

THE SEED ATLAS OF PAKISTAN-X. CUCURBITACEAE

RUBINA ABID, DURDANA KANWAL AND M. QAISER

Department of Botany, University of Karachi, Karachi-72570, Pakistan

Abstract

Seed morphological characters of 14 taxa of the family Cucurbitaceae distributed in 6 genera were examined using light and scanning microscopy. Seeds are obovate, oblanceolate, oblong, ovate or orbicular. Size ranges from 4-15 x 2-9mm. It is observed that seed morphology not only provides a beneficial tool for delimitation of taxa at various levels, but it can also be used to assess the phylogenetic relationship among different taxa.

Key words: Cucurbitaceae, Seed morphology, Phylogeny, Pakistan.

Introduction

The family Cucurbitaceae comprises 940 species distributed in 122 genera in tropical and warm temperate regions of the world (Mabberley, 2008). The family Cucurbitaceae has been splitted into two sub families viz., Nhandioboideae and Cucurbitoidae. Moreover, the sub family Cucurbitoidae is further splitted into 8 tribes viz., Melothricae, Jolifficae, Tricosantheae, Benincaseae, Cucurbitae, Cyclanthereae and Sicyocae (Jefferey, 2005). In Pakistan family Cucurbitaceae is represented by 17 genera and 32 species (Nazimuddin & Naqvi, 1984). Although seed morphology played a significant role for taxonomic delimitation of various families (Bergreen, 1981; Qaiser, 1987; Akbari & Azizian, 2006; Ather *et al.*, 2009, 2010; Kanwal *et al.*, 2009, 2012; Rajbhandary & Shrestha, 2010). However, concerning to the seed morphology of the family Cucurbitaceae, no attention has been paid except of the few scattered reports (Corner, 1976; Lu & Jeffery, 2002; Nazimuddin & Naqvi, 1984; Kirkbride *et al.*, 2006). However, Pollen morphology (Erdtman, 1952; Qaiser & Perveen, 1997; Perveen & Qaiser, 2008; Moore & Webb, 1978) and Molecular studies were carried out by various workers. Sanjur *et al.* (2001) examined the Phylogenetic relationship in the genus *Cucurbita* and used *mtDNA* gene in resolving interspecific and intraspecific taxonomic relationships Jobst *et al.* (1998) explained the evolutionary relationship within the family Cucurbitaceae with the help of sequence data of *ITS* nuclear region. Renner *et al.* (2007) studied the relationship in the genus *Cucumis* by using DNA sequences from chloroplast genes, intron and spacer regions. Moreover, Shaefer & Renner (2010) explained evolution of monoecy and dioecy in *Momordica* by using three genome phylogeny to trace out its origin. Likewise, Pasha & Seen (2003) after studying the seed protein pattern in the family Cucurbitaceae revealed that protein pattern helps in the delimitation of taxa at tribal and generic levels. The present study is based on detailed study of seed morphological characters with the intention to use this data in tracing out the phylogenetic relationship among different taxa of the family Cucurbitaceae.

Materials and Methods

Mature and healthy seeds of 14 taxa distributed in 6 genera were collected from herbarium specimens (List of voucher specimen is submitted in KUH). Mostly 10 plants/species and 15-20 seeds/plants were studied (depending on availability of material). The seeds were

examined for their morphological characters under stereomicroscope (SMZ800) 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 was in accordance to Berggren (1981) and Stearn (1983) with slight modifications. The following characters of seeds were studied: Presence of aril, size, colour, shape, surface (testa), and position of hilum (Table. 1).

Numerical analysis: Hierarchical clustering was performed by using Euclidean distance index and group strategy with the computer package (Anon., 2012). Each of the species was treated as operational taxonomic unit (OTU). 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 2 and 3).

Observations

General seed characters of the family Cucurbitaceae: Seeds 4-15 x 2-9 mm, elliptic, obovate with blunt base or pointed base, obliquely obovate, oblanceolate, oblong with pointed or blunt base, ovate or orbicular, compressed or not compressed, cream, light or dark brown, mustard brown, light yellow, golden, golden yellow, black, shiny, dull shiny or unshiny, granulate, reticulate, punctate, rugose, favulariate, lineate, lineolate, sulcate, pustulate, foveate, runcate or glebulate, hilum basal or lateral (Table 1; Figs. 1-9).

***Citrullus*:** Seeds 6-7 x 4-5 mm, obovate cream, light brown, unshiny, punctate, hilum basal (Table 1; Fig. 1A-B). Presently represented by a single species *Citrullus colocynthis* (L.) Schrad.

***Coccinia*:** Seeds 5-6 x 3-4 mm, obliquely obovate with blunt base, rugose, hilum basal (Table 1; Fig 1C-D). Represented by a single species *Coccinia cordifolia* (L.) Cogn.

