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A comparative study of seed morphology and seed coat anatomy of medicinal importance some species of Acanthaceae using SEM and their taxonomic significance

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Abstract
Seed shape, dimensions, surface texture and sculpture, Hilum shape and position were recorded for eleven species of Acanthaceae by using light microscope (LM), and scanning electron microscope (SEM). Seven patterns were recognized based on surface sculpturing pattern: reticulate undulate, papillate, rugose, heteromorphic tubercles, ruminate, moniliform cell, polygonal. Anatomical investigation using light microscope showed that the hypodermis is present in the outer integument of four species and absent in the rest. The inner integument is recorded two types. The data proved useful in the construction of a bracketed key to the species. The potential taxonomic value of the recorded characters is indicating by the richness of variation recorded in the limited sample of genera and species.

Keywords: acanthaceae; identification; seed morphology; seed coat anatomy; SEM

Introduction
Acanthaceae family is one of the 9th largest family of dicotyledons and includes several species are medicinal importance. Acanthaceae according to Cronquist (1981) [5] consists of about 250 genera and 2500 species widespread in tropical regions. In India about 70 genera and 340 species have been reported (Sharma, 2003) [9]. The features of the families include cystoliths usually on leaves and stem, explosively dehiscent capsule and seeds with endosperm lacking.

The morphology of seed coat is usually stable and is little influenced by external environmental conditions while the seeds developed and ripen within the fruit (Cole and Behnke, 1975; Barthlott, 1981) [4, 3]. Therefore seed characters can provide useful data in the delimitation and identification of species. The morphologic features of different seed structures provide a wide range of characters which can play an important role on the identification of taxa. The presence or absence of slime in the epidermal cells of seeds may have an inter or intra- specific taxonomic significance (Vaughan, 1968) [10] and have traditionally been used to solve systematic and phylogenetic problems. Micromorphology and ultra-structural data have contributed useful information for evolution and classification of seed plants and play an important role in the modern synthetic systems of Angiosperms (Dahlgren, 1980) [6]. Several studies focused on intragenic seed coat variation (Juan *et al.*, 2000; Segarra and Mateu, 2001) [7, 8] or on variation among several closely related genera and species (Al-Gohary and Mohamed, 2007; Abdel Khalik, 2013; Dalia Gabr, 2014) [2, 1]. Scanning electron microscope (SEM) provides deeper insight where gross morphology proves insufficient to analysis seed coat structure and surface sculpture. These two aspects are of a great taxonomic value at generic and infra-generic status (Brisson and Peterson, 1976, 1977).

Very little research has been undertaken of the seed coat surface of the species of Acanthaceae. In Maharashtra only a few species have been worked out for seed coat surface and anatomy are not yet correctly projected. The present work examined the morphological and micro-morphological characteristics of the seeds for eleven species in Maharashtra, in a search for taxonomic characters useful in distinguishing of the species.

Materials and Methods
The present study includes seeds of eleven species of Acanthaceae collected fresh from different localities (Table1) The Plant material brought to the laboratory and compared with herbarium specimens and studied thoroughly by using pertinent literature. Seeds taken from living plants were removed from fully or nearly dry capsules. The identification is also based on the of shrinkage of the intercostals regions of the fruit by desiccation. Fully dry capsules are evanescent, springing open and scattering and seeds at the slightest misdirected touch. Seeds were selected those appeared fully developed and undamaged.

For seed morphology, six to eight seeds were investigated to record their dimensions, shape, colour and surface texture. Dry seeds were cleaned of dust using a fine brush, mounted on the stub using double sided carbon tape. Details of seed sculpture were examined by FET Quanta – 200 scanning electron microscope operated at an accelerated voltage of 20 kV and photographed.

To examine the anatomy of the seed integuments, preserved mature and immature seeds in 70% FAA, dehydrated them in an ethanol series, embedded them in paraffin, then hand section at 10µm – 12µm in thickness take place. The sections were stained in saffranin, fast green, crystal violet and then photographed. (Plate III, Figs 1-9).

Table 1: List of studied species collected from all over Maharashtra.

