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Seed coat polymorphism in *Vigna* section *Aconitifoliae* in India

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ABSTRACT

The seed coat polymorphism of 50 accessions representing five species of *Vigna* section *Aconitifoliae* (subgenus *Ceratotropis*) was investigated using scanning electron microscopy (SEM) in order to evaluate the interspecific and intraspecific variations of various morphoanatomical seed traits. Seed shape, hilum structure aril and testa pattern were examined. The seed coat pattern was found to be a significant character for species delimitation. The testa cell size and ornamentation showed distinctive intra- and interspecific variations across the examined species. The results revealed that the wild accessions of *V. aconitifolia* (I-aco and II-aco), *V. indica* (I-ind and II-ind), *V. stipulacea* (I-sti, I-sti, II-sti, III-sti and VI-sti) and *V. trilobata* (I-tri, II-tri) have different seed coat types. In the present study, only a single seed coat pattern was recorded for the endemic, threatened species *V. khandalensis*. Finally, the congruency of seed coat patterns optimized onto an rDNA-ITS phylogeny was discussed.

1. Introduction

Vigna Savi is a pantropical genus that comprises 104 species (Lewis et al., 2005). The species of *Vigna* are known as an important source of food worldwide (Smart, 1990). The genus is divided into five subgenera, among which *Ceratotropis* (Piper) Verdc. is widely distributed in Asia and is also known as the Asian *Vigna* (Tomooka et al., 2002b). India is rich in species diversity and has 24 species of the subgenus *Ceratotropis* (Babu et al., 1985; Sanjappa, 1992; Bisht et al., 2005; Yadav et al., 2014). The subgenus *Ceratotropis* exhibits diverse morphological characteristics based on which it is divided into three sections, namely *Ceratotropis* Tomooka & Maxted, *Aconitifoliae* Tomooka& Maxted and *Angulares* Tomooka & Maxted (Tomooka et al., 2002a). Morphological and molecular phylogenetic studies of the species belonging to the subgenus *Ceratotropis* have confirmed the sectional classification proposed by several authors (Maréchal et al., 1978; Tomooka et al., 2002b; Bisht et al., 2005; Yadav et al., 2014; Takahashi et al., 2016; Umdale et al., 2017a, 2017b).

Vigna section *Aconitifoliae* consists of six species viz. *V. aconitifolia* (Jacq.) Maréchal, *V. aridicola* N. Tomooka et Maxted, *V. indica* T.M. Dixit, K.V. Bhat & S.R. Yadav, *V. khandalensis* (Santapau) Raghvan et Wadhwa, *V. trilobata* (L.) Verdc. and *V. stipulacea* (Lam.) Kuntze, among which two have been domesticated (*V. aconitifolia* (Jacq.) Maréchal and *V. stipulacea* Kuntze (Dixit, 2014). The species of section *Aconitifoliae* have been delimited on the basis of wide morphological variations. *Vigna aconitifolia* has linear to lanceolate lobed leaflets, *V. stipulacea* consists of large stipules and *V. khandalensis* shows foliaceous stipules and erect habit, which distinguish them from the other species of section *Aconitifoliae*. The seeds of *V. trilobata* possess a protruding hilum and a well-developed aril, whereas those of *V. indica* have rough, substriate testa and undeveloped aril (Tomooka et al., 2002b; Dixit et al., 2011; Umdale et al., 2017b).

The wild relatives of cultivated plants serve as potential genetic resources for improving crops. This requires to evaluate the intrinsic diversity and valuable genes of different populations. However, the inadequacy of information pertaining to the intraspecific micro-morphological polymorphism in section *Aconitifoliae* hampers the improvement and effective utilization of wild *Vigna* species. Hence, it is imperative to comprehensively assess the intraspecific diversity amongst the species of section *Aconitifoliae*.

The micro-morphological characters of the seed have offered unique and reliable data for species delimitation (Rajendra et al., 1979; Kumar and Rangaswamy, 1984; Chandel et al., 1991; Nath and Dasgupta, 2015; Umdale et al., 2017b) and intraspecific polymorphism in the

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genus *Vigna* (Sharma et al., 1983; Gopinathan and Babu, 1985; Ignacimuthu and Babu, 1985). Comprehensive seed morphological and micro-morphological study involving species of the subgenus *Ceratotropis* has been carried out (Chandel et al., 1991; Umdale et al., 2017b). Among the *Vigna* species, intraspecific variation has been reported in *V. radiata* (Gerstner et al., 1988; Saini et al., 2008; Raturi et al., 2011) and *V. mungo* (Souframanien et al., 2003) by analyzing the rDNA sequences. However, the seed coat polymorphism within section *Aconitifoliae* has never been explored. Therefore, an understanding of the interspecific and intraspecific variations is required to assess the genetic diversity and the different germplasms of the wild relatives existing in the section *Aconitifoliae*. In the present study, we examined the interspecific and intraspecific variations by using the micro-morphological characters of five species belonging to the section *Aconitifoliae*.

2. Materials and methods

2.1. Plant material

The mature seeds of 50 accessions representing five different species of the section *Aconitifoliae* (subgenus *Ceratotropis* of genus *Vigna*) were collected from the natural habitats and cultivated fields of India during the period 1997–2012 (Table 1; Fig. 1). All accessions were identified and the seeds were deposited in the Seed Genbank, NBPGR, New Delhi, India.

2.2. Morphological analyses

Twenty-five seeds from each accession were used to examine the morphological and micro-morphological characters, including seed size, shape and color, hilum shape and length–width and testa characteristics (Tables 2 and 3). Initially, 2–4 seeds of each accession were thoroughly cleaned using 70% ethanol, air dried and mounted on brass stubs using double-adhesive carbon tape. The seed-mounted stubs were then coated uniformly with a thin layer of gold using ion sputter JEOL JFC-100. Subsequently, the coated seeds were examined using a scanning electron microscope (JEOL JSM-840A) with accelerating voltages of 10–15 kV. The seeds were uniformly scanned at the hilum, and the surface was focused to examine the cellular and intercellular testa patterns. The terminologies proposed by Barthlott (1981, 1990) were adopted for describing the seed coat patterns. Fifteen mature seeds from each accession were used for measuring the parameters pertaining to size (seed length-width, hilum length-width, and testa cell length-width). The variation in seed length-width, hilum length-width and testa cell length-width among the different accessions were demonstrated as boxplots using R package ggplot2.

2.3. Molecular phylogeny

As a phylogenetic framework to analyse structural data, we generated a molecular-based analysis of ITS sequence data. The total genomic DNA was extracted from the dried leaves by using the CTAB method (Doyle, 1991). The rDNA-ITS region (ITS1, 5.8S gene, and ITS2) was amplified with pairs of universal primers ITS-1 and ITS-4 (White et al., 1990). The PCR reaction was performed with the aid of a thermal cycler (BIOR® 2700, Applied Biosystems), and it involved a total volume of 25 µl comprising 20 ng of genomic DNA as the template, 2 × PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µM of each primer (Synthesized by Xcelris Labs Ltd., India) and 1.0 U of Taq DNA Polymerase (Fermentas, Thermo Fisher Scientific, India). The reaction was programmed with an initial denaturation at 95 °C for 150 s, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 1–2 min, with a final extension at 72 °C for 5 min. The amplified products were electrophoresed on 1.5% agarose gel stained with 1% of 10 mg/ml ethidium bromide, and Generuler 100 bp DNA ladder (Thermo scientific, USA) was used to determine the band size of the amplified product. The product was visualized and documented using GeneSnap 4.00 Gene Genius Bio Imaging System (Syngene, LTD.). The nrDNA-ITS PCR products were purified using the AxyPrep PCR Clean-up Kit (AXYGENE Biosciences, USA) according to the manufacturer's instructions.

The DNA was sequenced with the ZBigDye Terminator Cycle Sequencing Kit version 3 (Sci Genom Labs Pvt. Ltd., Kerala, India). The sequenced product was examined using the forward primer with the help of the automated ABI 3730XL analyzer (Sci Genom Labs Pvt. Ltd., Kerala, India). The rDNA-ITS sequences were submitted to EMBL GenBank (Table 1). The raw sequence data were analyzed with the Ridom Trace Edit version 1.1.0 (Ridom GmbH, Germany). All sequences were aligned using the multiple sequence alignment tool ClustalX (Thompson et al., 1997). Phylogenetic analysis of the rDNA-ITS dataset was performed using maximum parsimony (MP) with MEGA6 software version 7.0.26 (Kumar et al., 2016). In this dataset, *Phaseolus vulgaris* (FJ172178) was treated as outgroup. The maximum parsimony tree was constructed using the Subtree-Pruning-Regrafting (SPR) algorithm using search level 3 in which the initial trees were obtained by the random addition of sequences (10 replicates). The pairwise sequence divergence rates between the accessions were measured using the Maximum Composite Likelihood method. The positions containing gaps and missing data were treated as complete deletions. The bootstrap values of the internal branches of the MP tree were determined through 1000 replications. The transition/transversion bias was estimated using the Kimura 2-parameter model with uniform rates. Tree length (TL), consistency index (CI), retention index (RI) and composite index were calculated using Sequence Data Explorer with default values in MEGA 7.0.26. The best-fit nucleotide substitution model was derived according to the Akaike Information Criterion. Models with the lowest BIC scores (Bayesian Information Criterion) were considered to best describe the substitution pattern (Kumar et al., 2016).

3. Results

3.1. Morphological analyses

3.1.1. Vigna aconitifolia (cultivated)

3.1.1.1. Type I-ac. The seeds are elongated with roundish ends and pale-brown in color. The texture of the testa is smooth and shiny. The seeds are variable in size, 2.43–4.36 (3.40 ± 0.12) mm long, 1.44–2.49 (2.03 ± 0.06) mm wide (Table 2). The hilum is central, oblong in shape, 0.85–1.27 (1.11 ± 0.02) mm long, 0.25–0.42 (0.35 ± 0.01) mm wide (Fig. 2a, Table 2). The aril is absent. The seed surface is colliculate-pusticulate, with broad polygonal testa cells (Fig. 2b). The testa cells are 5.04–9.74 (6.71 ± 0.19) µm long, 3.51–5.44 (4.34 ± 0.11) µm wide with an area of 14.71–33.18 (22.29 ± 0.85) µm (Table 2). The testa cells are slightly elongated and possess four to six sides with curved, undulate margins. The anticlinal walls are slightly raised and smooth within distinct thickness and slightly striated. The periclinal wall is unevenly granular (Fig. 2c).