***Corallocarpus*:** Seeds 4-5 x 3 mm, elliptic, obovate with blunt base, cream, light yellow mustard brown, dark brown, unshiny, favulariate or rugose, hilum basal (Table 1; Fig 1.E-H). Presently represented by 2 species viz., *Corallocarpus epigaeus* (Roettl.) Hook.f. and *C. shimperi* (Naud.) Hook.f.

Table 1. Seed morphology of the family Cucurbitaceae.

Name of taxa	Size(mm)	Shape	Color	Surface	Hilum
<i>Citrullus colocynthis</i>	6-7 x 4-5	Obovate	Cream-light brown unshiny	Faintly punctate	Basal
<i>Coccinia cordifolia</i>	5-6 x 3-4	Obliquely obovate with blunt base	Cream, unshiny	Rugose	Basal
<i>Corallocarpus epigaeus</i>	4-5 x 3	Obovate, with blunt base, compressed	Cream-light yellow unshiny	Favulariate	Basal
<i>C. shimperi</i>	4 x 3	Broadly elliptic compressed	Mustard brown dark brown, unshiny	Rugose	Basal
<i>Cucumis melo</i> ssp. <i>Melo</i> var. <i>melo</i>	4-5 x 3	Oblanceolate	Golden yellow shiny	Lineolate	Basal
<i>C. prophetarum</i>	4-6 x 2-3	Elliptic	Light yellow, dull shiny	Faintly lineate	Basal
<i>C. sativus</i>	4-5 x 2-3	Oblanceolate	Cream-light yellow shiny	Prominently lineate and sulcatus	Basal
<i>Ctenolepis cerasiformis</i>	9-11 x 5-7	Broadly obovate and planoconvex	Cream, shiny	Glebulate	Basal
<i>Luffa acutangula</i> var. <i>acutangula</i>	9-12 x 5-7	oblong with pointed base	Black and shiny	Reticulate, sparsely pusticulate and foveate	Basal
<i>L. acutangula</i> var. <i>amara</i>	9-12 x 5-7	Oblong with pointed base	Black and shiny	Reticulate, sparsely pusticulate and foveate	Basal
<i>Momordica balsamina</i>	9-10 x 5-7	Oblong	Cream-golden shiny	Ruminate at edges centrally granulate	Basal
<i>M. charantia</i>	12-15 x 8-9	Oblong	Light yellow, dull shiny	Ruminate at edges centrally granulate	Basal
<i>Mukia maderaspatana</i>	4-6 x 2.5-3	Broadly ovate and compressed	Black, unshiny	Pusticulate and foveate	Basal
<i>Solena heterophylla</i>	7-8 x 6-7	Orbicular	Cream, unshiny	Faintly reticulate	Lateral

Key to the genera

- 1 + Seeds orbicular, hilum lateral *Solena*
 - Seeds other than orbicular, hilum basal 2
 2 + Seeds compressed 3
 Seeds not compressed 4
 3 + Seeds ovate, black, surface pusticulate and foveate *Mukia*
 Seeds elliptic-obovate, other than black, surface favulariate- rugose *Corallocarpus*
 4 + Seeds oblong 5
 Seeds elliptic, obovate or oblanceolate 6
 5 + Seeds with pointed base, black, surface reticulate *Luffa*
 Seeds with blunt base, cream, golden or light yellow, surface ruminate or rugose *Momordica*
 6 + Seeds oblanceolate-elliptic, surface lineate –lineolate *Cucumis*
 Seeds obovate, surface other than lineate-lineolate 7
 7 + Seeds planoconvex, 9-11 mm long, surface glebulate *Ctenolepis*
 Seeds biconvex, 5-7 mm long, surface punctuate or rugose 8
 8 + Seeds with pointed base, surface rugose *Coccinia*
 Seeds with rounded base, surface punctuate *Citrullus*

Key to the species

- 1 + Seeds obovate, cream-light brown, surface favulariate *C. epigaeus*
 - Seeds elliptic, mustard brown-dark brown, surface rugose *C. shimperii*

Cucumis: Seeds 4-6 x 2-3 mm, elliptic, oblanceolate, cream, light yellow, golden yellow, shiny or dull shiny, lineate, lineolate or sulcatus, hilum basal (Table 1; Fig. 11-L, Fig. 2A-B). Presently represented by 3 species viz., *Cucumis melo* L. ssp. *melo* var. *melo* *C. prophetarum* L. and *C. sativus* L.