Name of Taxa	Locality and date
Andrographis paniculata (Burm. f.) Wall.	Wardha, Nursery, 2/ 2013
Asystasia gangetica (L.) T. Anders	Daryapur, Garden, 3/2014
Barleria cristata L.	Kolhapur, University Garden, 2/2013
Barleria prionitis L.	Kolhapur, University Garden, 3/2014
Crossandra infundibuliformis (L.) Nees.	Daryapur, Various houses Garden, 3/2013
Dipteracanthus prostratus (Poir.*)* Nees.	Wardha, Open grass Land, 2/2014
Ecbolium virid (Forsk) Alston.	Aurangabad, University Garden, 3/2013
Hygrophila schulli (Buch.-Ham) Almeida.	Wardha, Water logged area, 3 /2014
Justicia quinqueangularis Koen.	Daryapur, Field or farm, 2/2014
Lepidagathis cristata Wild.	Wardha, Open Barren land, 3/2014
Rungia repens (L.) Nees.	Wardha, Weed in Cultivated field, 2/2014

Result and discussion

Examination of available specimens yielded a wealth of information concerning seed morphology, sculpture of seed surface and anatomy of seed coat. Variation in these aspects among the species is listed in Table 2 and recorded comparatively for individual species in Table 3. Although the characters and their status are self-explanatory, they are illustrated in Plates 1-3 for further clarification. Most of the characters and their status as defined in Tables 2 are recorded for the first time for the species included in the study.

The data recorded in Table 3 were used to construct the following bracketed key for the 11 species of Acanthaceae that it might help in the confirmation of their identification.

1	Seed hairy	
2	Seed tuberculat	
3	Seed triangular elongated and seed coat reticulate- ribbed.....	*Lepidagathis cristata*
4	Seed oblong with median furrow and seed coat tuberculate.....	*Andrographis paniculata*
5	Seeds clothed	
6	Seeds not clothed	
7	Seed texture hairy at margins and seed coat papillate.....	*Dipteracanthus prostratus*
8	Seed texture not hairy; coat not papillate	
9	Seed texture smooth and seed coat reticulate	
10	Seed stexture tuberculate and seed coat undulate.....	*Ecbolium viride*
11	Seed coat tuberculate and surface are Striate-regate with strips.....	*Rungia repen*
12	Seed coat composed monilifrom cells and Flakes of wax like deposition.....	*Barleria cristata*
13	Seeds globose	
14	Seeds flattened	
15	Seed Colour yellow and seed coat composed	
	fringed Scale	Crossandra infundibuliformis
16	Seed colour otherwise and seed coat reticulate	
17	Seed colour brown and more than 7 mm.....	*Barleria prionitis*
18	Seed colour black and seed size 1-1.5 mm.....	*Justica quinqueangularis*
19	Seed ovate and seed coat heteromorphy tuberculate.....	*Asystasia gangetica*
20	Seed discoid and seed coat reticulate ribbed.....	*Hygrophila schulli*

Table 2: List of characters of seed morphology and anatomy recorded comparatively for 11 species of Acanthaceae. Illustrations of characters-states are shown in figures of plate 1-3. Distribution of characters and character-states among the species in given in Table 3.

No. Part	Characters	Characters-states	Illustrations	
		And symbols	plate	Figure
1 Seed	Colour	1- Yellow	1	1
		2- Brown	1	4
		3- Gray	1	3
		4- Black	1	9
		5- Cream	1	7
		6- Dark-Brown	1	8
2 Seed	Texture	1- Warty	1	11
		2- Tuberculate	1	1
		3- Clothed	1	3
		4- Fringed scale	1	5
		5- Soft	1	6
3 Seed	Outline	1- Wavy	1	2
		2- Ovate	1	7
		3- Oblique	1	1
		4- Ttrangular	1	10
		5- Globular	1	6
4 Seed	Shape	1 – Orbicular	1	6
		2 – Sub-orbacular	1	11
		3- Discoid	1	8
		4- Ovate	1	4
5 Hilum	Position	1- Apical	1	5
		2- Basal	1	9
6 Hilum	Shape	1- Elliptic	1	5
		2- Conical	1	9
		3- Linear	1	10
7 Hilum	Level	1- Depressed	1	7
		2- Semi-depressed	1	2
		3- Superficial	1	11
8 Seed surface	Sculpture	1- Reticulate	2	10
		2- Moniliform cells	2	8
		3- Polygonal	2	19
		4- Papillae	2	13
		5- Tuberculate	2	5
		6- Concentric furrows	2	22
9 Seed surface	Secondary	1- Present	2	1
	Ornamentation	2- Absent	2	6
10 Seed surface	Middle lamella	1- Conspicuous	2	23
		2- Inconspicuous	2	16
11 Seed surface	Wax deposition	1- Present	2	21
		2- Absent	2	12

