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THE SEED ATLAS OF PAKISTAN-XII. NYCTAGINACEAE

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ABSTRACT

Seed macro and micro morphological characters of nine species distributed in three genera of the family Nyctaginaceae were studied using stereo and scanning electron microscopy, and these characters have been utilized to trace out inter relationships between species by hierarchical clustering using SPSS computer program. Five different parameters of seeds were used including seed size, shape, colour, surface and position of hilum. Seed are found to be angular, non-angular, orbicular, elliptic, or oblanceolate. The seed morphology not only plays a useful role in the delimitation of taxa at the generic or specific levels but it can also be used to assess the phylogenetic relationship among different taxa.

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

INTRODUCTION

The family Nyctaginaceae is commonly known as 4'O clock family, comprises 39 genera and 390 species distributed in tropical and warm regions of the world especially in America, while few species are found in temperate regions (Mabberley, 2008). In Pakistan it is represented by 5 genera distributed in 11 species (Nasir, 1977).

Previously, Bittrich and Khun (1993) splitted the family Nyctaginaceae into 6 tribes viz., Boldoeae, Leucastereae, Nyctagineae, Abronieae, Bougainvilleeae and Pisonieae . The tribe Nyctagineae is further divided into 4 sub tribes viz., Coligoniinae, Boerhaviinae, Nyctagininae, Phaeoptilinae. The genera *Boerhavia*, and *Commicarpus* are placed under the subtribe Boerhaviinae, while the genus *Mirabilis* is placed in the subtribe Nyctagininae of the tribe Nyctagineae.

According to APG III (2009), the family Nyctaginaceae has been divided into 7 tribes and most of the tribes are similar to that of Bittrich and Khun (1993), but without further sub division of any tribe. In Pakistan it is represented by 3 tribes viz., Bougainvilleae (Bougainvilla), Pisoneae (Pisonia) and Nyctagineae (*Boerhavia, Commicarpus* and *Mirabilis*).The studied genera viz., *Boerhavia, Commicarpus* and *Mirabilis* are directly placed in the tribe Nyctaginae (APG III, 2009).

Previously the family Nyctaginaceae was studied for its pollen morphology (Erdtman 1952; Perveen and Qaiser, 2001) and few reports are also available on its phylogeny (Levin, 2000; Douglas and Manos, 2007). Moreover, importance was also given to the anthocarp but no detail study was carried out for the seeds (Corner, 1976; Spellenberg, 2003; Dequan and Gilbert, 2004; Kirkbride *et al*., 2006; Bojnansky and Fargasova, 2007). Seed morphological characters can be used as a beneficial tool for systematic of angiosperms (Duke, 1969; Corner, 1976; Berggren, 1981; Ahmad and Qaiser, 1989) and to recognize phylogenetic relationship among different taxonomic groups (Hufford, 1995; Tsutsumi *et al*., 2007; Fawzi *et al*., 2010). Presently seed morphology of 9 species of the family Nyctaginaceae has been carried out in order to provide the additional micromorphological characters for taxonomic delimitation both at generic and specific levels. Secondly these characters are used to trace out phylogenetic relationship among the taxa of family Nyctaginaceae from Pakistan.

MATERIALS AND METHODS

Mature and healthy seeds of nine species distributed in three genera were collected from herbarium specimens (Appendix-I). Mostly 20 seeds per plant and 10 plants per species were studied. Seed morphological characters were examined under sterio microscope (Nikon Type 102) and scanning electron microscope (JSM-6380A) by teasing the anthocarps to obtain seeds with the help of needle. For scanning electron microscopy dry seeds were directly mounted on metallic stubs using double adhesive tape and coated with gold for a period of 6 minutes in a sputtering chamber and observed under SEM. The terminology used is in accordance of Lawrence (1970), Bergreen (1981) and Stearn (1983) with slight modifications. The following characters of seeds were studied: size, colour, shape, surface (testa), and position of hilum.