3.1.2. V. aconitifolia (wild)

3.1.2.1. Type I-aw. The seeds are elongated with roundish ends and pale-brown to yellowish-brown in color. The texture of the testa is smooth and shiny. The seeds are variable in size, 1.98–3.15 (2.59 ± 0.17) mm long, 1.77–1.87 (1.82 ± 0.01) mm wide (Table 2). The hilum is central, oblong in shape, 0.60–0.73 (0.72 ± 0.03) mm long, 0.23–0.41 (0.32 ± 0.02) mm wide (Fig. 2d, Table 2). The aril is absent. The seed surface is colliculate-pusticulate, with irregularly arranged polygonal testa cells (Fig. 2e). The testa cells are polygonal with undulate margins. The anticlinal walls are distinctly raised, smooth wavy. The testa cells are 3.40–5.81 (4.80 ± 0.21) µm long, 2.38–3.07 (2.75 ± 0.08) µm wide with an area of 7.13–12.66 (9.46 ± 0.57) µm (Table 2). The periclinal wall is unevenly granular

Table 1
List of accessions of section *Aconitifoliae* species studied with Number of accessions, site of collection, date, voucher information and GenBank accession number for rDNA-ITS sequences.

Sr. No	Species	Accession No.	Site of Collection	Date of Collection	N	E	Elevation (ft)	Seed type	GenBank accession number
1	*V. aconitifolia* (Cult)	RMO-257	NBPGR, Regional Station, Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	LT717350
2	*V. aconitifolia* (Cult)	IC-439425	NBPGR, Regional Station, Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	LT717351
3	*V. aconitifolia* (Cult)	IC-439426	NBPGR, Regional Station Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	LT717352
4	*V. aconitifolia* (Cult)	IC-439448	NBPGR, Regional Station Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	LT717353
5	*V. aconitifolia* (Cult)	IC-439449	NBPGR, Regional Station Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	*
6	*V. aconitifolia* (Cult)	IC-439451	NBPGR, Regional Station Jodhpur, Rajasthan	05.10.2006	26°24'	72°99'	623	I-ac	*
7	*V. aconitifolia* (Wild)	BB-2639	Rajgurunagar-Nashik road Pune, Maharashtra	04.11.2006	19°08'	74°02'	2130	I-aw	LT717354
8	*V. aconitifolia* (Wild)	SUK-26	Kavita, Udaipur, Rajasthan	04.10.2009	24°41'	73°38'	640	I-aw	LT717355
9	*V. aconitifolia* (Wild)	BBYD-2714	Udaipur, Rajasthan	03.10.2009	24°28'	73°40'	1955	II-aw	LT717356
10	*V. aconitifolia* (Wild)	BBYD-2718	Oma, Mount Abu, Sirohi, Rajasthan	04.10.2009	24°63'	72°28'	2536	II-aw	LT717357
11	*V. indica*	BB-2616	Jawai Bandh outer site, Pali, Rajasthan	05.10.2006	25°49'	73°11'	703	I-ind	LT717358
12	*V. indica*	BB-2620	Ranakpur Temple, Pali, Rajasthan	06.10.2006	25°47'	73°52'	771	I-ind	LT717359
13	*V. indica*	BB-2626	Juntra, Rajsamund, Rajasthan	06.10.2006	25°73'	73°52'	1944	I-ind	LT717360
14	*V. indica*	BB-2627	Beawar-Sendra Road, Pali, Rajasthan	07.10.2006	26°04'	73°13'	1015	I-ind	LT717361
15	*V. indica*	BBYD-2696	Ranthambhor, Sawai Madhopur, Rajasthan	03.10.2009	26°21'	76°46'	903	I-ind	LT717362
16	*V. indica*	BB-2714	Kavita, Udaipur, Rajasthan	03.10.2009	24°39'	73°73'	2033	I-ind	LT717363
17	*V. indica*	BBYD-2717	Udaipur, Rajasthan	03.10.2009	24°33'	73°40'	1943	I-ind	LT717364
18	*V. indica*	BBYD-2727	Jaswantgarh, Udaipur, Rajasthan	06.10.2009	24°39'	73°73'	1502	I-ind	LT717365
19	*V. indica*	BBYD-2730	Ranakpur, Pali, Rajasthan	07.10.2009	25°70'	73°52'	851	I-ind	LT717366
20	*V. indica*	BBYD-2733	Kavita, Udaipur, Rajasthan	04.10.2009	24°38'	73°72'	2030	I-ind	LT717367
21	*V. indica*	BBYD-2734	Lakheri to Bundi, Bundi, Rajasthan	08.10.2009	25°48'	75°79'	935	I-ind	LT717368
22	*V. indica*	BB-2614	Kalandri-Sirohi Road, 7 km from Sirohi, Rajasthan	05.10.2006	24°32'	73°29'	904	II-ind	LT717369
23	*V. indica*	BB-2618	Sumerpur, Pali, Rajasthan	06.10.2006	24°52'	72°24'	1002	II-ind	LT717370
24	*V. indica*	BB-2619	Sumerpur - Sanderao road, Sirohi, Rajasthan	06.10.2006	24°54'	72°48'	920	II-ind	LT717371
25	*V. indica*	SUK-40	Katraj, Maharashtra	22.10.2010	18°27'	73°51'	2117	I-ind	LT717372
26	*V. indica*	SUK-104	Shivaji University campus, Kolhapur, Maharashtra	31.12.2010	16°40'	74°15'	1988	I-ind	LT717373
27	*V. stipulacea*	BBD-20-01B	Raipur, Chattisgarh	31.10.2001	21°12'	81°37'	955	I-sti	LT717374
28	*V. stipulacea*	BB-2-2K	Raisen, Madhya Pradesh	25.09.2000	23°05'	81°03'	2427	I-sti	LT717375
29	*V. stipulacea*	TCR-327	NBPGR, Regional Station, Thrissur, Kerala	20.09.2009	10°33'	76°16'	99	I-sti	LT717376
30	*V. stipulacea*	TCR-319	NBPGR, Regional Station, Thrissur, Kerala	20.09.2009	10°33'	76°16'	100	I-sti	LT717377
31	*V. stipulacea*	BB-02-01	Udham Singh Nagar, Uttarakhand	03.11.2001	28°58'	79°33'	718	I-sti	LT717378
32	*V. stipulacea*	TCR-320	NBPGR, Regional Station, Thrissur, Kerala	20.09.2009	10°33'	76°16'	101	II-sti	LT717379
33	*V. stipulacea*	BBD-21-01B	Raipur, Chattisgarh	30.10.1997	21°41'	81°21'	920	II-sti	LT717380
34	*V. stipulacea*	TCR-192	NBPGR, Regional Station, Thrissur, Kerala	20.09.2009	10°33'	76°16'	100	II-sti	LT717381
35	*V. stipulacea*	BB-2611	Jalore-Bagra Road, Jalore, Rajasthan	05.10.2006	25°17'	72°37'	591	II-sti	LT717382
36	*V. stipulacea*	BB-23-01B	Bilaspur, Chattisgarh	01.11.2001	22°05'	82°07'	912	II-sti	LT717383
37	*V. stipulacea*	BBD-04-01B	Ganjam, Odisha	26.10.2001	19°23'	85°03'	21	II-sti	LT717384
38	*V. stipulacea*	BB-2610	Jalore fort, Jalore, Rajasthan	04.10.2006	25°20'	72°36'	1605	III-sti	LT717385
39	*V. stipulacea*	BBYD-2707	Sirohi-Pindwara route, Sirohi, Rajasthan	06.10.2009	24°63'	72°28'	930	III-sti	LT717386
40	*V. stipulacea*	BBD-03-01B	Khordha, Odisha	01.11.2001	20°09'	95°38'	214	IV-sti	LT717387
41	*V. stipulacea*	SUK-76	Madurai on NH-7, Madurai, Tamil Nadu	16.12.2009	09°49'	77°58'	123	IV-sti	LT717388
42	*V. stipulacea*	SDU-01	Miraj, Maharashtra	10.10.2012	16°48'	74°39'	1798	IV-sti	LT717389
43	*V. trilobata*	TCR-303	Kanyakumari, Tamil Nadu	22.08.2001	08°58'	77°32'	283	I-tri	LT717390
44	*V. trilobata*	SUK-78	Kuttalam, Tamil Nadu	27.12.2009	08°55'	77°15'	204	I-tri	LT717391
45	*V. trilobata*	TCR-02	Thrissur, Kerala	20.09.2009	10°33'	76°16'	99	II-tri	LT717392
46	*V. trilobata*	TCR-215	Thrissur, Kerala	20.09.2009	10°33'	76°16'	102	II-tri	LT717393
47	*V. khandalensis*	BB-2678	Panchgani, Satara, Maharashtra	08.11.2006	17°55'	73°50'	3033	kha	LT717394
48	*V. khandalensis*	BB-2688	Sinhagad, Pune, Maharashtra	10.11.2006	18°21'	73°44'	3022	kha	LT717395
49	*V. khandalensis*	BB-2661	Khed, Ratnagiri, Maharashtra	07.11.2006	18°05'	73°18'	509	kha	LT717396
50	*V. khandalensis*	BB-2683	Purander Fort, Pune, Maharashtra	09.11.2006	18°16'	73°58'	3757	kha	LT717397

* accessions excluded; ac- *Vigna aconitifolia* (cult); aw- *V. aconitifolia* (wild); ind- *V. indica*; sti- *V. stipulacea*; tri- *V. trilobata*; kha- *V. khandalensis*; I- type one; II-Type two; III- Type three, IV-type four.

(Fig. 2f).

