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THE SEED ATLAS OF PAKISTAN-XV. OLEACEAE

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ABSTRACT

Seed morphological characters of 6 taxa belonging to the family Oleaceae were numerically analyzed. Seed macro and micro characters were examined under light and scanning electron microscopy (SEM). A considerable variation was found in seed characters which could be significantly used as an additional tool for the generic and specific delimitation within the family Oleaceae.

Key-words: Oleaceae, Seed morphology, Numerical analysis, Pakistan

INTRODUCTION

The family Oleaceae comprises 24 genera and 615 species (Mabberley, 2008). In Pakistan the Olive family is represented by 8 genera and 8 species along with 22 cultivated species. The family is sub-cosmopolitan in distribution i.e. tropical, sub-tropical and temperate regions of the world, especially in tropical and temperate Asia. (Grohmann, 1974; Mabberley, 2008). According to Angiosperm phylogeny group system III (2009) and Olmstead *et al*. (2014) the family Oleaceae is divided into 5 tribes, out of which only two tribes viz., Myxopyreae (*Nyctanthes*) and Oleeae (*Fraxinus, Ligustrum* and *Olea*) are represented in Pakistan. In the present studies seed characters of the 4 genera viz., *Nyctanthes* L., *Fraxinus* L., *Ligustrum* L. and *Olea* L. have been observed. In the recent trend of plant systematics the seed morphology is effectively used as an additional tool for the delimitation of the various taxa (Amini *et al*., 2011; Mahdavi *et al*., 2012; Abid *et al*., 2015; Patil *et al*., 2015). Besides this few workers studied the seed characters of the family Oleaceae and the data was found significant for the generic delimitation (Grohmann, 1974; Corner, 1976; Rohwer, 1994 a, b; Chang *et al*., 1996). Moreover, Rohwer (1994 a, b; 1995) studied the seed characters of various genera of the family Oleaceae and data was correlated with evolutionary patteren. Inspite of the above information there is no detailed report available from any part of the world. The present study provides the seed morphological information of the family Oleaceae from Pakistan which may be utilized as an additional tool for the taxonomic delimitation of the family Oleaceae.

MATERIALS AND METHODS

Mature and healthy seeds of 6 taxa of the family Oleaceae were collected from herbarium specimens. Mostly 10 plants/species and 10 seeds/plant were studied (Appendix I) and examined under stereomicroscope (Nikon XN Model) and scanning electron microscope (JSM-6380A). For scanning electron microscopy dry seeds were directly mounted on metallic stub using double adhesive tape and coated with gold for a period of 6 minutes in sputtering chamber and observed under SEM. The terminology used is in accordance to Lawrence (1970), Radford *et al*., (1974) and Stearn (1983) with slight modifications. Numerical analysis was carried out by Hierarchical clustering using the Euclidean distance index with the computer package (IBM SPSS Statistics ver.19). Each taxon was treated as an operational taxonomic unit (OTU). Macro and micro morphological characters of seeds viz., size, shape, colour, surface patterns were used. Characters were recorded as presence or absence and coded as 1 or 0 respectively. The average values of the quantitative characters viz., seed length and breadth were directly used (Tables 1-3).

Observations

General seed characters of the family Oleaceae

Seeds 4-14 × 2-5 mm, angular or non-angular, compressed or non-compressed, linear, elliptic, norrowly elliptic, broadly obovate, apex acute, obtuse, rounded or beaked, base cuneate, rounded, oblique or with basal stipe, brown with yellow shade, dusty brown, orange brown, blackish brown, reddish brown, black, surface reticulate, rugosely reticulate, striately foveate, scalariform, glabrous, hilum basal in position (Table 1; Plt. 1).

Presently 2 tribes viz., Myxopyreae and Oleeae are investigated.

Key to the tribes

1 + Seeds broadly obovate ……. Myxopyreae

- Seeds, linear, narrow elliptic or elliptic…….Oleeae

General seed characters of the tribe Myxopyreae

Seeds 5-6×3-4 mm, non-angular, compressed, broadly obovate, apex rounded, base cuneate, black, surface reticulate, glabrous, hilum basal in position (Plt. 1 A-B).

