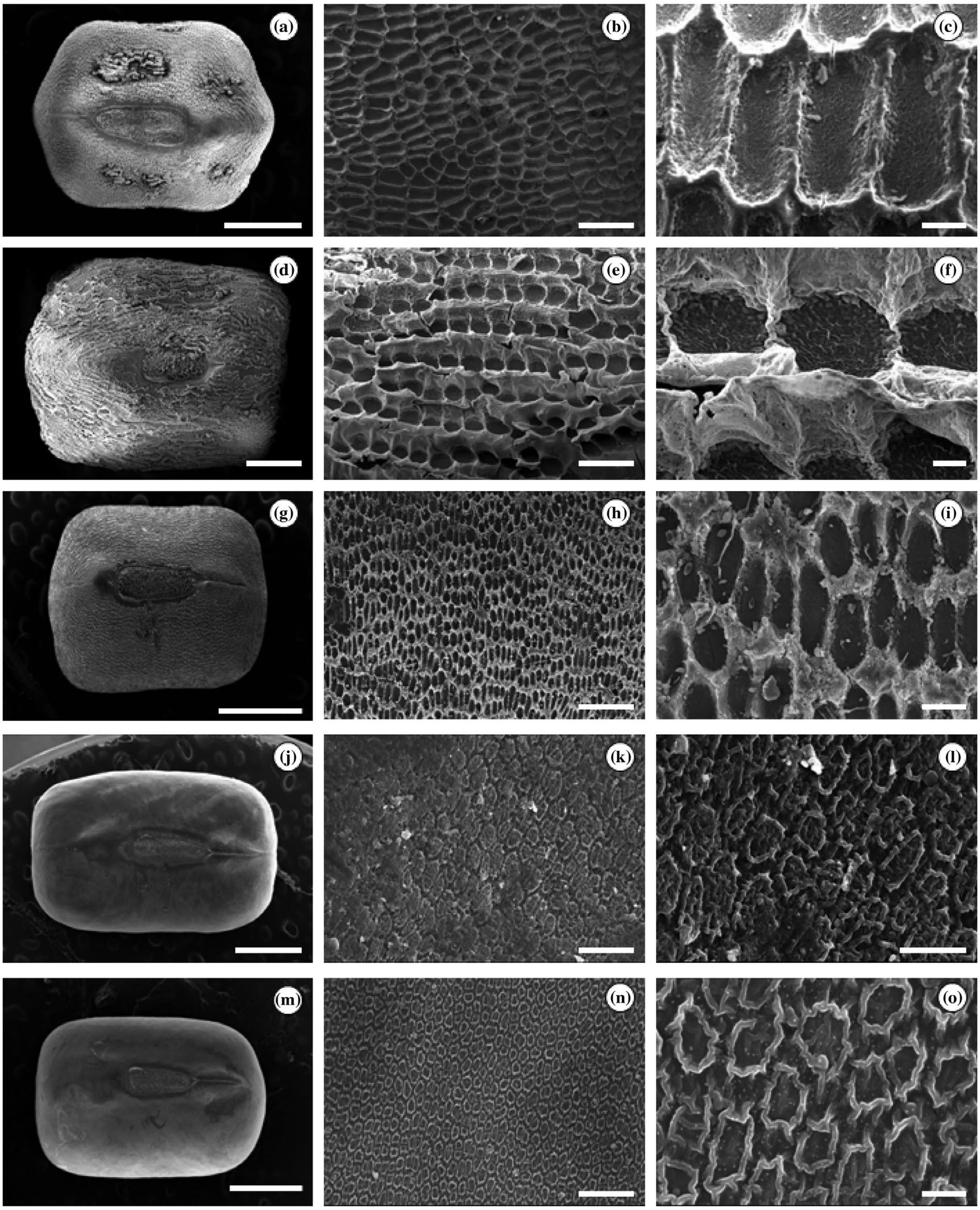

Figure 2. Scanning electron micrographs of seeds. **(A–C)** *Vigna konkanensis*; **(D–F)** *Vigna subramaniana*; **(G–I)** *Vigna hainiana*; **(J–L)** *Vigna aconitifolia* (cultivated); **(M–O)** *V. aconitifolia* (wild). Scale bars: A, D, G, J, M, 1 mm; B, E, H, K, N, 100 μm; C, F, I, L, O, 10 μm.

section *Angulares*: *V. angularis*, *V. umbellata* (wild), *V. umbellata* (cult.), *V. glabrescens*, *V. trinervia* var. *trinervia*, *V. trinervia* var. *bourneae* except *V. dalzelliana*, which was placed in cluster II along with *V. stipulacea* and *V. vexillata* of section *Plectotropis* and *V. unguiculata* of section *Catiang*. Cluster II comprised two subclusters; IIA and IIB. Subcluster IIA included the species of section *Aconitifoliae*: *V. trilobata*, *V. indica*, *V. aconitifolia* (wild), *V. aconitifolia* (cultivated), *V. stipulacea* and *V. khandalensis*. Subcluster IIB included the species of section *Ceratotropis*: *V. mungo*, *V. silvestris*, *V. sahyadriana*, *V. radiata*, *V. sublobata*, *V. konkanensis*, *V. subramaniana*, except *V. hainiana*, which was grouped in cluster IIA.

