

## Pollen morphology of Asian *Vigna* species (genus *Vigna*; subgenus *Ceratotropis*) from India and its taxonomic implications

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**Abstract:** The pollen morphology of 22 Asian *Vigna* [subgenus *Ceratotropis* (Piper) Verdc.] species, including wild and cultivated taxa from India, was investigated with light microscopy and scanning electron microscopy. The quantitative data were analyzed by descriptive statistics and multivariate statistics. The pollen grains are prolate spheroidal in shape with equatorial diameter of 36.06–116.33  $\mu\text{m}$  and polar diameter of 36.31–120.96  $\mu\text{m}$  in equatorial view. The small pollen dimensions were observed in *Vigna hainiana*, *V. trilobata*, and *V. indica* and the largest in *V. vexillata*. The pollen grains have reticulate-homobrochate and reticulate-heterobrochate types of pollen sculpture. The lumina are psilate or granulate with penta-hexagonal, penta-polygonal, and irregular shape. The muri are sinuous, straight, or narrow-straight. Pair group method using arithmetic averages analysis was used to divide the main pollen taxa into four groups based on pollen morphology. Statistically distinctive taxa were identified using principal component analysis. In the present investigation, the taxa studied demonstrated sufficient pollen polymorphism in their sizes, equatorial views, lumina shapes, and muri widths.

**Key words:** *Ceratotropis*, palynology, principal component analysis, pollen morphology, *Vigna*

### 1. Introduction

The genus *Vigna* Savi consists of 104 species that are distributed throughout the world (Lewis et al., 2005). The genus *Vigna* is divided into five subgenera: *Ceratotropis* (Piper) Verdc., *Haydonia* (Wilczek) Verdc., *Lasiosporon* (Benth.) Verdc., *Plectotropis* (Schum.) Baker, and *Vigna* Savi (Verdcourt, 1970; Thulin et al., 2004; Delgado-Salinas et al., 2011). India, with 24 species (Sanjappa, 1992), represents a center of diversity for the subgenus *Ceratotropis* of genus *Vigna* (Arora, 1985; Babu et al., 1985; Bisht et al., 2005; Yadav et al., 2014). Recently, *V. trilobata* (L.) Verdc. var. *pusilla* Naik & Pokle was raised to the rank of species as *V. indica* by Dixit et al. (2011). One new species, *V. sahyadriana*, and one new combination, *V. silvestris* from varietal status *V. mungo* var. *silvestris*, were described by Aitawade et al. (2012), and in another report, Latha et al. (2014) described the new species *V. konkanensis* from the west coast of India.

The subgenus *Ceratotropis* has been divided into three sections, *Ceratotropis* Tomooka & Maxted, *Aconitifoliae*

Tomooka & Maxted, and *Angulares* Tomooka & Maxted, on the basis of habit and habitat, seedling characteristics, and size of floral parts (Tateishi 1996; Tomooka et al., 2002a). The sectional classification of subgenus *Ceratotropis* was confirmed by several researchers (Jaaska and Jaaska, 1990; Kaga et al., 1996; Doi et al., 2002; Konarev et al., 2002; Tomooka et al., 2002a, 2002b; Saini et al., 2008). Moreover, morphological studies and molecular approaches have been used efficiently for the taxonomy of the subgenus *Ceratotropis* (Verdcourt, 1970; Maréchal et al., 1978; Egawa and Tomooka, 1994; Lawn, 1995; Tateishi, 1996; Doi et al., 2002; Goel et al., 2002; Tateishi and Maxted, 2002; Tomooka et al., 2002b; Bisht et al., 2005; Tun and Yamaguchi, 2007; Saini et al., 2008; Saini and Jawali, 2009; Vir et al., 2010; Dixit et al., 2011; Javadi et al., 2011; Aitawade et al., 2012; Latha et al., 2014; Yadav et al., 2014).

Pollen morphological studies of the genus *Vigna* are deficient. Vishnu-Mittre and Sharma (1962), in their study on Indian pollen grains, investigated six species of the genus *Vigna* for the first time. Later on, pollen

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morphological studies of genus *Vigna* species were undertaken by Taylor (1966), Verdcourt (1970), Bronckers et al. (1972), Stainier (1974), Maréchal et al. (1978), and Wael (2009). The pollen sculpture characteristics in the subgenus *Ceratotropis* have never been described in detail and have not yet been explored for differentiation of species using scanning electron microscopy (SEM). The aim of this study was to carry out a detailed analysis of pollen grains of the subgenus *Ceratotropis* in India and to correlate the palynological data with the species relationships among sections *Ceratotropis*, *Aconitifoliae*, and *Angulares* of subgenus *Ceratotropis*.

## 2. Materials and methods

The wild and cultivated taxa of the subgenus *Ceratotropis* used in the present investigation were collected during 2008–2013 (Appendix). Pollen samples were collected from flowers and preserved in acetic acid. These specimens are maintained in the botanical garden of Shivaji University, Kolhapur. The seeds germplasms were deposited in the Seed Bank of the National Bureau of Plant Genetic Resources, New Delhi, India.

Pollen grains were prepared for light microscopy according to the technique of Erdtman (1943, 1952). The pollen grains were stained with safranin in glycerin gel. The slide was slightly heated and a cover-slip was placed on it. The pollen samples were examined with a Leica DM2000 microscope with a digital photomicrograph system. Each of the characteristics of polar diameter and equatorial diameter were measured for 20 pollen grains per sample of each specimen. The P/E ratios were calculated and results were provided as range, mean, and standard error.

For SEM, the pollen grains of each specimen were transferred onto aluminum stubs and coated with gold for 4 min in an Auto Fine JFC-1600 sputter coater. Micromorphological observations were made with a JEOL JSM-6360 scanning electron microscope at the SEM Laboratory, Department of Physics, Shivaji University, Kolhapur. Each of the characteristics of porus width, lumina diameter, muri width, and porus diameter were measured for 10 pollen grains per sample of each taxon. Terminologies were adopted from Punt et al. (1994, 2007) and Hesse et al. (2009) and pollen shape classification follows Kremp (1965) and Erdtman (1969) based on P/E ratios given in Table 1.

A total 13 characters were evaluated for each species, comprising seven morphological (qualitative) and six morphometrical (quantitative) characters. The species of subgenus *Ceratotropis* of genus *Vigna* were clustered based on pollen morphological and morphometrical characters. A dissimilarity index was calculated using the Euclidean distance coefficient and a dendrogram was developed from the unweighted pair group method

using arithmetic averages (UPGMA) clustering method. Principal component analysis (PCA) uses 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. These analysis were performed using PAST (Paleontological Statistics Software Package for Education and Data Analysis) software 3.01 (Hammer et al., 2001).

## 3. Result

Pollen grains of the subgenus *Ceratotropis* showed variation in their morphological characters. Detailed information acquired from light microscopy and SEM for pollen grains is given in Tables 1–3 and Figures 1a–1v, 2a–2l, 3a–3j, 4, and 5. Pollen parameters for studied taxa are described as follows.

### 3.1. Pollen size

The pollen grains of the studied taxa are classified into three groups according to Kremp (1965). Most of the studied pollens are medium and large in size, except *V. vexillata*, which has a very large pollen size. Medium pollens (25–50  $\mu\text{m}$ ) are observed in *V. mungo* (Figure 1a), *V. radiata* (Figure 1d), *V. sublobata* (Figure 1e), *V. konkanensis* (Figure 1f), *V. hainiana* (Figure 1h), *V. trilobata* (Figure 1i), *V. indica* (Figure 1j), *V. aconitifolia* (Figure 1k), and *V. umbellata* (Figures 1s and 1t); large (51–100  $\mu\text{m}$ ) in *V. silvestris* (Figure 1b), *V. sahyadriana* (Figure 1c), *V. subramaniana* (Figure 1g), *V. stipulacea* (Figure 1i), *V. khandalensis* (Figure 1m), *V. angularis* (Figure 1n), *V. dalzelliana* (Figure 1e), *V. glabrescens* (Figure 1p), *V. trinervia* var. *trinervia* (Figure 1q), *V. trinervia* var. *bourneae* (Figure 1r), and *V. unguiculata* (Figure 1v); and very large (101–200  $\mu\text{m}$ ) in *V. vexillata* (Figure 1u).