Presently only one species of *Nyctanthes* L. viz., *Nyctanthes arbor-tristis* L. is investigated.

General seed characters of the tribe Oleeae

Seeds 4-14×2-5 mm, angular or non-angular, non-compressed, linear, elliptic, norrowly elliptic, apex acute, obtuse, rounded or beaked, base oblique, cuneate, rounded or with basal stipe, dusty brown, orange brown, reddish brown, blackish brown or brown with yellow towards the margin, surface reticulate, rugosely reticulate, striately foveate, scalariform or glabrous, hilum basal in position (Plt. 1 C-L).

Presently 3 subtribes viz., Fraxininae, Ligustrinae and Oleinae are investigated.

Key to the subtribes

1 + Seeds non-angular, compressed, blackish bown or brown with yellow towards the margin, apically rounded or beaked……2

- Seeds angular, non-compressed, dusty brown, orange brown or reddish brown, apically acute or obtuse…….. Fraxininae

2 + Seeds blackish brown, base cuneate, surface rugosely reticulate…… Ligustrinae

- Seeds brown with yellow towards the margin, base rounded, surface reticulate…….. Oleinae

General seed characters of the subtribe Fraxininae

Seeds 9-14×2-5 mm, angular, non-compressed, linear or narrow elliptic, apex acute or obtuse, base oblique, cuneate or with basal stipe, dusty brown, orange brown or reddish brown, surface striately foveate, scalariform or reticulate, glabrous, hilum basal in position (Plt. 1 C-H).

Presently the genus *Fraxinus* L. with 3 taxa viz., *Fraxinus hookeri* Wenzig, *F. oxycarpa* Willd. var. *oligophylla* (Boiss.) Wenzig and *F. xanthoxyloides* (G. Don) DC. is investigated.

Key to the species

1 + Seeds linear apically acute…….. *F. hookeri*

- Seeds narrow elliptic, apically obtuse……..2

2 + Seeds orange brown with cuneate base…..*F. oxycarpa* var. *oligophylla*

- Seeds reddish brown with basal stipe…….. *F. xanthoxyloides*

General seed characters of the subtribe Ligustrinae

Seeds 4-6×2-3 mm, non-angular, non-compressed, elliptic, apex rounded, base cuneate, blackish brown, surface rugosely reticulate, glabrous, hilum basal in position (Plt. 1 -I).

Presently the genus *Ligustrum* L. with a single species viz., *Ligustrum ovalifolium* Hassk. is investigated.

General seed characters of the subtribe Oleinae

Seeds 4-6×2.5-3 mm, non-angular, non-compressed, elliptic, apex rounded-beaked, base rounded, brown with yellow shade, surface reticulate, glabrous, hilum basal in position (Plt. 1 J-L).

Presently the genus *Olea* L. with a single species viz., *Olea ferruginea* Royle is investigated.

RESULTS AND DISCUSSION

Although the family Oleaceae belongs to the most advance and diverse order Lamiales but mostly characterized by woody plants, larger (5-14 mm) seeds (Fig. 2) and tricolporate pollen grains (Kubitzki, 1990-onward; APG III, 2009). Previously Eames (1961) also indicated that larger seeds are associated with tree habit. The dendrogram of the family Oleaceae (Fig. 1) clearly reveals two distinct groups. Within the first group *Ligustrum ovalifolium, Nyctanthes arbor-tristis* and *Olea ferruginea* occupy the same cluster by having non angular, broadly obovate or elliptic seeds (Fig. 3) with reticulate or rugose surface patterns (Fig. 5) and entire leaflets with berry or drupe (Grohmann, 1974; Chang *et al*., 1996). Since long time the position of the genus *Nyctanthes* remained

