

SIGNIFICANCE OF SEED MICROMORPHOLOGICAL CHARACTERS AND SEED COAT ELEMENTS FOR THE TAXONOMIC DELIMITATION OF THE GENUS *CLEOME* L. (CLEOMACEAE) FROM PAKISTAN

SANA RIAZ AND RUBINA ABID*

Department of Botany, University of Karachi, Karachi-75270, Pakistan

*Corresponding author's email: rubinaku@yahoo.com

Abstract

Seed micromorphology and seed coat elements were studied in 10 species of the genus *Cleome* L. from Pakistan. Seed micromorphological characters such as shapes, colours, surfaces and seed coat elements were found to be significant to provide the strength to the taxonomic delimitation of the genus *Cleome*. The genus is characterized by the presence of cleft on the seeds and presence of carbon and oxygen in seed coats. Regarding to the seed shapes and surfaces a variety was observed but reniform and pyriform shapes and reticulate surface pattern were most frequent within the genus. While, on the other hand, seed micromorphological data could also be well correlated with qualitative and quantitative values of seed coat elements to strengthen the taxonomic decisions for the genus *Cleome*.

Key words: *Cleome*, Seed micromorphology, Elements, Pakistan,

Introduction

Seeds are produced by all spermatophytes in the process of reproduction and seed morphological data could be significantly used in plant diagnosis and systematics (McClure, 1957; Berggren, 1962, 1969; Lawrence, 1970; Corner 1976; Hufford, 1995; Otto, 2002; Black, 2007; Kanwal *et al.*, 2009; Abid *et al.*, 2016). Seed morphological characters are also important to trace out the phylogenetic relationship and history of plants (Otto, 2002). Concerning to the seed morphology of the family Capparidaceae there are various reports available such as; Sanchez-Acebo (2005) studied 35 species of *Cleome* for their seed characteristics and these seed characters were significantly used in systematics of the genus *Cleome*. Similarly, the seeds of 35 species of *Cleome* were studied by Inda *et al.* (2008) and they gave the importance to seed coat, aril and seed cleft. They used this data along with molecular data to study phylogeny of *Cleome*. Kamel *et al.* (2010) studied 10 species of *Cleome* from Egypt, where they also used seed characters like seed shape and surface to describe the species. Iltis *et al.* (2011) gave a brief account on seeds of Brassicaceae, Capparaceae and Cleomaceae and this data was used for the recognition of these three families as separate entities. Besides this various workers also paid the attention to seed morphology for the taxonomic treatment of the genus *Cleome* in various Floras (Oliver, 1868; Hooker, 1875; Bobrov, 1939; Hedge & Lamond, 1970; Sharma & Balakrishnan, 1993).

These reports did not include the detailed seed morphology and even not covered all the species found in Pakistan. Present studies are first of its kind to utilize the seed morphological characters and seed coat elements for taxonomic delimitation of the genus *Cleome* from the area under consideration.

Materials and Methods

Mature and healthy seeds of 10 species of the genus *Cleome* were collected from herbarium specimens (Appendix I) and studied under stereomicroscope (Nikon XN model) and scanning electron microscope (JSM-6380 A). For scanning electron microscopy the mature dried seeds were mounted on metallic stub using double adhesive tape, then gold plated in sputtering chamber for a period of 6 minutes and observed under scanning electron microscope. Seed characters like seed size, shape, colour, surface, and hilum were studied. The terminology used is in accordance with Lawrence (1970), Radford *et al.* (1974) and Stearn (1983) with slight modifications. Seed coat elements were detected by energy-dispersive X-ray spectroscopy (EDS) using EDS detector EX-54175jMU. ZAF method standard less quantitative analysis.

Observations and Results

General Seed characteristics of the genus *Cleome* L.