12 Seed coat	Anticlinal wall	1- Straight	2	9
		2- Undulate	2	10
		3- Concave	2	19
13 Seed coat	Anticlinal wall	1- Thick	2	10
	Thickness	2- Thin	2	21
14 Seed coat	Periclinal wall	1- Smooth	2	7
	Texture	2- Rough	2	24
15 Seed coat	Periclinal wall	1- Flat	2	20
		2- Tuberculate	2	4
		3- Warty	2	24
16 Seed coat	Outer integuments	1- Thick	3	1
Anatomy	cuticle thickness	2- Thin	3	8
17 Seed coat	Outer integuments	1- Smooth	3	3
Anatomy	cuticle surface	2- Warty	3	2
18 Seed coat	Outer integuments	1- One-Two	3	9
	Layers	2- Many	3	5
19 Seed coat	Epidermis cells	1- present	3	1
Anatomy	have dense cytoplasm	2-Absent	3	2
2o Seed coat	Inner integument	1-Present	3	4
		2- Absent	3	6

Table 3: Comparative recording of the (20) characters listed in Table 2 for eleven species of each of the Acanthaceae. Serial number of characters and symbols denting characters-states are those assigned to them in Table 2.

Name of Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Andrographis paniculata	1	1	3	2	2	2	2	5	2	2	2	2	1	2	2	1	2	2	1	1
Asystasia gangetica	6	1	1	2	2	1	2	5	1	1	2	2	1	2	8	1	2	2	2	1
Barleria cristata	3	3	4	2	1	1	2	2	2	2	1	1	2	1	1	1	1	2	1	1
Barleria prionitis	2	3	2	4	1	1	2	2	2	2	1	1	2	1	1	1	1	2	1	1
Crossandra infudibulifromis	1	4	5	2	1	3	3	5	1	1	1	2	1	2	2	1	2	2	2	1
Dipteracanthus prostratus	5	5	5	1	1	1	3	4	1	1	1	-	-	-	-	-	-	-	-	-
Ecbolium viride	5	2	3	2	2	2	1	5	2	1	1	2	1	2	2	1	2	1	2	2
Hygrophila schulli	6	3	3	3	2	1	2	1	2	2	1	1	2	1	1	2	1	1	2	2
Justica quinqueangularis	4	1	3	3	2	2	2	3	1	1	2	3	1	2	3	1	-	-	-	-
Lepidagathis cristata	3	3	4	4	1	3	2	2	2	2	1	1	2	1	1	2	1	2	2	2
Rungia repens	6	1	5	2	1	2	3	6	1	1	1	2	1	2	3	1	2	1	2	1