### 3.2. Shape

All taxa studied show a prolate-spheroidal pollen shape. The smallest pollen dimensions were observed in *V. trilobata*, *V. hainiana*, and *V. indica* and the largest in *V. unguiculata* and *V. vexillata* (Table 2).

### 3.3. Aperture

The pollen grains of studied taxa show a triporate type of aperture. Porus diameter varies from 5.85 to 12.20  $\mu\text{m}$ . The smallest porus diameter is observed in *V. indica* and the largest in *V. vexillata* (Table 1).

### 3.4. Exine ornamentation

All taxa studied show a reticulate type of exine ornamentation. Most of the *Vigna* species evaluated show a reticulate heterobrochate type of ornamentation. The reticulate homobrochate type of ornamentation is observed in *V. radiata* (Figure 2d), *V. sublobata* (Figure 2e), *V. konkanensis* (Figure 2f), *V. stipulacea* (Figure 2i), *V. trinervia* var. *trinervia* (Figure 3e), and *V. trinervia*

**Table 1.** Pollen morphometric characteristics and measurements ( $\mu\text{m}$ ) of the pollen grains of species of subgenus *Ceratotropis* (n = 20).

Taxon	PD		ED		PD/ED	LD		MW		PRD	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE		Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
<i>V. mungo</i>	46.25–48.92	47.32 $\pm$ 0.5	43.94–47.32	45.47 $\pm$ 0.5	1.041	5.08–5.99	5.71 $\pm$ 0.1	0.63–0.74	0.68 $\pm$ 0.02	6.33–6.93	6.70 $\pm$ 0.2
<i>V. silvestris</i>	48.26–51.20	50.10 $\pm$ 0.5	46.98–49.77	47.94 $\pm$ 0.5	1.043	3.01–3.67	3.34 $\pm$ 0.1	0.74–0.79	0.76 $\pm$ 0.01	6.16–6.64	6.33 $\pm$ 0.2
<i>V. sahyadriana</i>	55.25–59.69	57.31 $\pm$ 0.8	53.05–54.29	53.86 $\pm$ 0.2	1.064	4.75–5.28	5.10 $\pm$ 0.1	0.46–0.51	0.48 $\pm$ 0.01	9.33–9.52	9.44 $\pm$ 0.1
<i>V. radiata</i>	45.09–48.64	46.98 $\pm$ 0.6	42.17–47.39	44.88 $\pm$ 0.8	1.047	5.17–6.49	5.70 $\pm$ 0.1	0.56–0.62	0.59 $\pm$ 0.01	8.34–8.98	8.66 $\pm$ 0.2
<i>V. sublobata</i>	45.91–52.07	48.98 $\pm$ 0.8	42.55–46.48	44.81 $\pm$ 0.6	1.093	8.12–9.65	8.88 $\pm$ 0.2	1.07–0.89	0.96 $\pm$ 0.03	7.35–8.69	8.08 $\pm$ 0.3
<i>V. konkanensis</i>	45.89–49.15	47.55 $\pm$ 0.5	42.56–44.46	43.78 $\pm$ 0.3	1.086	6.14–8.62	7.57 $\pm$ 0.2	0.88–0.98	0.92 $\pm$ 0.02	8.48–9.52	8.78 $\pm$ 0.3
<i>V. subramaniana</i>	53.30–55.46	54.33 $\pm$ 0.4	49.68–53.35	51.61 $\pm$ 0.6	1.053	3.29–4.83	4.12 $\pm$ 0.1	0.91–0.99	0.95 $\pm$ 0.01	7.68–7.99	7.81 $\pm$ 0.1
<i>V. hainiana</i>	38.29–42.00	39.73 $\pm$ 0.8	36.48–38.29	37.18 $\pm$ 0.3	1.069	2.33–2.99	2.70 $\pm$ 0.1	1.01–1.29	1.14 $\pm$ 0.06	7.04–7.17	7.12 $\pm$ 0.1
<i>V. trilobata</i>	36.31–39.83	38.57 $\pm$ 0.5	36.06–36.71	36.34 $\pm$ 0.2	1.061	3.57–4.45	4.07 $\pm$ 0.1	0.43–0.57	0.50 $\pm$ 0.02	6.09–7.28	6.59 $\pm$ 0.3
<i>V. indica</i>	40.86–41.68	41.34 $\pm$ 0.1	38.42–40.06	38.84 $\pm$ 0.3	1.064	2.41–3.33	3.07 $\pm$ 0.1	0.59–0.67	0.65 $\pm$ 0.02	5.70–5.98	5.85 $\pm$ 0.1
<i>V. aconitifolia</i>	47.68–48.56	48.03 $\pm$ 0.2	42.78–45.62	44.62 $\pm$ 0.6	1.076	7.60–9.14	8.40 $\pm$ 0.3	0.64–0.74	0.70 $\pm$ 0.02	6.31–6.53	6.45 $\pm$ 0.1
<i>V. stipulacea</i>	52.00–53.79	52.92 $\pm$ 0.3	48.63–51.81	50.36 $\pm$ 0.5	1.051	3.98–5.71	4.84 $\pm$ 0.2	0.57–0.64	0.60 $\pm$ 0.01	6.29–6.68	6.52 $\pm$ 0.1
<i>V. khandalensis</i>	53.18–55.02	54.09 $\pm$ 0.3	46.69–51.33	49.19 $\pm$ 0.9	1.099	4.23–4.79	4.63 $\pm$ 0.1	0.61–0.77	0.69 $\pm$ 0.03	7.02–7.71	7.31 $\pm$ 0.2
<i>V. angularis</i>	51.20–54.14	52.48 $\pm$ 0.4	48.53–51.92	50.57 $\pm$ 0.5	1.038	12.02–14.33	12.80 $\pm$ 0.3	0.92–0.95	0.94 $\pm$ 0.01	10.27–10.64	10.44 $\pm$ 0.2
<i>V. dalzelliana</i>	52.50–57.78	55.22 $\pm$ 0.7	50.21–54.79	52.66 $\pm$ 0.7	1.049	4.56–5.31	4.94 $\pm$ 0.1	0.90–0.97	0.92 $\pm$ 0.01	8.70–8.95	8.81 $\pm$ 0.1
<i>V. glabrescens</i>	60.20–64.97	62.61 $\pm$ 0.9	56.58–65.08	60.51 $\pm$ 1.2	1.035	4.31–5.78	4.99 $\pm$ 0.2	0.70–0.71	0.71 $\pm$ 0.01	7.88–8.67	8.15 $\pm$ 0.3
<i>V. trinervia</i> var. <i>trinervia</i>	59.24–60.81	59.95 $\pm$ 0.3	55.27–58.88	57.15 $\pm$ 0.6	1.049	4.21–6.05	5.51 $\pm$ 0.2	0.74–0.79	0.76 $\pm$ 0.01	7.34–7.94	7.60 $\pm$ 0.2
<i>V. trinervia</i> var. <i>bourneae</i>	57.30–59.20	58.43 $\pm$ 0.3	54.09–58.37	55.82 $\pm$ 0.8	1.047	4.62–5.87	5.30 $\pm$ 0.1	0.72–0.76	0.74 $\pm$ 0.01	7.21–7.91	7.54 $\pm$ 0.2
<i>V. umbellata</i> (cult.)	46.83–50.98	48.26 $\pm$ 0.6	42.69–48.65	45.94 $\pm$ 0.9	1.050	5.09–7.64	6.05 $\pm$ 0.3	0.78–0.82	0.79 $\pm$ 0.01	8.05–8.20	8.14 $\pm$ 0.1
<i>V. umbellata</i> (wild)	45.17–48.98	46.09 $\pm$ 0.6	40.36–43.62	42.43 $\pm$ 0.7	1.086	5.33–6.83	5.92 $\pm$ 0.2	0.70–0.81	0.78 $\pm$ 0.02	8.00–8.19	8.07 $\pm$ 0.1
<i>V. vexillata</i>	113.66–120.96	116.96 $\pm$ 1.3	107.35–116.54	111.77 $\pm$ 1.6	1.046	12.27–15.52	13.87 $\pm$ 0.3	1.03–1.19	1.09 $\pm$ 0.03	12.12–12.25	12.20 $\pm$ 0.1
<i>V. unguiculata</i>	90.95–96.76	94.35 $\pm$ 0.8	83.78–92.80	90.18 $\pm$ 1.35	1.046	8.13–14.83	12.86 $\pm$ 0.7	0.81–0.85	0.83 $\pm$ 0.01	11.12–11.58	11.37 $\pm$ 0.1

SE: Standard error, ED: equatorial diameter, PD: polar diameter, PD/ED: polar diameter/equatorial diameter, LD: lumina diameter, MW: muri width, PRD: porus diameter.