Numerical analysis

Hierarchical clustering was performed by using Euclidean distance index and group strategy with the computer package (SPSS 18, 2012) .Each of the species is treated as operational taxonomic unit (OTU). Nineteen characters were recorded in binary state (Table 2) and coded as presence or absence (1 and 0 respectively). The average value of the quantitative characters viz., seed length and breadth were directly used.

OBSERVATIONS

General seed characters of the family Nyctaginaceae

Seeds 1-4.5 × 0.5-5mm, angular or non-angular, dorsiventral or iso-bilateral, orbicular, oblanceolate , elliptic or oblong-elliptic, black at the corners or not, light brown, dark brown or black, shiny or not shiny, surface crenatus and irregularly ribbed, lineate, lineolate-lineate, lineate and reticulate, lineolate and reticulate, ruminate and lineolate, reticulate, or sclariform, hilum basal or indistinct (Table 1; Fig. 1-6).

Key to the genera
1+ Seeds orbicular, 4.2-4.5 mm long, hilum distinct.....*Mirabilis*
-Seeds oblong,elliptic or oblanceolate, 1-3.5mm long, hilum indistinct.....2
2+ Seeds angular or non-angular, isobilateral or dorsiventral.....*Boerhavia*
-Seeds non-angular, isobilateral.....*Commicarpus*

Boerhavia Linn.

Seeds 1-2 × 0.5-1 mm, angular or non-angular, dorsiventral or iso bilateral, oblanceolate, elliptic, oblong-elliptic, light brown, black towards the margins, shiny, surface lineate, lineate-lineolate, reticulate or sclariform, hilum indistinct.

It is represented by 5 species viz., *Boerrhavia diandra* Linn., *B. diffusa* Linn., *B. procumbens* Banks ex Roxb., *B. repens* Linn., *B. rubicunda* Steud.

Key to the species
1+ Seeds angular.....3
-Seeds non-angular.....2
2+ Seeds oblanceolate.....*B. repens*
-Seeds elliptic.....*B. procumbens*
3+Seeds dorsiventral, surface lineate-lineolate.....*B. diffusa*
-Seeds isobilateral, surface reticulate or sclariform.....4
4+ Seeds surface reticulate.....*B. diandra*
-Seeds surface sclariform.....*B. rubicunda*

Commicarpus Standely

Seeds 2.8-3.5 × 1-1.3 mm, non-angular, oblanceolate, light brown,dark brown and black towards the margins and shiny, surface lineate-reticulate, lineolate- reticulate or ruminate-lineolate, hilum indistinct.

It is represented by 3 species viz., *Commicarpus boissieri* (Heimerl) Cufod., *C. helenae* (Roem and Schultes) Meikle, *C. stenocarpus* (Chiov.) Cufod.

Key to the species
1+ Seeds dark brown, surface lineate and reticulate or lineolate and ruminate.....2
-Seeds light brown, surface lineolate and reticulate.....*C. helenae*
2+Seeds surface lineate along with the reticulate pattern.....*C. boissieri*
-Seeds surface lineolate along with the ruminate pattern.....*C. stenocarpus*

Mirabilis Linn

Seeds 4.2-4.5 × 4-5 mm, orbicular, light brown and unshiny surface crenatus and irregularly ribbed, hilum basal.

It comprises single species viz., *Mirabilis jalapa* Linn.

RESULTS AND DISCUSSION

The fruit of the family Nyctaginaceae is named as anthocarp (Bogle, 1974; Douglas and Manos, 2007). These anthocarps are variable within the family Nyctaginaceae (Nasir, 1977; Willson and Spellenberg, 1977; Bittrich and Kuhn, 1993) and have been used as a significant character for taxonomic delimitations (Bittrich and Kuhn, 1993). But not a due attention was paid to the seeds inside the anthocarp. The present work is the first for examining the seeds within the anthocarps in the family Nyctaginaceae from Pakistan. In contrast to the uniform pollen (Erdtman, 1952; Perveen and Qaiser, 2001) the family Nyctaginaceae having the seeds with exclusive features.