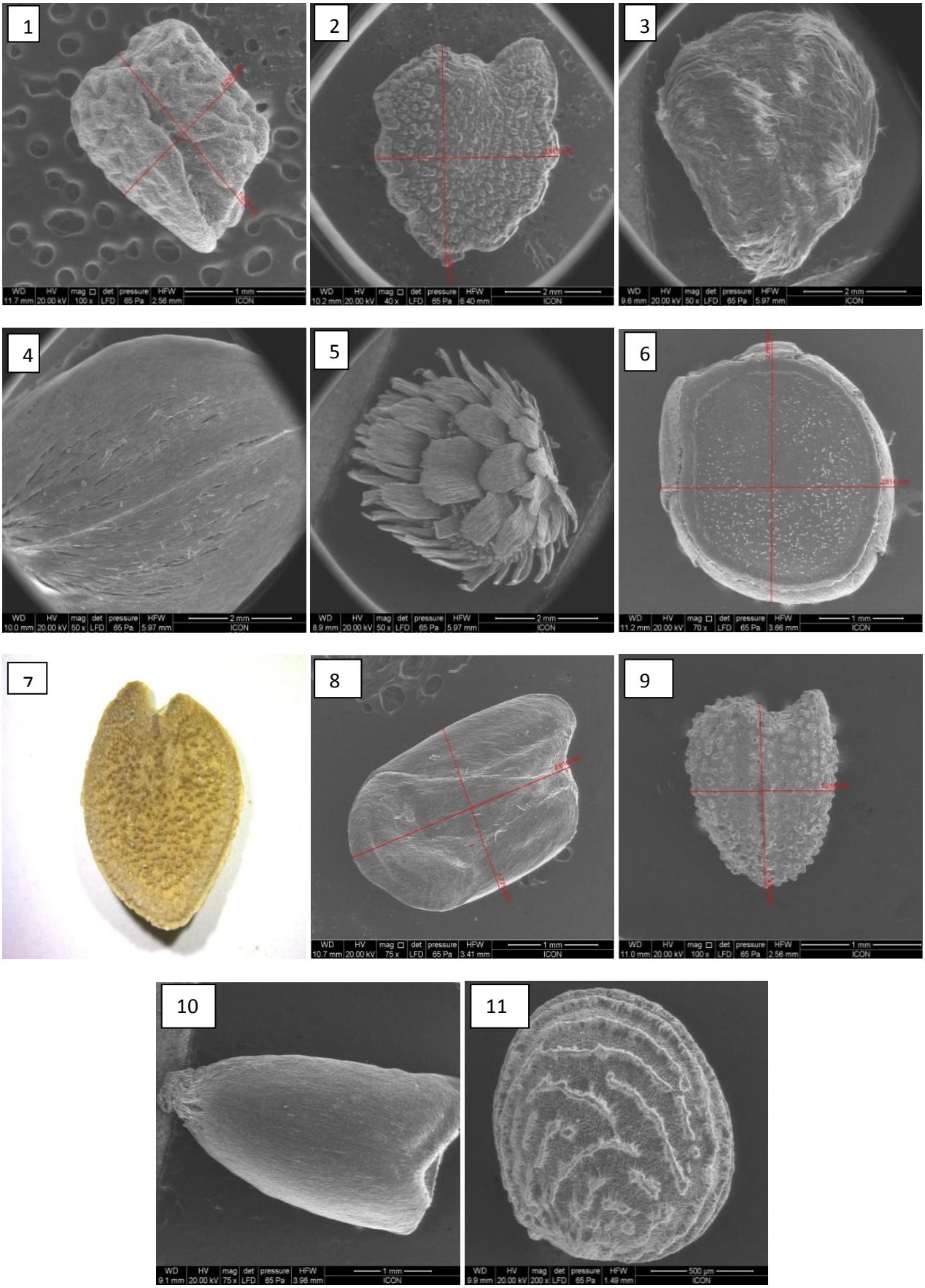

Plate 1: Seed morphology of 11 species of Acanthaceae as revealed by SEM. 1 *Andrographis paniculata*; 2. *Asystasia gangetica*; 3. *Barleria cristata*; 4. *Barleria prionitis*,; 5. *Crossandra infundibulifrom*; 6. *Dipteracanthus prostratus*; 7. *Ecbolium viride*; 8. *Hygrophila schulli;*; 9. *Justicia quinqueangularis*; 10. *Lepidagathis cristata*; 11. *Rungia repens*.

