

LM AND SEM STUDIES OF SPERMODERM PATTERNS IN SOME CUCURBITACEAE

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LM and SEM studies of spermoderm patterns have been undertaken in 19 genera and 36 species of Cucurbitaceae. In all, eight patterns viz., simple reticulate, multi-reticulate, lophate, substriate, rugulate, simple-foveolate, multi-foveolate and undulate are observed. The most common pattern seen is simple reticulate, while multi-reticulate is in four, lophate and sub-striate in two each and rugulate, multi-foveolate, simple-foveolate, as well as undulate patterns are in one taxon each. The present study reveals that the testa features differ significantly across the taxa investigated and found useful as micro-morphological markers for their identification.

Key words: Cucurbitaceae, SEM, spermoderm.

Scanning Electron Microscope (SEM) has been extensively used to study the seed coat ornamentation and considered useful for the taxonomic considerations (Heywood 1971, Barthlott 1984, Subba Rao and Shanmukha Rao 1992, Shanmukha Rao and Leela 1993, Manju Sharma *et al.* 2012) besides solving a variety of problems in the identification of hybrids, testing seed quality and understanding the structural variation associated with pest and disease resistance (Trivedi and Gupta 1988) and in ecology and evolution (Brisson and Peterson 1977). The seed characters also have importance in pharmacognostic studies, adulteration and quality determination of various economically important and commercial crops.

Cucurbitaceae with 800 species under 130 genera is one among the economically most important plant families (Kocyan *et al.* 2007 Schaefer and Renner 2011) and Indian representation being 31 genera and 94 species (Renner and Pandey 2013). SEM studies of the seed coat morphology and spermoderm patterns of cucurbits are very meagre so far and confined to a few taxa (Lott 1973 Ali and Pandey 2005-06 Jana Murovec *et al.* 2012 Ali *et al.* 2013). Hence this investigation dealing with both LM and SEM studies of spermoderm patterns of 19 genera and 36 species, of which 22 taxa are studied for the first time.

MATERIALS AND METHODS

The exomorphological characters of seeds and

the spermoderm patterns as observed under LM and SEM have been presented in Table 1. For SEM studies, the seeds were cleaned in petroleum ether for 2-3 hours. Then, the whole or portions of large seeds were mounted on aluminium stubs with silver paint and coated with a thin layer of gold. Observations were made at 10 Kv, 15 Kv and 20 Kv with a Hitachi S 520 Scanning Electron Microscope at Indian Institute of Chemical Technology (IICT), Hyderabad. For the purpose of uniformity, the data from the hylar region has been presented.

Terminology used to describe the spermoderm patterns is after Trivedi *et al.* (1978), Lersten (1981) and Barthlott (1984).

RESULTS AND DISCUSSION

LM Description

In the exomorphic characters of seed, shape is considered as an additional diagnostic feature in plant taxonomy (Corner 1976). Presently, the shape ranges from oblong, ovate, obovate, sub-rectangular, and triangular to pyriform (Table 1).

The colour of seeds varies from black, dark grey, white, whitish yellow, pale ash, yellowish, grey, creamy brownish, brownish, brownish yellow, whitish grey, red, greenish and blackish grey (Table 1). However, In *Citrullus lanatus*, different shades of black and red are observed and this is in tune with the earlier observations (Harper *et al.* 1970, Chakravarty 1982, Whitaker 1990).