uncertain and included in the family Verbenaceae however, various morphological and molecular reports evident the placement of *Nyctanthes* within the family Oleaceae (APG III, 2009; Olmstead *et al*., 2014). Present findings based on seed characters also strengthen the previous finding of Olmstead *et al*. (2014) as *Nyctanthes* shows affinity within the family Oleaceae. Amongst the species of first group, *Ligustrum ovalifolium* falls separatly by having rugosely reticulate seeds with drupe resembling berry (Grohmann, 1974; Chang *et al*., 1996). While, *Nyctanthes arbor-tristis* and *Olea ferruginea* are coupled due to the presence of reticulated seeds with drupe. However, both the taxa remain distinct with each other as *Nyctanthes arbor-tristis* could be distinguished by having broadly obovate and black seeds (Fig. 4) with larger pollen grains (Zhang, 1982) while, *Olea ferruginea* is characterized by elliptic brown seeds with yellow spots and smaller pollen grains (Zhang, 1982). The second group comprises the taxa of the genus *Fraxinus* viz., *F. hookeri*, *F. oxycarpa* var. *oligophylla* and *F. xanthoxyloides* due to the presence of angular, linear or narrowly elliptic seeds with striate surface pattern and serrate and crenate leaflets with samara fruits (Grohmann, 1974; Chang *et al*., 1996).

Table 1. Seed morphological characters of the family Oleaceae.

Name of taxa	Size (mm)		Angular/ Compressed	Shape	Apex	Base	Colour	Surface
	Length	Breadth						
Tribe-Myxopyreae								
Nyctanthes arbor-tristis	5-6	3-4	Non-angular, compressed	Broadly obovate	Rounded	Cuneate	Black	Reticulate
Tribe-Oleeae, Subtribe-Fraxininae								
Fraxinus hookeri	9-10	2-3	Angular, non-compressed	Linear	Acute	Oblique	Dusty brown	Striately foveate
F. oxycarpa* var. *oligophylla	13-14	4-5	Angular, non-compressed	Narrow elliptic	Obtuse	Cuneate	Orange brown	Striately scalariform
F. xanthoxyloides	9-10	2-3	Angular, non-compressed	Narrow elliptic	Obtuse	Small stiped	Reddish brown	Striately reticulate
Subtribe-Ligustrinae								
Ligustrum ovalifolium	4-6	2-3	Non-angular, non-compressed	Elliptic	Rounded	Cuneate	Blackish brown	Rugosely reticulate
Subtribe-Oleinae								
Olea ferruginea	4-6	2.5-3	Non-angular, non-compressed	Elliptic	Rounded-beaked	Rounded	Brown with yellow shade	Reticulate

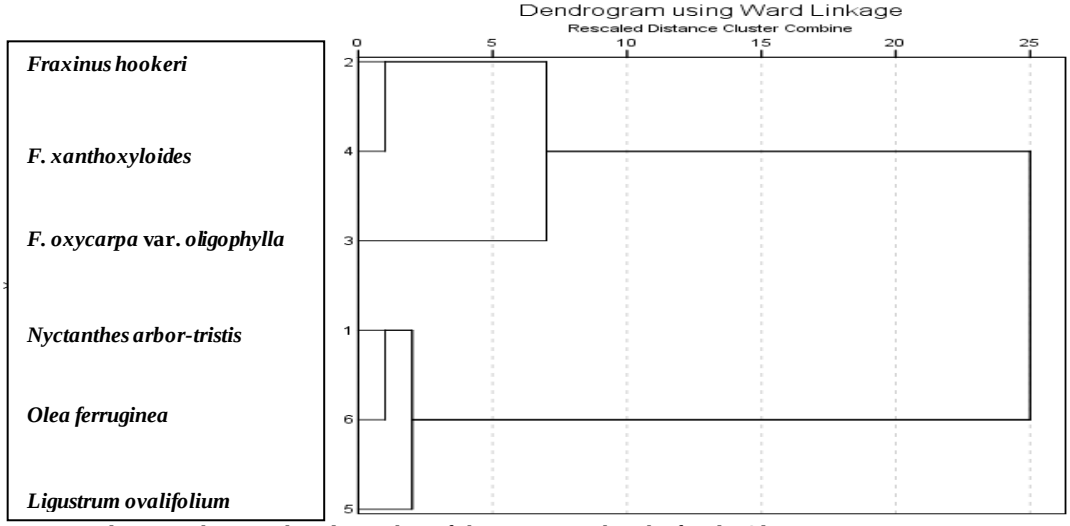

Fig. 1. Dendrogram showing the relationship of the species within the family Oleaceae.

Table 2. List of characters, scored for the cluster analysis for the taxa of the family Oleaceae listed in Table 3.