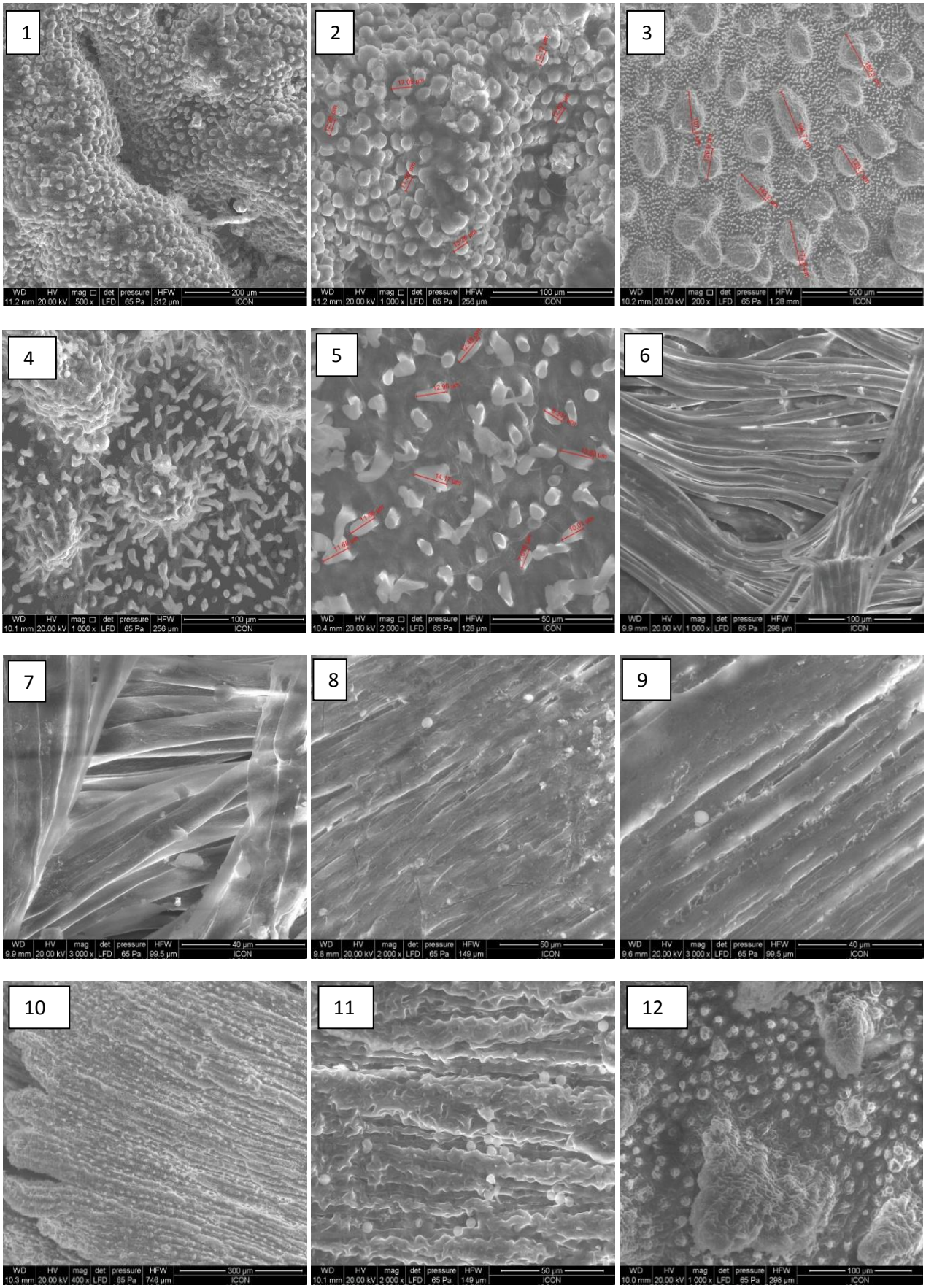

Plate 2: Seed sculpture of seed of 11 species of Acanthaceae as revealed by SEM. *Andrographis paniculata*, (1-2*)*, *Asystasia gangetica*,(3-5), *Barleria cristata*,(6-7), *Barleria prionitis*,(8-9*)*, *Crossandra infundibulifrom*,(10-12).