Appendix I.

Taxon	**Collector, number and herbarium**
Boerhavia diandra	*S. M. H. Jafri* 376, 4017 (KUH). *Y. Nasir* 795 (KUH); *S. M. H. Jafri s.n.* (KUH); *S. Abedin* 774 (KUH); *R. R. Stewart* 2264 (RAW).
B. diffusa	*S. I. Ali* 870 (KUH); *S. Omer* and *Rizwan Yousuf* 1947c (KUH) *Y. Nasir* 441 (KUH); *R. R. Stewart* 22651 (RAW).
B. procumbens	*Tahir Ali et al.,* 21 (KUH); *S. Abedin* 8513, 8349, 3699 (KUH); *M. Qaiser* and *A. Ghafoor* 487, 6419, 1643 (KUH); *M. Qaiser* 8533 (KUH); *Stewart* 6080 (RAW).
B. repens	*Kamal A. Malik et al.,* 2495 (KUH); *S. Abedin* 5096 (KUH); *M. Qaiser* and *S. Abedin* 8617, 5530 (KUH); *Stewart* 680,682 (RAW).
B. rubicunda	*Kamal A. Malik et al.,* 2158 (KUH); *S. A. Khan s.n.* (PPFI-B); *S. Abedin* and *Abrar Hussain* 6474 (KUH); *A. Ghafoor* and *M. Qaiser* 243 (KUH).
Commicarpus boissieri	*Kamal A. Malik et al.,* 2045 (KUH); *S. Abedin* 4268, 5575, 5586 (KUH); *M. Qaiser* 2560 (KUH); *M. Qaiser* 2560 (KUH); *M. Qaiser et al.,* 590, 530 (KUH); *A. Ghafoor* and *M. Qaiser* 3667, 4032 (KUH); *S. I. Ali et al.,* 191 (KUH).
C. helenae	*S. Khatoon* 291, 292 (KUH); *M. Qaiser* and *Abrar Hussain* 648 (KUH); *S. Hussain s.n.* (KUH); *S. Abedin* and *A. Hussain* 7292 (KUH); *S. Akhter s.n*. (KUH).
C. stenocarpus	*S. Abedin* and *Abrar Hussain* 6365, 6363 (KUH); *S. I. Ali* and *S. A. Farooqi* 1150, 1161 (KUH); *S. Abedin* 1151 (KUH). *A. Ghafoor* and *M. Qaiser* 367, 492 (KUH).
Mirabilis jalapa	*D. Kanwal* 01, 02, 03 (KUH); *D. Kanwal* and *A.Ather* 03, 04, 05 (KUH).