S. No.	Characters description
1	Seed length (mm)
2	Seed breadth (mm)
	Shape
3	Angular/Non-angular : Non-angular (0), Angular (1)
4	Compressed/Non-compressed : Non-compressed (0), Compressed (1)
5	Linear : Absent (0), Present (1)
6	Narrow elliptic : Absent (0), Present (1)
7	Elliptic : Absent (0), Present (1)
8	Broadly obovate : Absent (0), Present (1)
	Apex
9	Acute : Absent (0), Present (1)
10	Obtuse : Absent (0), Present (1)
11	Rounded : Absent (0), Present (1)
12	Beaked : Absent (0), Present (1)
	Base
13	Cuneate : Absent (0), Present (1)
14	Rounded : Absent (0), Present (1)
15	Oblique : Absent (0), Present (1)
16	Basal stipe : Absent (0), Present (1)
	Colour
17	Lighth brown : Absent (0), Present (1)
18	Yellow : Absent (0), Present (1)
19	Dusty brown : Absent (0), Present (1)
20	Orange brown : Absent (0), Present (1)
21	Blackish brown : Absent (0), Present (1)
22	Reddish brown : Absent (0), Present (1)
23	Black : Absent (0), Present (1)
	Surface
24	Reticulate : Absent (0), Present (1)
25	Rugose : Absent (0), Present (1)
26	Striate : Absent (0), Present (1)
27	Foveate : Absent (0), Present (1)
28	Scalariform : Absent (0), Present (1)

Table 3. Data matrix of the family Oleaceae scored for 28 characters present in Table 2.

Name of Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tribe Myxopyreae														
Nyctanthes arbor-tristis	5.6	3.6	0	1	0	0	0	1	0	0	1	0	1	0
Tribe Oleeae, Subtribe Fraxininae														
Fraxinus hookeri	9.6	2.6	1	0	1	0	0	0	1	0	0	0	0	0
F. oxycarpa* var. *oligophylla	13.6	4.6	1	0	0	0	1	0	0	1	0	0	1	0
F. xanthoxyloides	9.6	2.6	1	0	0	0	1	0	0	1	0	0	0	0
Subtribe Ligustrinae														
Ligustrum ovalifolium	5	2.6	0	0	0	1	0	0	0	0	1	0	1	0
Subtribe Oleinae														
Olea ferruginea	5	2.7	0	0	0	1	0	0	0	0	1	1	0	1
Name of Taxa	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Tribe Myxopyreae														
Nyctanthes arbor-tristis	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Tribe Oleeae, Subtribe Fraxininae														
Fraxinus hookeri	1	0	0	0	1	0	0	0	0	0	0	1	1	0
F. oxycarpa* var. *oligophylla	0	0	0	0	0	1	0	0	0	0	0	1	0	1
F. xanthoxyloides	0	1	0	0	0	0	0	1	0	1	0	1	0	0
Subtribe Ligustrinae														
Ligustrum ovalifolium	0	0	0	0	0	0	1	0	0	1	1	0	0	0
Subtribe Oleinae														
Olea ferruginea	0	0	1	1	0	0	0	0	0	1	0	0	0	0

Appendix I. List of voucher specimens.

Taxa	Collector, number and herbarium
Family Oleaceae, Tribe Myxopyreae	
Nyctanthes arbor-tristis	*S. Qureshi s.n.* (KUH); *A. Ghafoor* 914 (KUH).
Tribe Oleeae, Subtribe Fraxininae	
Fraxinus hookeri	*K. A. Malik* & *S. Nazimuddin* 1572 (KUH); *S. Omer* 228 (KUH); *M. Qaiser et. al.* 8313 (KUH).
F. oxycarpa* var. *oligophylla	*M. Qaiser* & *A. Ghafoor* 4391 (KUH); *F. Garhman* 6939 (RAW).
F. xanthoxyloides	*A. Ghafoor* & *R. Yousuf* 1255 (KUH); *M. Qaiser* & *A. Ghafoor* 1314, 9670 (KUH); *Nazim* & *Iqbal* 357 (KUH).
Subtribe Ligustrinae	
Ligustrum ovalifolium	*Coll. ignot s.n.* (KUH).
Subtribe Oleinae	
Olea ferruginea	*S. I. Ali* 903 (KUH); *M. Qaiser* & *A. Ghafoor* 1536 (KUH); *M. Qaiser* 198 (KUH); *Z. Ali* & *Y. Nasir s.n.* (KUH); *S. W. Khan* & *S. Hussain* 508 (KUH); *T. Ali* & *Tufail Ahmed* 1652, 1746 (KUH); *S. Abedin* 8192 (KUH).