Seeds 0.75–2.5 x 0.5–2.25 mm, angular or not angular; reniform, retortiform, obovate, elliptic-pyriform, ob-elliptic pyriform or ovoid, groove or cleft size varies from very short to long; light brown, dull brown, rust brown, dust brown, mustard brown, orange brown, golden brown, chocolate brown, dark brown, maroon, blackish brown, black or metal green; reticulate, appressedly reticulate, faintly reticulate, alveolate, foveate, favulariate, pusticulate or concentrically ridged, glabrous or hairy; pillose, tomentose, papillate or lineate, hilum lateral, (Table 1, Figs. 1-2) a number of elements like, carbon, oxygen, magnesium, potassium, copper, zinc, chlorine, calcium, sodium, silicon, aluminum and sulphur were detected in seed coats in varying concentrations (Table 2, Figs. 3-4).

Appendix-I. List of voucher specimens.

S. No.	Taxa	Collector, number and herbarium
1.	<i>C. ariana</i>	S. Abedin 8003 (KUH); M. Qaiser & A. Ghafoor 6592 (KUH); Kamal A. Malik & S. Abedin 1473 (KUH); Haider Ali 2063 , 6633, 6439, 1379 (KUH); A. Ghafoor & S. Omer 3019 (KUH)
2.	<i>C. brachycarpa</i>	M. Qaiser & A. Ghafoor 3 (KUH); S.M.H Jafri 813 (KUH); Shamim Akhter s.n. (KUH)
3.	<i>C. dolichostyla</i>	A.Ghafoor & S. Omer 1863 (KUH); A.G. Miller & J.A. Nyberg M9569 (KUH); M.Qaiser & Asad Raza Abbas 1043 (KUH)
4.	<i>C. fimbriata</i>	S.I.Ali 540 (KUH)
5.	<i>C. oxypetala</i>	Herbier J. Leonard 5856 (KUH); S.I. Ali <i>et al.</i> , 1033 (KUH)
6.	<i>C. pakistanica</i>	V. P. Dutta 5 (KUH)
7.	<i>C. rupicola</i>	Sultan-ul-Abedin & Abrar Hussain 6943, 6801 (KUH); S. Nazim Uddin <i>et al.</i> , 557 (KUH); S. M. H Jafri 2689 (KUH)
8.	<i>C. scaposa</i>	Nazim <i>et al.</i> , 63 (KUH); Kamal Akhter & Nazim Uddin 702 (KUH); Kamal Akhter <i>et al.</i> , 2123 (KUH); Sultan-ul-Abedin & M. Qaiser 9328 (KUH); A. Ghafoor & M. Qaiser 386 (KUH); Jafri <i>et al.</i> , s.n. (KUH); Sadiq Masih 10 (KUH); A. Ghafoor & Tahir Ali 3620 (KUH); A. Ghafoor & Steve A. Goodman 4938 (KUH); M. Qaiser 2563 (KUH)
9.	<i>C. spinosa</i>	Jafri 4010 (KUH)
10.	<i>C. viscosa</i>	M. Qaiser & A. Ghafoor 4867 (KUH); Sultan-ul-Abedin 5059, 4007, 4008, 4009 (KUH); A. Ghafoor & Tahir Ali 3739 (KUH)

Key to species

- 1 + Seeds angular 2
 - Seeds not angular 4
 2 + Seeds retortiform *C. spinosa*
 - Seeds reniform, ob-elliptic pyriform or elliptic pyriform 3
 3 + Seeds densely pubescent, silicon present *C. pakistanica*
 - Seeds appressedly reticulate, glabrous, silicon absent *C. dolichostyla*
 4 + Seeds hairy 5
 - Seeds glabrous 7
 5 + Seeds ob-elliptic pyriform, groove narrow towards the edges *C. ariana*
 - Seeds obovate or reniform groove uniform throughout the entire length 6
 6 + Seeds obovate, tomentose, groove very short, calcium present *C. oxypetala*
 - Seeds reniform, pillose, groove long, calcium absent *C. rupicola*
 7 + Seeds retortiform *C. viscosa*
 - Seeds elliptic pyriform, ovoid or reniform 8
 8 + Seeds elliptic-pyriform or ovoid, sulphur present *C. brachycarpa*
 - Seeds reniform, sulphur absent 9
 9 + Seeds metal green or black, alveolate, magnesium present *C. scaposa*
 - Seeds mustard brown, reticulate and pusticulate , magnesium absent *C. fimbriata*

Table 1. Seed morphological characteristics of genus *Cleome* L.