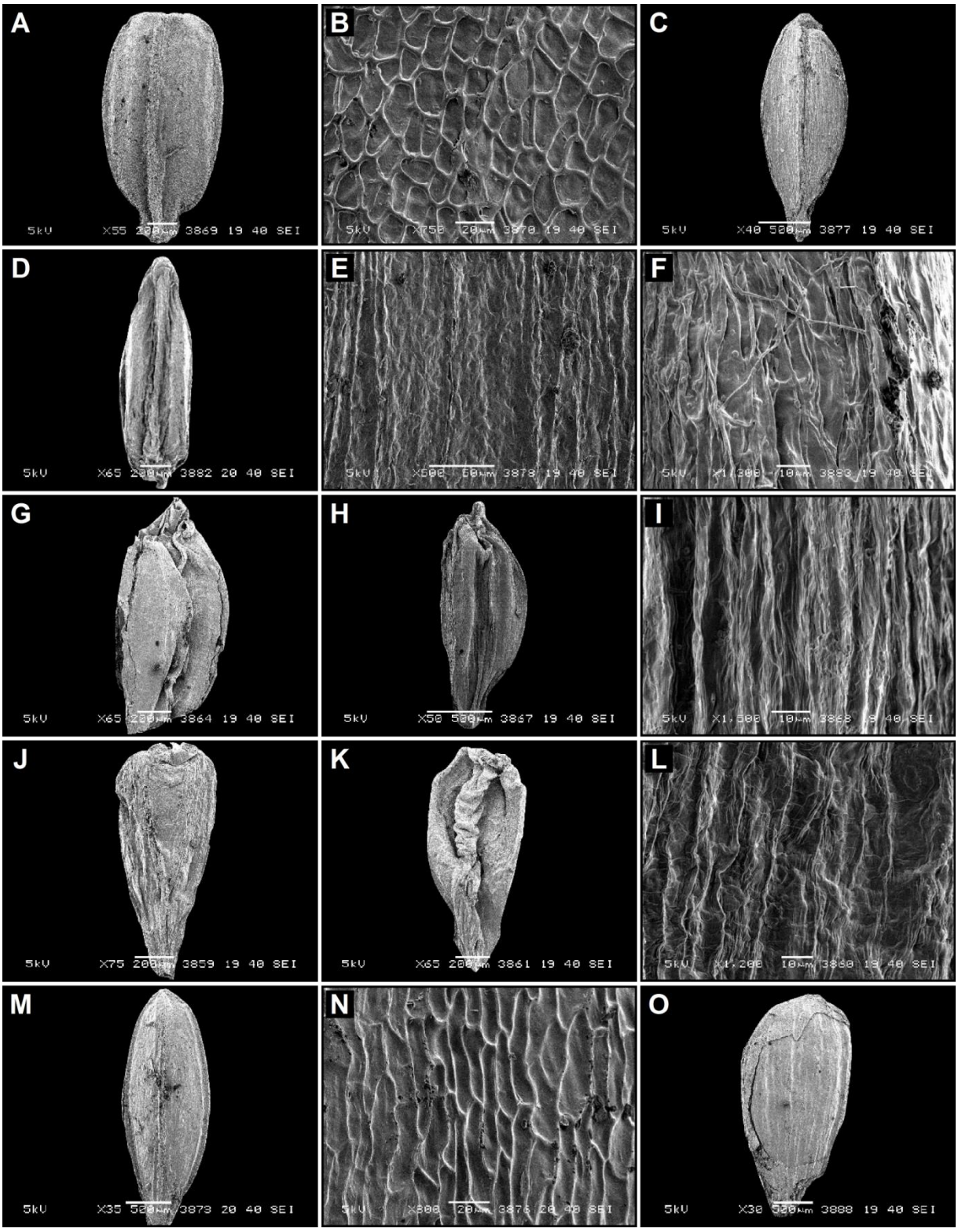
A
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Fig. 1. Scanning electron micrographs. *Boerhavia diandra*: A, seed; B, surface. *B. diffusa*: C, D, seed; E, F, surface. *B. procumbens*: G, H, seed; I, surface. *B. repens*: J, K, seed; L, surface. *B. rubicunda*: M, seed; N, surface. *Commicarpus boissieri*: O, seed.

