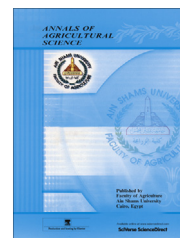




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Seed morphology and seed coat anatomy of some species of Apocynaceae and Asclepiadaceae



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Abstract Seed shape, dimensions, surface texture and sculpture, hilum shape and position were recorded for seven species of each of the Apocynaceae and Asclepiadaceae by using light microscope (LM) and scanning electron microscope (SEM). Seven patterns were recognized based on surface sculpturing pattern: reticulate (with five subtypes), striate, ruminant, papillate, colliculate, aculeate and rugose.

Anatomical investigation using light microscope showed that the hypodermis is present in the outer integument of two 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 indicated by the richness of variation recorded in the limited sample of genera and species.

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Introduction

The Apocynaceae and Asclepiadaceae are relatively large families comprising a total of approximately 5000 species of mainly tropical or subtropical plants with abundant latex. The seed features of the two families include wings, comas, and arils.

The morphology of seed coat is usually stable and is little influenced by external environmental conditions while the seeds develop and ripen within the fruit (Heywood, 1971; Cole and Behnke, 1975; Barthlott, 1981). 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 (Vaughan, 1968) 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). Several studies focused on intragenic seed coat variation (Juan et al., 2000; Segarra and Mateu, 2001) or on variation among several closely related genera (Zainhasan and Lester, 1990; Karam, 1997; Kanak Sahai et al., 1997; Koul et al., 2000; Abdel Khalik and Vander Maesen, 2002; Al-Gohary and Mohamed, 2007; Abdel Khalik, 2013). Scanning electron microscopy (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).

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Very little research has been undertaken on the seed coat surface of the species of Apocynaceae and Asclepiadaceae as (Pichon, 1948, 1949; Leeuwenberg, 1994; Omlor, 1998; Dlcdm et al., 2010 and Kumar et al., 2011). In Egypt only a few Egyptian species have been worked out for seed coat surface as; Al Nawaihi et al., 2006 and the anatomy are not yet correctly projected. The present work examined the morphological and micro-morphological characteristics of the seeds for 14 species in Egypt, in a search for taxonomic characters useful in distinguishing of the species.

Materials and methods

The present study included seeds of seven species of Apocynaceae and seven species of Asclepiadaceae collected fresh from different localities in Egypt (Table 1). The materials studied were identified by means of comparison with specimens kept in the herbarium of the Agricultural Museum (CAIM). In addition, keys of Bailey (1916), Lindley (1932), Metcalfe and Chalk (1979), Hutchinson and Dalziel (1963), Täckholm (1974), Davis (1975), Pandey (1997), Endress and Bruyns (2000) and Boulos (2000). Reference herbarium specimens of studied species were prepared and kept in the herbarium of Botany and Microbiology Department, Faculty of Science (Girls Branch) Al-Azhar University. For seed morphology, five to seven seeds were investigated to record their dimensions, shape, color and surface texture. Details of seed sculpture were examined by a JEOL JSM – 5500 scanning electron microscope operated at an accelerated voltage of 20 kV and photographed. The terminology of Barthlott (1981, 1990) and Stearn (1992) was adopted to describe the SEM aspects of the seed coat.

Table 1 Collection data of the species included in the present study; all localities are in Egypt.

Family	Species	Locality and date	
Apocynaceae	<i>Acokanthera spectabilis</i> Hook.	El-Orman Garden, 3/2009	
	<i>Carissa spinarum</i> L.	The Zoo, 3/2009	
	<i>Thevetia peruviana</i> (Pers.) K. Schum.	Agriculture Museum Garden, 3/2009	
	<i>Vinca rosea</i> L.	The Zoo, 3/2009	
	<i>Alstonia scholaris</i> (L.) R. Br.	The Zoo, 3/2009	
	<i>Nerium oleander</i> L.	El-Kobba Palace, 3/2009	
	<i>Mascarenhasia elastica</i> K. Schum.	Asswan Botanical Garden, 4/2009	
	Asclepiadaceae	<i>Leptadenia arborea</i> (Forssk.) Schweinf.	Asswan, 4/2010
		<i>Leptadenia pyrotechnica</i> (Forssk.) Decne.	Wadi Hagol, 3/2010
		<i>Calotropis procera</i> (Aiton) W. T. Aiton.	Al-Azhar University, 6/2009
<i>Cynanchum acutum</i> L.		El-Fayoum, 3/2009	
<i>Solenostemma argel</i> (Delile) Hayne.		Asswan, 4/2010	
<i>Asclepias curassavica</i> L.		Asswan Botanical Garden, 4/2009	
<i>Cryptostegia grandiflora</i> R.Br.		Agriculture Museum Garden, 3/2009	