Table 1: Seed Characteristics and Spermoderm Patterns in Cucurbitaceae#							
Name of the Taxon	LM Description			Dimension in mm			Spermoderm pattern
	Shape	Colour	Surface	Length	Width	L/W	
Sub family: Nhandiroboideae (Zanonioidae)							
Tribe 1: <i>Zanonieae</i>							
<i>*Actinostemma lobata</i> Maxim	Ovate	Brownish	Rough	13	8	1.6	Simple reticulate
<i>*Gynostemma pedata</i> Bl.	Oblong	Black	Rough	4	3	1.3	Simple reticulate
Sub family: Cucurbitoidae							
Tribe 1: <i>Melothriaceae</i>							
<i>*Cucumis callosus</i> (Rottl.) Cogn.	Oblong	White	Smooth	5	2	2.5	Lophate
		Whitish					
<i>*C. melo</i> L.	Oblong	yellow	Smooth	10	4	2.5	Substrate
<i>*C. prophetarum</i> L.	Oblong	Pale ash, yellowish	Rough	4	2	2	Simple foveolate
<i>C. sativus</i> L.	Oblong	Whitish yellow	Smooth	8	3	2.66	Substrate
<i>Melothria maderaspatana</i> (L.) Cogn.	Ovate, oblong	Grey, Yellowish	Smooth	5	3	1.66	Simple reticulate
<i>*M. mucronata</i> (Bl.) Cogn.	Obovate, oblong	Pale grey	Rough	4	3	1.33	Simple reticulate.
<i>*Zehneria maysorensis</i> (W & A) Arn.	Ovate	Whitish, yellowish	Smooth	5	3	1.66	Simple reticulate.
Tribe 2: Joliffieae							
<i>*Momordica charantia</i> L.	Sub-	Creamy,	Rough	15	18	1.87	Simple reticulate
var. <i>charantia</i> (Karela)	rectangular	brownish					
<i>*M. charantia</i> L.	Ovate,sub-	Yellowish	Rough	12	8	1.5	
var. <i>muricata</i> (Willd.) Uchchhe.	rectangular						Simple reticulate
<i>M. dioica</i> Roxb. ex Willd.	Ovate	Pale yellow	Rough	7	5	1.4	Simple reticulate
Tribe 3: <i>Trichosantheae</i>							
<i>*Gymnopetalum cochinchinense</i> (Loux.)Kurz.	Oblong	Black	Rough	8	3	2.6	Simple reticulate

<i>Trichosanthes cucumerina</i> L.								
<i>var. anguina</i> (L.) Haines	Oblong	Brownish	Rough	18	8	2.25	Multi-reticulate	
* <i>T. cucumerina</i> L.	Ovate,	Brownish						
<i>var. cucumerina</i> L.	oblong	yellow	Rough	12	6	2	Multi-reticulate	
* <i>T. palmata</i> Roxb.	Ovate,	Whitish						
	oblong	grey	Smooth	8	5	1.6	Simple reticulate	
Tribe 4: Benincaseae								
<i>Benincasa hispida</i> (Thunb.) Cogn.	Ovate	Yellowish, white	Smooth	12	7	1.71	Simple reticulate	
							page 2	
<i>Citrullus colocynthis</i> (L.) Schrad.	Ovate,	Yellowish	Smooth	6	3	2	Rugulate	
	oblong	brown						
<i>C. lanatus</i> (Thunb.) Mats. & Nakai	Ovate	Black, red, different shades	Smooth	8	4	2	Simple reticulate	
* <i>C. lanatus var. fistulosus</i> (Stocks) Chakravarty*	Ovate	Black	Smooth	13	7	1.85	Simple reticulate	
* <i>Coccinia cordifolia</i> (L.) <i>var. wightiana</i> (Roem.) Cogn.	Oblong	Yellowish	Rough	6	3	2	Simple reticulate	
<i>Coccinia grandis</i> (L.) Voigt	Oblong	Yellowish	Smooth	6	3	2	Lophate.	
* <i>Ecballium elaterium</i> (L.) A. Rich.	Ovate	Black	Rough	6	3	2	Multi-foveolate	
<i>Lagenaria siceraria</i> (Mol.) Standl.	Obovate, oblong, triangular	Yellowish	Smooth	16	7	2.28	Undulate	
* <i>Luffa acutangula</i> (L.) Roxb. <i>var. acutangula</i> L.	Ovate	Black	Rough	12	7	1.71	Simple reticulate	
* <i>L. acutangula</i> (L.) Roxb.	Ovate	Black, white, greenish	Rough	9	6	1.5	Simple reticulate	