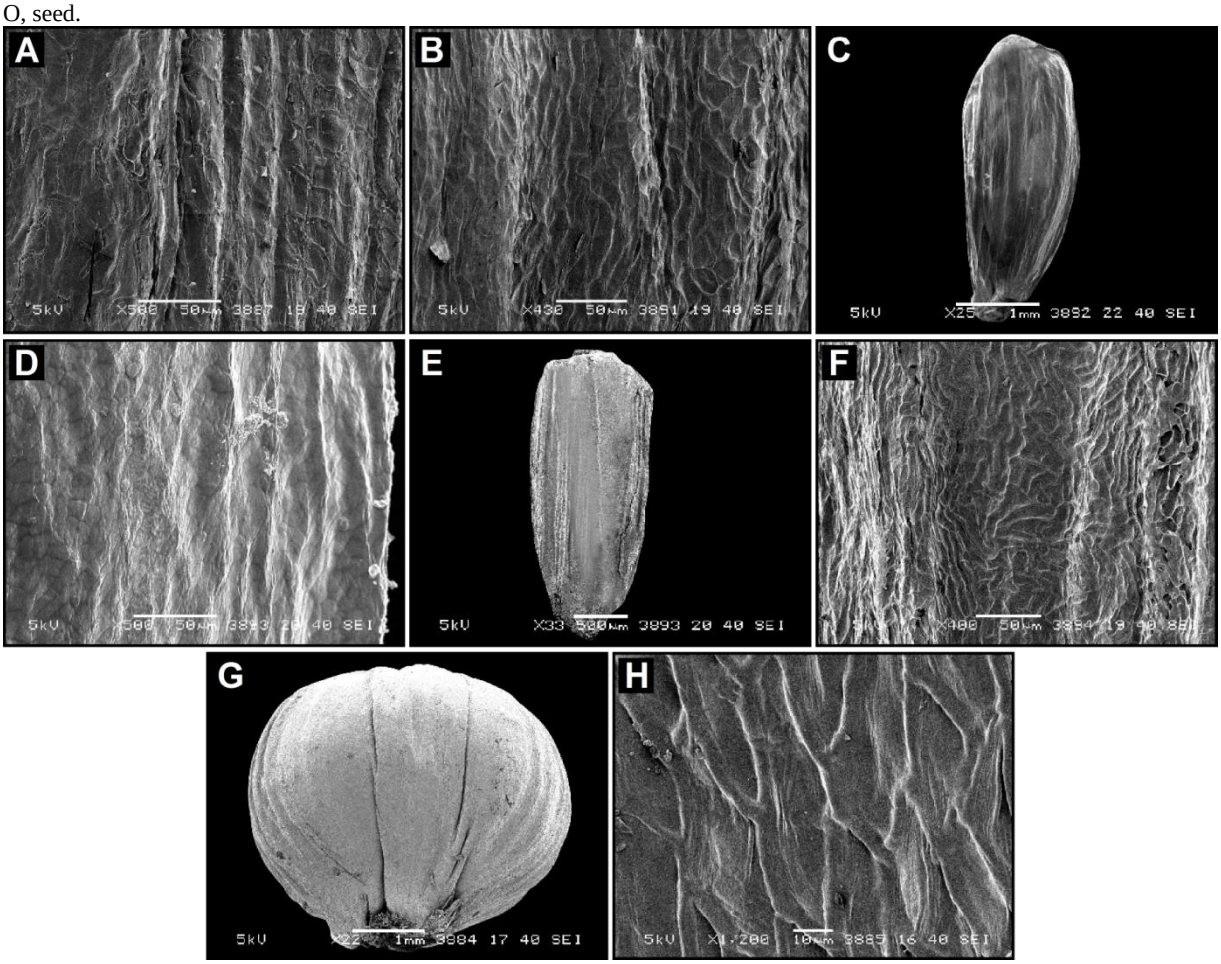

Fig. 2. Scanning electron micrographs. *Commicarpus boissieri*: A,B, surface. *C. helenae*: C, seed; D, surface. *C. stenocarpus*: E, seed; F, surface. *Mirabilis jalapa*: G, seed; H, surface.