3.1.2.2. Type II-aw. The seeds are elongated with roundish ends and greenish-brown in color. The testa texture is smooth and shiny. The seeds are 2.14–3.38 (2.73 ± 0.17) mm long, 1.78–0.02 (1.94 ± 0.02) mm wide (Table 2). The hilum is central, oblong in shape (Fig. 2g). The hilum is 0.54–0.94 (0.75 ± 0.05) mm long and 0.26–0.44 (0.36 ± 0.02) mm wide (Table 2). The aril is absent. The testa is colliculate-pusticulate, with irregularly arranged broad polygonal testa cells (Fig. 2h). The testa cells are 3.85–5.64 (4.75 ± 0.18) μm long,

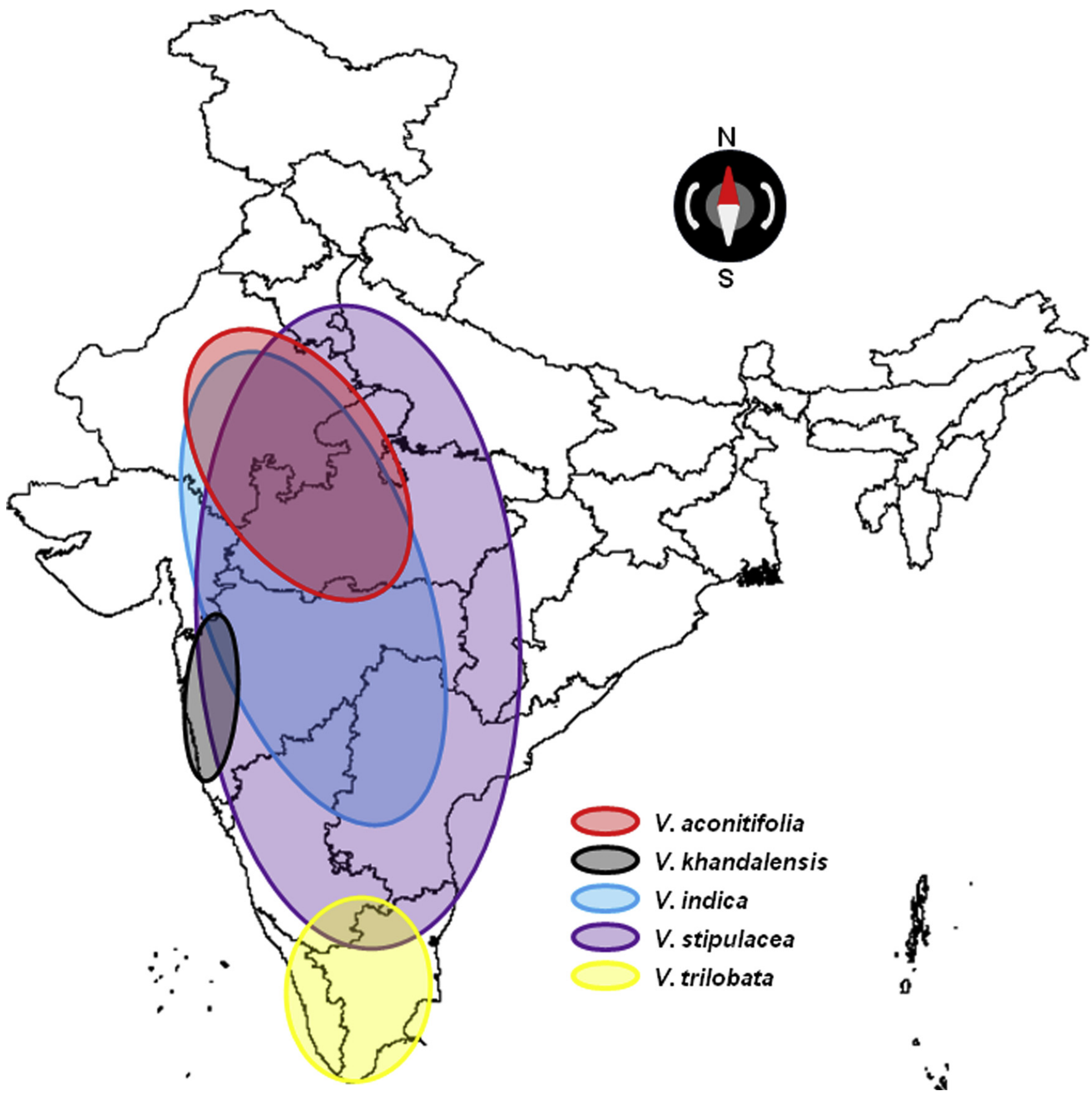

Fig. 1. Map of India showing the distribution of species of *Vigna* section *Aconitifoliae*.

2.56–3.97 (3.17 ± 0.14) µm wide with an area of 8.53–15.91 (11.81 ± 0.80) µm (Table 2). The anticlinal walls are not raised and wavy. The periclinal wall is raised or unevenly granular (Fig. 2i).

3.1.3. Vigna indica

3.1.3.1. Type I-ind. The seeds are cylindrical with truncate ends and yellowish-brown in color. The testa texture is rough. The seeds are variable in size, 2.58–3.53 (3.05 ± 0.03) mm long, 1.40–2.53 (1.94 ± 0.03) mm wide (Table 2). The hilum is sub-central, round and rectangular in shape (Fig. 3a). The length of the hilum is 0.29–0.57 (0.43 ± 0.01) mm and width is 0.21–0.31 (0.26 ± 0.01) mm (Table 2). The aril is absent. The seed surface is sub-striate, with broad polygonal testa cells arranged in rows (Fig. 3b). The testa cells are 30.23–53.26 (41.72 ± 0.82) µm long, 28.00–46.73 (36.67 ± 0.60) µm wide with an area of 906.51–2097.82 (1491.93 ± 38.37) µm (Table 2). The testa cells are polygonal with intercellular spaces. The anticlinal walls are raised, sharp and with thick deposition of wax. The periclinal wall is flat with compact reticulation (Fig. 3c).

3.1.3.2. Type II-ind. The seeds are cylindrical with truncate ends and greenish-brown in color. The testa texture is rough and dull. The seeds are variable in size, 2.57–3.85 (3.08 ± 0.13) mm long, 1.44–2.60 (1.98 ± 0.09) mm wide (Table 2). The hilum is sub-central, rounded rectangular in shape (Fig. 3d). The hilum is 0.46–0.59 (0.51 ± 0.01) mm long and 0.22–0.29 (0.25 ± 0.01) mm wide (Table 2). The aril is absent. The seed surface is sub-striate, with broad polygonal testa cells arranged in rows (Fig. 3e). The testa cells are 28.52–37.93 (32.49 ± 0.73) µm long, 22.53–35.96 (29.42 ± 0.91) µm wide with an area of 620.68–1207.41 (876.41 ± 43.39) µm (Table 2). The testa cells are polygonal with intercellular space. The anticlinal walls are raised, sharp and have thick wax deposition. The periclinal wall is flat with compact reticulation (Fig. 3f).

3.1.4. Vigna stipulacea

3.1.4.1. Type I-sti. The seeds are rectangular with roundish ends and brown in color. The texture of the testa is smooth. The seeds are variable in size, 2.24–2.80 (2.57 ± 0.03) mm long, 1.83–2.21 (2.02 ± 0.02) mm wide (Table 2). The hilum is central, ellipsoidal, oblong in shape and distinctly protruding (Fig. 4a). The hilum is 1.23–1.47 (1.35 ± 0.01) mm long and 0.56–0.67 (0.63 ± 0.01) mm wide (Table 2). The aril is moderately developed. The seed surface is reticulate-foveate to reticulate, with broad polygonal or circular testa cells (Fig. 4b). The testa cells are 7.85–13.02 (11.23 ± 0.25) µm long, 6.15–9.90 (8.28 ± 0.25) µm wide with 48.51–116.72 (80.58 ± 3.14) µm area (Table 2). The arrangement of polygonal testa cells is irregular.

Table 2
Seed testa characters of examined species of *Vigna* section *Aconitifoliae*.

Taxon	Type	Seed Shape	Seed Colour	Testa Texture	Hilum Position	Hilum Shape	Aril	Testa pattern	Testa Cell	Anticlinal wall	Periclinal Wall	Figures
V. aconitifolia (Cultivated)	I-ac	Elongated with roundish ends	Pale brown	Smooth, shiny	Central	Oblong	Absent	Colliculate-pusticulate	Broad polygonal	Not raised, wavy with indistinct thickness	Unevenly granular	2 a, b, c
V. aconitifolia (Wild)	I-aw	Globular to elongated with roundish ends	Pale brown, yellowish brown	Smooth, shiny	Central	Oblong	Absent	Colliculate-pusticulate	Polygonal, irregularly arranged	Raised, wavy	Unevenly granular	2 d, e, f
V. aconitifolia (Wild)	II-aw	Elongated with roundish ends	Greenish brown	Smooth, shiny	Central	Oblong	Absent	Colliculate-pusticulate	Broad polygonal, irregularly arranged	Not raised, wavy	Unevenly granular, raised	2 g, h, i
V. indica	I-ind	Cylindrical with truncate ends	Yellowish brown	Rough	Subcentral	Rounded rectangular	Absent	Sub-striate	Broad polygonal arranged in rows	Raised, sharp and thick wax deposition on wall	Flat, compact-reticulate	3 a, b, c
V. indica	II-ind	Cylindrical with truncate ends	Greenish brown	Rough	Subcentral	Rounded rectangular	Absent	Sub-striate	Broad polygonal arranged in rows	Raised, sharp and thick wax deposition on wall	Flat, loosely reticulate	3 d, e, f
V. stipulacea	I-sti	Rectangular with roundish ends	Brown	Smooth	Central	Ellipsoidal oblong, protruding	Moderately developed	Reticulate - foveate to reticulate	Polygonal or circular irregularly arranged	Raised, undulate, thick	Concave, loosely reticulate	4 a, b, c
V. stipulacea	II-sti	Rectangular with roundish ends	Brown	Smooth	Central	Ellipsoidal oblong, protruding	Moderately developed	Reticulate - foveate to reticulate	Polygonal or circular irregularly arranged	Raised, undulate, thick	Concave, micro-reticulate	4 d, e, f
V. stipulacea	III-sti	Rectangular with roundish ends	Greenish brown	Smooth	Central	Ellipsoidal oblong, protruding	Moderately developed	Reticulate - foveate to reticulate	Polygonal or circular irregularly arranged	Raised, sharp	Concave, compact reticulate	5 a, b, c
V. stipulacea	IV-sti	Rectangular with roundish ends	Greenish brown	Smooth	Central	Ellipsoidal oblong, protruding	Moderately developed	Reticulate - foveate to reticulate	Polygonal or circular irregularly arranged	Raised, sharp	Concave, compact reticulate with pustules	5 d, e, f
V. trilobata	I-tri	Rectangular to globular with roundish ends	Brown, yellowish brown with black spots	Smooth	Central	Broadly ovate, protruding	Well developed	Reticulate	Broad polygonal, arranged in rows	Thick longitudinal wall and thin transverse wall, disappearing at centre	Concave, compact reticulate	6 a, b, c
V. trilobata	II-tri	Rectangular to globular with roundish ends	Brownish with black spots	Smooth	Central	Broadly ovate, protruding	Well developed	Reticulate	Broad polygonal, arranged in rows with intercellular space	Thick longitudinal and thin transverse wall	Concave, centrally pitted, compact reticulate	6 d, e, f
V. khandalensis	kha	Rectangular to oblong	Light to dark black	Rough	Central	Oblong	Absent	Reticulate	Broad polygonal arranged in rows with intercellular space	Irregularly thick	Flat to concave, compact reticulate	7 a, b, c

Table 3
Morphometrical characters among the different populations of examined taxa.