S. No.	Name of taxa	Size (mm) L X b	Angular Or Not angular	Colour	Shape	Surface	Hilum
1.	<i>Cleome ariana</i>	1.5 X 1.0	Not angular	Light brown/ chocolate brown	Ob- elliptic pyriform, small groove is present narrow from edge	Papillate, Papilae lineate	Lateral
2.	<i>C. brachycarpa</i>	0.85 X 0.85	Not angular	Rust brown/ Maroon	Elliptic Pyriform / Ovoid, very small groove is present	Reticulate, Favulariate	Lateral
3.	<i>C. dolichostyla</i>	0.95 X 0.9	Angular	Light brown / Maroon	Reniform/ Ob-Elliptic Pyriform, extreme narrow groove like a slit	Appressedly reticulate	Lateral
4.	<i>C. fimbriata</i>	0.8 X 0.5	Not angular	Musturd brown	Reniform, wide somewhat circular groove present	Reticulate, Pusticulate	Lateral
5.	<i>C. oxypetala</i>	2.5 X 2.2	No tangular	Golden brown / Dull brown	Obovate, minute uniformly wide groove	Tomentose hairy	Lateral
6.	<i>C. pakistanica</i>	1.5 X 1	Angular	Orange - brown	Elliptic Pyriform with a small groove	Densely pubescent, Hairs lineate	Lateral
7.	<i>C. rupicola</i>	2.5 X 2.25	Not angular	Dust brown	Reniform with wide uniform long groove	Pillose hairy	Lateral
8.	<i>C. scaposa</i>	0.75 X 0.7	Not angular	Metal green / black	Reniform with small narrow groove	Alveolate	Lateral
9.	<i>C. spinosa</i>	1.0 X 1.0	Angular	Blackish brown	Retortiform with a wide rectangular groove	Faintly reticulate	Lateral
10.	<i>C. viscosa</i>	2.0 X 1.5	Not angular	Rust brown/ Dark brown	Retortiform, groove is widen, beaked	Concentrically ridged, Foveate	Lateral