The dendrogram based on seed morphological characters (Fig. 7) shows that the genus *Mirabilis* occupies the basal position among the remaining genera viz., *Boerhavia* and *Commicarpus*. The genus *Mirabilis* remains distinct from the above two genera due to the presence of showy flowers with prominent involucral bracts (Nasir, 1977; Bittrich and Kuhn, 1993; Douglas and Manos, 2001; Spellenberg, 2003; Dequan and Gilbert, 2004) and larger orbicular seeds with crenatus and ridged surface and distinct hilum. According to Presal (1843) and Ahmad and Qaiser (1989) hilum may be used for the identification of different taxa. The distinct hilum is found only in the genus *Mirabilis* which evidently makes its position very distinct within the family Nyctaginaceae. The distinction of the genus *Mirabilis* is also supported by molecular data of the family Nyctaginaceae by Douglas and Manos (2001) who observed the chloroplast loci (*ndhF*, *rps16*, *rpl16*, and nrITS) in the family Nyctaginaceae and place the genus *Mirabilis* separately in the clade of subtribe Nyctaginae. While the remaining two genera viz., *Boerhavia* and *Commicarpus* were placed within a clade of subtribe Boerhaviinae of the tribe Nyctagineae (Bittrich and Kuhn, 1993).

Similarly the remaining taxa clearly form two separate groups. The first group includes all species of the genus *Boerhavia*. This group is characterized with 1-2mm long seeds along with reticulate, lineate, lineate-lineolate or sclariform surface (Plate I), and 5-ribbed anthocarps (Nasir, 1977; Bittrich and Kuhn, 1993; Spellenberg, 2003; Dequan and Gilbert, 2004). On the other hand species of the genus *Commicarpus* are included in the second group and this group can be distinguished on the basis of 2.8-3.5mm long seeds with lineate-reticulate, lineolate-reticulate

or ruminate-lineolate surface (Plate II), and 10-ribbed anthocarps (Nasir, 1977; Bittrich and Kuhn, 1993; Spellenberg, 2003; Dequan and Gilbert, 2004).