Taxon	Type	Seed length (mm) m - M ($\bar{x} \pm s\bar{x}$)	Seed width (mm) m - M ($\bar{x} \pm s\bar{x}$)	Hilum length (mm) m - M ($\bar{x} \pm s\bar{x}$)	Hilum width (mm) m - M ($\bar{x} \pm s\bar{x}$)	Testa cell length (μm) m - M ($\bar{x} \pm s\bar{x}$)	Testa cell width (μm) m - M ($\bar{x} \pm s\bar{x}$)	Testa cell area (μm) m - M ($\bar{x} \pm s\bar{x}$)
V. aconitifolia (Cultivated)	I-ac	2.43 - 4.36 (3.40 ± 0.12)	1.44 - 2.49 (2.03 ± 0.06)	0.85 - 1.27 (1.11 ± 0.02)	0.25 - 0.42 (0.35 ± 0.01)	5.04 - 9.74 (6.71 ± 0.19)	3.51 - 5.44 (4.34 ± 0.11)	14.71 - 33.18 (22.29 ± 0.85)
V. aconitifolia (Wild)	I-aw	1.98 - 3.15 (2.59 ± 0.17)	1.77 - 1.87 (1.82 ± 0.01)	0.60 - 0.73 (0.72 ± 0.03)	0.23 - 0.41 (0.32 ± 0.02)	3.40 - 5.81 (4.80 ± 0.21)	2.38 - 3.07 (2.75 ± 0.08)	7.13 - 12.66 (9.46 ± 0.57)
V. aconitifolia (Wild)	II-aw	2.14 - 3.38 (2.73 ± 0.20)	1.78 - 2.02 (1.94 ± 0.02)	0.54 - 0.94 (0.75 ± 0.05)	0.26 - 0.44 (0.36 ± 0.02)	3.85 - 5.64 (4.75 ± 0.18)	2.56 - 3.97 (3.17 ± 0.14)	8.53 - 15.91 (11.81 ± 0.80)
V. indica	I-ind	2.58 - 3.53 (3.05 ± 0.03)	1.40 - 2.53 (1.94 ± 0.03)	0.29 - 0.57 (0.43 ± 0.01)	0.21 - 0.31 (0.26 ± 0.01)	30.23 - 53.26 (41.72 ± 0.82)	28.00 - 46.73 (36.67 ± 0.60)	906.51 - 2097.82 (1491.93 ± 38.37)
V. indica	II-ind	2.57 - 3.85 (3.08 ± 0.13)	1.44 - 2.60 (1.98 ± 0.09)	0.46 - 0.59 (0.51 ± 0.01)	0.22 - 0.29 (0.25 ± 0.01)	28.52 - 37.93 (32.49 ± 0.73)	22.53 - 35.96 (29.42 ± 0.91)	620.68 - 1207.41 (876.41 ± 43.39)
V. stipulacea	I-sti	2.24 - 2.57 (2.80 ± 0.03)	1.83 - 2.21 (2.01 ± 0.02)	1.23 - 1.47 (1.35 ± 0.01)	0.56 - 0.67 (0.63 ± 0.01)	7.85 - 13.02 (11.23 ± 0.25)	6.15 - 9.90 (8.28 ± 0.25)	48.51 - 116.72 (80.58 ± 3.14)
V. stipulacea	II-sti	2.29 - 2.66 (2.39 ± 0.02)	1.62 - 2.04 (1.83 ± 0.02)	1.15 - 1.46 (1.32 ± 0.02)	0.59 - 0.73 (0.66 ± 0.01)	13.67 - 18.84 (16.03 ± 0.30)	7.54 - 12.24 (10.13 ± 0.28)	93.25 - 188.05 (142.71 ± 4.77)
V. stipulacea	III-sti	2.36 - 2.69 (2.57 ± 0.03)	1.86 - 2.05 (1.93 ± 0.02)	1.18 - 1.42 (1.30 ± 0.03)	0.53 - 0.64 (0.60 ± 0.01)	8.39 - 15.06 (11.53 ± 0.66)	6.88 - 11.12 (9.30 ± 0.47)	59.64 - 155.99 (91.15 ± 9.90)
V. stipulacea	IV-sti	1.99 - 2.91 (2.35 ± 0.09)	1.65 - 2.41 (1.92 ± 0.08)	1.10 - 1.54 (1.26 ± 0.04)	0.52 - 0.74 (0.64 ± 0.01)	9.28 - 16.49 (12.61 ± 0.54)	5.88 - 10.77 (8.44 ± 0.04)	48.19 - 153.16 (94.16 ± 7.71)
V. trilobata	I-tri	1.85 - 2.94 (2.34 ± 0.18)	1.55 - 1.84 (1.68 ± 0.03)	0.69 - 1.18 (0.92 ± 0.07)	0.65 - 1.06 (0.84 ± 0.06)	22.62 - 46.87 (37.74 ± 2.28)	15.15 - 28.60 (19.87 ± 1.62)	648.98 - 977.71 (868.94 ± 34.53)
V. trilobata	II-tri	2.67 - 3.00 (2.84 ± 0.05)	1.67 - 1.86 (1.77 ± 0.02)	1.08 - 1.17 (1.13 ± 0.01)	0.90 - 0.97 (0.94 ± 0.01)	24.44 - 39.23 (30.11 ± 1.49)	17.16 - 24.28 (20.42 ± 0.72)	474.78 - 807.47 (644.93 ± 33.03)
V. khandalensis	kha	3.24 - 4.41 (3.79 ± 0.08)	2.74 - 3.76 (3.21 ± 0.07)	1.20 - 1.69 (1.43 ± 0.03)	0.45 - 0.68 (0.57 ± 0.01)	23.75 - 44.55 (34.63 ± 1.10)	20.83 - 34.95 (24.69 ± 0.65)	374.07 - 875.15 (654.76 ± 26.32)

Minimum m; maximum M; arithmetic mean $\bar{x}$; standard deviation $s\bar{x}$.

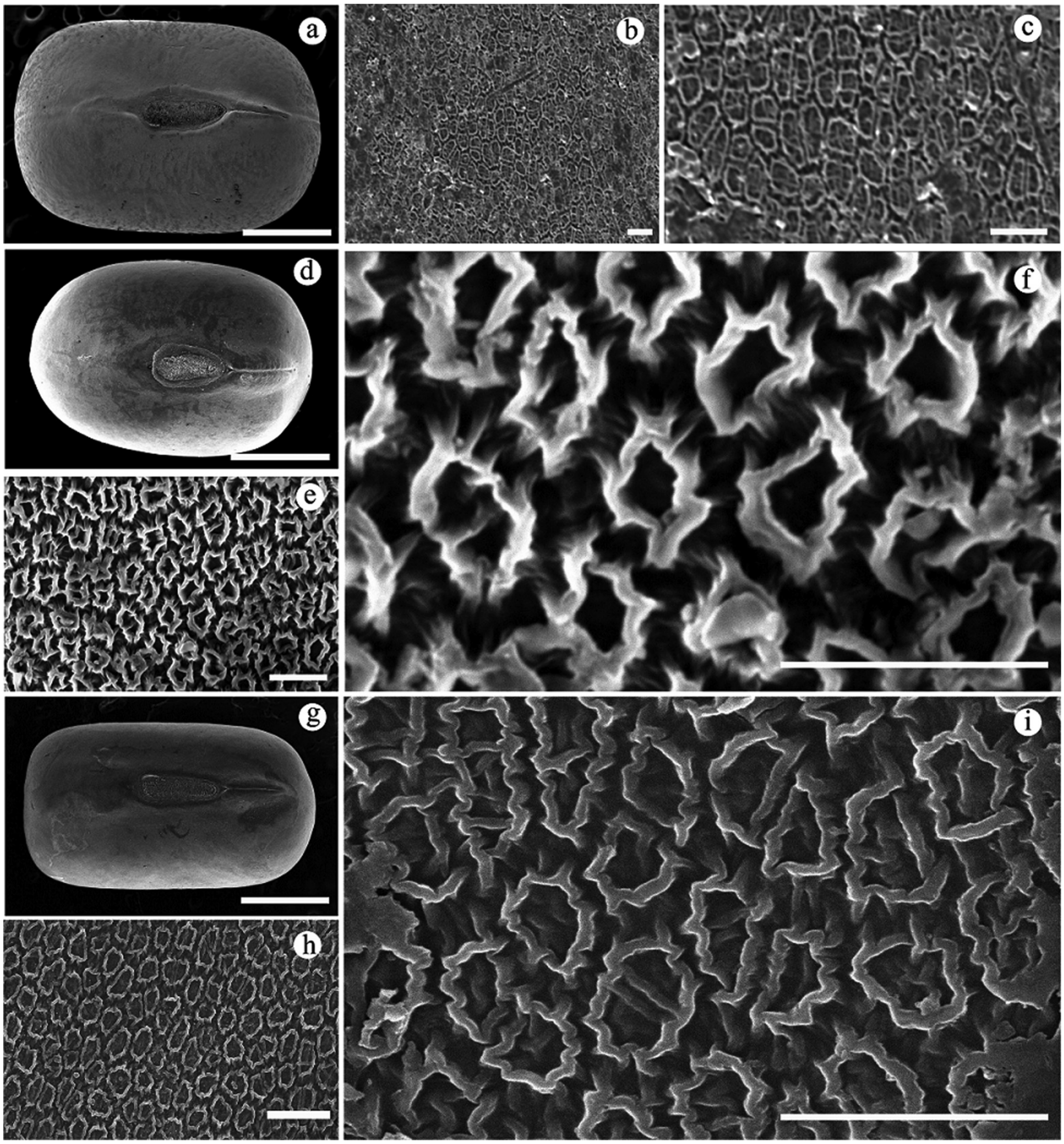

Fig. 2. Scanning electron micrographs of seeds. (a–c) *Vigna aconitifolia* (cult), I-ac type; (d–f) *Vigna aconitifolia* (wild), I-aw type; (g–i) *Vigna aconitifolia* (wild), II-aw type; Scale bars: a, d, g - 1 mm; b, e, h - 100 µm; c, f, i - 10 µm.