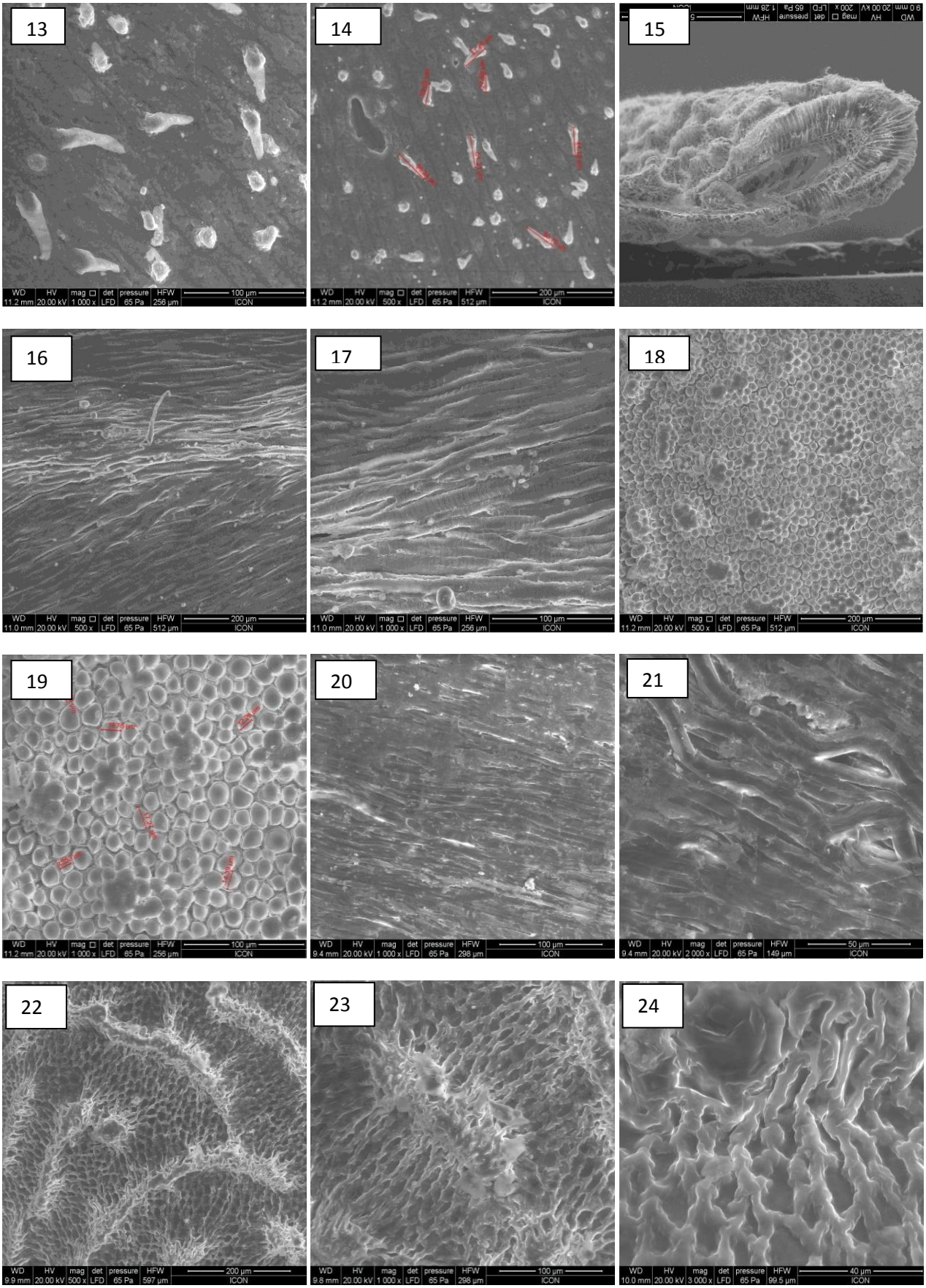

Fig 2: (continued) Surface sculpture of seeds of 11 species of Acanthaceae as revealed by SEM. *Dipteracanthus prostratus*, (13-14), *Ecbolium viride*,(15), *Hygrophila schulli*, (16-17), *Justicia quinqueangularis*,(18-19), *Lepidagathis cristata*, (20-21), *Rungia repens* (22-24).