For seed anatomy matured seed was soaked in boiling water, then hand section at 20–30 µm in thickness take place. The sections were stained according to Dilcher (1974) in safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) then photographed.

Results 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 three aspects among the species is listed in Table 2 and recorded comparatively for individual species in Table 3. Although the characters and their states are self explanatory, they are illustrated in Plates 1–3 for further clarification. Most of the characters and their states as defined in Table 2 are recorded for the first time for the species included in the present study.

The data recorded in Table 3 were used to construct the following bracketed key to the 14 species of Apocynaceae and Asclepiadaceae that it might help in the confirmation of their identification.

1. Seeds folded	2
1. Seeds flat	
2. Seed elliptic and seed coat reticulate-undulate	<i>Carissa spinarum</i>
2. Seeds flask-shaped; seed coat irregular reticulate. . .	<i>Solenostemma argel</i>
3. Seed winged	4
3. Seed wingless	
4. Seed texture hairy with papillate coat	<i>Calotropis procera</i>
4. Seed texture not hairy; coat not papillate	
5. Seed texture smooth and seed coat reticulate	<i>Leptadenia pyrotechnica</i>
5. Seed texture tuberculate and seed coat not so	
6. Seed coat ruminant	<i>Leptadenia arborea</i>
6. Seed coat colliculate	<i>Cynanchum acutum</i>
Seed globose	3
7. Seed flattened	5
Seed color off white and seed coat striate	<i>Thevetia peruviana</i>
8. Seed color otherwise and seed coat reticulate	
9. Seed color brown and seed size more than 10 mm	<i>Acokanthera spectabilis</i>
9. Seed color black and seed size 1–2 mm	<i>Vinca rosea</i>
10. Seed texture hairy or warty	
10. Seed texture tuberculated	
11. Seed texture hairy and seed coat reticulate-rugose. . .	<i>Nerium oleander</i>
11. Seed texture warty and seed aculeate	<i>Asclepias curassavica</i>
12. Seed oblong and coma persistent	<i>Alstonia scholaris</i>
12. Seed elliptic or long ovate and coma deciduous	
13. Seed elliptic and seed coat reticulate-verruculate	<i>Mascarenhasia elastica</i>
13. Seed long ovate; seed coat rugose	<i>Cryptostegia grandiflora</i>

Table 2 List of characters of seed morphology and anatomy recorded comparatively for 14 species of Apocynaceae and Asclepiadaceae. Illustrations of character-states are shown in figures of [Plates 1–3](#). Distribution of characters and character-states among the species is given in [Table 3](#).

No.	Part	Character	Character-states and symbols	Illustrations	
				Plate	Figure(s)
1	Seed	Color	1 – Brown	1	1
			2 – Off white	1	3
			3 – Black	1	4
2	Seed	Texture	1 – Warty	1	13
			2 – Tuberculate	1	5
			3 – Irregular striated	1	3
			4 – Hairy	1	10
			5 – Smooth	1	9
3	Seed	Outline	1 – Elliptic	1	7
			2 – Ovate	1	8
			3 – Oblong	1	5
			4 – Flask-shaped	1	12
4	Seed	Shape	1 – Globose	1	1
			2 – Flattened	1	14
5	Seed	Margin	1 – Wingless	1	3
			2 – Winged	1	10
			3 – Folded	1	2
			4 – Hairy	1	5
6	Seed	Size	1 – 10–14 mm	1	1
			2 – 4–8 mm	1	8
			3 – 1–2 mm	1	4
7	Hilum	Position	1 – Terminal	1	3
			2 – Central	1	2
8	Hilum	Shape	1 – Oblong	1	1
			2 – Elliptic	1	5
			3 – Conical	1	3
			4 – Linear	1	14
			5 – Oblong-ovate	1	10
9	Hilum	Level	1 – Depressed	1	2
			2 – Semi-depressed	1	7
			3 – Elevated	1	4
			4 – Superficial	1	14
10	Coma	Color	1 – Brown	1	5
			2 – Off-white	1	14
			3 – Absent	1	1
11	Coma	Duration	1 – Deciduous	1	10
			2 – Persistence	1	6
			3 – Absent	1	3
12	Coma	Position ^A	1 – Terminal	1	14
			2 – Peripheral	1	5
			3 – Covering all seed surface	1	6
			4 – Absent	1	2
13	Seed surface	Sculpture	1a – Reticulate–regular	2	1
			1b – Reticulate–undulate	2	2a
			1c – Reticulate–rugose	2	6
			1d – Reticulate–verruculate	2	7
			1e – Reticulate–irregular	2	12a
			2 – Striate	2	3
			3 – Ruminant	2	8a
			4 – Papillate	2	10a
			5 – Colliculate	2	11a
			6 – Aculeate	2	13
			7 – Rugose	2	14