The anticlinal walls are raised, undulate with thick wax deposition. The periclinal walls are concave with loose reticulations (Fig. 4c).

3.1.4.2. Type II-sti. The seeds are rectangular with roundish ends and brown in color. The texture of the testa is smooth. The seeds are variable in size, 2.29–2.66 (2.39 ± 0.02) mm long, 1.62–2.04 (1.83 ± 0.02) mm wide (Table 2). The hilum is central, ellipsoidal, oblong in shape and distinctly protruding (Fig. 4d). The hilum is 1.15–1.46 (1.32 ± 0.02) mm long and 0.59–0.73 (0.66 ± 0.01) mm wide (Table 2). The aril is moderately developed. The seed surface is reticulate-foveate to reticulate, with broad polygonal or circular testa cells (Fig. 4e). The testa cells are 13.67–18.84 (16.03 ± 0.30) µm long, 7.54–12.24 (10.13 ± 0.28) µm wide with an area of 93.25–188.05 (142.71 ± 1.77) µm (Table 2). The arrangement of polygonal testa cells is irregular. The anticlinal walls are raised, undulate with thick wax deposition. The periclinal walls are concave with loosely arranged micro-reticulations (Fig. 4f).

3.1.4.3. Type III-sti. The seeds are rectangular with roundish ends and brown in color. The texture of the testa is smooth. The seeds are variable in size, 2.36–2.69 (2.57 ± 0.03) mm long, 1.83–2.05 (1.93 ± 0.02) mm wide (Table 2). The hilum is central, ellipsoidal, oblong in shape and distinctly protruding (Fig. 5a). The hilum is 1.18–1.42 (1.30 ± 0.03) mm long and 0.53-0.64 (0.60 ± 0.01) mm wide (Table 2). The aril is moderately developed. The seed surface is reticulate-foveate to reticulate, with broad polygonal or circular testa cells (Fig. 5b). The testa cells are 8.39–15.06 (11.53 ± 0.66) µm long, 6.88–11.12 (9.30 ± 0.47) µm wide with an area of 59.64–155.99 (91.15 ± 9.90) µm (Table 2). The arrangement of polygonal testa cells is irregular. The anticlinal walls are raised, sharp and have thick wax deposition. The periclinal walls are concave with compact reticulate surface (Fig. 5c).

3.1.4.4. Type IV-sti. The seeds are rectangular with roundish ends and brown in color. The texture of the testa is smooth. The seeds are variable in size, 1.99–2.91 (2.35 ± 0.09) mm long, 1.65–2.41 (1.92 ± 0.08) mm wide (Table 2). The hilum is central, ellipsoidal, oblong in shape and distinctly protruding (Fig. 5d). The hilum is

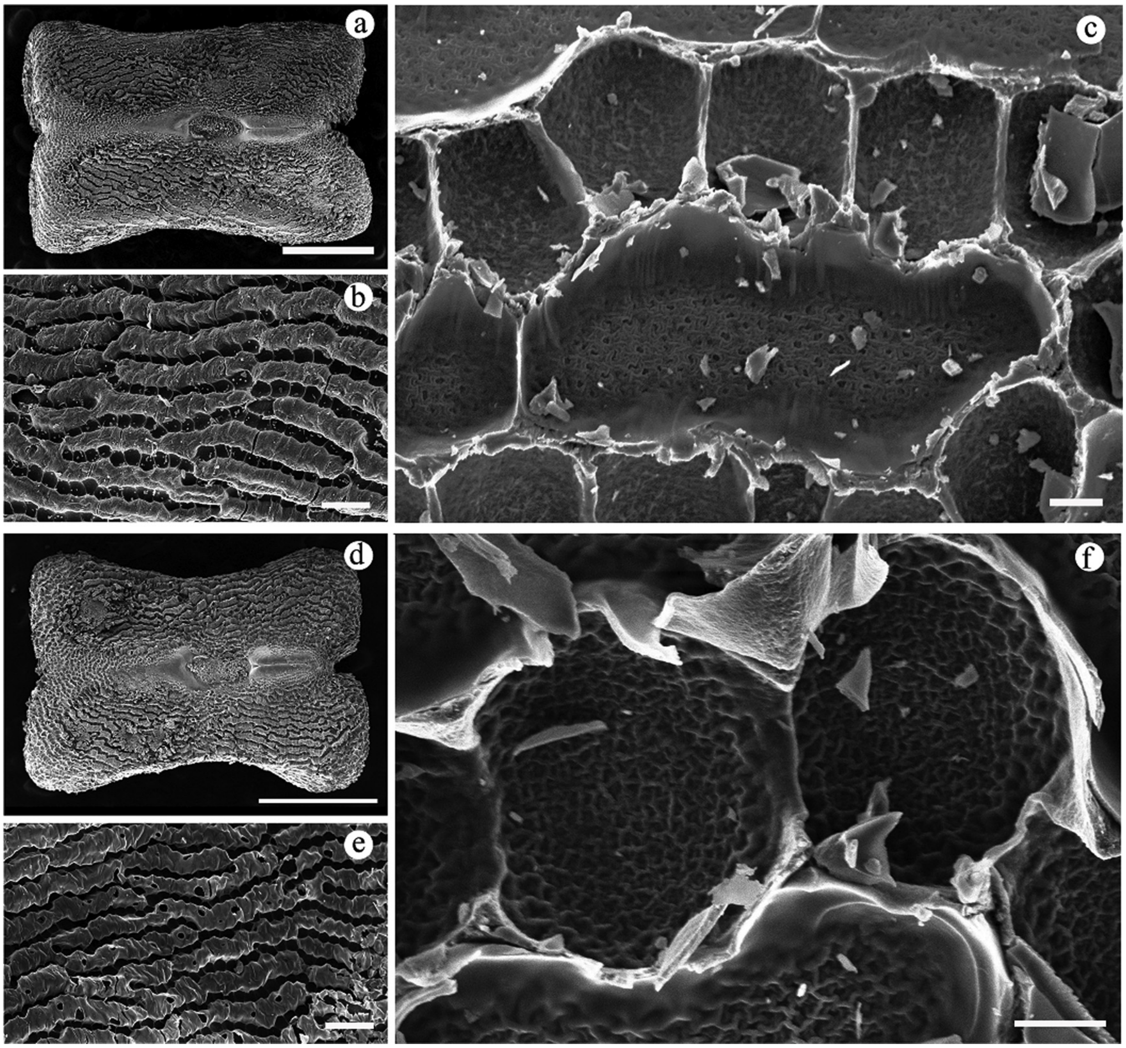

Fig. 3. Scanning electron micrographs of seeds. (a–c) *Vigna indica*, I-ind type; (d–f) *Vigna indica*, II-ind type (taken from Umdale et al., 2017b); Scale bars: a, d - 1 mm; b, e - 100 µm; c, f - 10 µm.

1.10–1.54 (1.26 ± 0.04) mm long and 0.52–0.74 (0.64 ± 0.01) mm wide (Table 2). The aril is moderately developed. The seed surface is reticulate-foveate to reticulate, with broad polygonal or circular testa cells (Fig. 5e). The testa cells are 9.28–16.49 (12.61 ± 0.54) µm long, 5.88–10.77 (8.44 ± 0.04) µm wide with an area of 48.19–153.16 (94.16 ± 7.71) µm (Table 2). The arrangement of polygonal testa cells is irregular. The anticlinal walls are raised, undulate with thick wax deposition. The periclinal walls are concave and compact reticulate with pustules (Fig. 5f).

3.1.5. *Vigna trilobata*

3.1.5.1. Type I-tri. The seeds are globular with roundish ends and yellowish brown with black spots. The testa texture is smooth and shiny. The seeds are variable in size, 1.85–2.94 (2.34 ± 0.18) mm long, 1.55–1.84 (1.68 ± 0.03) mm wide (Table 2). The hilum is central, broadly ovate in shape and distinctly protruding (Fig. 6a). The hilum is 0.69–1.18 (0.92 ± 0.07) mm long and 0.65–1.06 (0.84 ± 0.06) mm wide (Table 2). The aril is well developed. The seed surface is reticulate, with broad polygonal testa cells arranged in rows (Fig. 6b). Testa cell are 22.62–46.87 (37.74 ± 2.28) µm long, 15.15–28.60 (19.87 ± 1.62) µm wide with an area of 648.98–977.71 (868.94 ± 34.53) µm (Table 2). The testa cells are polygonal with thick longitudinal wall and thin transverse wall, disappearing at center. The periclinal walls are concave with compact reticulations (Fig. 6c).

3.1.5.2. Type II-tri. The seeds are rectangular to globular with roundish ends. The seed surface is brown with black spots. The texture of the testa is smooth and shiny. The seeds are variable in size, 2.67–3.00 (2.84 ± 0.05) mm long and 1.67–1.86 (1.77 ± 0.02) mm wide (Table 2). The hilum is central, broadly ovate in shape and distinctly protruding (Fig. 6d). The hilum is 1.08–1.17 (1.13 ± 1.01) mm long and 0.90–0.97 (0.94 ± 0.01) mm wide (Table 2), and the aril is well developed. The seed surface is reticulate. The broad polygonal testa cells are arranged in rows with intercellular space (Fig. 6e). The testa cells are 24.44–39.23 (30.11 ± 1.49) µm long, 17.16–24.28 (20.42 ± 0.72) µm wide with an area of 474.78–807.47 (644.93 ± 33.03) µm (Table 2). The testa cells are polygonal with thick longitudinal walls and thin transverse walls. The periclinal walls exhibit a densely reticulate surface with central pit (Fig. 6f).