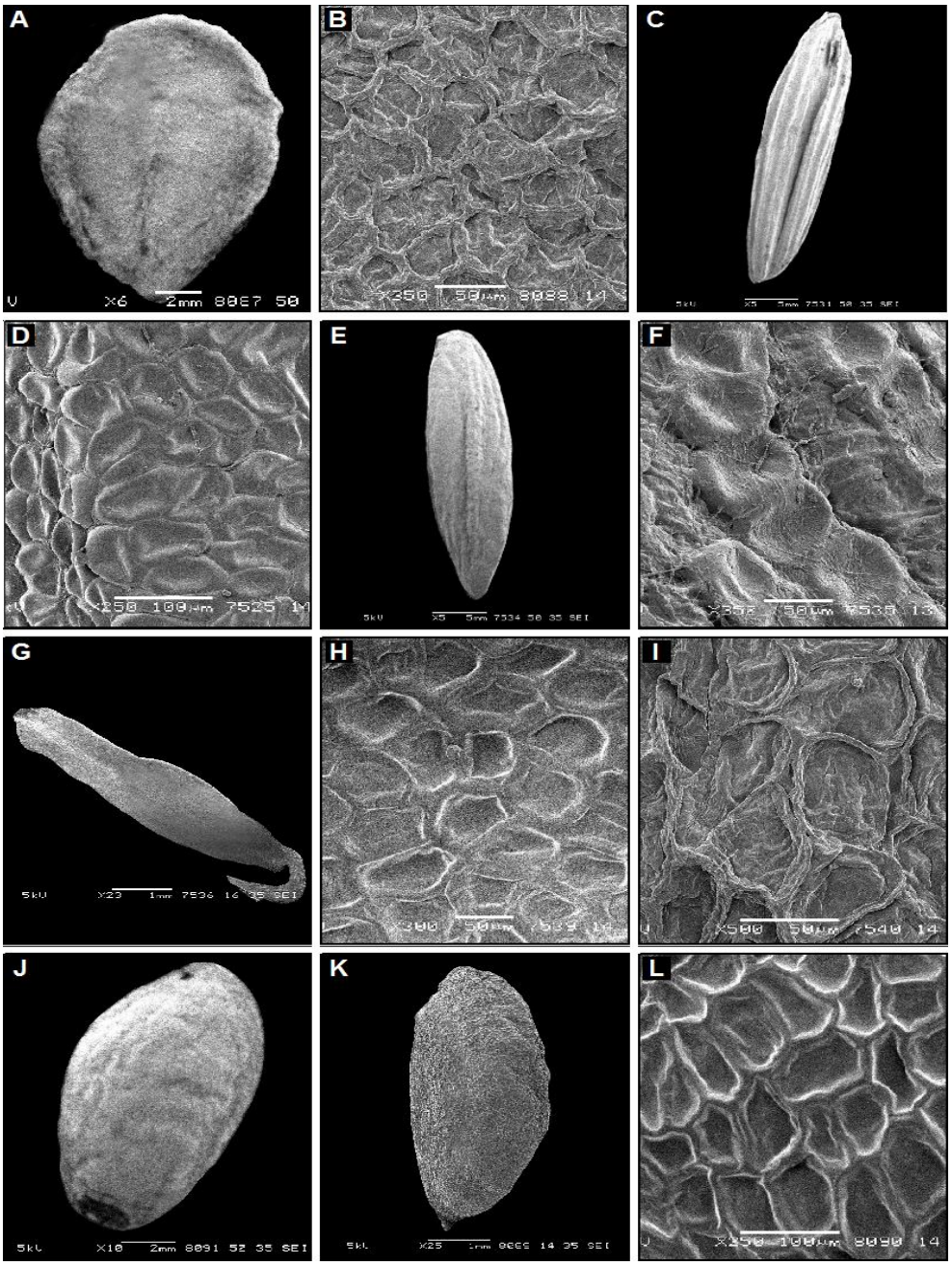

Plate 1. Scanning electron micrographs. *Nyctanthes arbox-tristis*: A, seed; B, surface. *Fraxinus hookeri*: C, seed; D, surface. *F. oxycarpa* var. *oligophylla*: E, seed; F, surface. *F. xanthoxyloides*: G, seed; H, surface. *Ligustrum ovalifolium*: I, surface. *Olea ferruginea*: J, K, seeds; L, surface (Scale bars: C, E= 5 mm; A, J= 2 mm; G, K= 1 mm; D, L= 100 µm; B, F, H, I= 50 µm).

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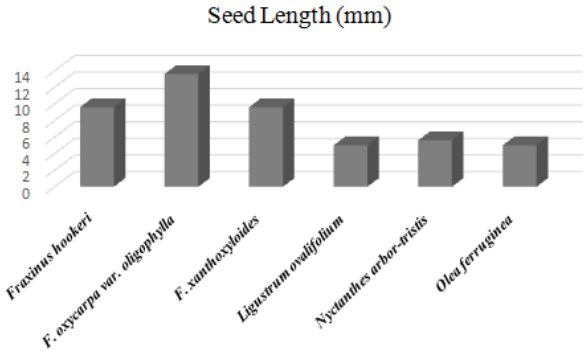

Fig.2.