(continued on next page)

Table 2 (continued)

No.	Part	Character	Character-states and symbols	Illustrations	
				Plate	Figure(s)
14	Seed coat	Anticlinal walls: shape	1 – Wavy	2	1
			2 – Undulate	2	10a
			3 – Undulate with hairs	2	6
			4 – Spiral	2	3
15	Seed coat	Anticlinal walls: thickness	1 – Thick	2	2a
			2 – Thin	2	5
16	Seed coat	Anticlinal walls: level	1 – Raised	2	12a
			2 – Grooved	2	11a
17	Seed coat	Periclinal walls: level	1 – Concave	2	6
			2 – Convex	2	14
18	Seed coat	Periclinal walls: texture	1 – Smooth-finely striated	2	1
			2 – Smooth	2	2a
			3 – Smooth with wax	2	6
			4 – Tuberculate	2	10a
			5 – ± Warty	2	12a
19	Seed wing	Sculpture ^A	1 – Reticulate	2	9b
			2 – Reticulate foveate	2	2b
			3 – Tuberculate	2	10b
			4 – Colliculate	2	11b
			5 – Colliculate with secondary roseate structure	2	12b
20	Seed wing	Anticlinal walls: shape ^A	1 – Irregular round	2	2b
			2 – Round-polygonal	2	8b
			3 – Undulate	2	9b
			4 – ± Round	2	11b
			5 – Round	2	12b
21	Seed wing	Anticlinal walls: thickness ^A	1 – Thick	2	2b
			2 – Thin	2	12b
22	Seed wing	Anticlinal walls: level ^A	1 – Raised	2	8b
			2 – Grooved	2	10b
23	Seed wing	Periclinal walls: level ^A	1 – Concave	2	2b
			2 – Convex	2	12b
24	Seed wing	Periclinal walls: texture ^A	1 – Smooth	2	12b
			2 – Smooth with wax	2	11b
			3 – Tuberculate	2	10b
			4 – Warty with wax	2	2b
25	Seed coat anatomy	Outer integument: cuticle thickness	1 – Thick	3	2
			2 – Thin	3	1
26	Seed coat anatomy	Outer integument: cuticle surface	1 – Smooth	3	10
			2 – Warty	3	6
27	Seed coat anatomy	Epidermis	1 – Radial	3	4
			2 – Tangential	3	6
			3 – Radial + tangential	3	7
28	Seed coat anatomy	Hypodermis	1 – Present	3	10
			2 – Absent	3	12
29	Seed coat anatomy	Inner integument	1 – Layers of crushed parenchyma cells	3	1
			2 – One layer of thick square cell followed by crushed parenchyma cells	3	8
30	Seed coat anatomy	Endosperm shape	1 – Angular	3	1
			2 – Elongated	3	2
			3 – Round-polygonal	3	4
			4 – Round	3	10
			5 – Polygonal	3	13
31	Seed coat anatomy	Endosperm thickness	1 – Thick	3	8
			2 – Thin	3	1

^A Inapplicable state if part is absent.

Table 3 Comparative recording of the [31] characters listed in Table 2 for seven species of each of the Apocynaceae and Asclepiadaceae. Serial numbers of characters and symbols denoting character-states are those assigned to them in Table 2.