**Table 2.** Pollen micromorphological characters in species of the subgenus *Ceratotropis* of genus *Vigna*.

Taxon	Size	Equatorial view	Exine sculpture	Lumina shape	Lumina texture	Muri
<i>V. mungo</i>	Medium	Elliptical	Reticulate-heterobrochate	Polygonal	Psilate	Narrow-straight
<i>V. silvestris</i>	Large	Elliptical	Reticulate-heterobrochate	Polygonal	Psilate	Straight
<i>V. sahyadriana</i>	Large	Elliptical	Reticulate-heterobrochate	Polygonal	Granulate	Sinuuous
<i>V. radiata</i>	Medium	Elliptical	Reticulate-homobrochate	Pentagonal-hexagonal	Granulate	Narrow-straight
<i>V. sublobata</i>	Medium	Elliptical	Reticulate-homobrochate	Pentagonal-hexagonal	Granulate	Narrow-straight
<i>V. konkanensis</i>	Medium	Elliptical	Reticulate-homobrochate	Pentagonal-hexagonal	Granulate	Narrow-straight
<i>V. subramaniana</i>	Large	Circular	Reticulate-heterobrochate	Pentagonal-polygonal	Granulate	Sinuuous
<i>V. hainiana</i>	Medium	Circular	Reticulate-heterobrochate	Polygonal	Psilate	Sinuuous
<i>V. trilobata</i>	Medium	Circular	Reticulate-heterobrochate	Polygonal	Psilate	Sinuuous
<i>V. indica</i>	Medium	Circular	Reticulate-heterobrochate	Polygonal	Psilate	Sinuuous
<i>V. aconitifolia</i>	Medium	Circular	Reticulate-heterobrochate	Irregular	Psilate	Sinuuous
<i>V. stipulacea</i>	Large	Elliptical	Reticulate-homobrochate	Pentagonal-polygonal	Granulate	Straight
<i>V. khandalensis</i>	Large	Elliptical	Reticulate-heterobrochate	Pentagonal-polygonal	Granulate	Straight
<i>V. angularis</i>	Large	Elliptical	Reticulate-heterobrochate	Polygonal	Granulate	Straight
<i>V. dalzelliana</i>	Large	Circular	Reticulate-heterobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. glabrescens</i>	Large	Elliptical	Reticulate-heterobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. trinervia</i> var. <i>trinervia</i>	Large	Circular	Reticulate-homobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. trinervia</i> var. <i>bourneae</i>	Large	Circular	Reticulate-homobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. umbellata</i> (cult.)	Medium	Elliptical	Reticulate-heterobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. umbellata</i> (wild)	Medium	Elliptical	Reticulate-heterobrochate	Pentagonal-polygonal	Psilate	Straight
<i>V. vexillata</i>	Very large	Elliptical	Reticulate-heterobrochate	Pentagonal-hexagonal	Granulate	Narrow-straight
<i>V. unguiculata</i>	Large	Elliptical	Reticulate-heterobrochate	Polygonal	Granulate	Sinuuous

**Table 3.** Factor loadings showed the most intrinsic characters that enhanced separations of the studied subgenus *Ceratotropis* species.

Variables	Factor loadings				
	Principal components				
	PC 1	PC 2	PC 3	PC 4	PC 5
PS	0.018	-0.066	0.089	-0.174	-0.037
PV	-0.005	-0.066	-0.034	0.000	0.000
SC	0.002	-0.017	-0.142	-0.027	0.182
LU	0.007	0.085	0.025	0.021	-0.158
LS	0.016	0.209	0.059	0.313	0.408
LSH	0.000	-0.067	0.758	0.550	0.380
MU	0.000	0.014	-0.590	0.168	0.286
PD	0.714	-0.046	0.036	-0.146	-0.317
ED	0.692	-0.096	-0.042	0.007	0.013
PD/ED	0.000	0.001	0.001	0.205	-0.169
LD	0.089	0.920	0.090	-0.010	0.040
MW	0.002	0.017	0.006	-0.636	0.641
POD	0.051	0.279	-0.142	-0.040	0.027
Percentage of total variation for the first three factors: 99.55%					

PS: Pollen size, PV: polar view, SC: pollen sculpture, LU: lumina diameter, LS: lumina size, LSH: lumina shape, MU: muri, PD: polar diameter, ED: equatorial diameter, PD/ED: polar diameter/equatorial diameter, LD: lumina diameter, MW: muri width, POD; pollen diameter.

var. *bourneae* (Figure 3f). Lumina shape is irregular in *V. aconitifolia* (Figure 2k); pentagonal-hexagonal in Figures 2d–2f and 3i; pentagonal to polygonal in Figures 2g, 2i, 3a, and 3c–3h; and polygonal in Figures 2a–2c, 2h–2j, 3b, and 3j. Among the species studied, lumen diameter is smallest in *V. hainiana* (Figure 2h), *V. indica* (Figure 2j), and *V. silvestris* (Figure 2b), whereas it is largest in *V. angularis* (Figure 3b), *V. unguiculata* (Figure 3j), and *V. vexillata* (Figure 3i). Lumina are granulate in Figures 2c–2g, 2i, 3a, 3b, 3i, and 3j and psilate in Figures 2a, 2b, 2h–2k, and 3c–3h. The largest lumina are found in the central region of the pollen grain in all the species and lumen diameters decrease towards the pore.