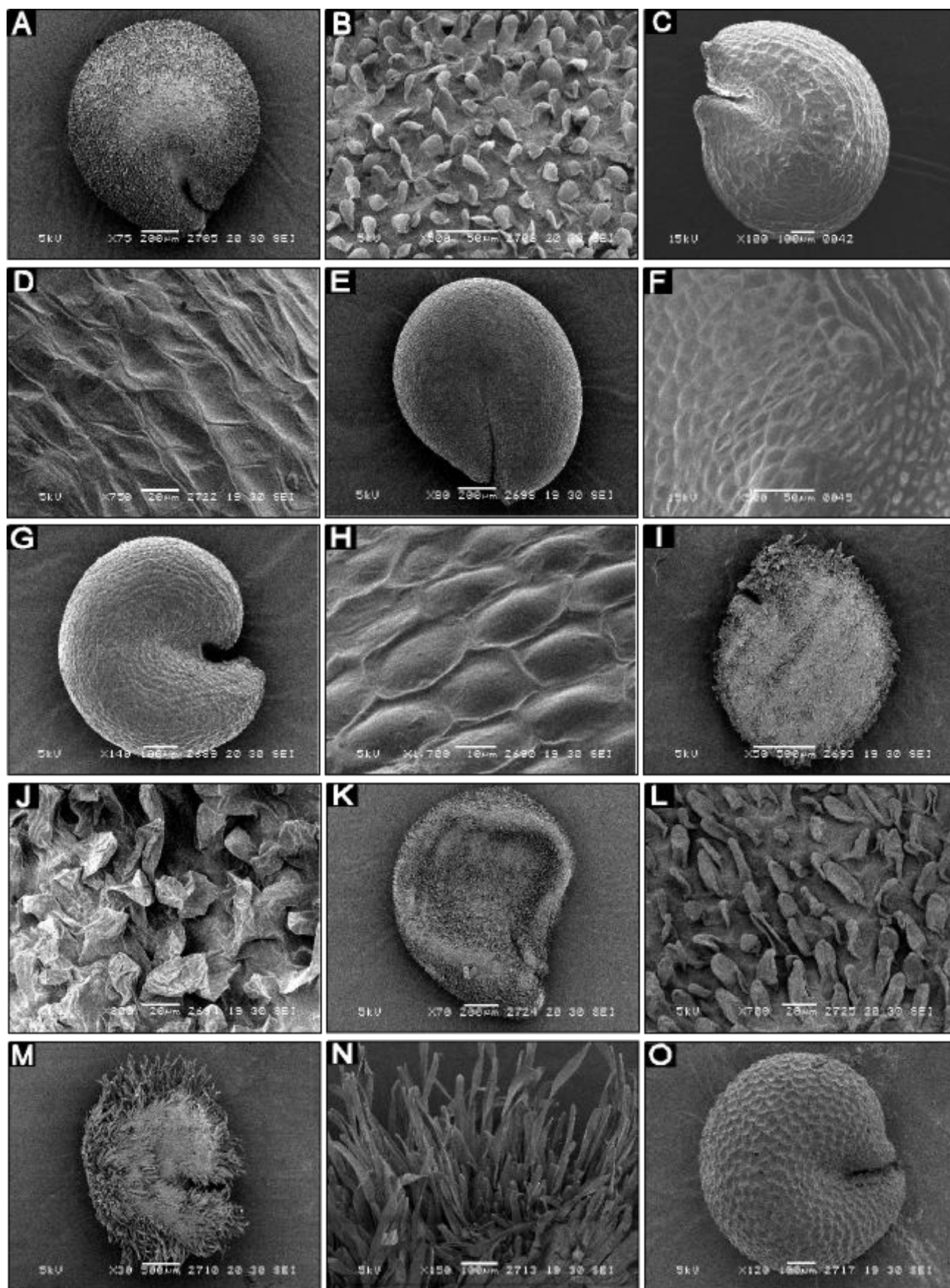


Fig. 1. Scanning electron micrographs. *Cleome ariana*: A, seed; B, seed surface. *C. brachycarpa*: C, seed; D, seed surface. *C. dolichostyla*: E, seed; F, seed surface. *C. fimbriata*: G, seed; H, seed surface. *C. oxypetala*: I, seed; J, seed surface. *C. pakistanica*: K, seed; L, seed surface. *C. rupicola*: M, seed; N, seed surface. *C. scaposa*: O, seed. (Scale bars: A, E, K = 200 µm; B, F = 50 µm; C, G, N, O = 100 µm; D, J, L = 20 µm; H = 10 µm; I, M = 500 µm).