Species	Characters																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19 ^a	20 ^a	21 ^a	22 ^a	23 ^a	24 ^a	25	26	27	28	29	30	31	
<i>Acokanthera spectabilis</i>	1	1	2	1	1	1	2	1	3	3	3	4	1a	1	2	1	1	1	–	–	–	–	–	–	2	1	3	2	1	1	2	
<i>Carissa spinarum</i>	1	2	1	2	3	2	2	2	1	3	3	4	1b	2	1	1	1	2	2	1	1	1	1	4	1	1	1	2	1	2	1	
<i>Thevetia peruviana</i>	2	3	3	1	1	1	1	3	4	3	3	4	2	4	2	1	1	2	–	–	–	–	–	–	2	1	1	2	1	3	2	
<i>Vinca rosea</i>	3	2	2	1	1	3	2	2	3	3	3	4	1a	2	2	1	1	2	–	–	–	–	–	–	2	1	1	2	1	3	2	
<i>Alstonia scholaris</i>	1	2	3	2	4	2	2	2	2	1	2	2	1a	2	2	1	1	2	–	–	–	–	–	–	1	1	1	2	1	3	2	
<i>Nerium oleander</i>	1	4	2	2	1	2	2	2	3	1	2	3	1c	3	1	1	1	3	–	–	–	–	–	–	2	2	2	2	1	3	1	
<i>Mascarenhasia elastica</i>	1	2	1	2	1	2	2	1	2	1	1	1	1d	3	1	1	1	5	–	–	–	–	–	–	1	2	3	2	1	3	1	
<i>Leptadenia arborea</i>	1	2	2	2	2	2	2	4	4	2	1	1	3	3	2	1	1	1	2	2	1	1	1	1	1	2	3	2	2	3	1	
<i>Leptadenia pyrotechnica</i>	1	5	2	2	2	1	2	4	2	2	1	1	1a	2	2	1	1	4	1	3	2	1	1	3	2	1	3	2	1	3	2	
<i>Calotropis procera</i>	1	4	2	2	2	2	2	5	2	2	1	1	4	2	2	2	2	4	3	2	2	2	2	3	2	1	1	1	1	4	2	
<i>Cynanchum acutum</i>	1	2	2	2	2	2	2	4	4	2	1	1	5	2	2	2	2	5	4	4	2	2	2	2	2	1	2	3	2	1	1	1
<i>Solenostemma argel</i>	1	2	4	1	3	2	2	4	1	2	1	1	1e	2	1	1	2	5	5	5	2	2	2	2	1	2	1	3	2	2	2	1
<i>Asclepias curassavica</i>	1	1	2	2	1	2	2	4	4	2	1	1	6	2	2	2	2	2	–	–	–	–	–	–	1	2	2	1	2	5	2	
<i>Cryptostegia grandiflora</i>	1	2	2	2	1	2	2	4	4	2	1	1	7	2	2	2	2	5	–	–	–	–	–	–	1	2	1	2	1	2	1	

^a Inapplicable character-state.



Plate 1 Seed morphology of 14 species of Apocynaceae (1–7) and Asclepiadaceae (8–14) as revealed by light microscopy: 1. *Acokanthera spectabilis*; 2. *Carissa spinarum*; 3. *Thevetia peruviana*; 4. *Catharanthus roseus*; 5. *Alstonia scholaris*; 6. *Nerium oleander*; 7. *Mascarenhasia elastica*; 8. *Leptadenia arborea*; 9. *Leptadenia pyrotechnica*; 10. *Calotropis procera*; 11. *Cynanchum acutum*; 12. *Solenostemma argel*; 13. *Asclepias curassavica*; 14. *Cryptostegia grandiflora*.