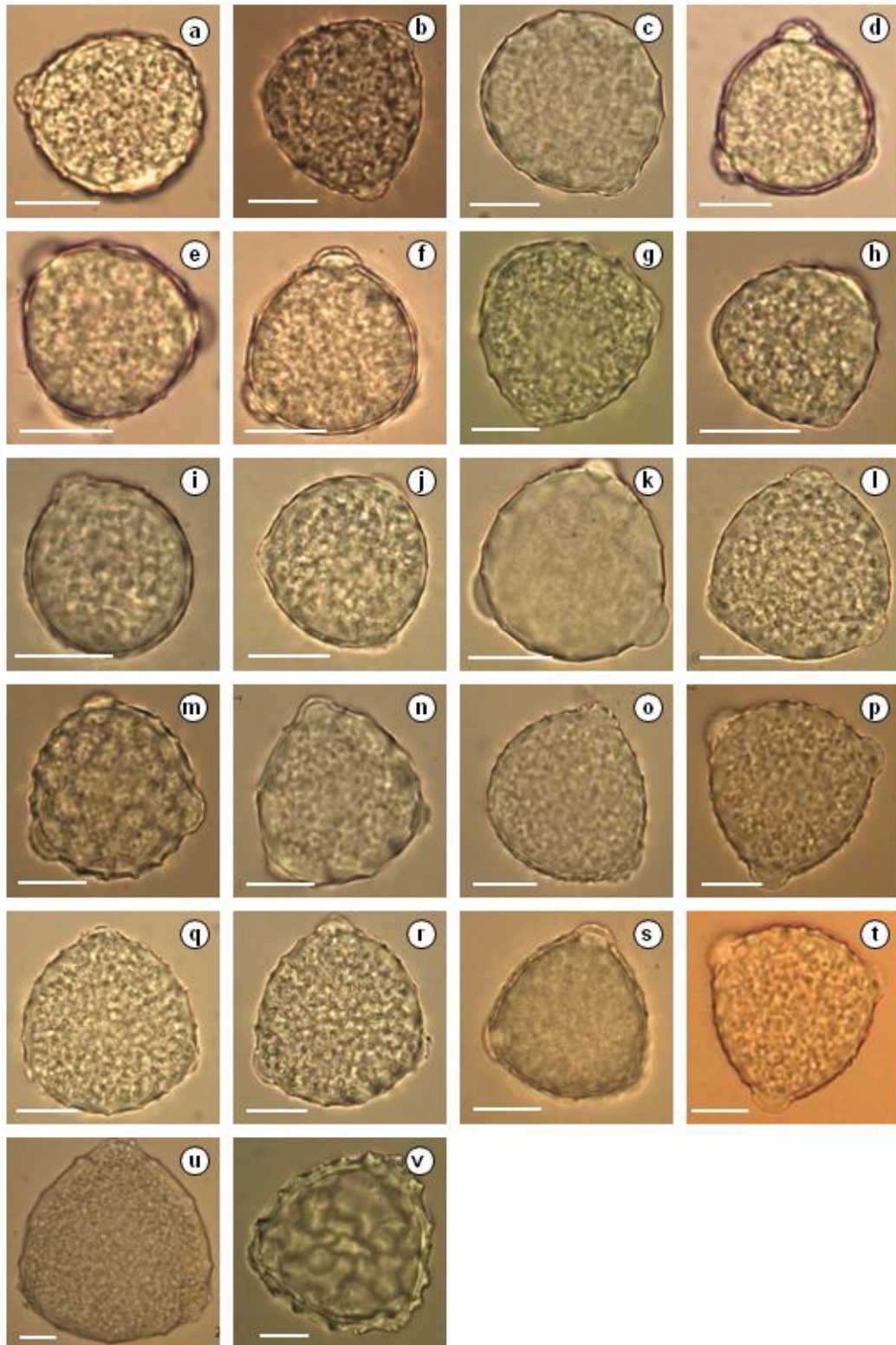
The muri are sinuous in *V. sahyadriana* (Figure 2c), *V. subramaniana* (Figure 2g), *V. hainiana* (Figure 2h), *V. trilobata* (Figure 2i), *V. indica* (Figure 2j), *V. aconitifolia* (Figure 2k), and *V. unguiculata* (Figure 3j); narrow-straight in *V. mungo* (Figure 2a), *V. radiata* (Figure 2d), *V. sublobata* (Figure 2e), *V. konkanensis* (Figure 2f), and *V. unguiculata* (Figure 3j); and straight in *V. silvestris* (Figure 2b), *V. stipulacea* (Figure 2l), *V. khandalensis* (Figure 3a), *V. angularis* (Figure 3b), *V. dalzelliana* (Figure 3c), *V. glabrescens* (Figure 3d), *V. trinervia* var. *trinervia* (Figure 3e), *V. trinervia* var. *bourneae* (Figure 3f), and *V. umbellata* (Figures 3g and 3h). The muri diameter varies from 0.48

to 1.14  $\mu\text{m}$ . Among the taxa studied the muri diameter is small in *V. sahyadriana* and *V. trilobata*. Muri diameter is largest in *V. vexillata* and *V. hainiana* (Table 1).

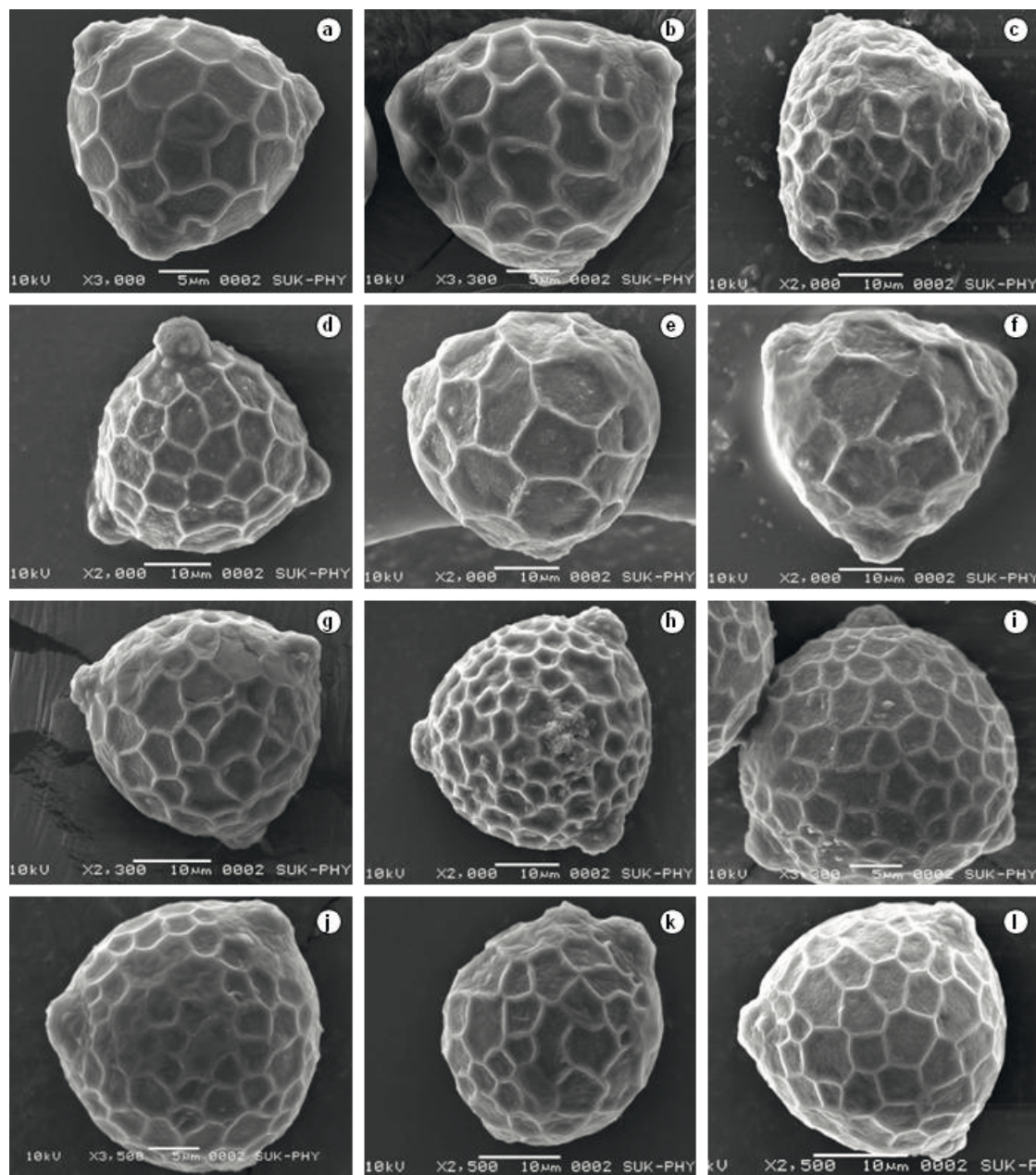
### 3.5. PCA

PCA analysis was done based on 12 character states of 22 taxa (operational taxonomic units) that became evident during palynological investigation. A clear separation of several *Vigna* species was found in PCA. In PCA, the first ten components explained 99.98% of the total variance in the studied taxa. The first three principal components accounted for 99.63% of the variance, in which maximum variability was contributed by the first component (99.48%), followed by the second component (0.94%) and third component (0.21%). The first principle component was most highly influenced by polar diameter, equatorial diameter, lumina diameter, and porus diameter (Table 3). In the second principle component, the traits contributing to the total variability of pollen were lumina diameter, porus diameter, and lumina size. The third principle component was influenced by lumina shape, lumina diameter, and pollen size.