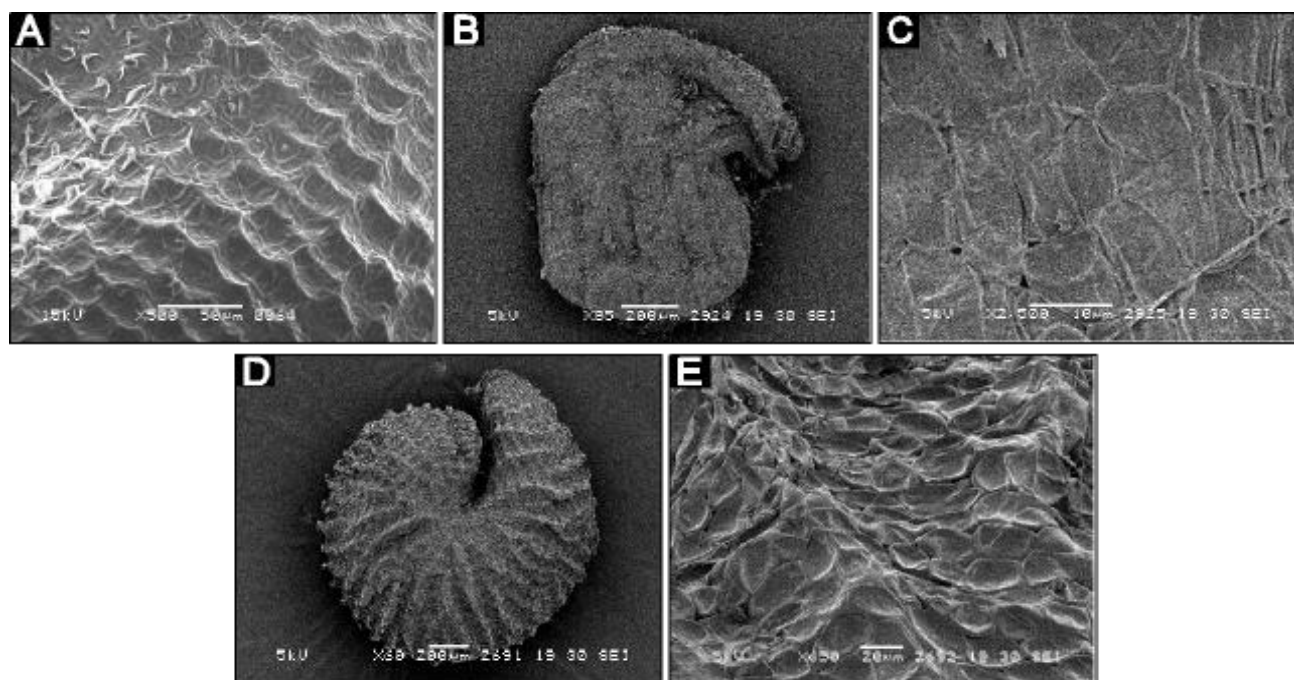


Fig. 2. Scanning electron micrographs. *C. scaposa*: A, seed surface. *C. spinosa*: B, seed; C, seed surface. *C. viscosa*: D, seed; E, seed surface. (Scale bars: A = 50 μ m; B, D = 200 μ m; C = 10 μ m; E = 20 μ m).

Table 2. Elemental composition of seed coat determined using energy-dispersive X-ray spectroscopy (EDS) and the associated analytical program EDS Analysis Station.

S. No.	Name of species	Percentage of elements in seed coat											
		C	O	Mg	K	Cu	Zn	Cl	Ca	Na	Si	Al	S
1.	<i>Cleome ariana</i>	56.58	37.68	0.60	0.96	1.31	-	-	0.49	-	-	2.39	-
2.	<i>C. brachycarpa</i>	52.05	42.03	0.63	3.07	-	-	-	1.83	-	-	-	0.39
3.	<i>C. dolichostyla</i>	56.70	26.22	0.67	2.09	8.73	4.76	0.82	-	-	-	-	-
4.	<i>C. fimbriata</i>	66.38	18.09	-	-	9.89	5.09	-	0.55	-	-	-	-
5.	<i>C. oxypetala</i>	51.94	42.12	1.01	2.13	2.12	-	-	0.69	-	-	-	-
6.	<i>C. pakistanica</i>	71.13	18.14	0.56	2.05	3.58	-	1.75	0.95	1.40	0.43	-	-
7.	<i>C. rupicola</i>	51.74	31.65	2.88	4.75	5.38	2.75	0.86	-	-	-	-	-
8.	<i>C. scaposa</i>	38.65	24.29	1.55	4.64	12.07	6.07	0.52	12.2	-	-	-	-
9.	<i>C. spinosa</i>	55.01	32.62	1.24	0.48	3.39	1.57	0.51	1.28	3.89	-	-	-
10.	<i>C. viscosa</i>	50.2	41.8	-	2.54	2.29	1.41	-	0.65	-	-	0.62	0.49