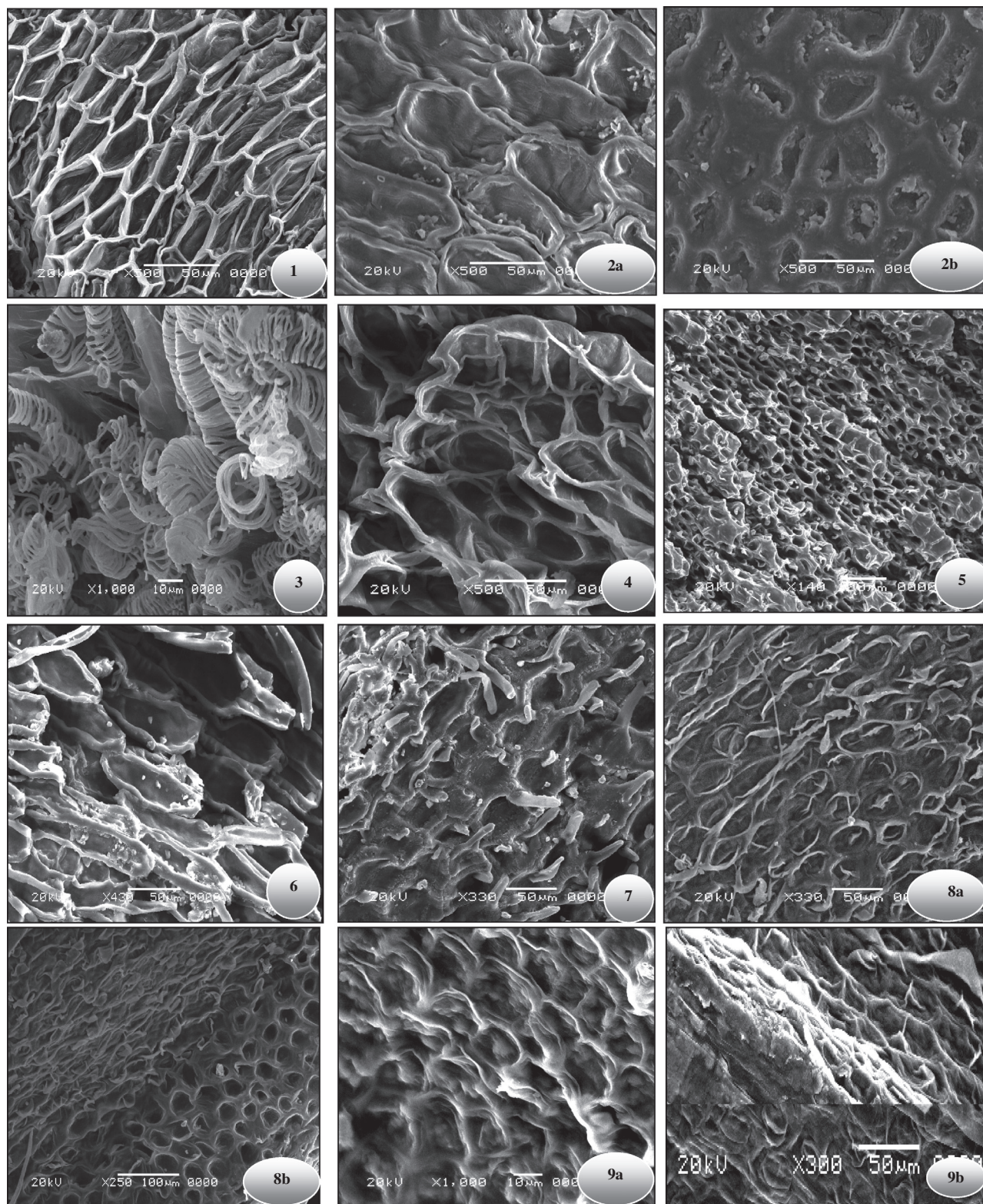


Plate 2 Surface sculpture of seeds of 14 species of Apocynaceae (1–7) and Asclepiadaceae (8–14) as revealed by SEM. 1. *Acokanthera spectabilis*; 2. *Carissa spinarum*; 3. *Thevetia peruviana*; 4. *Catharanthus roseus*; 5. *Alstonia scholaris*; 6. *Nerium oleander*; 7. *Mascarenhasia elastica*; 8. *Leptadenia arborea*; 9. *Leptadenia pyrotechnica*.