3.1.6. *Vigna khandalensis*

The seeds are rectangular to oblong in shape and light to dark black in color. The texture of the testa is rough. The seeds are variable in size, 3.24–4.41 (3.79 ± 0.08) mm long, 2.74–3.76 (3.21 ± 0.07) mm wide (Table 2). The hilum is central, oblong in shape (Fig. 7a). The hilum is 1.20–1.69 (1.43 ± 0.03) mm long and 0.45-0.68 (0.57 ± 0.01) mm wide (Table 2). The aril is absent. The seed surface is reticulate. The testa cells are arranged in rows with intercellular spaces (Fig. 7b). The testa cells are 23.75–44.55 (34.63 ± 1.10) µm long, 20.83–34.95 (24.69 ± 0.65) µm wide with an area of 374.07–875.15

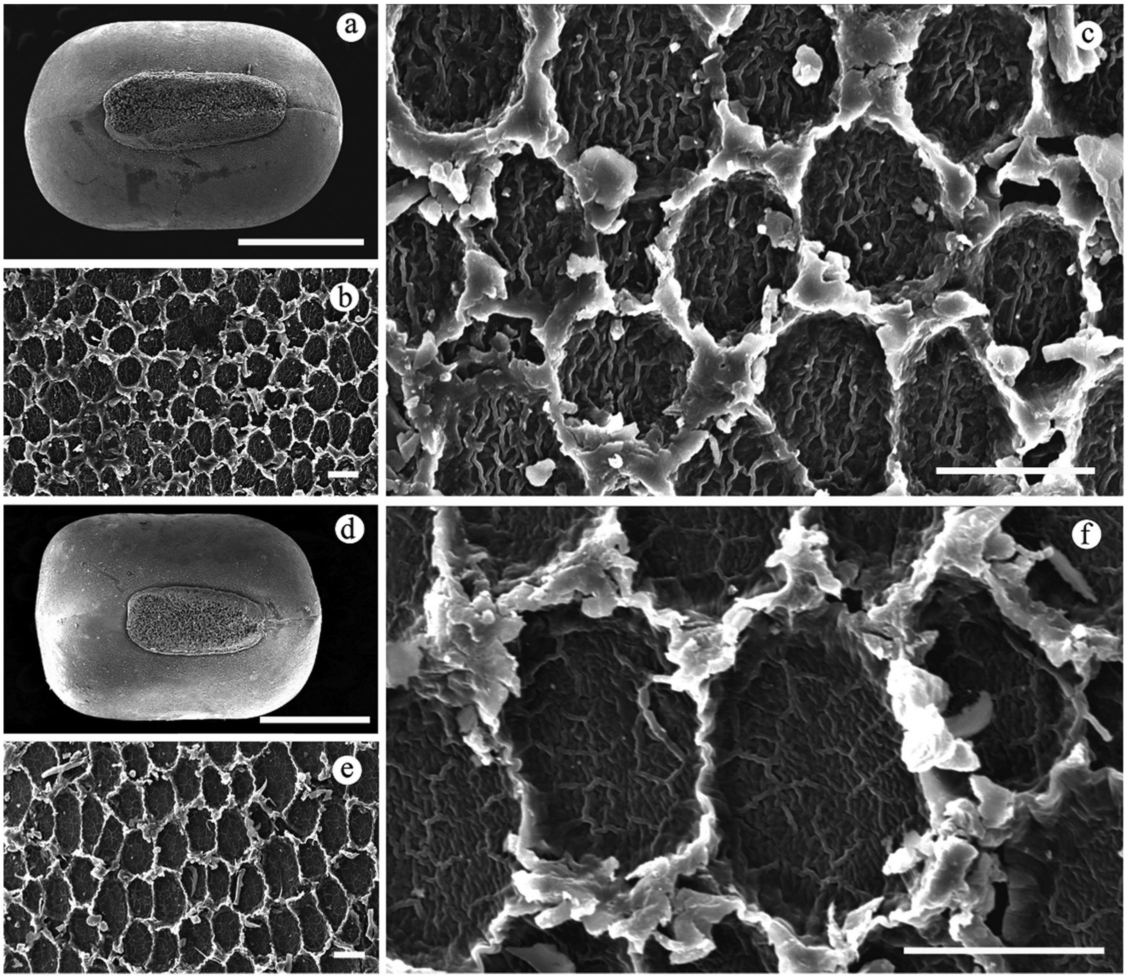

Fig. 4. Scanning electron micrographs of seeds. (a–c) *Vigna stipulacea*, I-sti type (taken from Umdale et al., 2017b); (d–f) *Vigna stipulacea*, II- sti type; Scale bars: a, d - 1 mm; b, e - 100 µm; c, f - 10 µm.

(654.76 ± 26.32) µm (Table 2). The testa cells are broad and polygonal with irregularly thick anticlinal walls. The periclinal walls are flat and exhibit a compact reticulate surface (Fig. 7c).

3.2. Molecular phylogeny

The rDNA-ITS region amplified with primer pairs ITS1 and ITS4 yielded a PCR product of approximately 630 bp. The aligned dataset of nrDNA-ITS (ITS1, 5.8 s, ITS2) sequences ranged from 602 to 618 bp and 602 to 631 bp in the aligned matrix of 647 characters with the outgroup (Supplementary data S1).The rDNA-ITS region contained 144 variable sites, 476 conserved sites, 34 singletons and 110 parsimony informative sites. The overall mean sequence distance was 6.61%. The overall transition/transversion bias (R) was 1.75, which was calculated by applying the Kimura 2-parameter model. Parsimony analysis of the rDNA-ITS data matrix resulted in six most parsimonious trees of length 205 with consistency index (CI) of 0.781, retention index (RI) of 0.961 and composite index of 0.750 (Supplementary data S1).

A strict consensus trees derived from MP analysis is depicted in Fig. 8. The K2 + G model is selected as the best model based on the hierarchical likelihood ratio test and the Akaike Information Criterion (AIC). The maximum parsimony analysis recovers three major clades within the section *Aconitifoliae* with high bootstrap support (Fig. 8). The clade I comprised two species *V. indica* and *V. aconitifolia* (BS = 97). All 16 accessions of *V. indica* were clustered in two groups, among which the first cluster included all 14 accessions of type I-ind seeds and the second cluster included two accessions with type II-ind seeds with high bootstrap support. Furthermore, all the eight accessions of *V. aconitifolia* were distinctly resolved into three clusters with high bootstrap support (BS = 85): four accessions with type I-ac seeds, two accessions with type I-aw seeds and two accessions with type II-aw seeds. Clade II included two species, namely *V. trilobata* and *V. stipulacea* and its bootstrap support is high (BS = 89) (Fig. 8). Within clade II, two subclades were observed, among which the first cluster comprised four accessions of *V. trilobata* and the second one encompassed 16 accessions of *V. stipulacea*. Within *V. trilobata*, two accessions with type I-tri seeds and two with type II-tri seeds constituted two distinct clusters. The 16 accessions of all four seed types (I-sti, II-sti, III-sti and IV-sti) of *V. stipulacea* were grouped into one major cluster. The clade III included all four accessions of *V. khandalensis* (Fig. 8).

4. Discussion

The seed micro-morphological characters, such as structure of hilum, development of aril and ornamentation of testa, have played a significant role for species delimitation within subgenus *Ceratotropis* (Sharma et al., 1983; Chandel et al., 1984; Dixit et al., 2011; Umdale et al., 2017b). The present investigation constitutes the first comprehensive evaluation of intraspecific variation of the cell types of the testa. We determined that each seed coat type has a distinctive cell wall pattern. The intraspecific seed coat polymorphism of the genus *Vigna* has been studied by several researchers (Sharma et al., 1983; Ignacimuthu and Babu, 1985). Sharma et al. (1983) assessed the seed coat polymorphism in 12 different natural populations of *Phaseolous*

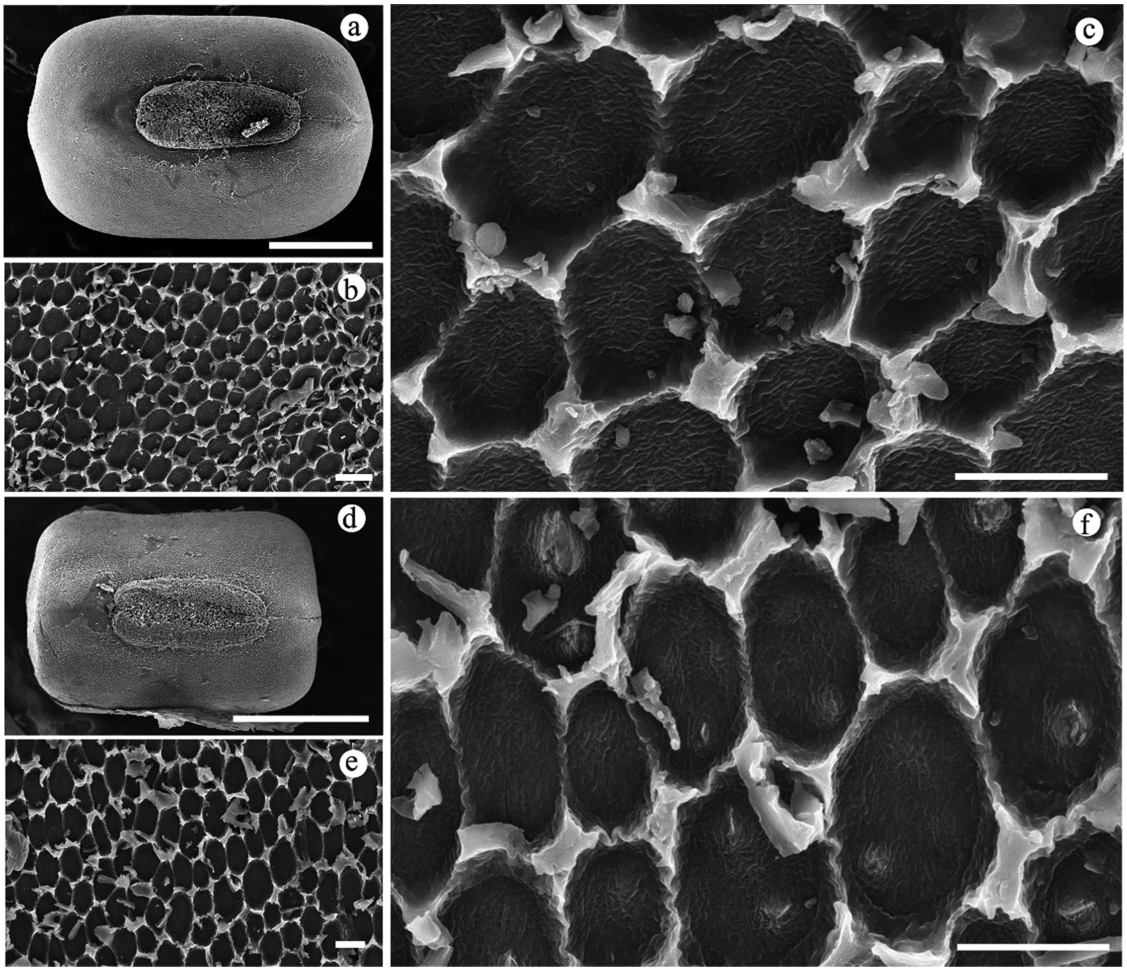

Fig. 5. Scanning electron micrographs of seeds. (a–c) *Vigna stipulacea*, III-sti type; (d–f) *Vigna stipulacea*, IV- sti type; Scale bars: a, d - 1 mm; b, e - 100 µm; c, f - 10 µm.

sublobatus Roxb. (= *Vigna sublobata*). The populations displayed five remarkable seed coat patterns, and the results were confirmed by multivariate statistical analyses. Similarly, Ignacimuthu and Babu (1985) detected two different seed coat types and subtypes across eight different *V. sublobata* populations collected from Palani hills of the Western Ghats in Tamilnadu, India.