Discussion

Genus *Cleome* has been classified on the basis of gross morphology by different taxonomists but seed characteristics and their utilization for taxonomic treatment did not get much attention except that of few reports (Sanchez-Acebo, 2005; Inda *et al.*, 2008; Kamel *et al.*, 2010). Presently the seed morphological characters of this genus could be significantly used in delimiting the taxa within genus *Cleome*. The genus *Cleome* is characterized by the presence of cleft on the seeds and presence of carbon and oxygen in their seed coats. Regarding to the shapes of seeds there is a variety like; reniform, retortiform, elliptic pyriform, ovoid, obovate and ob-elliptic pyriform are observed. Amongst them reniform is most dominant shape found in *C. dolichostyla*, *C. fimbriata*, *C. rupicola* and *C. scaposa* and these findings could be supported by the observations of Iltis *et al.* (2011) where they observed that this shape was most common within the taxa of family Cleomaceae. Genus

Cleome can be bifurcated on the basis of angular or not angular seeds. Angular seeds are present in *C. dolichostyla*, *C. spinosa* and *C. pakistanica*. While, *C. spinosa* remains distinct from other two species by having retortiform seeds. *C. pakistanica* and *C. spinosa* are also characterized by the presence of sodium in their seed coats. *Cleome dolichostyla* having appressedly reticulate and glabrous seeds and *C. pakistanica* has densely pubescent surface with lineate hairs and silicon is present in seed coat which distinguishes it from all the other species. Seeds are not angular in *C. ariana*, *C. rupicola*, *C. oxypetala*, *C. viscosa*, *C. brachycarpa*, *C. scaposa* and *C. fimbriata*. This group could be further divided in to two subgroups on the basis of hairy or glabrous seeds. *C. ariana*, *C. rupicola* and *C. oxypetala* have hairy seeds while the remaining species having glabrous seeds. The species with hairy seeds could be further differentiated from each other due to the presence of different seed shapes and surface patterns such as, *C. ariana* has ob-elliptic pyriform seeds with papillate surface and could be

separated from both species by having aluminum in its seed coats. *C. oxypetala* having obovate seeds with tomentose hairs while, *C. rupicola* has reniform seeds with pillose hairs and it can be differentiated from other two species due to the complete absence of calcium in seed coats. Another group with glabrous seeds including *C. viscosa*, *C. brachycarpa*, *C. scaposa* and *C. fimbriata* could be further divided on the basis of different seed shapes. Amongst them *C. viscosa* having retortiform seeds, *C. brachycarpa* having elliptic-pyriform or ovoid seeds while, *C. scaposa* and *C. fimbriata* possessing

reniform seeds. These species could be further splitted on the basis of different surface patterns i.e. *C. scaposa* having alveolate surface and *C. fimbriata* possess reticulate pusticulate surface. Further, *C. brachycarpa* lacks copper in seed coat which is present in the seed coats of all other species of the genus *Cleome*.

Besides, the presence of elements, their quantities may also vary from species to species (Table 2) and seed micromorphological data could also be well correlated with qualitative and quantitative elemental data for the delimitation of the genus *Cleome* from Pakistan.