<i>L. cylindrica</i> (L.) Roem.	Ovate	White	Rough	12	8	1.5	Simple reticulate
		brownish					
		black					
* <i>L. tuberosa</i> Roxb.	Ovate	Blackish	Smooth	8	5	1.6	Simple reticulate
		grey					
Tribe 5: Cucurbitaceae							
<i>Bryonopsis laciniosa</i> (L.) Naud.	Pyriform	Grey	Rough	5	3	1.66	Simple reticulate
<i>Cucurbita maxima</i> Duch. ex Lam.	Ovate	White or	Smooth	19	11	1.72	Simple reticulate
		yellowish					
<i>C. moschata</i> (Duch. ex Lam.) Duch. ex Poir.	Ovate	Yellow	Smooth	12	7	1.71	Multi- reticulate
		Whitish					
<i>C. pepo</i> L.	Ovate	yellow	Smooth	7	3	2.3	Simple reticulate
Tribe 6: Sicyeae							
* <i>Cyclanthera brachystachya</i> (Ser.) Cogn.	Ovate	Black	Rough	13	8	1.62	Simple reticulate
* <i>C. hystrix</i> (Gill) Arn. & Hooker	Ovate	Black	Rough	10	5	2	Simple reticulate
* <i>Sechium edule</i> (Jacq.) Swartz.	Ovate	Yellow	Smooth	35	20	1.75	Multi- reticulate

Treatment of sub families and Tribes is after jeffrey (2005)

* These taxa have been studied for the first time.

The texture of testa is smooth in 18 taxa, whereas in others it is rough (Table 1). Always, the hilum is inconspicuous and terminal in position.

Quantitative parameters like length, width, thickness and length/width ratio have been investigated and the length ranges from 4 mm to 35 mm, width 2mm to 20 mm and the thickness 1 to 4 mm (Table 1). The length/width ratio of the seeds ranges from 1.3 to 2.66.

SEM Description

Presently, a total of eight patterns viz., rugulate (irregularly roughened), substriate (short parallel ridges), simple reticulate (meshwork of ridges enclosing single cells), multi-reticulate (primary plus secondary ridges), simple-foveolate (single cell ends isolated by grooves), multi-foveolate (unit of several cells

surrounded by grooves), lophate (short ridges with irregular sides) and undulate (wavy) patterns are recognisable and the dominant pattern being simple reticulate as witnessed in 24 taxa (Table 1). Of the rest, multi-reticulate is in four, lophate and substriate in two each and rugulate, multi-foveolate, simple-foveolate, as well as undulate patterns are seen in one taxon each (Table 1).

The cell shape, cellular arrangements, relief of outer cell walls and anticlinal wall nature of the spermoderm patterns are not uniform among the taxa studied. The simple reticulate pattern has either polygonal or hexagonal cells and the anticlinal walls are either straight (Fig. 1C), wavy (Fig. 3H) or reticulations are of U or V or Omega shaped (Fig. 1F). The substriate pattern shows parallel rows of linear cells (Fig. 2H) and the simple-foveolate pattern, is with grooves isolated by single epidermal cells (Fig. 2A). On