Key to the species

- 1 + Seeds elliptic *C. prophetarum*
 - Seeds oblanceolate 2
 2 + Seeds golden yellow, surface lineolate *C. melo*
 - Seeds rugose *C. sativus*

Ctenolepis: Seeds 9-11 x 5-7 mm, obovate and planoconvex, cream, shiny, glebulate, hilum basal (Table 1; Fig. 2C-D). Represented by single species *Ctenolepis cerasiformis* (Stocks) Hook.f.

Luffa: Seeds 9-12 x 5-7 mm, oblong with pointed base, black and shiny, reticulate, pusticulate, foveate, hilum basal (Table 1; Fig. 2E-F). Presently represented by single species with 2 varieties viz., *Luffa acutangula*

var. *acutangula* (L.) Roxb. and *L. acutangula* var. *amara* (Roxb.) Clarke.

Momordica: Seeds 9-15 x 5-9 mm, oblong, cream, golden, light yellow, shiny or dull shiny, ruminant and granulate, hilum basal (Table 1; Fig. 2G-H). Presently represented by 2 species *Momordica balsamina* L., and *M. charantia* L.

Key to the species

- 1 + Seeds cream-golden, 9-10 mm long *M. balsamiana*
 - Seeds light yellow, 12-15 mm long *M. charantia*

Mukia: Seeds 4-6 x 2.5-3, ovate, compressed, black unshiny, pusticulate and foveate, hilum basal (Table 1; Fig. 2I-J). Represented by single species *Mukia maderaspatana* (L.) M.J. Roem.

Solena: Seeds 7-8 x 6-7 mm, orbicular, cream unshiny, reticulate, hilum lateral (Table 1; Fig. 2K-L). Represented by a single species *Solena heterophylla* (Lour.) Cogn.

Table 2. List of characters, scored for cluster analysis. For taxa of family Cucurbitaceae listed on Table 3.

No.	Character description
1.	Length(mm):
2.	Breadth(mm):
3.	Compressed or non compressed: non compressed (0) compressed (1)
Shape	
4.	Orbicular: absent (0), present (1)
5.	Oblong: absent (0), present (1)
6.	Oblanceolate: absent (0), present (1)
7.	Obovate: absent (0), present (1)
8.	Elliptic: absent (0), present (1)
9.	Ovate: absent (0), present (1)
Colour	
10.	Cream: absent (0), present (1)
11.	Cream-light brown: absent (0), present (1)
12.	Cream-light yellow: absent (0), present (1)
13.	Cream-golden: absent (0), present (1)
14.	Light yellow: absent (0), present (1)
15.	Golden yellow: absent (0), present (1)
16.	Mustard brown-dark brown: absent (0), present (1)
17.	Black: absent (0), present (1)
Surface	
18.	Lineate: absent (0), present (1)
19.	Lineolate: absent (0), present (1)
20.	Rugose: absent (0), present (1)
21.	Favulariate: absent (0), present (1)
22.	Glebulate: absent (0), present (1)
23.	Punctate: absent (0), present (1)
24.	Reticulate: absent (0), present (1)
25.	Lineate and sulcatus: absent (0), present (1)
26.	Reticulate, pusticulate and foveate: absent (0), present (1)
27.	Ruminant and granulate: absent (0), present (1)
28.	Pusticulate and foveate: absent (0), present (1)
29.	Hilum: basal (1), lateral (2)

Results and Discussion

The family Cucurbitaceae is a Eurosperous family as considerable variation has been observed in seed characters especially in seed shape and surface patterns (Figs. 6 & 8). It is observed that obovate and oblong seeds

are most frequent in all the studied taxa followed by elliptic and oblanceolate seeds, while ovate and orbicular shape is less frequent (Fig. 6). Presently seed morphological data have been used to assess the phylogenetic relationship among different taxa, and the seed characters have been well correlated with morphological and palynological data.

Dendrogram (Fig. 3) of the family Cucurbitaceae clearly forms two broad groups. The first group comprises 3 genera viz., *Ctenolepis*, *Momordica* and *Luffa*. This group is characterized by having monoecious-dioecious or monoecious plants with simple palmately lobed or 5-lobbed leaves, oblong or sub-globose ovary (Nazimuddin & Naqvi, 1984) tricolpate pollen grains (Perveen & Qaiser, 2008) along with oblong or obovate seeds. This group occupies the basal position in dendrogram and taxa of this group are considered to be primitive as they have larger seeds as compared to the taxa of other group. Among the taxa of first group *Momordica charantia* falls separately from the remaining taxa by having light yellow 8-9mm broad seeds, while the remaining taxa have 5-7 mm broad cream, golden or black seeds. These taxa further form two sub groups. The first subgroup includes the genus *Luffa* and remains distinguished on the basis of polypetalous flowers (Nazimuddin & Naqvi, 1984) and black seeds. Likewise another sub group includes *Momordica balsamina* and *Ctenolepis cerasiformis*, and they are characterized due to the presence of gamopetalous flowers (Nazimuddin & Naqvi, 1984) and cream or golden seeds. However *Momordica balsamina* and the genus *Ctenolepis cerasiformis* are placed in a same cluster but both the taxa can be further separated by having flowers with sub-globose ovary (Nazimuddin & Naqvi, 1984) prolate-spheroidal pollen grains (Perveen & Qaiser, 2008) and obovate seeds with glebulate surface in *Ctenolepis cerasiformis*. While *Momordica balsamina* have flowers with oblong ovary (Nazimuddin & Naqvi, 1984) prolate pollen grains (Perveen & Qaiser, 2008) and oblong seeds with ruminant and granulate surface. The present findings are also supported by Lu & Jeffery (2002) who have also observed oblong seeds in *Momordica*.