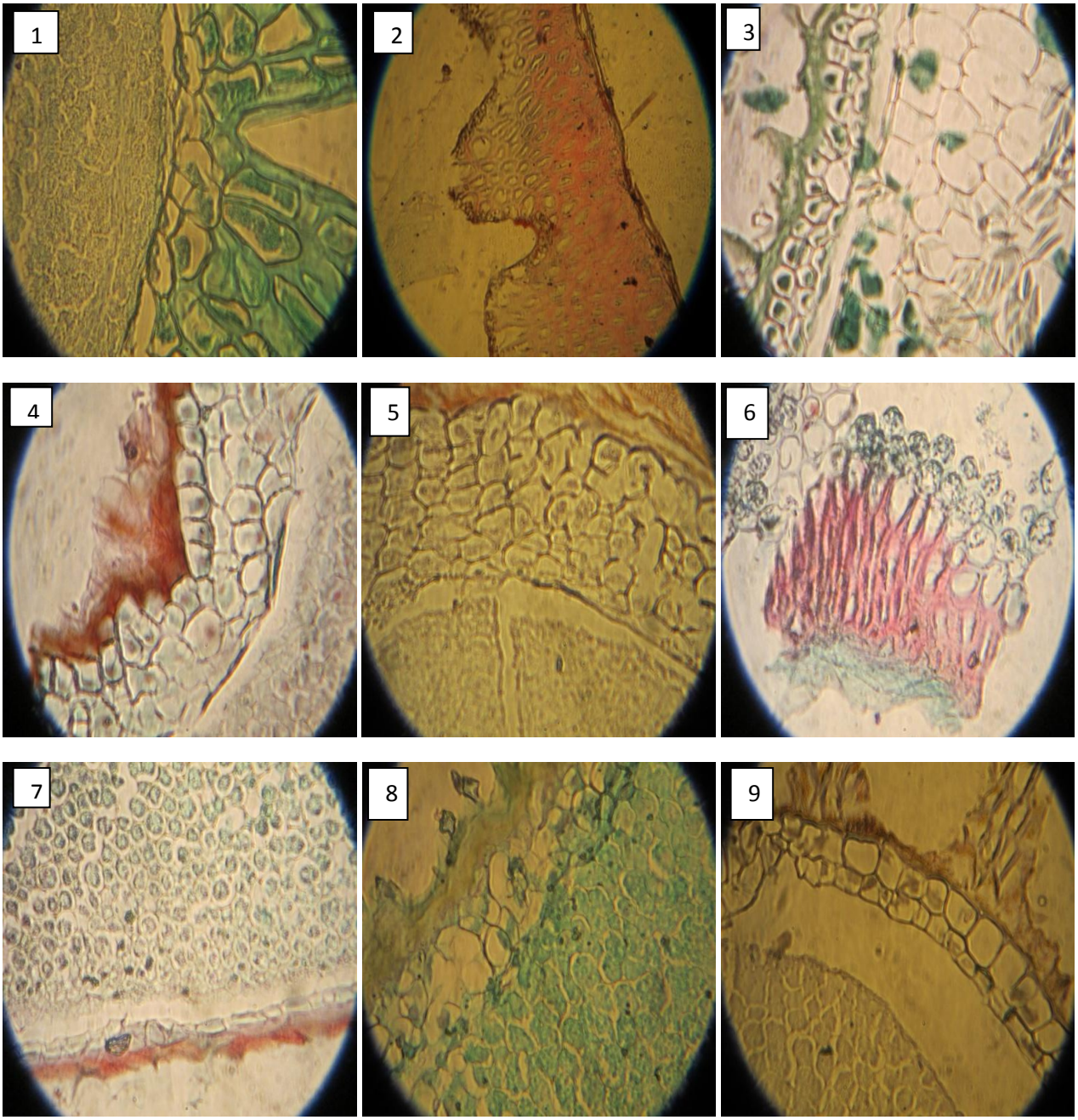

Plate 3: Photomicrograph of cross-section of seed coat of 11 species of Acanthaceae. 1. *Andrographis paniculata*; 2. *Asystasia gangetica*; 3. *Barleria cristata*,; 4. *Barleria prionitis*; 5. *Crossandra infundibulifrom;* 6. *Ecbolium viride*; 7. *Hygrophila schulli;* 8. *Lepidagathis cristata;* 9. *Rungia repens*.

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