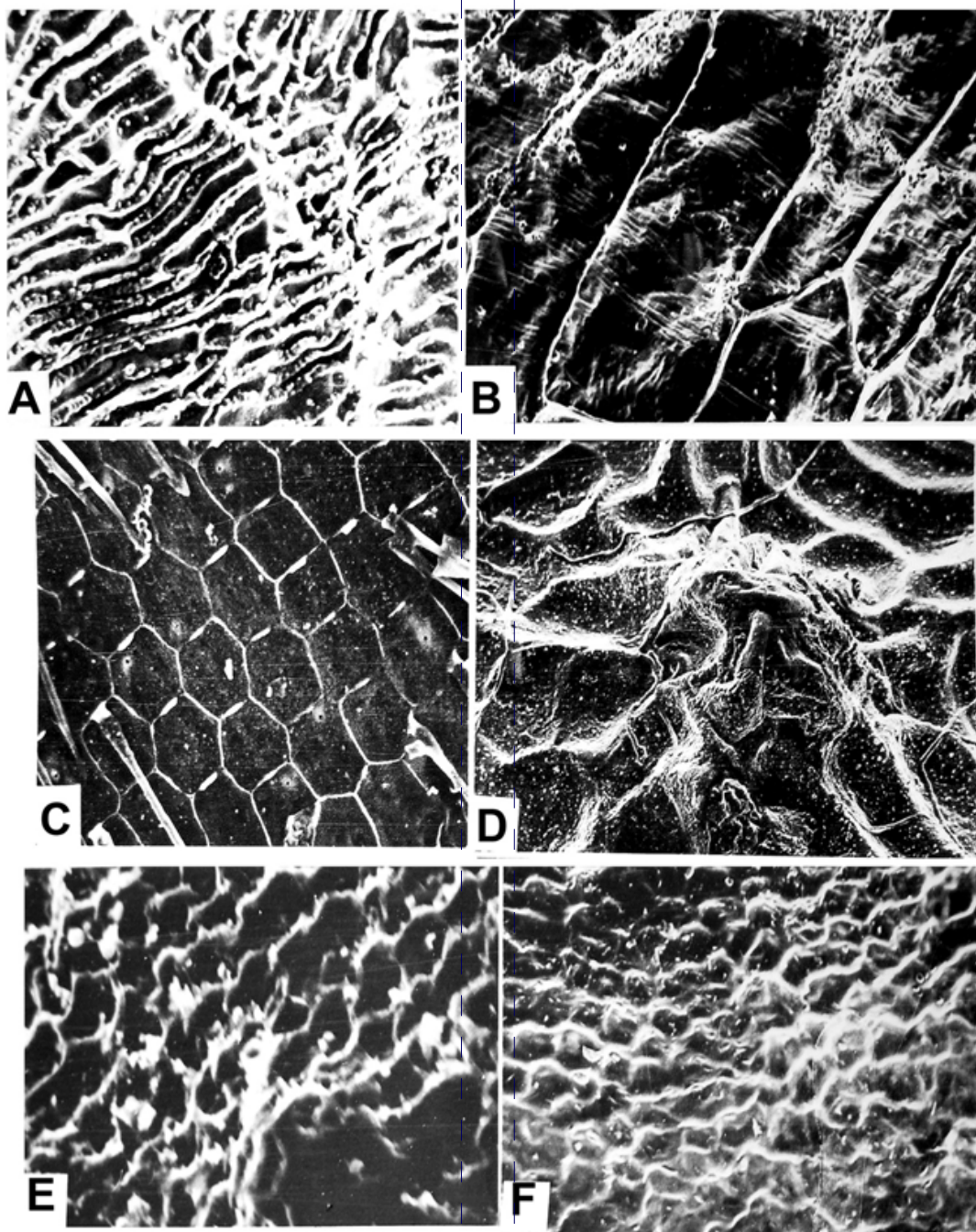


Figure - 1

1A-F. Spermoderm patterns in Cucurbitaceae. A,C-F. Simple reticulate. B. Multi reticulate. A. *Cyclanthera hystrix*, B. *Cucurbita moschata*, C. *Melothria maderaspatana*, D. *Gynostemma pedata*, E. *Luffa cylindrica*, F. *Momordica charantia* var. *Charantia*. (x 500).