In the PCA scatter plot, on the basis of small pollen size and P/E ratio, *Vigna radiata*, *V. mungo*, *V. sublobata*, *V. konkanensis*, *V. aconitifolia*, and *V. umbellata* (cultivated and wild) formed the first group. The second group



**Figure 1.** Light microscopy micrographs of pollen grains of: a. *V. mungo*; b. *V. silvestris*; c. *V. sahyadriana*; d. *V. radiata*; e. *V. sublobata*; f. *V. konkanensis*; g. *V. subramaniana*; h. *V. hainiana*; i. *V. trilobata*; j. *V. indica*; k. *V. aconitifolia*; l. *V. stipulacea*; m. *V. khandalensis*; n. *V. angularis*; o. *V. dalzelliana*; p. *V. glabrescens*; q. *V. trinervia* var. *trinervia*; r. *V. trinervia* var. *bourneae*; s. *V. umbellata* (cultivated); t. *V. umbellata* (wild); u. *V. vexillata*; v. *V. unguiculata*. Scale bars: 20  $\mu$ m.

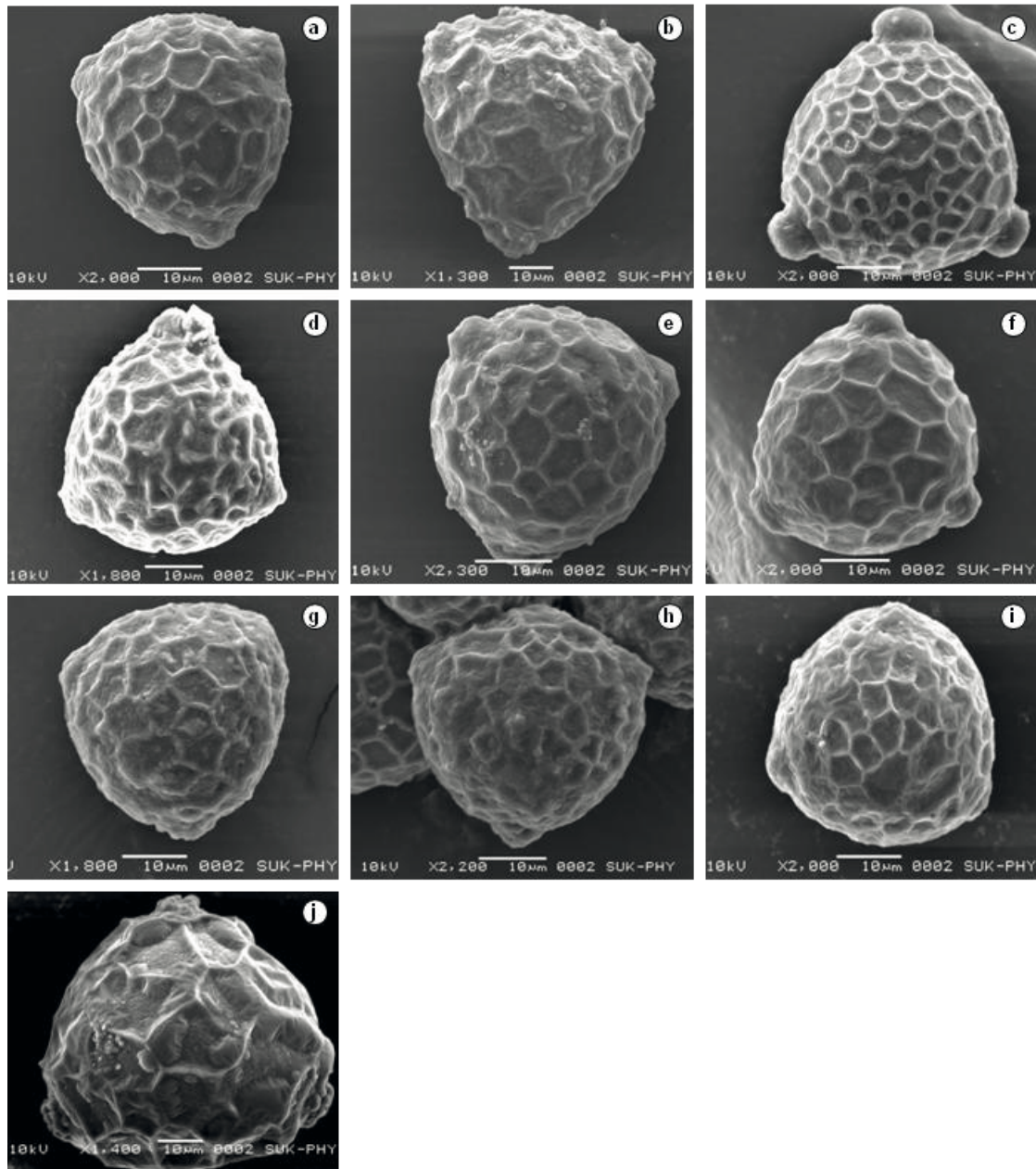


**Figure 2.** SEM micrographs of pollen grains of: a. *V. mungo*; b. *V. silvestris*; c. *V. sahyadriana*; d. *V. radiata*; e. *V. sublobata*; f. *V. konkanensis*; g. *V. subramaniana*; h. *V. hainiana*; i. *V. trilobata*; j. *V. indica*; k. *V. aconitifolia*; l. *V. stipulacea*.

comprised three species: *V. trilobata*, *V. hainiana*, and *V. indica*. *V. silvestris*, *V. sahyadriana*, *V. subramaniana*, *V. stipulacea*, *V. khandalensis*, *V. dalzelliana*, *V. glabrescens*, *V. trinervia* var. *trinervia*, and *V. trinervia* var. *bourneae* formed the third group. *V. angularis* was farthest from all groups due to higher lumina and porus diameter. The fourth group consisted of a species of subgenus *Vigna* Savi, *V. unguiculata*, and a species of subgenus *Plectotropis* (Schumach.) Baker, *V. vexillata*, which have positive values on both axes (Figure 4).

### 3.6. Cluster analysis

The generated UPGMA dendrogram grouped all 22 species into four major clusters (Figure 5) based on pollen morphological characters. The first cluster comprised seven species: *V. mungo*, *V. silvestris*, *V. sahyadriana*, *V. radiata*, *V. sublobata*, *V. konkanensis*, and *V. subramaniana*. The second cluster included three species: *V. hainiana*, *V. trilobata*, and *V. indica*. The third cluster included ten species: *V. aconitifolia*, *V. stipulacea*, *V. khandalensis*, *V. angularis*, *V. dalzelliana*, *V. glabrescens*, *V. trinervia*



**Figure 3.** SEM micrographs of pollen grains of: a. *V. khandalensis*; b. *V. angularis*; c. *V. dalzelliana*; d. *V. glabrescens*; e. *V. trinervia* var. *trinervia*; f. *V. trinervia* var. *bourneae*; g. *V. umbellata* (cult.); h. *V. umbellata* (wild); i. *V. vexillata*; j. *V. unguiculata*.

var. *trinervia*, *V. trinervia* var. *bourneae*, *V. umbellata* (cultivated), and *V. umbellata* (wild). The remaining two species, *V. unguiculata* and *V. vexillata*, were grouped in the fourth cluster.

On the basis of pollen features, main five types were recognized in Asian *Vigna* (subgenus *Ceratotropis*):