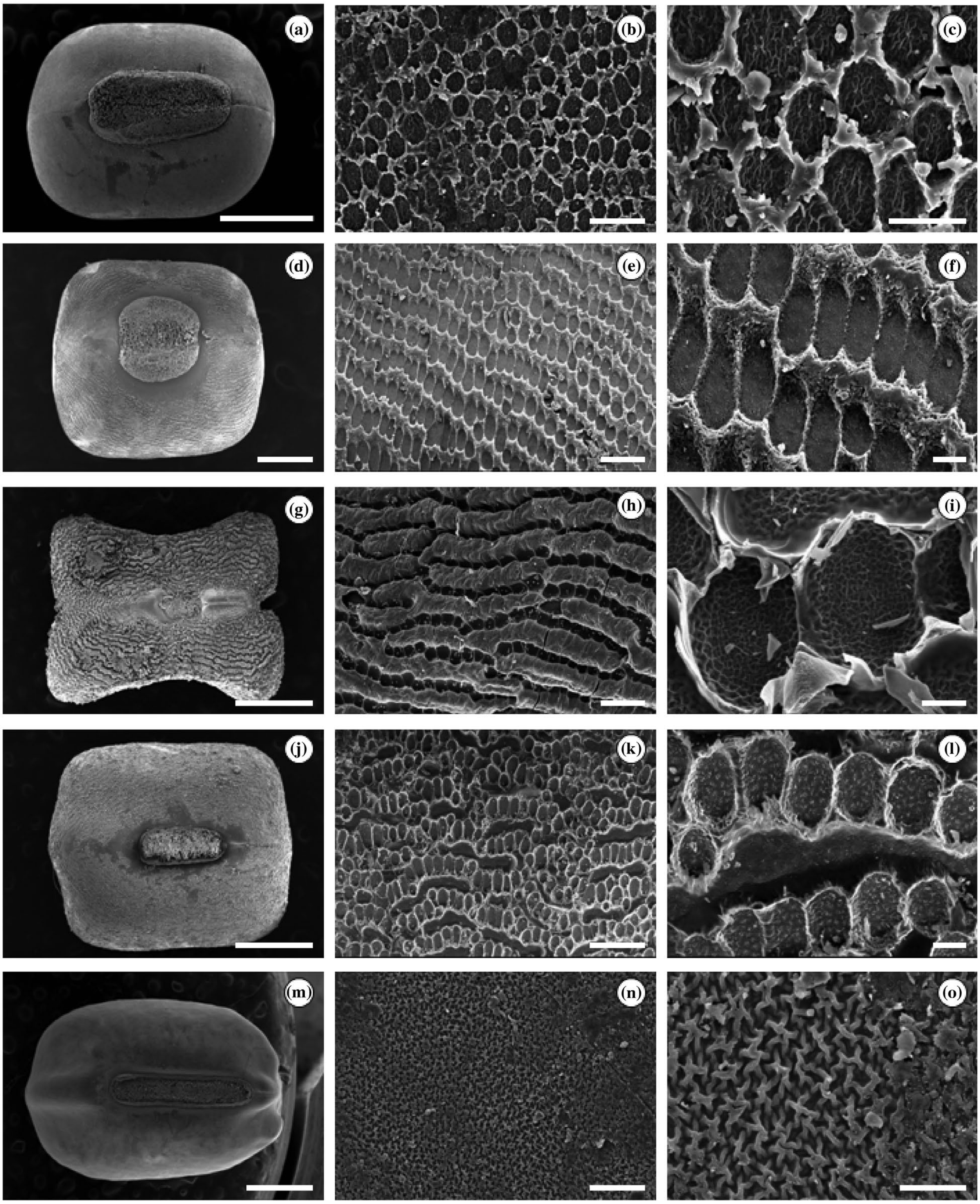

Figure 3. Scanning electron micrographs of seeds. **(A–C)** *Vigna stipulacea*; **(D–F)** *Vigna trilobata*; **(G–I)** *Vigna indica*; **(J–L)** *Vigna khandalensis*; **(M–O)** *Vigna angularis*. Scale bars: A, D, G, J, M, 1 mm; B, E, H, K, N, 100 μm; C, F, I, L, O, 10 μm.

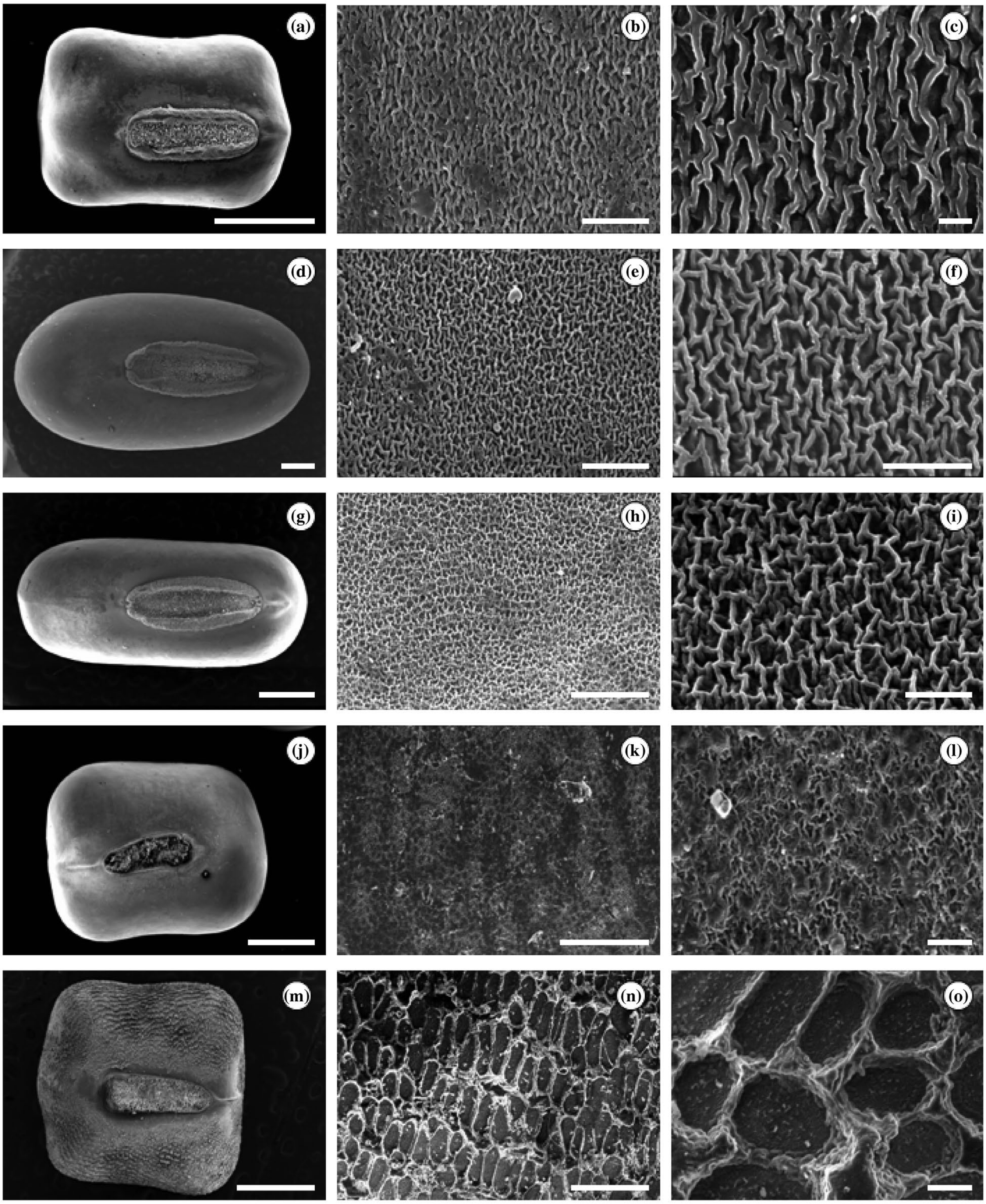

Figure 4. Scanning electron micrographs of seeds. **(A–C)** *Vigna dalzelliana*; **(D–F)** *Vigna umbellata* (cult.); **(G–I)** *Vigna umbellata* (wild); **(J–L)** *Vigna glabrescens*; **(M–O)** *Vigna trinervia* var. *trinervia*. Scale bars: A, D, G, J, M, 1 mm; B, E, H, K, N, 100 μm; C, F, I, L, O, 10 μm.