The present study establishes that all the species except *Vigna khandalensis* exhibit extensive seed coat polymorphism. The testa cell wall is less raised, wavy and of indistinct thickness in cultivated *V. aconitifolia*. However, two distinct (type I-aw and type II-aw) seed testa patterns were recognized in wild populations of *V. aconitifolia*. Type I-aw seeds were characterized by irregularly-arranged polygonal testa cells with raised, wavy anticlinal and unevenly granular periclinal walls. A similar seed testa pattern has been reported in the wild accession of *V. aconitifolia* (C-2515) (Chandel et al., 1991). Type II-aw seeds exhibit broad polygonal testa cells without protruding anticlinal walls. A comparable testa pattern for wild accessions has been reported by Kumar and Rangaswamy (1984).The accessions of the cultivated and wild forms showed remarkable variations in the size of the testa cells according to the seed type (Fig. 9a,b,c). The phylogenetic analysis used for reference indicates no geographical correlation of the two clusters of *V. aconitifolia* (Fig. 8).

Dixit et al. (2011) raised *Vigna trilobata* (L.) Verdc. var. *pusilla* Naik et Pokle, to the rank of species as *Vigna indica* T.M. Dixit, K.V. Bhat et S.R. Yadav. Takahashi et al. (2016) and Umdale et al. (2017b) recognized differences between *V. indica* from *V. trilobata* based on molecular data and seed testa pattern. In the present investigation, we have recorded two different seed testa patterns in *V. indica*. The type I-ind seeds showed large testa cells with flat, compactly reticulate periclinal walls, and the type II-ind seeds displayed small testa cells with flat, loosely reticulate periclinal walls. In *V. indica*, the testa cells of type I-ind seeds are larger than those of type II-ind seeds (Fig. 9a,b,c). The latter species is commonly distributed in the Western, Central and Northern India.

Furthermore, the rDNA ITS phylogenetic analysis recovers two different accession clusters in *Vigna indica*. Dixit et al. (2011) reported morphological differentiation among the different populations collected from Rajasthan, Maharashtra, and Karnataka. Dixit et al. (2011) stated that such variation is not sufficient to formally propose a particular intraspecific status. The result obtained in this study concurs with the observation of Dixit et al. (2011) regarding the absence of conclusive differential traits at an intraspecific level.

Vigna stipulacea can be easily distinguished from all other species of the section *Aconitifoliae* by the presence of large stipules (Tomooka et al., 2002b; Dixit et al., 2011; Dixit, 2014). Umdale et al. (2017b) documented the testa pattern with reticulate periclinal walls and raised, sharp anticlinal walls. In the present investigation, 16 accessions of *V. stipulacea* were differentiated into four seed testa patterns. Of these, type I seeds includes five, type II seeds includes six, type III seeds include two and type IV seeds includes three accessions (Table 1). The presence of warty, periclinal wall of the testa cells was observed by Ignacimuthu and Babu (1985) in *V. sublobata* populations. Out of the four seed testa patterns, type I-sti seeds and type II-sti seeds were the most common ones as they were found in 11 accessions. The phylogenetic tree showed no significant variation among different accessions of *V. stipulacea* (Fig. 8).

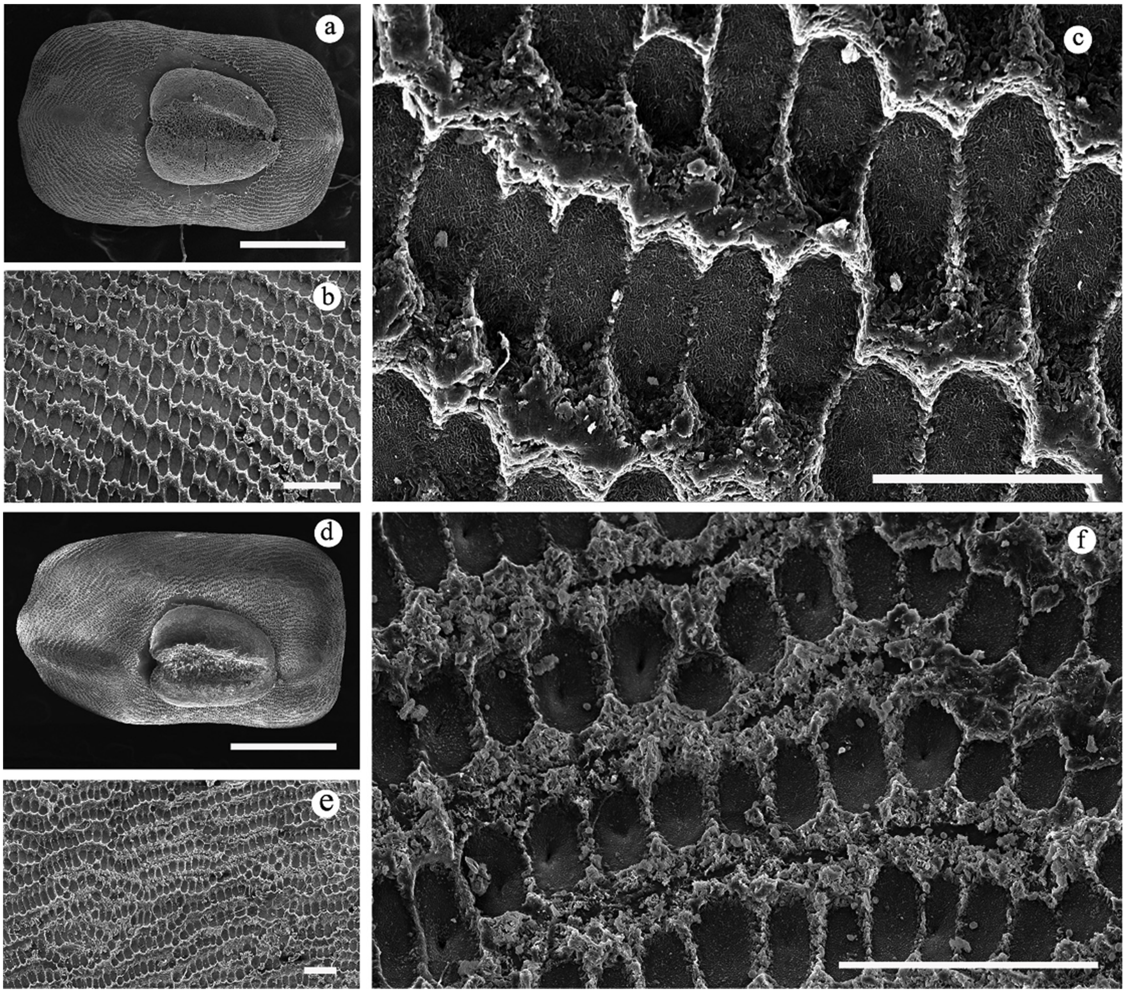

Fig. 6. Scanning electron micrographs of seeds.(a–c) *Vigna trilobata*, I-tri type; (d–f)*Vigna trilobata*, II-tri type; Scale bars: a, d - 1 mm; b, e - 100 μm; c, f - 10 μm.

Morphologically, *Vigna trilobata* and *V. stipulacea* are closely related (Tomooka et al., 2002b; Gore et al., 2019). *Vigna trilobata* has lobed and occasionally entire leaves, seeds with broadly ovate, protruding hilum and well-developed aril (Dixit et al., 2011; Dixit, 2014). In the seed gene bank of NBPGR, most of the collections of *V. trilobata* come from southern India. Out of the four accessions of *V. trilobata*, two from Tamil Nadu exhibit type I-tri seed coat pattern and two from Kerala possess type II-tri seeds. The type I-tri seed pattern was characterized by broad polygonal testa cells arranged in rows with a thin transverse wall disappearing at the center. Umdale et al. (2017b) has also reported a similar seed testa pattern. The type II-tri seed pattern is characterized by testa cells arranged in rows with intercellular spaces and centrally

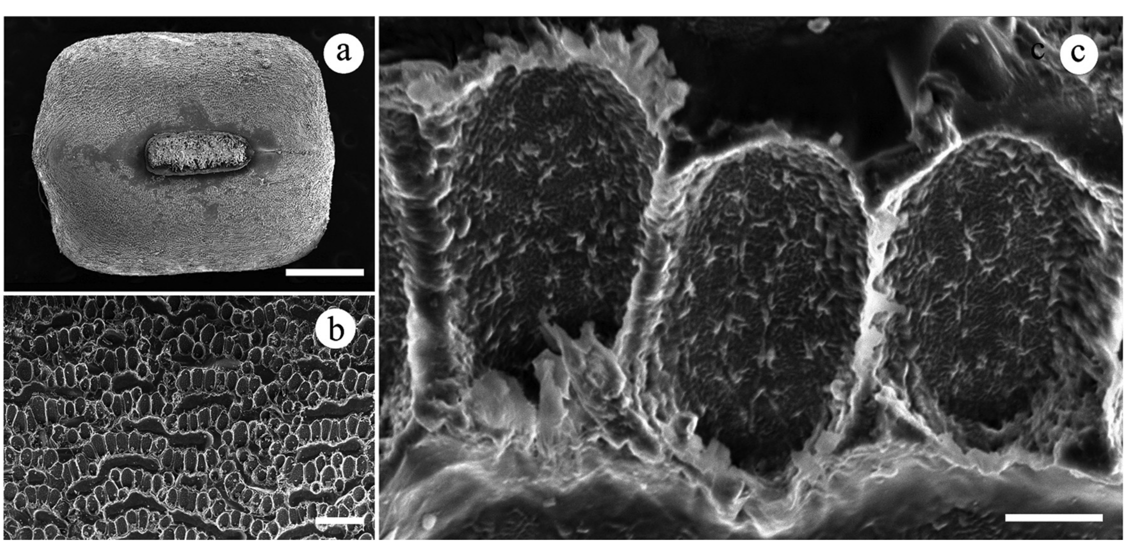

Fig. 7. Scanning electron micrographs of seeds. (a–c) *Vigna khandalensis*, kha type (taken from Umdale et al., 2017b); Scale bars: a-1 mm; b-100 μm; c-10 μm.