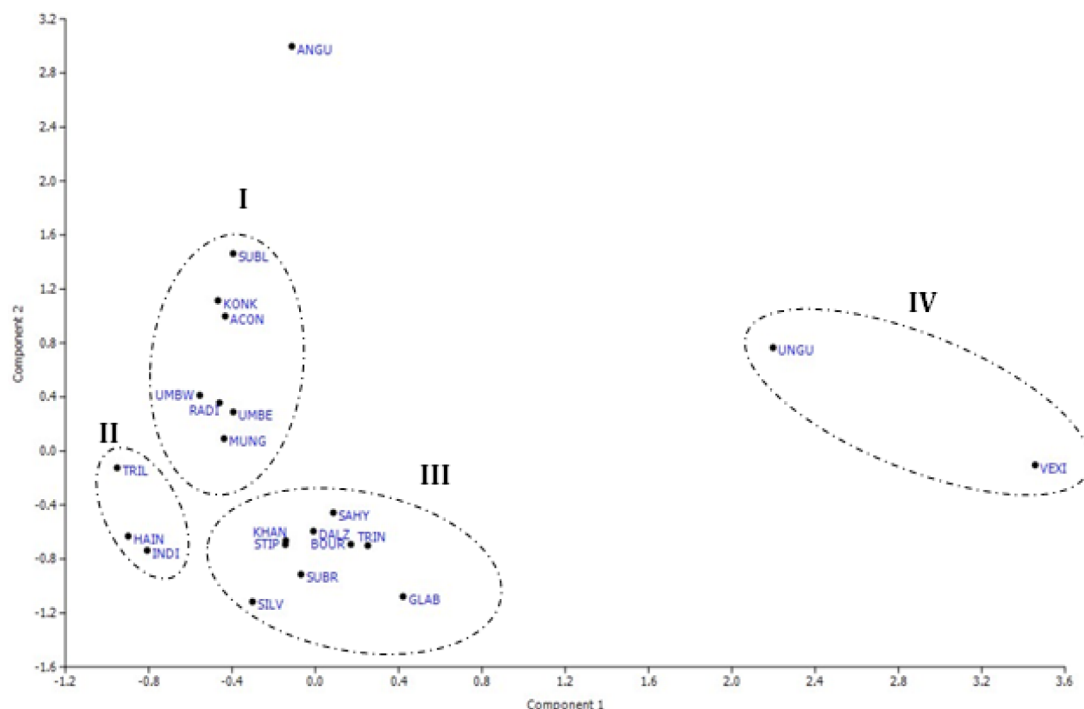
Type I- Pollens medium, elliptical in equatorial view- *V. mungo*, *V. radiata*, *V. sublobata*, *V. konkanensis*, *V. umbellata* (wild), *V. umbellata* (cultivated).

Type II- Pollens medium with sinuous muri- *V. hainiana*, *V. trilobata*, *V. indica*.

Type III- Pollens large with pentagonal to polygonal lumina- *V. silvestris*, *V. sahyadriana*, *V. subramaniana*, *V. stipulacea*, *V. khandalensis*, *V. dalzelliana*, *V. glabrescens*, *V. trinervia* var. *trinervia*, *V. trinervia* var. *bourneae*.

Type IV- Pollens large with granulate lumina- *V. unguiculata*, *V. vexillata*.

Type V- Pollens large with large pore size- *V. angularis*.



**Figure 4.** PCA scatterplot of the Asian *Vigna* species (subgenus *Ceratotropis*) based on pollen morphological characters.

#### 4. Discussion

Pollen morphology has played an important role in legume systematics (Erdtman, 1969; Bronckers et al., 1972; Ferguson and Skvarla, 1981; Kahraman et al., 2013; Ceter et al., 2013a, 2013b; Pinar et al., 2014). In previous studies, several authors observed the significant variation in pollen sculpture among species of the genus *Vigna* (Verdcourt, 1970; Bronckers et al., 1972; Stainier, 1974; Maréchal et al., 1978; Wael, 2009). The pollens of genus *Vigna* are stenopalynous (Bronckers et al., 1972; Stainier, 1974). A literature survey showed that the pollen morphological study of Asiatic *Vigna* species is deficient (Vishnu-Mittre and Sharma, 1962; Verdcourt, 1970; Maréchal et al., 1978; Wael, 2009).

Erdtman (1952), in his study on pollen morphology and taxonomy of angiosperms, concluded that all investigated *Vigna* species had a single pollen type. For the first time, Vishnu-Mittre and Sharma (1962) studied the pollen morphology of Indian *Vigna* species. In their studies on Indian pollen grains, four species out of six assumed species of *Vigna* were really *Vigna* and the rest of the species belonged to the genus *Phaseolus* (Stainier, 1974). Taylor (1966) described 13 species of the genus *Phaseolus*, of which seven species were of the genus *Vigna* and had Asian origins (Asiatic *Vigna*). Of the species of subgenus *Ceratotropis*, strongly reticulate pollen grains are present in *Phaseolus aconitifolius* (*V. aconitifolia*), *P. aureus* (*V. radiata*), *P. angularis* (*V. angularis*), *P. calcaratus*

(*V. umbellata*), and *P. mungo* (*V. mungo*). Verdcourt (1970), in his studies on the problem of distinction between *Phaseolus* and *Vigna*, found that species of the genus *Vigna* have pollen grains with wide, open reticulation and species of the genus *Phaseolus* showed very conspicuous reticulations. Bronckers et al. (1972) analyzed the palynological characteristics of 39 species, five varieties, and one form of the genus *Vigna* and classified them into four groups on the basis of pollen size, smooth or granular sculpture, pore size, and irregularity of meshes of exine reticulations. However, he chose these criteria for distinction of groups of species and not for individual species (Stainier, 1974).

Stainier (1974) studied pollen morphology of nine *Vigna* species and reported some characteristics of *Vigna* pollens: triporate, reticulate; pollens are equiaxed, the ectexine apparently structured and easily distinguishable from the endexine. The reticulum is a sequence of ridges usually narrow at the top, more or less enlarged at the base, with smooth depressions. In a monograph on the *Phaseolus-Vigna* complex, Maréchal et al. (1978) briefly investigated the palynological characters of *Vigna* species and recognized 17 species in the subgenus *Ceratotropis*. Recently, a SEM study of the pollen sculpturing of 11 species of the genus *Vigna* was carried out by Wael (2009), who concluded that the pollen grains of the genus *Vigna* varied in shape, size, and surface ornamentation.



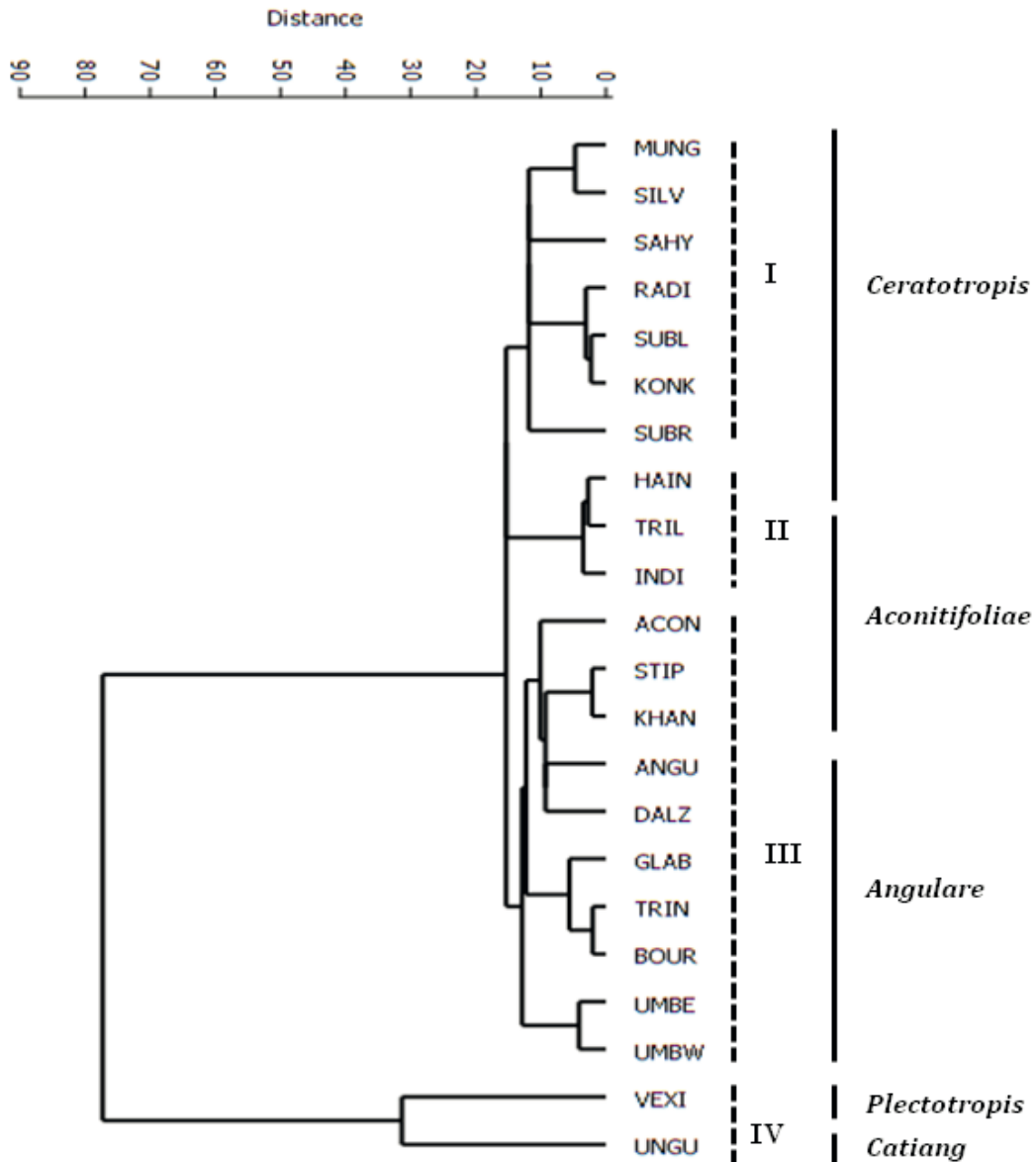


Figure 5. UPGMA clustering of the pollen similarities among the Asian *Vigna* species (subgenus *Ceratotropis*).