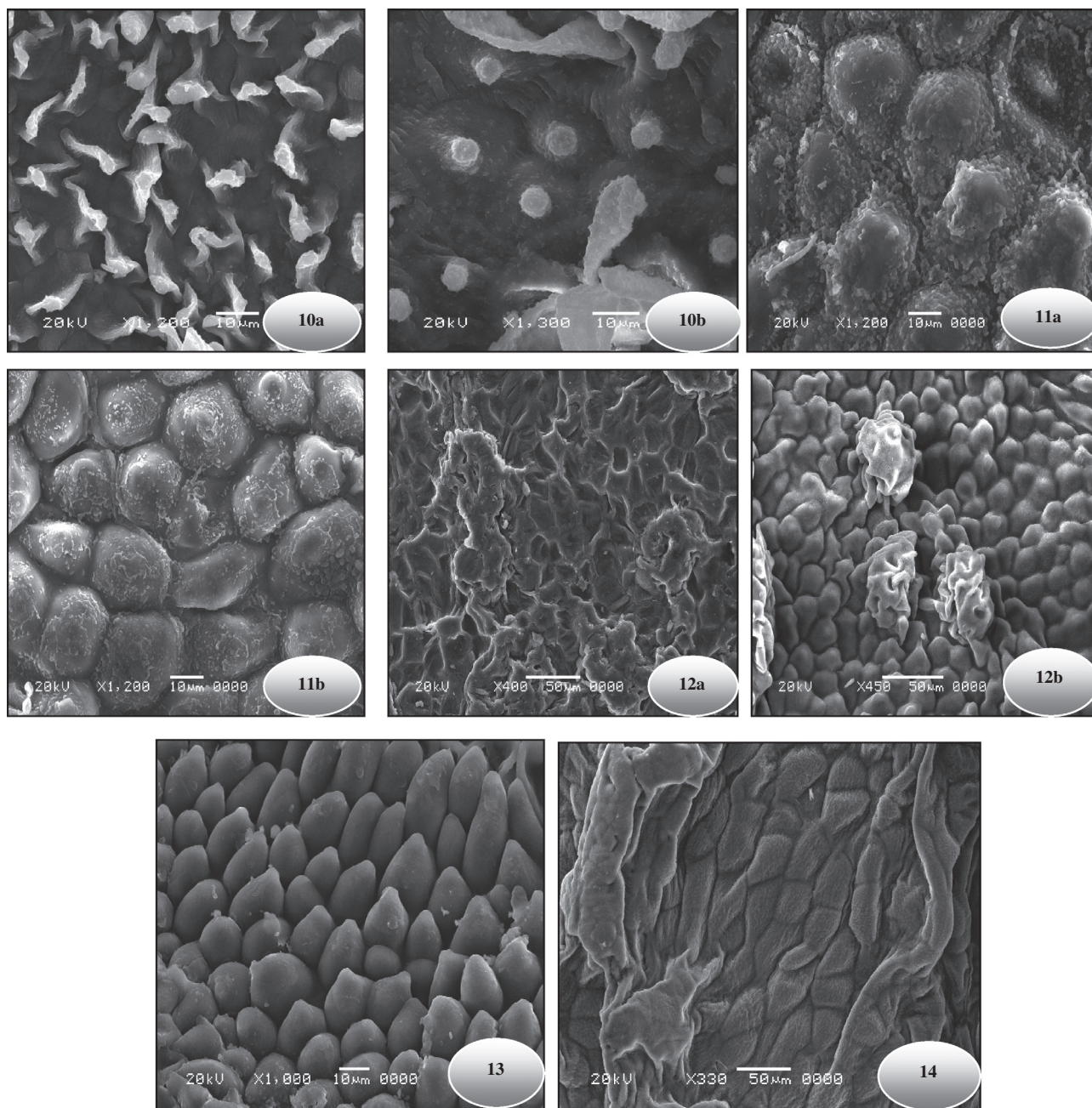


Fig. 2 (continued): Surface sculpture of seeds of 14 species of Apocynaceae (1–7) and Asclepiadaceae (8–14) as revealed by SEM. 10. *Calotropis procera*; 11. *Cynanchum acutum*; 12. *Solenostemma argel*; 13. *Asclepias curassavica*; 14. *Cryptostegia grandiflora*.