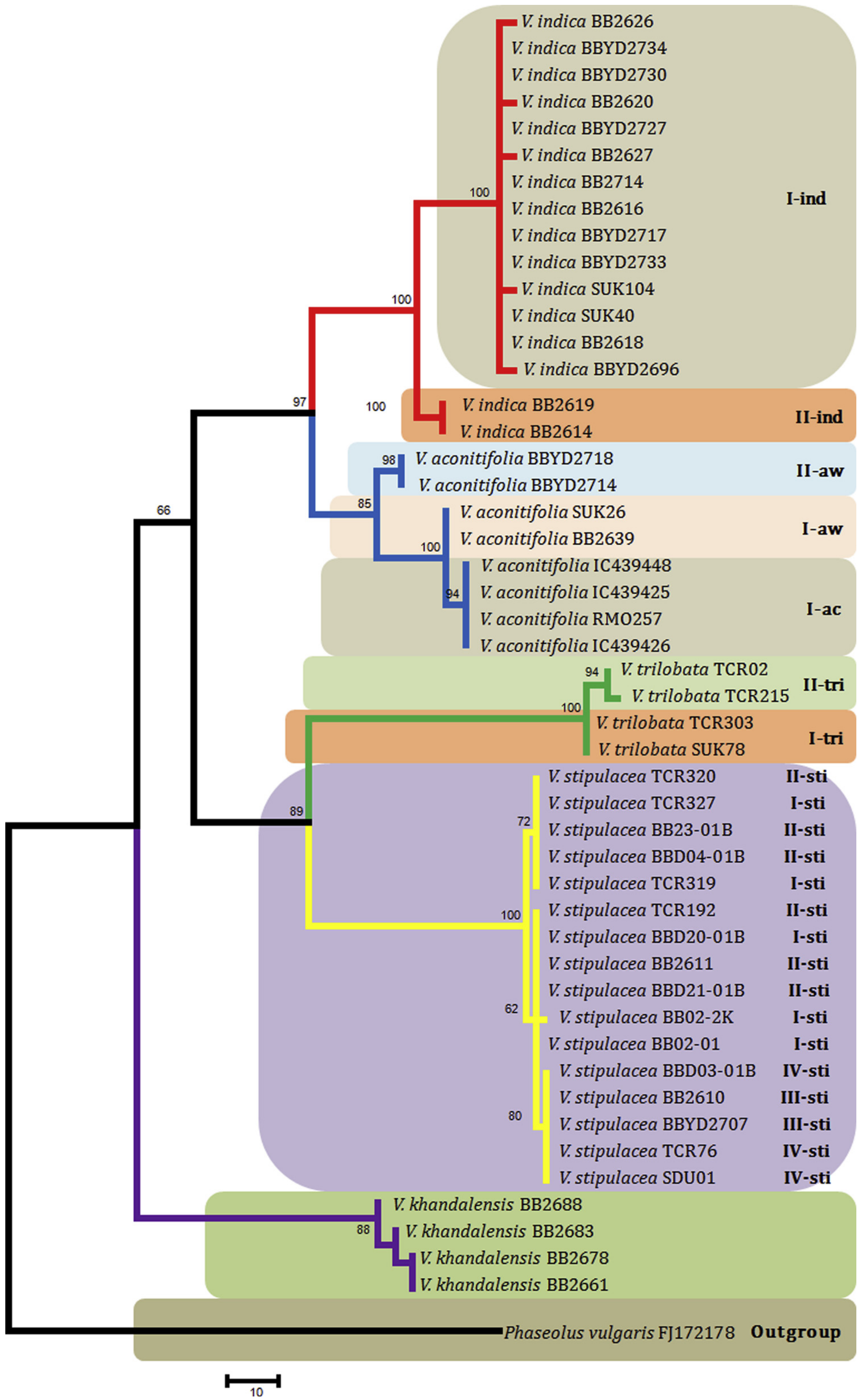

Fig. 8. A strict consensus tree resulting from parsimony analysis of the nrITS dataset including sequences of 48 accessions of species of *Vigna* section *Aconitifoliae*.

pitted, compact reticulate periclinal walls. In the phylogenetic analyses, four populations from Tamil Nadu and Kerala were included, which represented two different clusters.

Vigna khandalensis has an erect, robust habit. This species grows at high elevations and is endemic to the Northern part of the Western Ghats in India (Umdale et al., 2018). The robust habit and large foliaceous stipules set it apart from all other species of the section *Aconitifoliae*. The seeds of *V. khandalensis* are large and possess broad polygonal testa cells arranged in stalks with intercellular spaces (Umdale et al., 2018). In the present investigation, only a single seed coat pattern was recorded. The four populations of *V. khandalensis* are clustered together as one of the main clusters in the phylogenetic

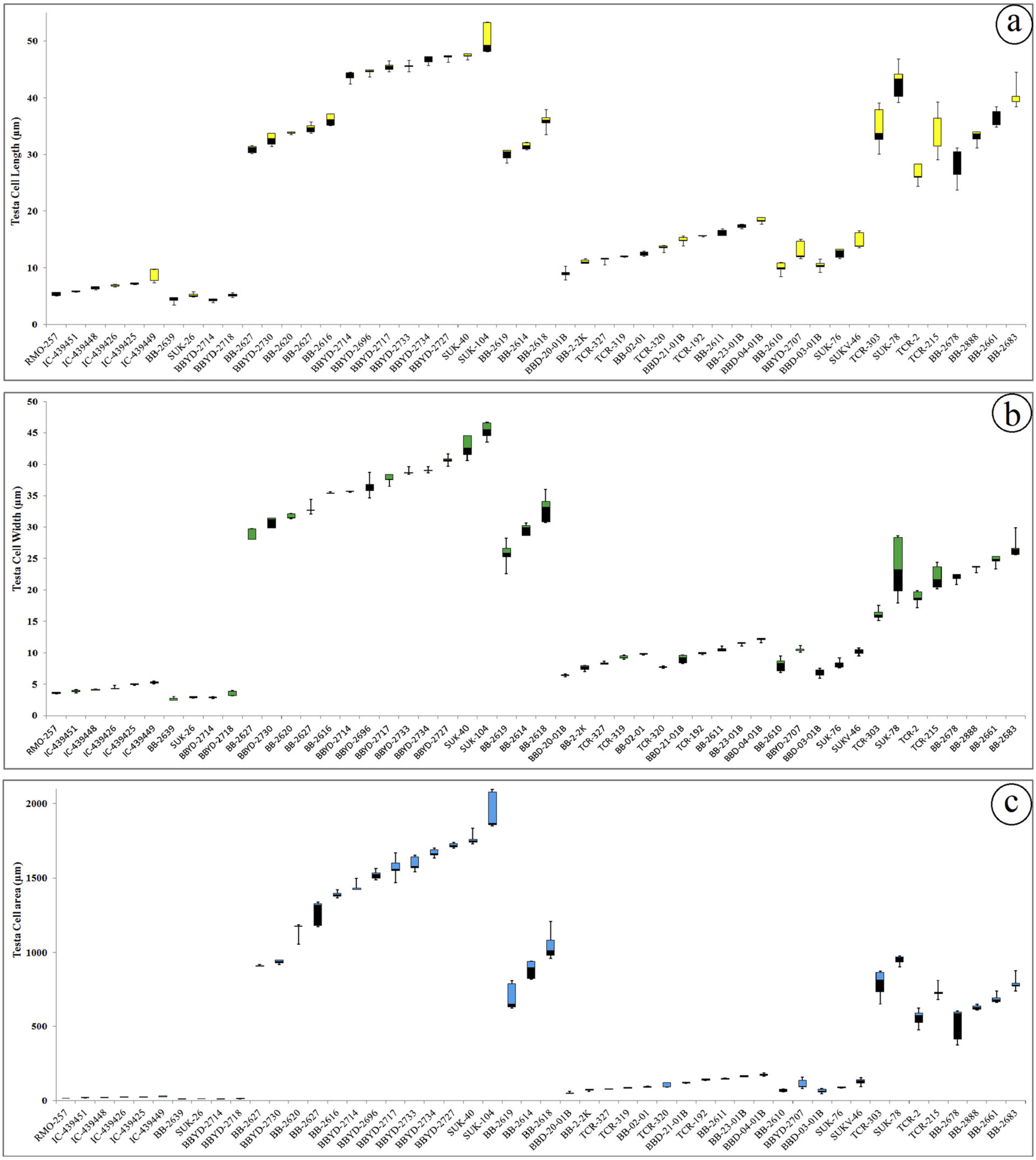

Fig. 9. Variation of (A) testa cell length (B) testa cell width (C) testa cell area ratio among species of section *Aconitifoliae* of genus *Vigna*. Rectangles define 25 and 75 percentiles; vertical lines indicate median; the whiskers are from 10 to 90 percentiles.

analysis (Fig. 8), which suggests its segregation as a distinct species.

5. Conclusion

The hilum, testa cell size, and ornamentation reveal the presence of distinctive features within the species of *Vigna* section *Aconitifoliae*. At the species level, the structure of the hilum and surface of seed coat can be diagnostic at species level within this section. However, at the intraspecific level, only the testa cell characters are significant. Therefore, the testa patterns offer a set of useful characters for the evaluation and management of diverse germplasm accessions belonging to the species of section *Aconitifoliae*.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.flora.2019.151458.

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