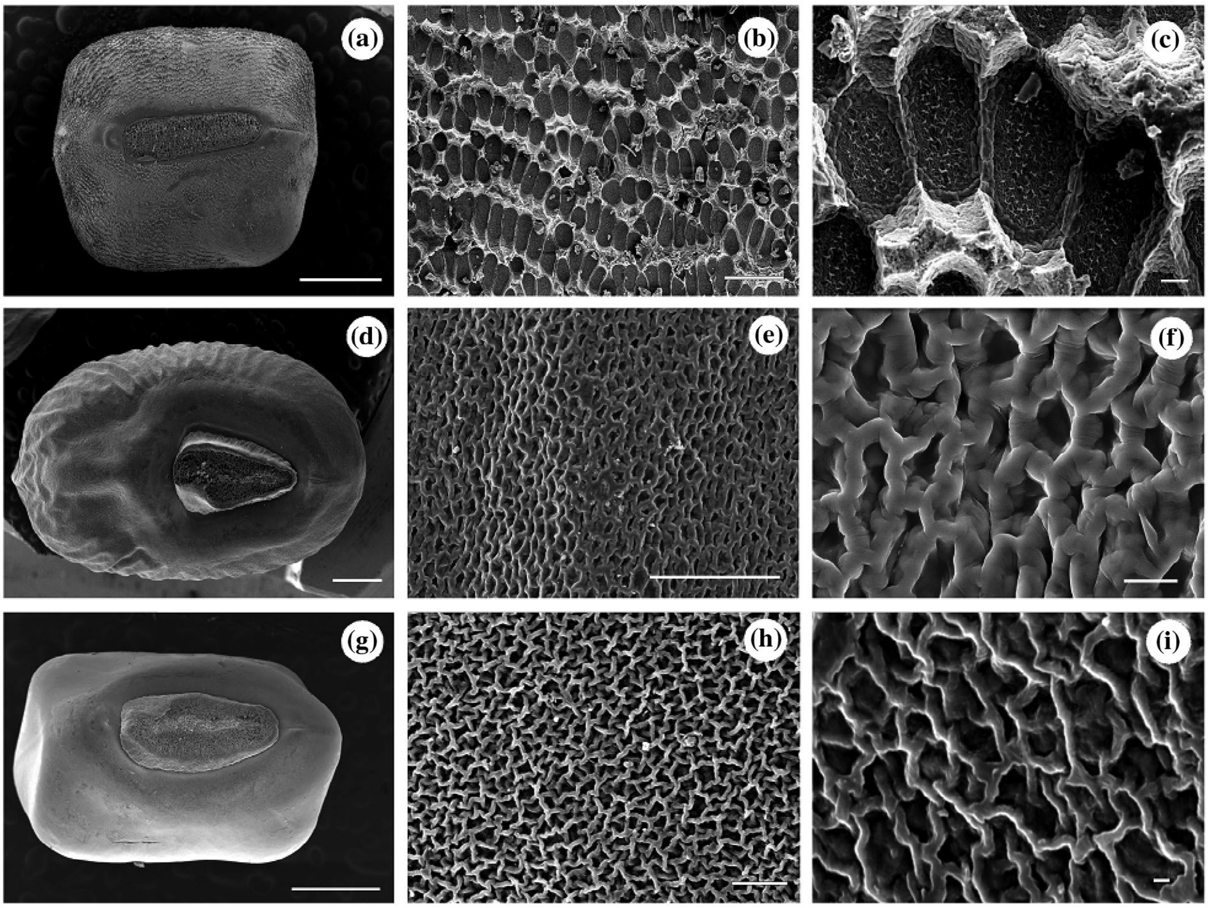

Figure 5. Scanning electron micrographs of seeds. **(A–C)** *Vigna trinervia* var. *bourneae*; **(D–F)** *Vigna unguiculata*; **(G–I)** *Vigna vexillata*. Scale bars: A, D, G, 1 mm; B, E, H, 100 µm; C, F, I, 10 µm.

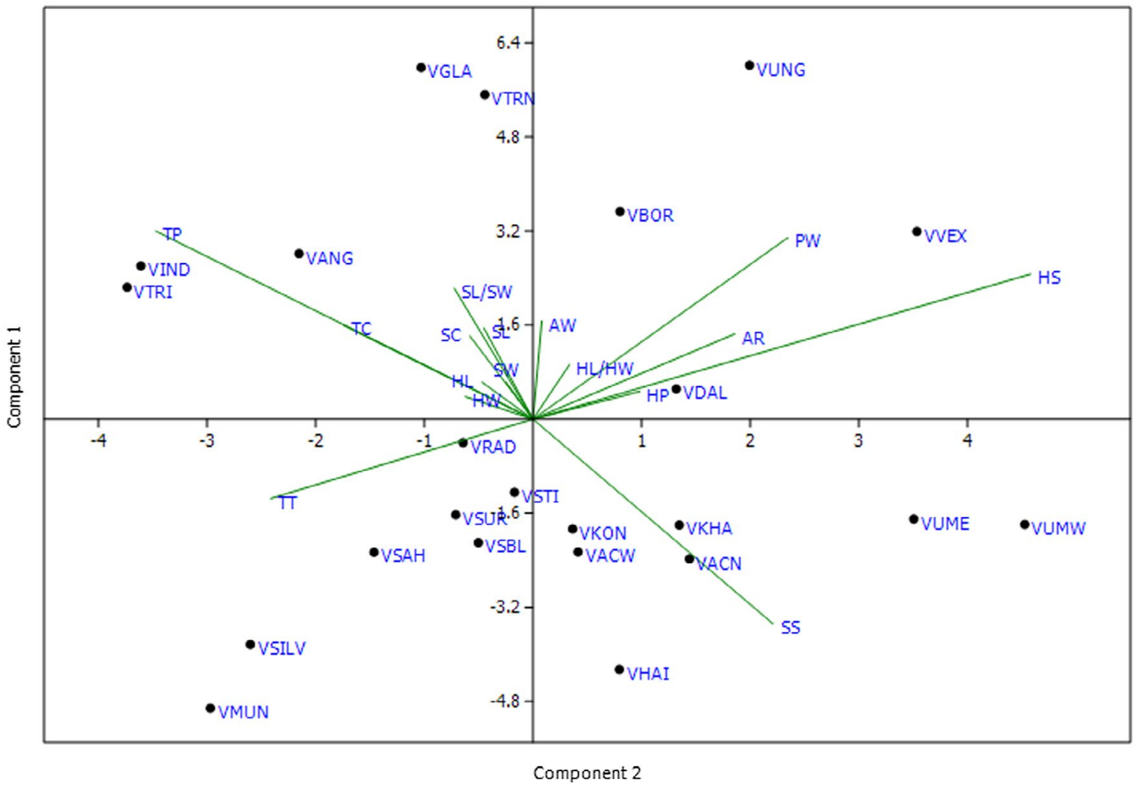

Figure 6. Principal component analysis of the Asian *Vigna* based on seed morphological characters.