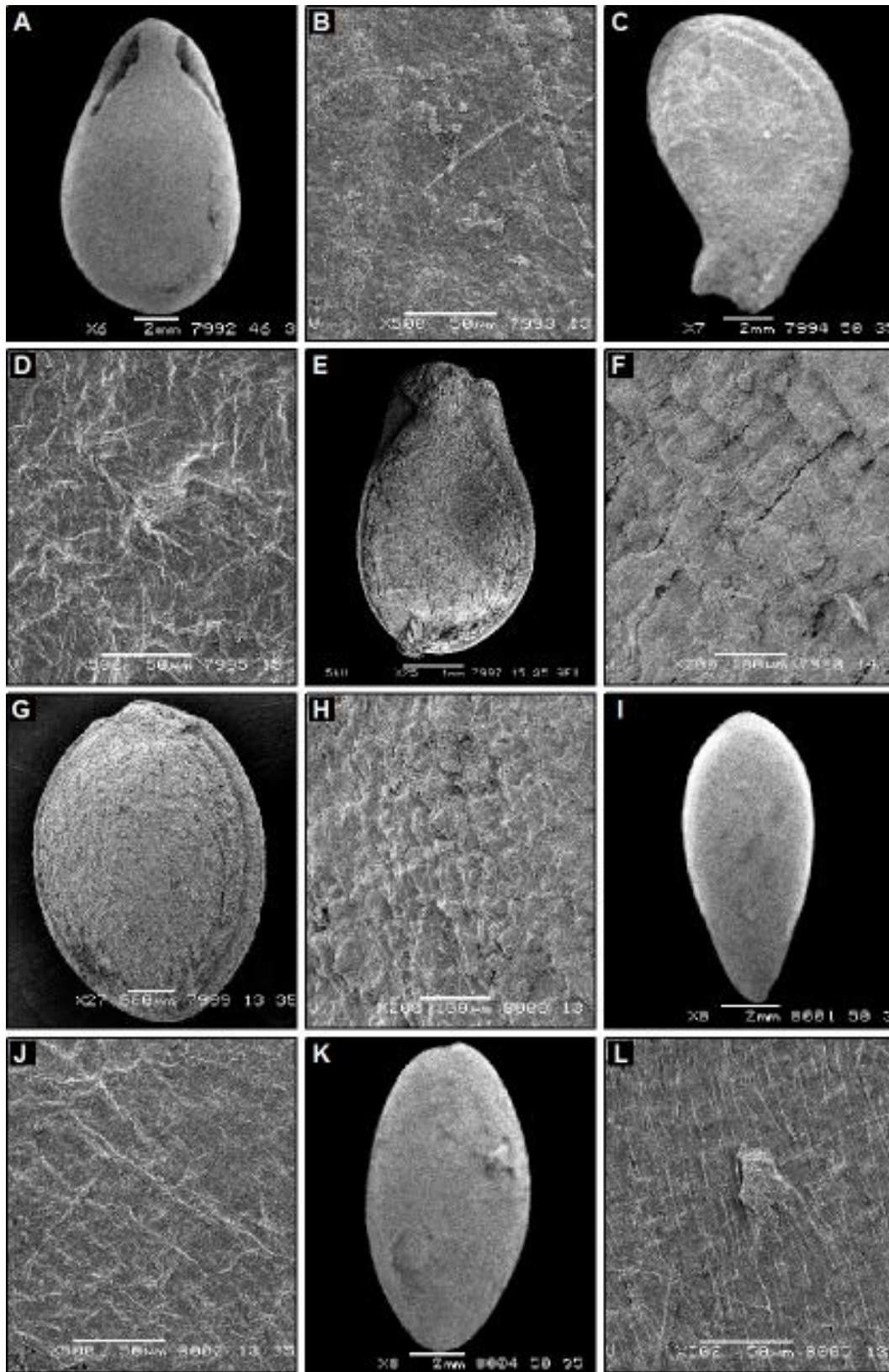


Fig. 1. *Citrullus colocynthis*: A, seed; B, surface. *Coccinia cordifolia*: C, seed; D, surface. *Corallocarpus epigaeus*: E, seed; F, surface. *C. shimperi*: G, seed; H, surface. *Cucumis melo* ssp. *melo* var. *melo*: I, seed; J, surface. *C. prophetarum*: K, seed; L, surface.