#### 4.1. Section *Ceratotropis*

The section *Ceratotropis* comprises eight species widely distributed throughout India (Aitawade et al., 2012). In the present study, the section *Ceratotropis* showed both large- and medium-sized pollens; prolate spheroidal shape; circular or elliptical in equatorial view; lumina heterobrochate or homobrochate; polygonal, pentagonal to hexagonal and pentagonal to polygonal lumina with psilate or granulate texture; muri narrow-straight or sinuous. In terms of pollen morphological characteristics, both *V. silvestris* and *V. sahyadriana* have large-sized prolate-spheroidal, reticulate heterobrochate pollen grains with polygonal lumina. However, *Vigna silvestris* showed psilate

lumina texture with straight muri, while *V. sahyadriana* has granulate lumina texture with sinuous muri. *V. mungo* is only cultivated species of the mungo group and has medium-sized pollens and psilate lumina texture with narrow straight muri as key characters as compared with *Vigna silvestris* and *V. sahyadriana* (Figures 2a–2c).

According to Bronckers et al. (1972) and Maréchal et al. (1978), *V. radiata* pollen grains display variable lumina size. On the contrary, in the present study, we observed reticulate homobrochate ornamentation. In morphometric analysis, *V. radiata* showed smaller lumina size than *V. sublobata*. *Vigna konkanensis* is morphologically similar to *V. sublobata* and they are often confused (Latha et al.,

2014). In terms of their pollen characters, the pollens of the two species are medium-sized, although they differ in terms of their lumina and pore size. *Vigna sublobata* showed comparatively smaller lumina, larger muri width, and larger porus diameter than *V. konkanensis* (Figures 2d–2f). The pollens of *V. subramaniana* are quite distinct; it has large-sized pollens, circular in equatorial view, reticulate-heterobrochate exine sculpture and pentagonal-polygonal lumina with sinuous muri. *V. hainiana* has small pollen with small, psilate lumina and large, sinuous type of muri (Figure 2h).

#### 4.2. Section *Aconitifoliae*

The section *Aconitifoliae* comprises five species, which have characteristic small flower size and wide spreading habit (Dixit et al., 2011; Yadav et al., 2014). The pollen grains of *V. aconitifolia* were described by Stainer (1974) as having reticulate exine sculpture and granular lumina. The data presented by Stainer (1974) for this species agree with our findings, differing only in the psilate lumina texture. Morphologically, *V. aconitifolia* is similar to *V. indica* in spreading habit and dissected leaflets. In terms of their pollen characteristics, both species have medium-sized pollens with reticulate-heterobrochate exine sculpture, psilate lumina, and sinuous muri. A key character aiding in differentiation of *V. aconitifolia* is pollen with irregular shape of lumina. The small pollen size, reticulate-heterobrochate sculpture, polygonal and psilate lumina, and sinuous muri in *V. indica* reveal that it is distant taxon in the section *Aconitifoliae*. Morphologically, *V. trilobata* and *V. stipulacea* are closely related. Probably due to incorrect identification based on seed morphological study, Kumar and Rangaswamy (1984) and Chandel et al. (1991) wrongly described *V. stipulacea* as *V. trilobata* (Dixit et al., 2011). The present investigation showed that *V. stipulacea* has large-sized pollen, pentagonal to polygonal lumina with granulate texture and straight muri, while *V. trilobata* showed medium apolar pollen, granular lumina, and sinuous muri. According to Stainer (1974), the pollens of *V. trilobata* were large (42–54  $\mu\text{m}$ ) with pore diameter of 6  $\mu\text{m}$ . Conversely, in the present study, we found small pollen diameters (36–38  $\mu\text{m}$ ), but similar pore diameters.

The erect habit and large foliaceous stipules of *V. khandalensis* are very distinct characters (Dixit et al., 2011). In terms of pollen characteristics, *V. khandalensis* pollen is relatively large in size, elliptical in equatorial view, pentagonal to polygonal lumina with granulate texture.

#### 4.3. Section *Angulares*

The species of the section *Angulares* are distinct from sections *Ceratotropis* and *Aconitifoliae* having large flowers, well developed keel pockets, and long style beaks (Tomooka et al., 2002b; Yadav et al., 2014). SEM studies of pollen surfaces show that large pollen size, pentagonal

to polygonal lumina shape, psilate lumina texture, and straight muri are distinct characters for delineation of species of the section *Angulares*. The pollen exine sculpture shows reticulate heterobrochate lumina, polygonal lumina shape with granulate texture, and large lumina diameter and porus diameter, which make *V. angularis* a distinct species in the section *Angulares*. According to Stainer (1974), *V. angularis* pollen has pore size of 5.5  $\mu\text{m}$ , but in present study we recognized much larger (10.44  $\mu\text{m}$ ) pores.

In the present study, we observed distinct pollen characters such as large pollens, circular in equatorial view, pentagonal to polygonal lumina of *V. dalzelliana* and small pollen size, pollen elliptical in equatorial view, and pentagonal-polygonal lumina in *V. umbellata*. Among the pollen characters, the cultivated form of *V. umbellata* has large pollens and lower P/E ratios. However, the wild form exhibits small pollen dimensions with higher P/E ratios (Table 1). The pollen dimension is the only parameter to differentiate the wild and cultivated forms of *V. umbellata*. According to Stainer (1974), *V. umbellata* (cultivated) has large pollens (60  $\mu\text{m}$ ). On the contrary, in the present investigation, we observed medium pollen diameters of 45.94–48.26  $\mu\text{m}$  in *V. umbellata* (cultivated). The two varietal forms of *Vigna trinervia*, *V. trinervia* var. *trinervia* and *V. trinervia* var. *bourneae*, are morphologically more similar. In morphometric analysis, *V. trinervia* var. *trinervia* shows comparatively larger pollens, lumina diameter, and muri width, which further confirms the distinguishing characters for delineation of these species. *Vigna glabrescens* is a tetraploid species (2n-44) within the section *Angulares*. Based on pollen characters, *V. glabrescens* differs from *V. trinervia* in having pollens elliptical in equatorial view, reticulate-heterobrochate exine sculpture, and small lumina size. The closeness of *V. glabrescens* and *V. trinervia* further supports the view that *V. trinervia* is one of the genome donors of tetraploid *V. glabrescens* (Tomooka et al., 2002b).