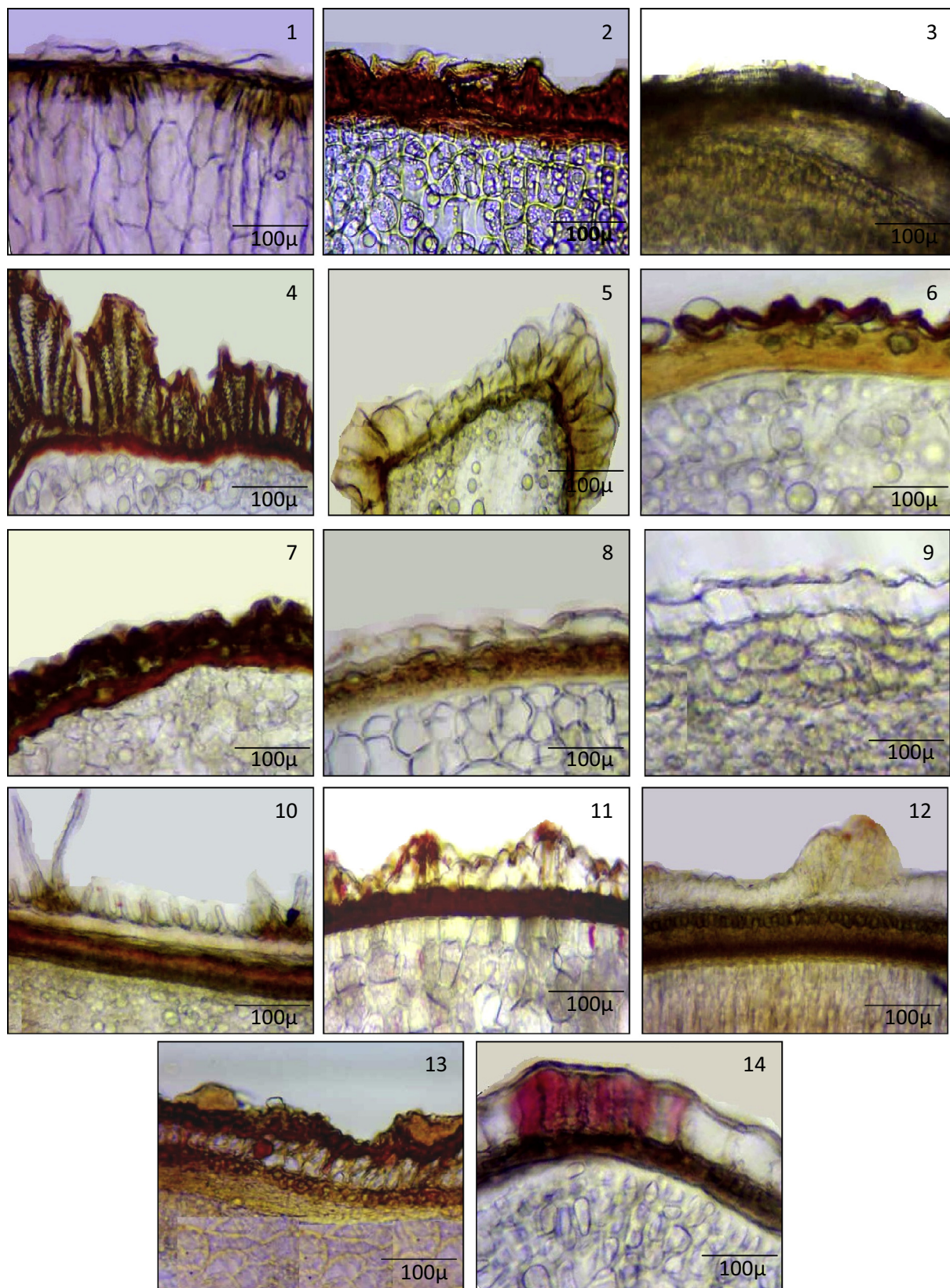


Plate 3 Photomicrographs of cross-sections of seed coat of 14 species of Apocynaceae (1–7) and Asclepiadaceae (8–14). 1. *Acokanthera spectabilis*; 2. *Carissa spinarum*; 3. *Thevetia peruviana*; 4. *Catharanthus roseus*; 5. *Alstonia scholaris*; 6. *Nerium oleander*; 7. *Mascarenhasia elastica*; 8. *Leptadenia arborea*; 9. *Leptadenia pyrotechnica*; 10. *Calotropis procera*; 11. *Cynanchum acutum*; 12. *Solenostemma argel*; 13. *Asclepias curassavica*; 14. *Cryptostegia grandiflora*.

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