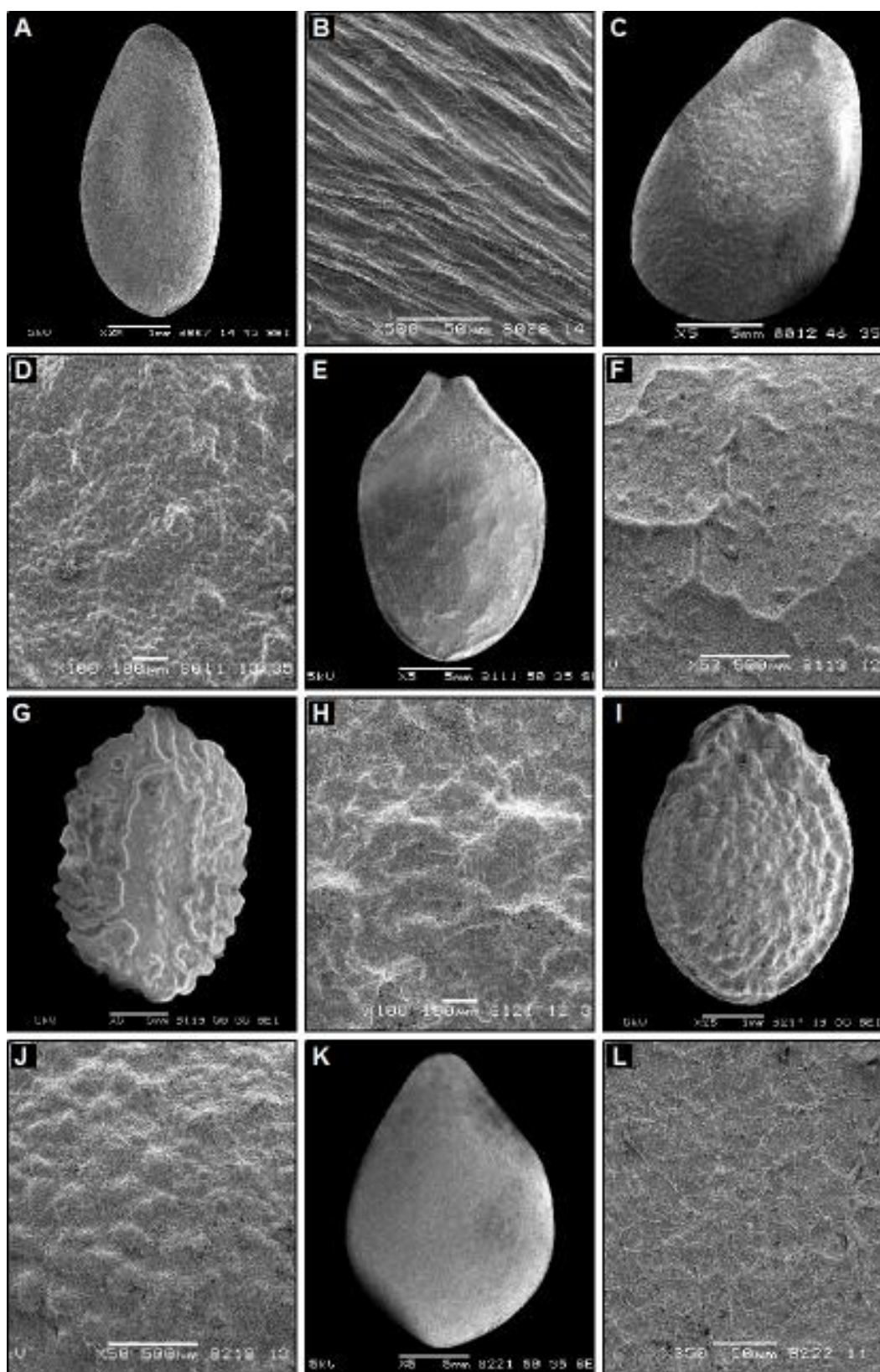


Fig. 2. *C. sativus*: A, seed; B, surface. *Ctenolepis cerasiformis*: C, seed; D, surface. *Luffa acutangula* var. *acutangula*: E, seed; F, surface. *Momordica balsamina*: G, seed; H, surface. *Mukia maderaspatana*: I, seed; J, surface. *Solena heterophylla*: K, seed; L, surface.