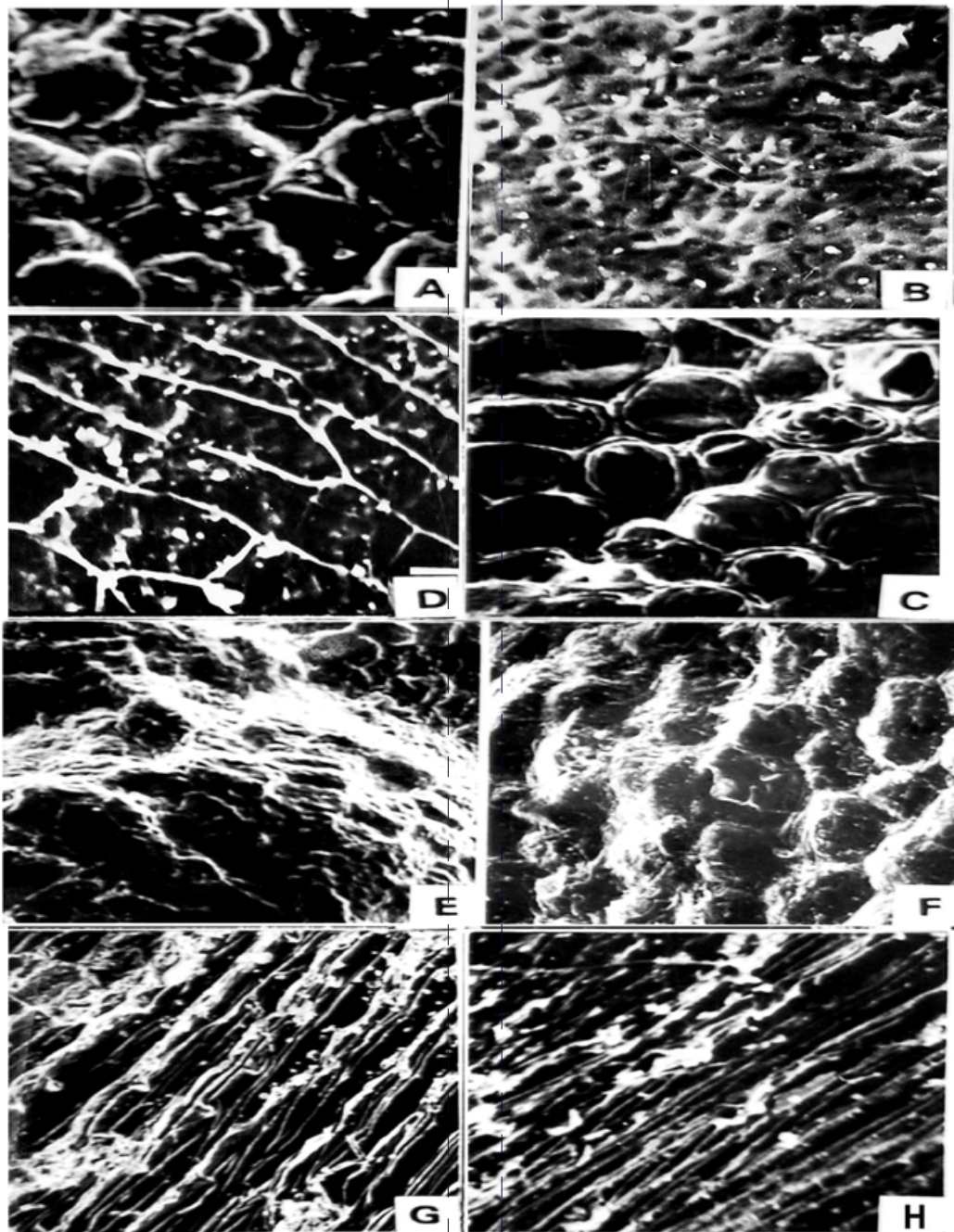


Figure - 2

2A-H. Spermoderm patterns in Cucurbitaceae. A. *Cucumis prophetarum* - simple foveolate, B. *Momordica dioca*- simple reticulate, C. *Ecballium elaterium*- multi-foveolate, D. *Benincasa hispida* - simple reticulate, E. *Cucumis callosus* – lophate, F. *Lagenaria siceraria* – undulate, G. *Cucumis sativus* – substriate, H. *Cucumis melo* - substriate. (x 500).