Table 5. Eigenvectors of morphological and morphometrical variables explained by the first five principal components.

Character states	PC 1	PC 2	PC 3	PC4	PC5
SS	−0.236	−0.083	−0.267	0.167	0.202
SC	0.127	0.066	0.339	−0.247	−0.363
TT	−0.327	0.325	0.168	−0.059	−0.007
HP	0.294	−0.248	−0.322	−0.027	−0.110
HS	0.280	−0.372	−0.030	0.001	0.091
AR	0.368	−0.179	−0.195	0.062	0.113
TP	0.165	0.054	0.499	0.109	0.448
TC	0.134	0.343	−0.024	0.307	0.019
AW	0.169	−0.064	0.286	−0.089	−0.511
PW	0.339	−0.064	−0.039	0.391	−0.184
SL	0.314	0.289	0.006	−0.176	−0.112
SW	0.242	0.319	−0.192	−0.330	−0.069
SL/SW	0.211	0.033	0.400	0.474	0.076
HL	0.245	0.328	−0.103	−0.161	0.339
HW	0.148	0.464	−0.277	0.225	−0.042
HL/HW	0.221	−0.115	0.160	−0.443	0.398

Notes: SS, seed size; SC, seed colour; TT, testa texture; HP, hilum position; HS, hilum shape; AR, aril; TP, testa pattern; TC, testa cell; AW, anticlinal wall; PW periclinal wall; SL, seed length; SW, seed width; SL/SW, seed length/seed width; HL hilum length; HW, hilum width; HL/HW, hilum length/hilum width.

Key to the species based on seed morphological characters

1. Hilum central..2
Hilum sub-central..18
2. Testa rough with reticulate pattern...................3
Testa smooth with reticulate, colliculate-pusticulate, ruminate pattern..12
3. Aril well developed, hilum elliptic to linear, protruding..4
Aril not developed, hilum linear to oblong, rectangular...6
4. Testa cells broad polygonal...............***V. silvestris***
Testa cells elongated polygonal................................5
5. Seed round, oblongoid, testa cell wall papillat e...***V. mungo* (cult.)**
Seed rectangular with roundish ends, testa wall undulate……………………….........***V. sahyadriana***
6. Hilum oblong..7
Hilum linear, rounded rectangular and obovate...10
7. Testa cells broad hexagonal to polygonal, arranged in tires..8
Testa cells broad hexagonal, arranged irregularly...9
8. Testa cell elongated with thick longitudinal thin transverse wall.......................................***V. sublobata***
Testa cell broad, anticlinal wall irregularly thick ....***V. khandalensis***
9. Anticlinal wall granulate with indistinct thickness, periclinal wall granulate................***V. hainiana***
Anticlinal wall papillate, periclinal wall compact-reticulate..........................***V. trinervia* var. *trinervia***
10. Hilum linear and periclinal wall loosely reticulate...................................***V. trinervia* var. *bourneae***
Hilum rectangular and obovate periclinal wall loose-reticulate..11
11. Seed ovoid, testa cell with thin longitudinal wall...***V. konkanensis***
Seed oblong, testa cell with thick longitudinal wall. ....***V. subramaniana***
12. Reticulate testa pattern..................................13
Colliculate-pusticulate, ruminate testa pattern....16
13. Seed with simple reticulate testa pattern......14
Seed with reticulate pusticulate, reticulate - foveate to reticulate testa...15
14. Aril well developed, hilum broadly ovate or orbicular, protruding................................***V. trilobata***
Aril not developed, hilum oblong...***V. radiata* (cult.)**
15. Hilum narrowly elliptic................***V. glabrescens***
Hilum oblong, protruding......................***V. stipulacea***
16. Ruminate testa, hilum linear.......................... ....***V. angularis* (cult.)**
Colliculate-pusticulate testa, hilum oblong............17
17. Testa cell wall not raised, wavy with indistinct thickness...................................***V. aconitifolia* (cult)**
Testa cell wall raised, wavy....***V. aconitifolia* (wild)**
18. Rough, sub-striate testa; aril not developed..... ....***V. indica***
Smooth testa with ruminate or reticulate pattern....19
19. Seed testa ruminate-channelled, ruminate-reticulate...20
Seed testa reticulate...22
20. Aril less developed, ruminate-channelled....... ....***V. dalzelliana***
Aril well developed, ruminate-reticulate...............21
21. Seeds large 5.68-7.25 × 2.93-3.41 mm............ ....***V. umbellata* (cult.)**
Seeds small 2.79-6.74 × 1.79-4.02 mm.................. ....***V. umbellata* (wild)**
22. Seed testa simple reticulate............................ ....***V. unguiculata* (cult.)**
Seed testa reticulate to scalariform..........***V. vexillata***

Discussion

The present study demonstrates that the seed surface of species of subgenus *Ceratotropis* has remarkable variations and adds some novel features to the structure described earlier. The results recorded in the present study were in consonance with the morphological studies of Maréchal, Mascherpa, and Stainier (1978), Tomooka, Maxted et al. (2002), Yadav et al. (2014), Umdale et al. (2017) and the molecular studies of Doi et al. (2002), Saini, Reddy, and Jawali (2008), Saini and Jawali (2009) and Javadi et al. (2011).

Section Ceratotropis

In India, the section *Ceratotropis* of subgenus *Ceratotropis* of genus *Vigna* is represented by eight species (Aitawade et al. 2012; Latha et al. 2014; Yadav et al.