Table 3. Data matrix of Cucurbitaceae scored for 29 characters present 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	21	22	23	24	25	26	27	28	29
<i>Citrullus colocynthis</i>	6.5	4.5	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>Coccinia cordifolia</i>	5.5	3.5	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>Corallocarpus epigaeus</i>	4.5	2.7	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>C.shimperi</i>	4.2	3.2	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>Cucumis melo ssp. Melo</i>	4.5	3.2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1
<i>C. prophetarum</i>	5	2.5	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
<i>C.sativus</i>	4.5	2.5	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
<i>Ctenolepiscerasiformis</i>	10	6	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>Luffa acutangula var. acutangula</i>	10.5	6	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>L.acutangula var. amara</i>	10.5	6	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>Momordicabalsamina</i>	9.5	6	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>M.charantia</i>	13.5	8.5	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>Mukia maderaspatana</i>	5	2.75	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
<i>Solena heterophylla</i>	7.5	6.5	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2

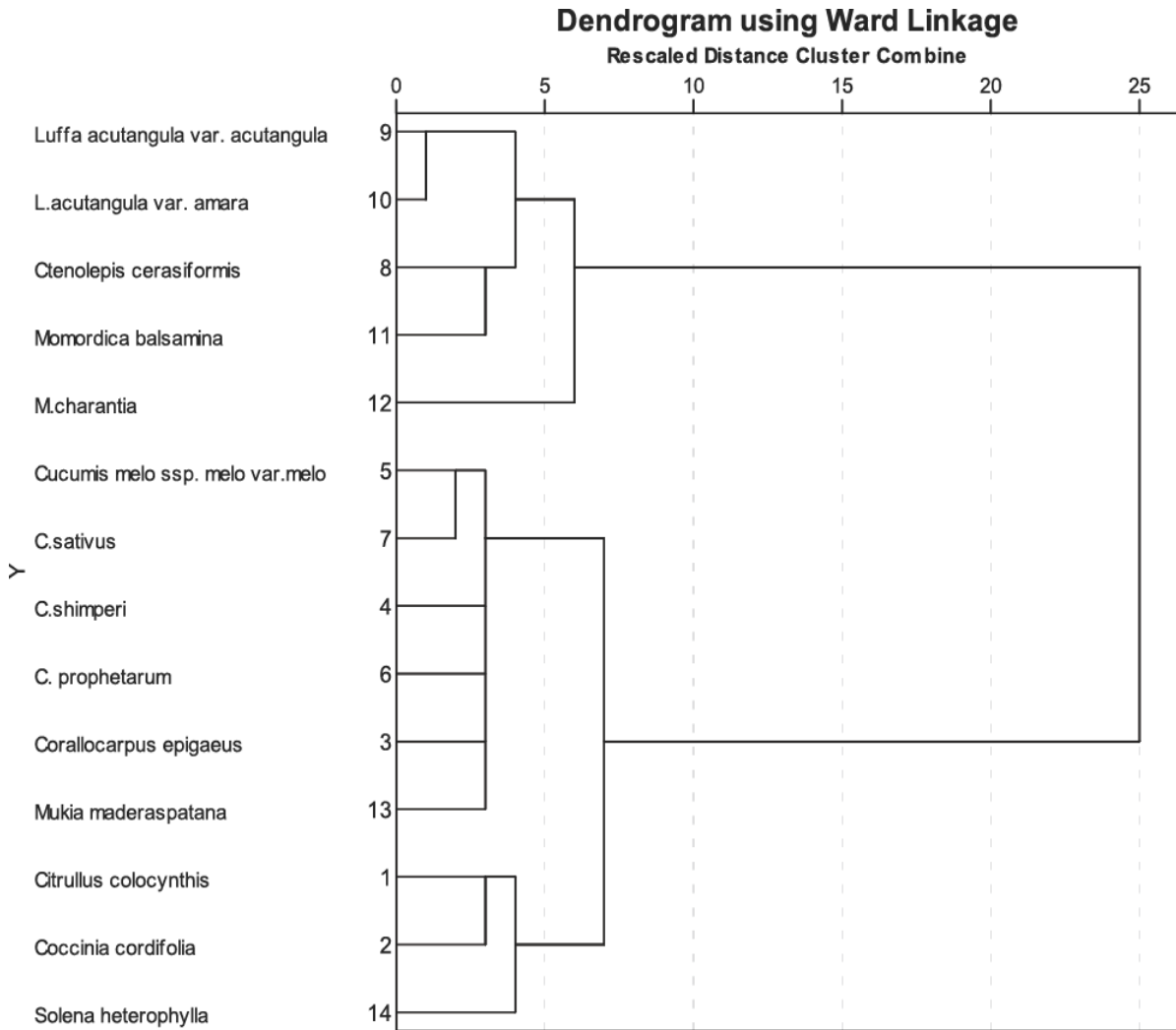


Fig. 3. Dendrogram showing relationship within the taxa of the family Cucurbitaceae.