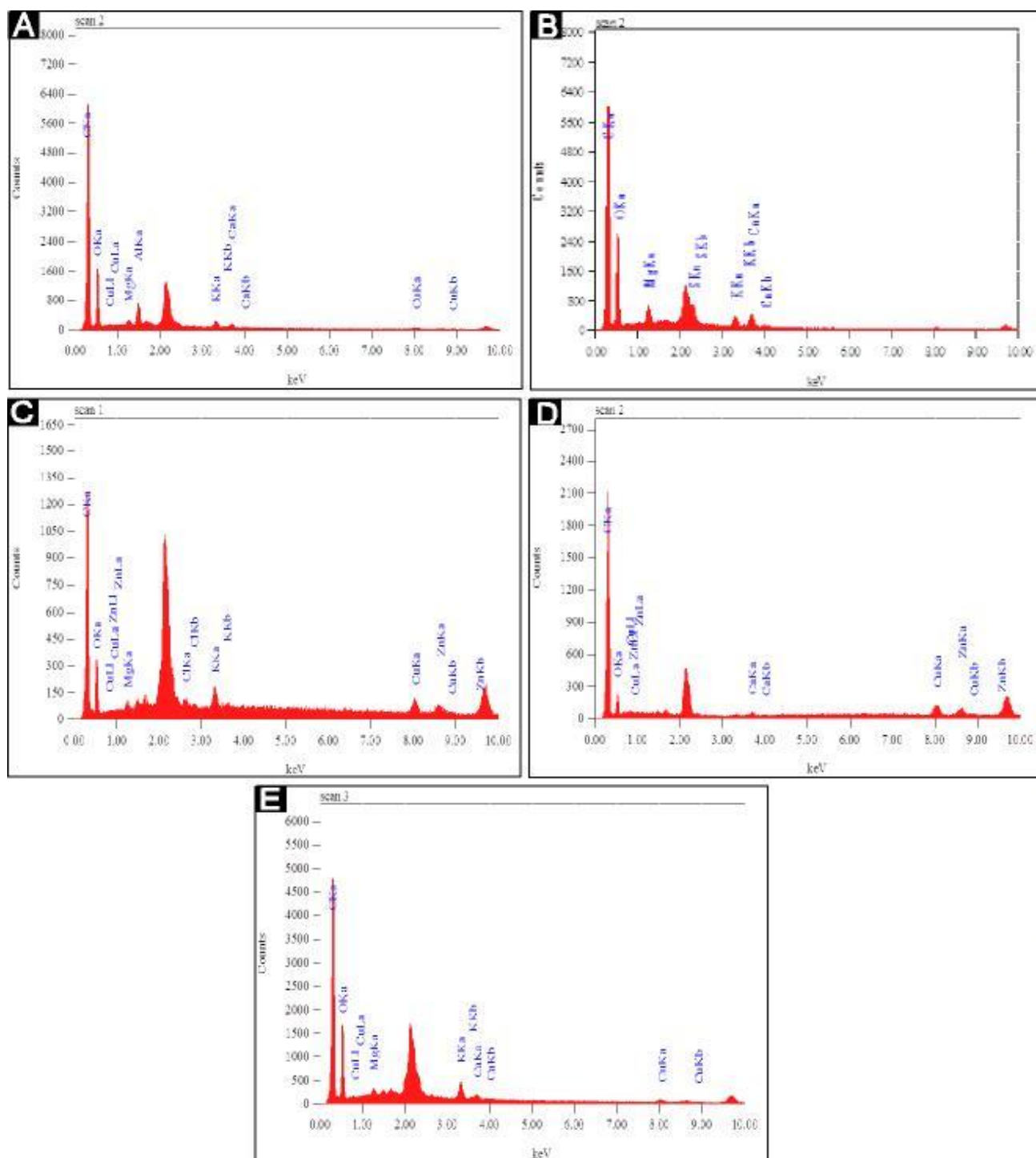


Fig. 3. Energy-dispersive X-ray spectroscopy (EDS) graphs. *Cleome ariana*: A, seed coat elemental composition. *C. brachycarpa*: B, seed coat elemental composition. *C. dolichostyla*: C, seed coat elemental composition. *C. fimbriata*: D, seed coat elemental composition. *C. oxypetala*: E, seed coat elemental composition.