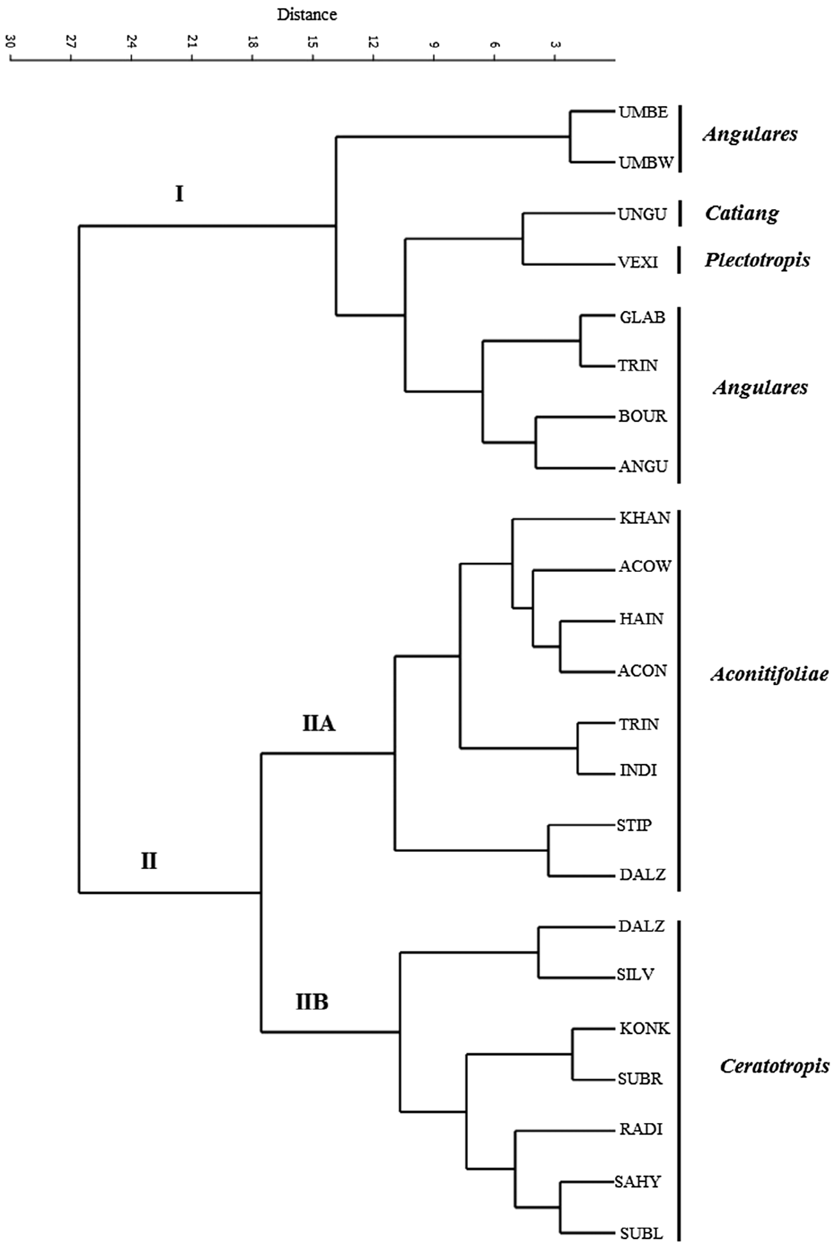

Figure 7. UPGMA dendrogram showing similarity distance of the examined taxa of Asian *Vigna*.

2014). The section *Ceratotropis* is divided into a Mungo group and a Radiata group based on isozyme markers (Jaaska and Jaaska 1990), proteinase inhibitor polymorphism (Konarev, Tomooka, and Vaughan 2002); rDNA internal transcribed spacers and *atp*B-*rbc*L sequence analysis (Doi et al. 2002) and random amplified polymorphic DNA analysis (Kaga et al. 1996; Vir, Bhat, and Lakhanpaul 2009).

The present study of the seed morphology of eight species of section *Ceratotropis* also suggests that these taxa could be differentiated into two groups: the Mungo group, which includes *V. mungo*, *V. silvestris* and *V. sahyadriana*, and the Radiata group, which includes *V. radiata*, *V. sublobata*, *V. subramaniana* and *V. konkanensis*. The species of the Mungo group have been well differentiated from the Radiata group by their unique hilum and aril structure. Among the species of the Mungo group, the cultivated species (*V. mungo*) has larger seeds than the wild species (*V. silvestris*). Both *V. sahyadriana* and *V. silvestris* are morphologically very similar and have similar geographical distribution.

According to Aitawade et al. (2012) and Takahashi et al. (2016) *V. sahyadriana* is distinct and closely related to *V. silvestris*. In the study, it was found that the seed morphologies of *V. silvestris* (Figure 1E, F) and *V. sahyadriana* (Figure 1H, I) were indeed very similar to each other except for the structure of the anticlinal and periclinal walls of the testa cell. Our seed morphology findings fully concur with those of Aitawade et al. (2012). The *V. sublobata* showed a very rough seed coat and rectangular seed, which differentiates it from *V. radiata*. Sharma, Babu, and Johri (1983) and Kumar and Rangaswamy (1984) noted polymorphic seed coat in *V. sublobata* accessions. In the present investigation, *V. sublobata* seeds collected from different eco-geographic regions of India have also been shown seed coat polymorphism.

Trivedi and Gupta (1985, 1986) reported a close relationship between *V. mungo*, *V. sublobata* and *V. radiata* on the basis of reticulate seed coat pattern. However, in the present study, *V. mungo* showed a distinct protruding hilum with well-developed aril (Figure 1C), from which we propose that the hilum structure plays a decisive role in distinguishing the Mungo group from the Radiata group.

Recently, Latha et al. (2014) described a new taxon *V. konkanensis* from the west coast of India, which is closely allied to *V. sublobata* and *V. hainiana*. The seeds of *V. konkanensis* are quite different from the raised, reticulate testa of *V. sublobata* (Latha et al. 2014) and the granulate waxy mounds with indistinct thickness in the anticlinal wall of the testa cell of *V. hainiana* (Figure 2I). The seeds of *V. konkanensis* were found to be different from those of *V. sublobata* in having an ovoid seed shape (Figure 2A), obovate hilum, testa cells that were irregularly arranged and broadly hexagonal testa cells with loosely reticulate periclinal wall (Figure 2D). Babu, Sharma, and Johri (1985) and Vir, Bhat, and Lakhanpaul (2009) suggested that *V. hainiana* could be the common progenitor of wild relatives of *V. mungo* and *V. radiata*. In the present study, *V. hainiana* showed intermediate characters between *V. radiata* and *V. mungo* with respect to the hilum structure and seed coat pattern, which further confirms the conclusion of Babu, Sharma, and Johri (1985) and Vir, Bhat, and Lakhanpaul (2009). In UPGMA cluster analysis, *V. hainiana* is placed in cluster IIA (Mungo group). The testa patterns of *V. subramaniana* showed less developed aril and loosely reticulate periclinal wall (like *V. radiata*) and support the view that *V. subramaniana* could be a species of the Radiata group (Tomooka, Vaughan et al. 2002).

Section *Aconitifoliae*

Dixit et al. (2011) were the first authors to study the morphology of five species of section *Aconitifoliae* occurring in India. They recorded crested and simple-reticulate testa pattern in species of section *Aconitifoliae* (Table 4).