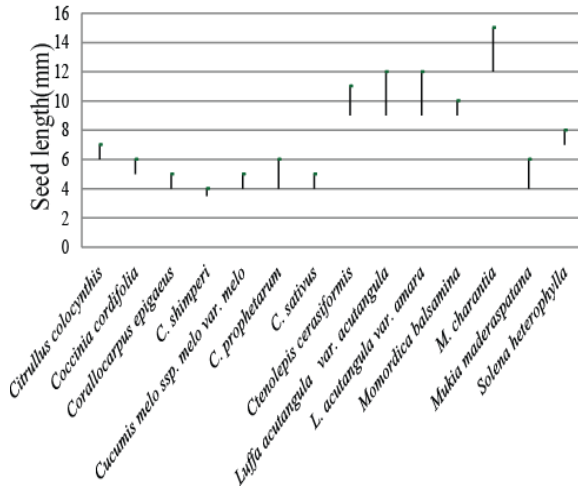


Fig. 4. Graph showing variation in seed length within the taxa of the family Cucurbitaceae.

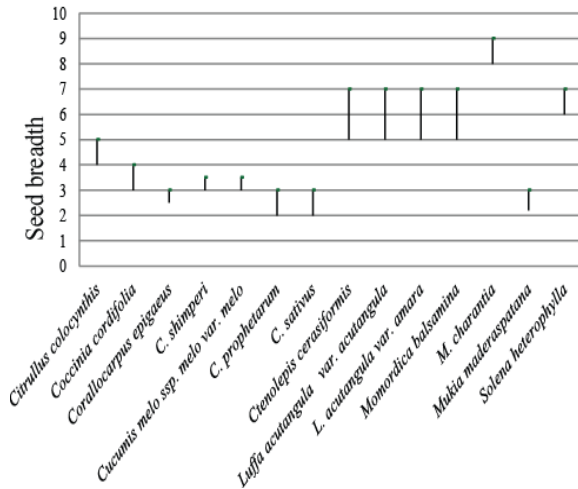


Fig. 5. Graph showing variation in seed breadth within the taxa of the family Cucurbitaceae.

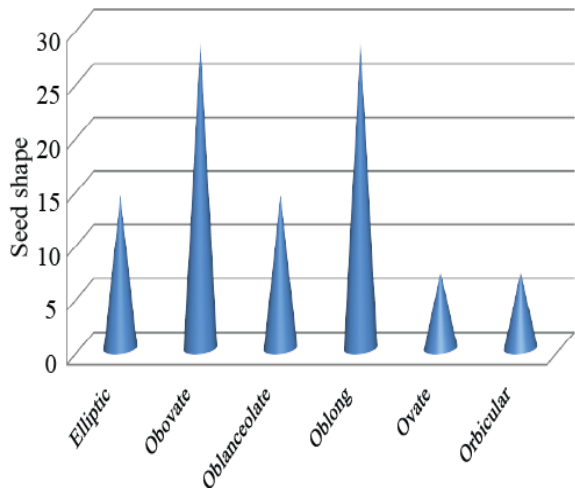


Fig. 6. Graph showing variation in seed shape within the taxa of the family Cucurbitaceae.

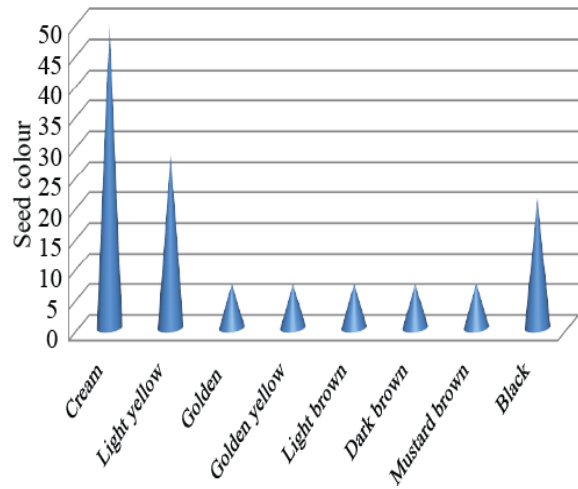


Fig. 7. Graph showing variation in seed colour within the taxa of the family Cucurbitaceae.