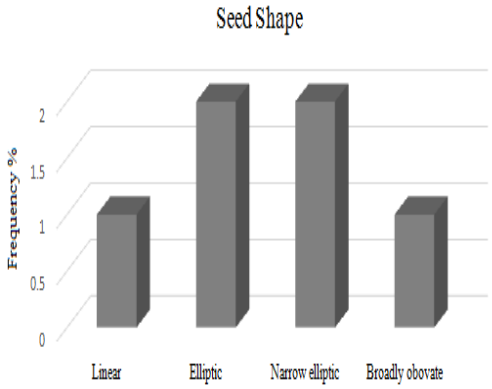

Fig.3.

Fig. 2. Bar diagram showing the variation in average seed length (mm) within different taxa of the family Oleaceae.
Fig. 3. Bar diagram showing the variation in seed shape within the family Oleaceae.

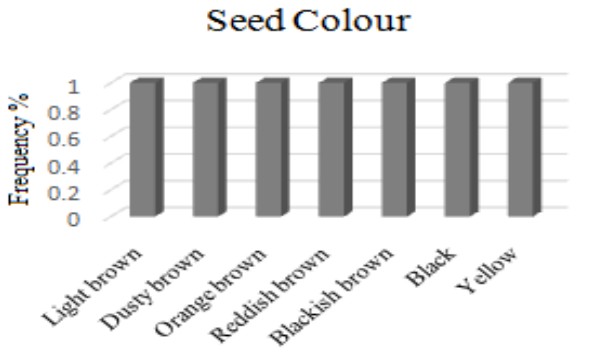

Fig. 4.

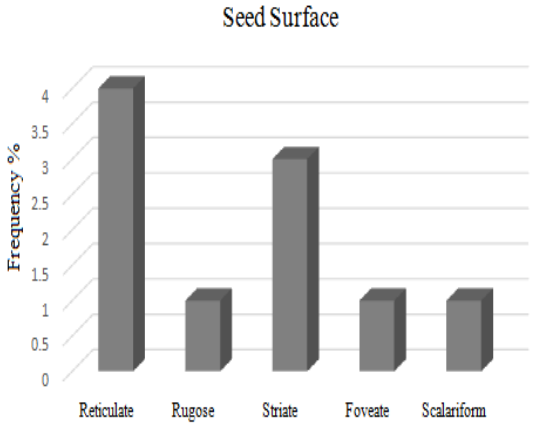

Fig. 5.

Fig. 4. Bar diagram showing the variation in seed colour within the family Oleaceae.
Fig. 5. Bar diagram showing the variation in seed surface within the family Oleaceae.

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