As in the study of Dixit et al. (2011), we confirmed that seed texture, presence or absence of an aril, and seed ornamentation can play a significant role in species delineation within the section *Aconitifoliae*. Among the five taxa studied, *V. aconitifolia* showed comparatively less raised, wavy and indistinct thickness of testa cell in cultigens (Figure 2L), and raised testa cells with wavy anticlinal wall in wild taxa (Figure 2O). *Vigna trilobata* (Figures 2A–C) and *V. stipulacea* (Figures 2M–O) are part of a species complex. Probably, due to incorrect identification, Kumar and Rangaswamy (1984); Chandel, Malik, and Nayar (1991) and Nath and Dasgupta (2015) wrongly described *V. stipulacea* as *V. trilobata*. Morphologically *V. trilobata* and *V. stipulacea* are closely related (Tomooka, Maxted et al. 2002). Dixit et al. (2011) were the first to highlight the difference between these two species in India.

In *V. stipulacea* seed testa cells are polygonal with raised sharp anticlinal walls and flat, reticulate periclinal wall, whereas in *V. trilobata* testa cells are broad polygonal, arranged in tiers with thick longitudinal and thin transverse walls, which are disappearing at the centre. A striking difference in the arrangement of testa cells was observed in *V. khandalensis* (Figures 3D, E) whereas *V. indica* presented a substriate testa pattern (Figure 3A), and subcentral hilum position (Figure 3B), which revealed that these taxa are far distant from the species of section *Aconitifoliae*. According to Takahashi et al. (2016), in the phylogenetic trees, *V. indica* showed the closest relationship with *V. aconitifolia*, and *V. khandalensis* was most closely related to *V. stipulacea*.

Section *Angulares*

The species of section *Angulares* are distinct and characterized by large flower, well-developed keel pocket, long style beak and distribution in high rainfall and high altitude (Tomooka, Vaughan et al. 2002). The smooth seed coat with ruminated testa pattern, comparatively large hilum length and hilum length : width ratio, smooth anticlinal wall of *V. angularis*, *V. dalzelliana* and *V. umbellata* indeed showed the distinctiveness of section *Angulares*. A similar type of pattern has been reported for *V. umbellata* by Kumar and Rangaswamy (1984); Gopinathan and Babu (1985) and Chandel, Malik, and Nayar (1991).

Morphologically, *V. dalzelliana* resembles *V. umbellata*, having glabrous or minutely hairy, slightly sickle-shaped pods with a characteristic long linear hilum (Bisht et al. 2005). However, in the present study, we observed distinct characters including small seeds, channelled testa cells with smooth anticlinal walls in *V. dalzelliana* (Figures 4A–C) and large seed, polygonal testa cell with smooth, undulate anticlinal walls in *V. umbellata* (Figure 4G–I). Cultivated and wild *V. umbellata* were differentiated by large seed size and loosely reticulate testa cell in cultigens (Figures 4D–F), against small seed size and compact reticulate testa cells in wild accessions of *V. umbellata* (Figure 4G–I).

The brown bristle-like hairs on mature pods and rough seed coat in *V. trinervia* showed resemblance with the section *Ceratotropis*, but hypogeal seed germination and petiolate first and second leaves are distinctive characters of section *Angulares* (Tateishi 1985; Tomooka, Vaughan et al. 2002). Similarly, the present study on seed morphology revealed a resemblance to species of section *Ceratotropis* with respect to the seed shape and arrangement of the testa cell (Figures 4 M–O, 5A–C). Tateishi (1985) suggested an intermediate position of *V. trinervia* between section *Ceratotropis* and section *Angulares*. Furthermore, he concluded that species of section *Angulares* were derived from section *Ceratotropis* through *V. trinervia*. The intermediate position *V. trinervia* has also been reported by Tomooka, Yoon et al. (2002) and Doi et al. (2002). The rectangular seeds with oblong hilum, irregular thickness of longitudinal and transverse walls in *V. trinervia* var. *trinervia*, and the rectangular seeds with roundish ends, long linear hilum, testa cell arranged in tiers, thick longitudinal and thin, sharp transverse wall (Figure 5A–C) in *V. trinervia* var. *bourneae*, further confirmed varietal status of the taxa, as reported by Tateishi and Maxted (2002) and Tomooka, Maxted et al. (2002). Another confused species distinction between *V. glabrescens* and *V. sublobata* has been resolved. The unique reticulate-pusticulate testa pattern (Figure 1N) of *V. glabrescens* can be easily differentiated from reticulate testa (Figure 4K) of *V. sublobata*.

Vigna vexillata (wild) belonging to subgenus *Plectotropis* and *V. unguiculata* (cultivated) of subgenus *Vigna* are frequently distributed in India. Both *V. unguiculata* and *V. vexillata* were clearly distinguished from species of subgenus *Ceratotropis* by large seed size (Table 2), oblong to reniform seed shape (Figure 5D, G), subcentral hilum and distinct reticulate to scalariform seed testa pattern (Figure 5F, I). Similar seed morphological features have been reported by Kumar and Rangaswamy (1984) for *V. vexillata* and *V. unguiculata*.

Conclusion

The present study demonstrated that species of subgenus *Ceratotropis* exhibit high seed coat polymorphism. The seed characters, which include seed shape, testa texture, hilum, aril and testa pattern, were identified as taxonomically significant. Seed data were congruent with classification of Tomooka, Vaughan et al. (2002) for delineation of existing sections in subgenus *Ceratotropis*. As a very first attempt so far, our investigation described unique hilum structure and periclinal wall of testa cell of *V. trilobata*, *V. khandalensis*, *V. indica*, *V. hainiana*, *V. sahyadriana*, *V. subramaniana* and *V. konkanensis*. The periclinal wall of the testa cell showed species-specific features and, further supported by hilum structure, proved to be important for taxonomic delineation of species in subgenus *Ceratotropis*. The present investigation highlighted the presence of intraspecific variations in *V. sublobata* and *V. trinervia*, as reported in earlier studies. Hence the macromorphology and micromorphology can significantly contribute to further taxonomic and phylogenetic studies of the subgenus *Ceratotropis* of genus *Vigna*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Notes on contributors

Suraj D. Umdale Assistant professor (YCIS, Satara), research interests in plant molecular taxonomy, plant tissue culture and authentication of traditional Indian medicinal herbs. *Contribution:* laboratory experiments, interpretation of the observations and manuscript writing.

Pravin D. Patil Researcher (NBPGR), research interests in plant taxonomy and pollen pistil interaction studies among crop plants. *Contribution*: laboratory experiments and analysis of data.

Surendra K. Malik Principle scientist at Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, New Delhi, India. *Contribution*: laboratory experiments and provided scanning electron microscopy facilities.

M. Latha Principle scientist at NBPGR, Regional station, Thrissur, Kerala. Her specialization is economic botany and she has immensely contributed to the conservation and characterization of crop wild relatives of *Vigna*, *Cucumis* and *Abelmoschus*. *Contribution*: statistical analysis.