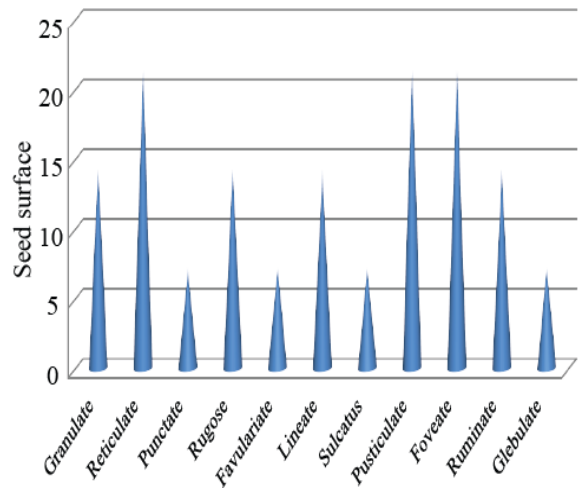


Fig. 8. Graph showing variation in seed surfaces within the taxa of the family Cucurbitaceae.

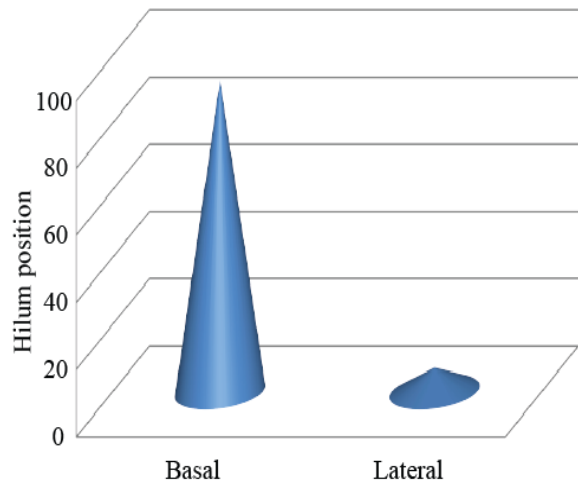


Fig. 9. Graph showing variation in position of hilum on seeds within the taxa of the family Cucurbitaceae.

The second group includes 6 genera viz., *Cucumis*, *Corallocarpus*, *Coccinia*, *Citrullus*, *Mukia* and *Solena* and this group remains distinct from the first group by having monoecious, dioecious or rarely monoecious-dioecious plants with simple, 3-7-lobed, bi-pinnately lobed or 5-lobbed leaves, ovoid, globose-sub-globose, oblong or oblong-linear ovary (Nazimuddin & Naqvi, 1984) tricolpate-porate, oblate, sub-oblate, oblate-spheroidal or prolate pollen grains (Perveen & Qaiser, 2008) and 2-7 mm broad elliptic, oblanceolate, obovate, ovate or orbicular seeds. This group may also be separated into two sub groups. The first sub group includes genera *Cucumis*, *Corallocarpus* and *Mukia*. This group is distinguished on the basis of bi-pinnately lobed or 5-lobbed leaves (Nazimuddin & Naqvi, 1984) oblate-suboblate pollen grains (Perveen & Qaiser, 2008) and ovate, elliptic or oblanceolate seeds. All the 6 taxa of first subgroup occupy same cluster in the dendrogram by having elliptic, obovate, ovate or oblanceolate seeds. While the taxa *Cucumis melo* and *C. sativus* are more closely related by having oblanceolate seeds. While the remaining 4 taxa viz., *Corallocarpus epigaeus*, *C. shimperi*, *Cucumis prophetarum* and *Mukia maderaspatana* have elliptic, obovate or ovate seeds. However, all the above taxa can be further distinguished from each other by having different seed surface patterns. Similarly 3 genera viz., *Citrullus*, *Coccinia* and *Solena* falls in another subgroup and remains distinct by having plants with simple or 3-7 lobbed leaves (Nazimuddin & Naqvi, 1984) tricolpate, oblate-spheroidal or prolate pollen grains (Perveen & Qaiser, 2008) and obovate or orbicular seeds with punctuate, rugose or reticulate surface. The genus *Solena* may be separated from the remaining genera by having flowers with oblong ovary (Nazimuddin & Naqvi, 1984) prolate pollen grains and orbicular seeds with lateral hilum. In contrast to the present findings Nazimuddin & Naqvi (1984) observed oblong seeds but present findings are supported by Lu and Jeffery (2002) where orbicular seeds were also observed. While, the remaining 2 genera *Citrullus* and *Coccinia* are related to each other by having obovate seeds. But both genera remains distinct due to the presence of plants with sub-globose ovary (Nazimuddin & Naqvi, 1984) oblate-spheroidal pollen grains (Perveen & Qaiser, 2008) and seeds with punctuate surface in *Citrullus*, while in *Coccinia* plants are with ovoid, oblong-linear ovary (Nazimuddin & Naqvi, 1984) prolate pollen grains (Perveen & Qaiser, 2008) and seeds with rugose surface patterns.

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