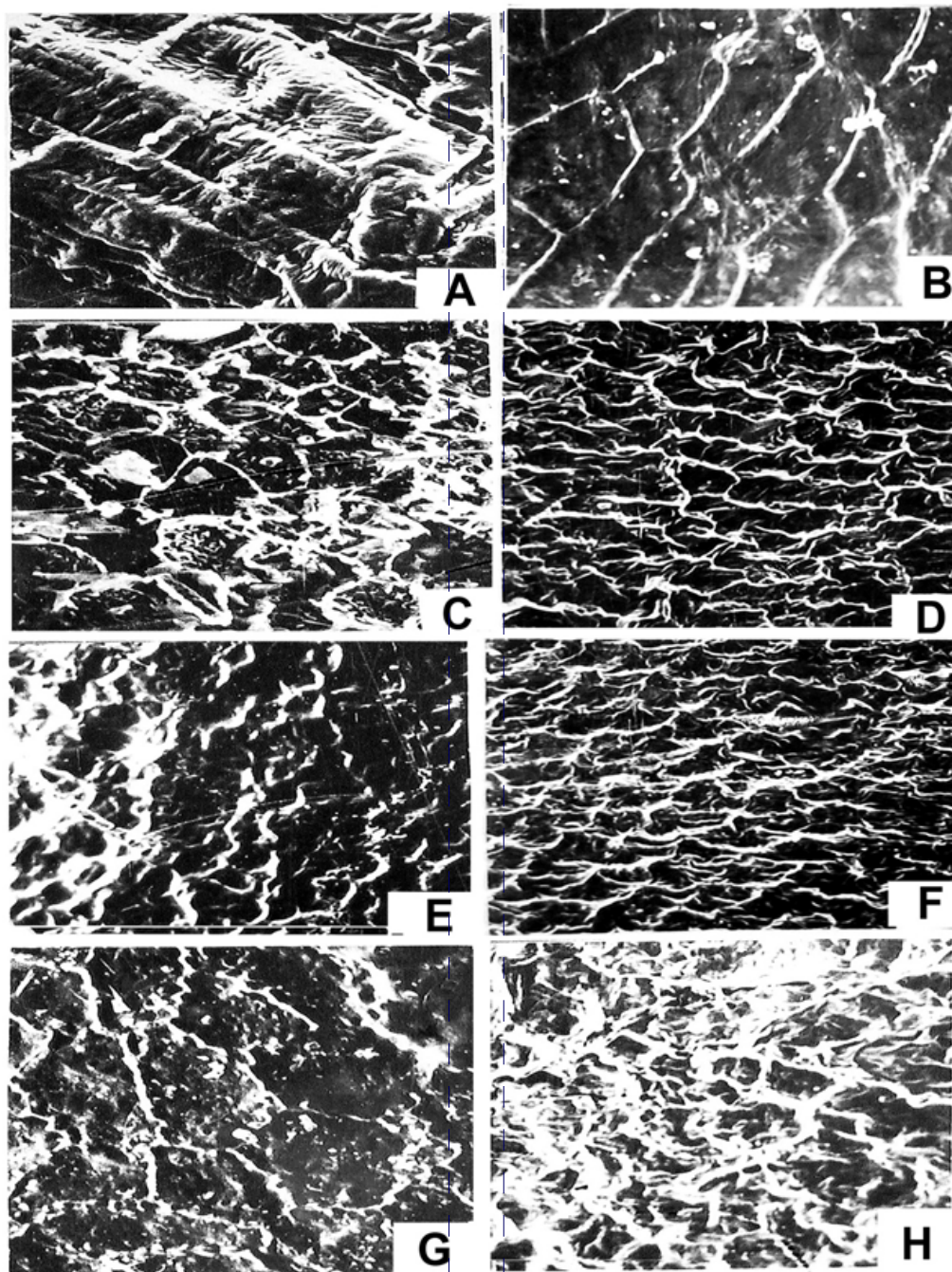


Figure - 3

3A-H. Spermoderm patterns in Cucurbitaceae. A. Multi reticulate. B-D, F-H. Simple reticulate. E. Rugulate. A. *Sechium edule*. B. *Cucurbita pepo* C. *Coccinia cordifolia* var. *wightiana*. D. *Luffa acutangula* var. *acutangula* E. *Citrullus colocynthis*. F. *Luffa acutangula* var. *amara*. G. *Luffa tuberosa*. H. *Zehneria maysorensis*. (x 500).

the other hand, multi-reticulate pattern shows parallel cells connected to each other by cross ridges as seen in *Sechium edule* (Fig. 3A).