Satyawada R. Rao Presently, Professor and Head, Department of Biotechnology & Bio-Informatics, North Eastern Hill University, Shillong, Meghalaya (India). He specializes in plant biotechnology, cellular and molecular cytogenetics, conservation biology. *Contribution*: collection of *Vigna* germplasm and analysis of seed morphological data.

Shrirang R. Yadav Professor, Department of Botany, Shivaji University, Kolhapur, Maharashtra, India. His specialization is angiosperm taxonomy. *Contribution*: adoption of seed terminologies.

Nikhil B. Gaikwad Associate professor at the Department of Botany, Shivaji University, Kolhapur, Maharashtra, India. His specialization is cytogenetics and plant breeding and he has contributed to the conservation and phytochemical characterization of rare, endemic and endangered medicinal plants from the Western Ghats of India. *Contribution*: experiment design.

Kangila V. Bhat Principle scientist and officer In-charge at Division of Genomic Resources, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. He specializes in the conservation and characterization of crop wild relatives and molecular cytogenetics and conservation biology. *Contribution*: designing experiment and supervision of the research project.

ORCID

Suraj D. Umdale http://orcid.org/0000-0002-9319-5892

References

Aitawade, M. M., S. P. Sutar, S. R. Rao, S. K. Malik, S. R. Yadav, and K. V. Bhat. 2012. "Section *Ceratotropis* of Subgenus *Ceratotropis* of *Vigna* (Leguminosae-Papilionoideae) in India with a New Species from Northern Western Ghats." *Rheedea* 22 (1): 20–27.

Babu, C. R., S. K. Sharma, and B. M. Johri. 1985. "Leguminosae-Papilionoideae: Tribe-Phaseoleae." *Bulletin of the Botanical Survey of India* 27: 1–28.

Barthlott, W. 1981. "Epidermal and Seed Surface Characters of Plants: Systematic Applicability and Some Evolutionary Aspects." *Nordic Journal of Botany* 1 (3): 345–355.

Barthlott, W. 1990. "Scanning Electron Microscopy of the Epidermal Surface in Plants." In *Scanning Electron Microscopy in Taxonomy and Functional Morphology*, edited by D. Claugher, 69–94. Oxford: Clarendon.

Bisht, I. S., K. V. Bhat, S. Lakhanpaul, M. Latha, P. K. Jayan, B. K. Biswas, and A. K. Singh. 2005. "Diversity and Genetic Resources of Wild *Vigna* Species in India." *Genetic Resources and Crop Evolution* 52 (1): 53–68.

Chandel, K. P. S. 1981. "Wild *Vigna* Species in the Himalayas." *Plant Genetic Resources Newsletter* 45: 17–19.

Chandel, K. P. S., R. N. Lester, and R. J. Sterling. 1984. "The Wild Ancestors of Urid and Mung Beans (*Vigna. mungo* (L.) Hepper and *V. radiata* (L.) Wilczek)." *Botanical Journal of the Linnean Society* 89 (1): 85–96.

Chandel, K. P. S., S. K. Malik, and E. R. Nayar. 1991. "SEM Studies of Seed Coat in Some Asiatic *Vigna* Species and Their Taxonomic and Phylogenetic Significance." *Indian Journal of Plant Genetic Resources* 4 (1): 1–11.

de Queiroz, R. T., A. M. Gaulart de Azevedo Tozzi, and G. P. Lewis. 2013. "Seed Morphology: An Addition to the Taxonomy of *Tephrosia* (Leguminosae, Papilionoideae, Millettieae) from South America." *Plant Systematics and Evolution* 299 (2): 459–470.

Delgado-Salinas, A., M. Thulin, R. Pasquet, N. Weeden, and M. Lavin. 2011. "*Vigna (*Leguminosae*) sensu lato:* The Names and Identities of the American Segregate Genera." *American Journal of Botany* 98 (10): 1694–1715.

Dixit, T. M., S. P. Sutar, S. R. Yadav, K. V. Bhat, and S. R. Rao. 2011. *Vigna indica*, a New Name for *Vigna trilobata* var. *pusilla* and a Note on Section *Aconitifoliae* in India. *Rheedea* 21 (1): 1-7.

Doi, K., A. Kaga, N. Tomooka, and D. A. Vaughan. 2002. "Molecular Phylogeny of Genus *Vigna* Subgenus *Ceratotropis* Based on rDNA ITS and *atp*B-*rbc*L Intergenic Spacer for cpDNA Sequences." *Genetica* 114 (2): 129–145.

Gopinathan, M. C., and C. R. Babu. 1985. "Structural Diversity and Its Adaptive Significance in Seeds of *Vigna minima* (Roxb.) Ohwi and Ohashi and Its Allies (Leguminosae-Papilionoideae)." *Annals of Botany* 56 (6): 723–732.

Ignacimuthu, S., and C.R. Babu. 1985. Significance of Seed Coat Pattern in *Vigna radiata* var. *sublobata*. *Proceedings of The National Academy of Sciences, India,* 94 (4): 561–566.

Jaaska, V., and V. Jaaska. 1990. "Isoenzyme Variation in Asian Beans." *Botanica Acta* 103: 281–290.

Javadi, F., Y. Tun, M. Kawase., K. Guan, and H. Yamaguchi. 2011. "Molecular Phylogeny of the Subgenus *Ceratotropis* (Genus *Vigna*, Leguminosae) Reveals Three Eco-Geographical Groups and Late Pliocene-Pleistocene Diversification: Evidence from Four Plastid DNA Region Sequences." *Annals of Botany* 108: 367–380.

Kaga, A., N. Tomooka, Y. Egawa, K. Hosaka, and O. Kamijima. 1996. "Species Relationships in the Subgenus *Ceratotropis* (Genus *Vigna*) as Revealed by RAPD Analysis." *Euphytica* 88 (1): 17–24.

Kirkbride, J. H., C. R. Gunn, and A. L. Weitzman. 2003. *Fruits and Seeds of Genera in the Subfamily Faboideae (Fabaceae)*. vol. 1. Washington: United States Department of Agriculture, Agriculture Research Service, Technical Bulletin No. 1890.

Konarev, A. V., N. Tomooka, and D. A. Vaughan. 2002. "Proteinase Inhibitor Polymorphism in the Genus *Vigna* Subgenus *Ceratotropis* and Its Biosystematic Implications." *Euphytica* 123 (2): 165–177.

Koul, K. K., R. Nagpal, and N. Raina. 2000. "Seed Coat Microsculpturing in *Brassica* and Allied Genera (Subtribes *Brassicinae, Raphaninae, Moricandiinae*)." *Annals of Botany* 86 (2): 385–397.

Kumar, D., and N. S. Rangaswamy. 1984. "SEM Studies on Seed Surface of Wild and Cultivated Species of *Vigna* Savi." *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences* 93 (1): 35–42.