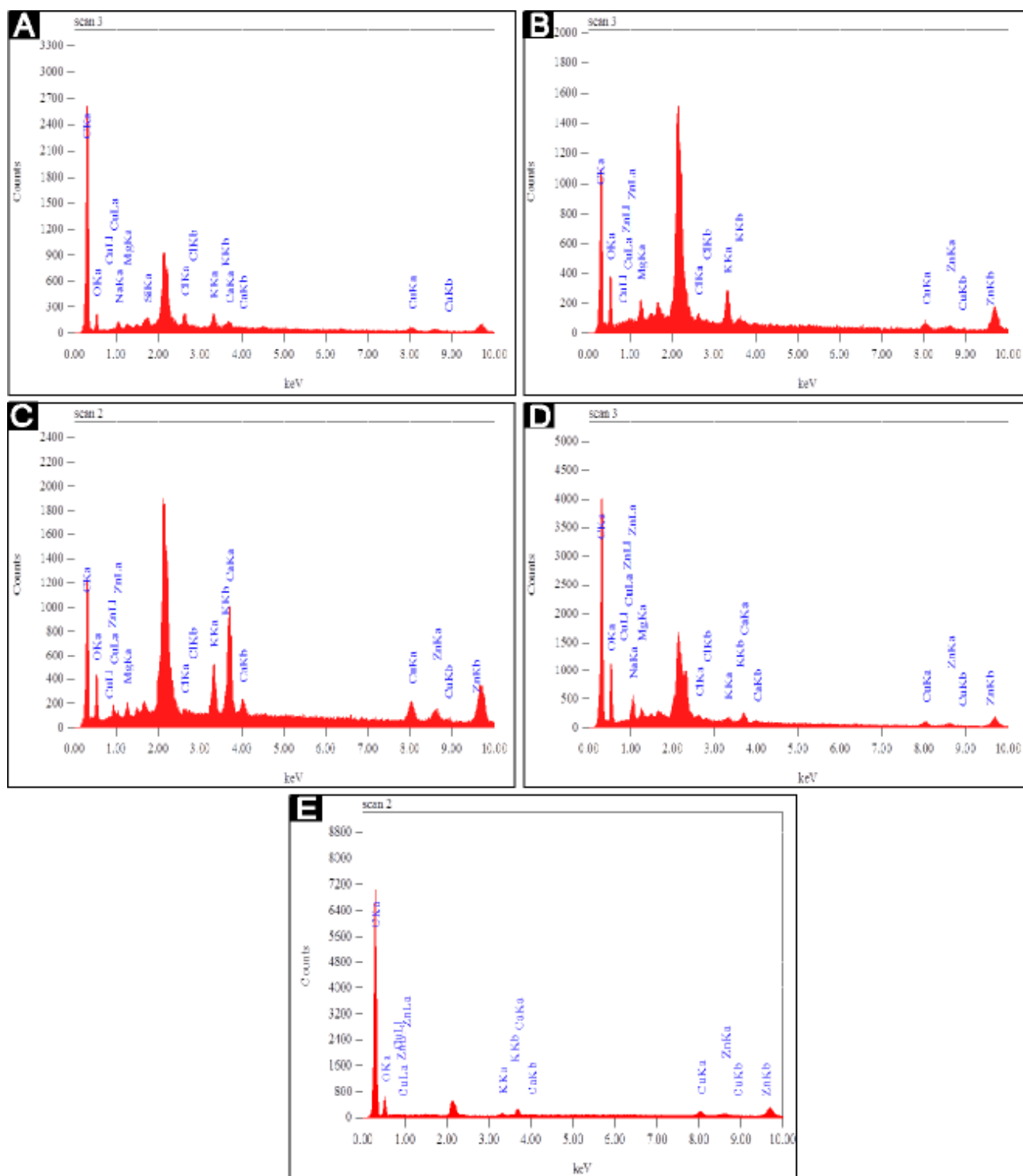


Fig. 4. Energy dispersive X-ray spectroscopy (EDS) graphs. *Cleome pakistanica*: A, seed coat elemental composition. *C. rupicola*: B, seed coat elemental composition. *C. scaposa*: C, seed coat elemental composition. *C. spinosa*: D, seed coat elemental composition. *C. viscosa*: E, seed coat elemental composition.

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