Ali and Pandey (2005-06) reported the spermoderm pattern to be rugulate in *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*, *Lagenaria siceraria* and *Trichosanthes cucumerina* var. *anguina* while in *Citrullus lanatus* and *Coccinia grandis* it is retico-rugulate, reticulate in *Diplocyclos palmatus*, *Melothria maderaspatana*, *Momordica dioica* and it is tuberculate in *Luffa cylindrica*. However, the present observations are at variance with the above ones and show the following patterns: substriate in *Cucumis sativus* (Fig. 2G), simple reticulate in *Citrullus lanatus*, *Cucurbita maxima*, *Cucurbita pepo* (Fig. 3B), *Diplocyclos palmatus*, *Melothria maderaspatana* (Fig. 1C), *Momordica dioica* (Fig. 2B), *Luffa cylindrica* (Fig. 1E), multi-reticulate in *Cucurbita moschata* (Fig. 1B), as well as *Trichosanthes cucumerina* var. *anguina*, undulate in *Lagenaria siceraria* (Fig. 2F) and lophate in *Coccinia grandis* (Table 1). Further, Ali *et al.* (2013) reported the spermoderm pattern to be rugulate in *Benincasa hispida*, reticulate in *Citrullus colocynthis*. However, the present study reveals that it is reticulate in *Benincasa hispida* (Fig. 2D) and rugulate in *Citrullus colocynthis* (Fig. 3E).

The following taxa are studied for their spermoderm for the first time and the observation are simple reticulate, the cells are polygonal or hexagonal and the anticlinal walls are straight in *Actinostemma lobata*, *Gynostemma pedata* (Fig. 1D), *Gymnopetalum cochinchinense*, *Citrullus lanatus* var. *fistulosus*, *Coccinia cordifolia* var. *wightiana* (Fig. 3C), *Cyclanthera hystrix* (Fig. 1A), *Melothria maderaspatana* (Fig. 1C) or wavy in *Ctenolepis garcinii*, *Melothria mucronata*, *Zehneria maysorensis* (Fig. 3H), *Luffa tuberosa* and *Cyclanthera brachystachya* but reticulations of U or V or Omega shape in *Momordica charantia* var. *charantia* (Fig. 1F), *Momordica charantia* var. *muricata*, *Trichosanthes palmata*, *Luffa acutangula* var.

acutangula, (3 D) *Luffa acutangula* var. *amara* (Fig. 3F), lophate in *Cucumis callosus* (Fig. 2E) substriate with parallel rows of linear cells in *Cucumis melo* (Fig. 2H) simple-foveolate with grooves isolate with single epidermal cells in *Cucumis prophetarum* (Fig. 2A), multi-reticulate in which parallel cell are connected to each other by cross ridges in *Trichosanthes cucumerina* var. *cucumerina* and *Sechium edule* (Fig. 3A) multi-foveolate in *Ecballium elaterium* (Fig. 2C).

An attempt has been made presently to look into spermoderm pattern of the two sub-families recognized in general in Cucurbitaceae (Jeffrey 2005, Kocyan *et al.* 2007 Schaefer and Renner 2011). *Gymnostemma pedata* and *Actinostemma lobata*, both belonging to Nhandiroboideae (=Zanonioideae) showed only simple reticulate pattern, whereas in the sub-family Cucurbitoideae, the normal tendency is to show more than one spermoderm pattern excepting in the tribe Joliffieae (Table 1). Though the present sample is limited to a few taxa, it would be worthwhile to study a larger sample and to assess if Nhandiroboideae shows constantly simple reticulate pattern in comparison to the presence of multiple patterns in Cucurbitoideae.

Six of the seven tribes of the sub-family Cucurbitoideae (Jeffrey 2005) have been analysed with reference to their spermoderm patterns. They reveal certain correlations. The tribe Joliffieae possess simple reticulate pattern, whereas Trichosanthaeae, Cucurbitaeae and Sicyeae show simple reticulate and multi-reticulate patterns. On the other hand, Benincaseae reveals simple reticulate, rugulate, lophate, multi-foveolate and undulate patterns whereas Melothrieae displays simple reticulate, lophate, substriate and simple-foveolate patterns.

At the generic level, the monotypic *Ecballium* (Benincaseae) is unique by the exclusive presence of multi-foveolate spermoderm pattern and appear distinct from rest of the taxa presently studied.

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