Latha, M., S. Scariah, M. V. Krishnaraj, K. T. Presannakumari, K. V. Bhat, I. S. Bisht, and K. John. 2014. "*Vigna Konkanensis* (Fabaceae: *Papilionoideae*) a New Species from the West Coast of India." *Webbia: Journal of Plant Taxonomy and Geography* 69 (1): 49-52.

Leese, B. M. 1958. "Identification of Asiatic Species of *Phaseolus* by Seed Characters." *The American Midland Naturalist* 60 (1): 132–144.

Lewis, G., B. Schrire, B. MacKinder, and M. Look, eds. 2005. *Legumes of the World*. Kew, UK: Royal Botanical Gardens.

Manning, J. C., and J. V. van Staden. 1987. "The Functional Differentiation of the Testa in Seed of *Indigofera parviflora* (Leguminosae: Papilionoideae)." *Botanical Gazette* 148 (1): 23–34.

Maréchal, R., J. M. Mascherpa, and F. Stainier. 1978. "Étude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques traitées par l'analyse informatique [Taxonomic Study of One Complex Group of Species from the *Phaseolus-Vigna* Genera Based on Morphology and Palynology Data Treated to Computer Analysis]." *Boissiera* 28: 1–273.

Nath, D., and T. Dasgupta. 2015. "Study of Some *Vigna* Species following Scanning Electron Microscopy (SEM)." *International Journal of Scientific and Research Publications* 5 (9): 1–6.

Patil, P., S. K. Malik, S. Sutar, S. R. Yadav, J. John, and K. V. Bhat. 2015. "Taxonomic Importance of Seed Macro- and Micro-Morphology in *Abelmoschus* (Malvaceae)." *Nordic Journal Botany* 33 (6): 696–707.

Rajendra, B. R., K. A. Mujeeb, and L. S. Bates. 1979. "Genetic Analysis of Seed-Coat Types in Interspecific *Vigna* Hybrids via SEM." *Journal of Heredity* 70 (4): 245–249.

Saini, A., and N. Jawali. 2009. "Molecular Evolution of 5S rDNA Region in *Vigna* Subgenus *Ceratotropis* and Its Phylogenetic Implications." *Plant Systematics and Evolution* 280: 187–206.

Saini, A., S. K. Reddy, and N. Jawali. 2008. "Inter-Individual and Intra-Species Heterogeneity in Nuclear rDNA ITS Region of *Vigna* Species from Subgenus *Ceratotropis*." *Genetics Research* 90: 299–316.

Sharma, S. K., C. R. Babu, B. M. Johri, and A. Hopworth. 1977. "SEM Studies on Seed Coat Patterns in *Phaseolus mungo-radiatus-sublobatus* Complex." *Phytomorphology* 27: 106–111.

Sharma, S. K., C. R. Babu, and B. M. Johri. 1983. "Scanning Electron Microscopic Studies on Seed-Coat Polymorphism in the *Phaseolus sublobatus* Roxb. Alliance (Leguminosae-Papilionoideae)." *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences* 49: 41–49.

Stearn, W. T. 1966. *Botanical Latin*. London: Thomas Nelson and Sons.

Takahashi, Y., P. Somta, C. Muto, K. Iseki, K. Naito, M. Pandiyan, S. Natesan, and N. Tomooka. 2016. "Novel Genetic Resources in the Genus *Vigna* Unveiled from Gene Bank Accessions." *PLoS ONE* 11 (1): e0147568.

Tateishi, Y. 1985. *A Revision of the Azuki Bean Group, the Subgenus Ceratotropis of the Genus Vigna (Leguminosae)*. PhD diss., Tohoku University.

Tateishi, Y., and N. Maxted. 2002. "New Species and Combinations in *Vigna* Subgenus *Ceratotropis* (Piper.) Verdc. (Leguminosae, Phaseoleae)." *Kew Bulletin* 57 (3): 625–633.

Thulin, M., M. Lavin, R. Pasquet, and A. Delgado-Salinas. 2004. "Phylogeny and Biogeography of *Wajira* (Leguminosae): A Monophyletic Segregate of *Vigna* Centered in the Horn of Africa Region." *Systematic Botany* 29: 903–920.

Tomooka, N., D. Vaughan, H. Moss, and N. Maxted. 2002. *The Asian Vigna: Genus Vigna Subgenus Ceratotropis Genetic Resources*. Dordrecht: Kluwer Acadmic.

Tomooka, N., N. Maxted, C. Thavarasook, and A. H. M. Jayasuriya. 2002. "Two New Species, Sectional Designation and New Species Combination in *Vigna* Subgenus *Ceratotropis* (Piper) Verdc. (Leguminosae, Phaseoleae)." *Kew Bulletin* 57 (3): 613–624.

Tomooka, N., M. S. Yoon, K. Doi, A. Kaga, and D. Vaughan. 2002. "AFLP Analysis of Diploid Species in the Genus *Vigna* Subgenus *Ceratotropis*." *Genetic Resources and Crop Evolution* 49 (5): 521–530.

Trivedi, B. S., and M. Gupta. 1985. "SEM Studies on the Seed Structure of Some Varieties of Mung Bean (*Vigna radiata* (L.) Wilczek)." In *Trends in Plant Research*, edited by C. M Govil, and V. Kumar, 252-261. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Trivedi, B. S., and M. Gupta. 1986. "SEM Studies on Seed Coat Structure of Some Varieties of *Vigna mungo* (L) Hepper." *Phytomorphology* 36: 271–282.

Trivedi, B. S., G. D. Bagchi, and U. Bajpai. 1979. "Scanning Electron Microscopic Studies on the Spermoderm of Some *Mimosoideae* (Leguminoceae)." *Phytomorphology* 29 (16): 211–218.

Umdale, S. D., M. M. Aitawade, N. B. Gaikwad, L. Madhavan, S. R. Yadav, S. R. Rao, and K. V. Bhat. 2017. "Pollen Morphology of Asian *Vigna* Species (Genus *Vigna*; Subgenus *Ceratotropis*) from India and Its Taxonomic Implications." *Turkish Journal of Botany* 41 (1): 75–81.

Verdcourt, B. 1970. "Studies in the Leguminosae-*Papilionoideae* for the 'Flora of Tropical East Africa: IV'." *Kew Bulletin* 24: 507–569.

Vir, R., K. V. Bhat, and S. Lakhanpaul. 2009. "Analysis of Population Substructure, Genetic Differentiation and Phylogenetic Relationship among Selected Asiatic *Vigna* Savi Species." *Genetic Resources and Crop Evolution* 56 (6): 783–795.

Yadav, S. R., M. M. Aitawade, S. Shrikant, K. V. Bhat, M. Latha, J. K. John, S. K. Malik, S. D. Umdale, and S. R. Rao. 2014. *Genus Vigna Savi in India: An Illustrated Guide for Species Identification*. New Delhi: NBPGR.