Another two species of the genus *Vigna*, *V. vexillata* (wild) from subgenus *Plectotropis* and *V. unguiculata* (cultivated) of subgenus *Vigna*, are widely distributed in India (Yadav et al., 2014). The pollens of species of the section *Ceratotropis* are small or medium in size (40–60  $\mu\text{m}$ ), whereas *V. vexillata* pollens are very large in size (116.96  $\mu\text{m}$ ). Wael (2009) studied the pollen morphology of *V. vexillata* and *V. unguiculata*. Those data diverged from the present study in terms of pollen size and type of aperture. According to Wael (2009), *V. vexillata* exhibits a dicolpate aperture and *V. unguiculata* pollen prolate tricolporate apertures. In the present study, the pollen grains of *V. vexillata* were described as triporate pollen with the relatively largest pollen dimensions among the studied taxa; in *V. unguiculata*, it was triporate with large pollen dimensions. Furthermore, the present investigation

concur with Wael (2009) in terms of general shape and P/E ratios of pollens of *V. vexillata* and *V. unguiculata*. *V. unguiculata* (cultivated) is a species of the subgenus *Vigna* that has African origins and is widely cultivated in India as a food and fodder crop. The pollen grains of *V. unguiculata* are large-sized, with prolate spheroidal shape, large polygonal lumina with granulate texture, sinuous muri, and large porus diameter.

The pollen grains of *V. radiata* and *V. trilobata* were studied by Maréchal et al. (1978) and Wael (2009). These authors described prolate tricolpate apertures, reticulate sculpture, and diameters ranging between 27 and 31.5 µm for *V. radiata* and oblate tricolpate aperture, ellate shape, and diameters ranging between 30 and 35.5 µm for *V. trilobata*. Our results agree with those of Maréchal et al. (1978) and Wael (2009) only regarding the pollen size of *V. trilobata*. We noticed triporate pollen, reticulate homobrochate, and diameter ranging between 44.88 and 46.98 µm in *V. radiata* and triporate, reticulate heterobrochate in *V. trilobata*. In his study, Wael (2009) did not discuss muri structure or measure values of lumina and pore size.

Morphological characteristics of pollen such as size, equatorial view, lumina shape, and muri width were the most variable characters and useful for delimitation of species of the subgenus *Ceratotropis*. The results of the PCA scatter plot and UPGMA clustering based on qualitative and quantitative characteristics are partially in common. In the PCA scatterplot, we recognized five types of pollens, of which type II, type IV, and type V were constant in the UPGMA dendrogram. The species of type I, such as *V. aconitifolia*, *V. umbellata* (wild), and *V. umbellata* (cultivated), meanwhile, were grouped in cluster III. Similarly, the species of type III, *V. silvestris*,

*V. sahyadriana*, and *V. subramaniana*, were grouped in cluster I. *V. angularis* was placed far in the PCA scatter plot (Figure 4) due to the large lumina size and porus diameter. *V. unguiculata* (subgenus *Vigna*) and *V. vexillata* (subgenus *Plectotropis*) are clustered together distantly from species of the subgenus *Ceratotropis* due to large pollen size.

In conclusion, we suggest that detailed and descriptive analysis of the characteristics of pollen is very important for understanding taxa. The subgenus *Ceratotropis* is stenopalynous; however, we recognized unique pollen attributes that allow for distinction between species. The present study showed that pollen attributes such as pollen size, shape, exine sculpture, lumina, and muri will lead to a better understanding of the species and aid in better contributions in future taxonomic studies of the subgenus *Ceratotropis*. At the same time, the present investigation also shed light on the species relationships within the subgenus *Ceratotropis*. In the present investigation, arrangements of species in specific clusters in the PCA scatter plot and UPGMA clustering showed the distinctive range of palynological variations and indicated their significance in species delimitation.

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**Appendix.** Specimens examined.

*Vigna mungo* (L.) Hepper. Karnataka: Halayandi near Udupi, 13°02.113'N, 74°47.604'E, 2 m, 5 December 2009, SUK-55 (MUNG).

*Vigna silvestris* (Lukoki, Marechal et Otoul) Aitawade, K.V. Bhat & S.R. Yadav. Maharashtra: Khopoli, 18°47.722'N, 73°19.359'E, 92 m, 18 October 2009, SUK-49 (SILV).

*Vigna sahyadriana* Aitawade, M. A., K.V. Bhat & S.R. Yadav. Maharashtra: Pasarani Ghat, 18°46.951'N, 73°21.061'E, 779 m, 21 October 2011, SUK-156 (SAHY).

*Vigna radiata* (L.) R. Wilkzek. Odisha: Sambalpur to Buranpur road, 19°24.399'N, 84°31.097'E, 88 m, 13 October 2011, SUK-120 (RADI).

*Vigna sublobata* (Roxb.) Babu & S.K. Sharma. Madhya Pradesh: Dumna reserve forest, 23°07.264'N, 79°49.352'E, 387 m, 18 October 2011, SUK-143 (SUBL).

*Vigna konkanensis* Latha K, K.V. Bhat, I. S. Bisht, Scariah, Joseph John & Krishnaraj. Maharashtra: Ratnagiri, 16°58.233'N, 73°19.314'E, 78 m, 18 December 2012, TCR-268 (KONK).

*Vigna subramaniana* (Babu ex Raizada) M. Sharma. Kerala: Mannuthy Nursery, Thrissur, 10°32.221'N, 76°16.733'E, 33 m, 4 October 2011, TCR-170 (SUBR).

*Vigna hainiana* Babu, Gopin. & S.K.Sharma. Rajasthan: Ranathambore, 26°02.036'N, 76°26.352'E, 275 m, 30 September 2009, SUK-25 (HAIN).

*Vigna trilobata* (L.) Verdc. Tamil Nadu, Kuttalam, 8°55.801'N, 77°15.366'E, 204 m, 27 December 2009, SUK-78 (TRIL).

*Vigna indica* T.M. Dixit, K.V. Bhat & S.R. Yadav. Rajasthan Bansi-Udaipur, 24°32.513'N, 74°09.932'E, 507 m, 3 October 2009, SUK-32 (INDI).

*Vigna aconitifolia* (Jacq.) Marechal., Rajasthan: Barodiya, Banswara, 24°41.609'N, 73°38.078'E, 640 m, 4 October 2009, SUK-26 (ACON).

*Vigna stipulacea* (Lam.) Kuntz. Maharashtra: Nanded, 19°07.420'N, 77°18.079'E, 367 m, 22 September 2011, SUK-105 (STIP).

*Vigna khandalensis* (Santapau) Raghavan & Wadhwa. Maharashtra: Junnar, 19°13.220'N, 73°44.332'E, 965 m, 16 August 2009, SUK-22 (KHAN).

*Vigna angularis* (Willd.) Ohwi & Ohashi. Himachal Pradesh: Solan, 30°50.218'N, 77°05.456'E, 1143 m, 16 October 2012, Hpu-51 (ANGU).

*Vigna dalzelliana* (Kuntz.) Verdc. Maharashtra: Tilari Ghat, 15°48.279'N, 74°10.601'E, 705 m, 22 November 2009, SUK-52 (DALZ).

*Vigna glabrescens* Marechal, Mascherpa & Stainier. Madhya Pradesh: Haringhat, 22°12.234'N, 78°12.332'E, 418 m, 12 August 2002, TCR-20 (GLAB).

*Vigna trinervia* var. *trinervia* (Heyne ex Wall) Tateishi & Maxted, Orissa: Jaypure to Koraput road, Koraput, 18°49.976'N, 82°47.505'E, 762 m, 15 October 2011, SUK-128 (TRIN).

*Vigna trinervia* var. *bourneae* (Gamble) Tateishi & Maxted, Karnataka: Udupi-Mangalore Road, Sattapatte, 13°23.210'N, 74°44.353'E, 45 m, 10 September 2011, SUK-101 (BOUR).

*Vigna umbellata* (Thunb.) Ohwi & Ohashi. Meghalaya: Mawkhar, Shillong, 25°35.352'N, 91°55.186'E, 1436 m, 18 November 2010, TCR-348 (UMBE).

*Vigna umbellata* (Thunb.) Ohwi & Ohashi. Meghalaya: Shillong, 25°36.155'N, 91°54.235'E, 1447 m, 18 November 2010, SKM-09 (UMBW).

*Vigna vexillata* (L.) A. Rich, Goa: Lolium Plateau, 14°56.228'N, 74°03.628'E, 57 m, 2 November 2009, SUK-48 (VEXI).

*Vigna unguiculata* (L.) Walp. Odisha: Gajapati, 18°84.667'N, 84°10.588'E, 96 m, 19 October 2010, V-240 (UNGU).