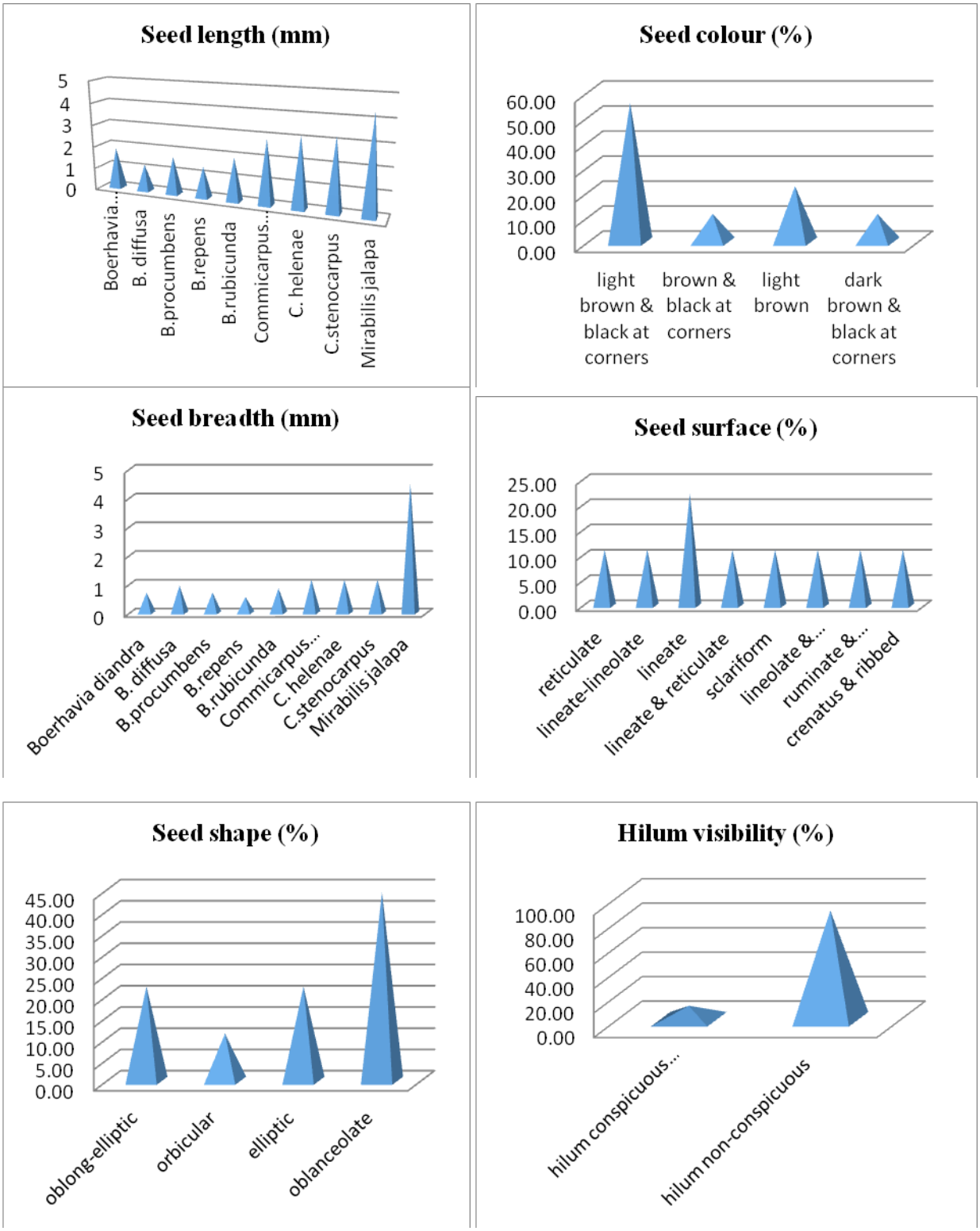

Fig. 3. Bar diagram showing morphological characters of seeds within the taxa of the family Nyctaginaceae.

Table 1. Seed morphology of the family Nyctaginaceae.

Name of taxa	Size (mm)		Shape	Color	Surface	Hilum
	length	breadth				
Boerhavia diandra	1.7-2(±0.06)	0.6-0.8(±0.04)	Angular, oblong-elliptic	Light brown & black at corners & shiny	Reticulate	Indistinct
B. diffusa	1-1.3(±0.06)	0.9-1(±0.03)	Angular, oblong-elliptic dorsiventral	Light brown & black at corners & shiny	Lineate- lineolate	Indistinct
B. procumbens	1.6-1.8(±0.03)	0.6-0.8(±0.03)	Elliptic, dorsiventral	Light brown & black at corners & shiny	Lineate	Indistinct
B. repens	1.3-1.5(±0.09)	0.5-0.6(±0.02)	Oblanceolate,dorsi ventral	Light brown & black at corners & shiny	Lineate	Indistinct
B. rubiunda	1.9-2(±0.02)	0.8-1(±0.04)	Elliptic, angular	Light brown & black at corners & shiny	Sclarifrom	Indistinct
Commicarpus boissieri	2.8-3(±0.09)	1-1.3(±0.06)	Oblanceolate	Brown & black at corners & shiny	Lineate & reticulate	Indistinct
C. helenae	3-3.3(±0.09)	1-1.3(±0.06)	Oblanceolate	Light brown & shiny	Lineolate & reticulate	Indistinct
C. stenocarpus	3-3.5(±0.23)	1-1.3(±0.06)	Oblanceolate	Dark brown & black at corners & shiny	Ruminate & lineolate	Indistinct
Mirabilis jalapa	4.2-4.5(±0.06)	4-5(±0.18)	Orbicular	Light brown & un shiny	Crenatus & irregularly ribbed	Basal

Table 2. list of characters, scored for cluster analysis for the taxa of family Nyctaginaceae listed on Table3.

No.	Character description
1.	Length (mm)
2.	Breadth (mm)
3.	Angular or non-angular: Non-angular (0), Angular (1)
	Shapes
4.	Elliptic: absent(0), present(1)
5.	Oblong-elliptic: absent(0), present(1)
6.	Oblanceolate: absent(0), present(1)
7.	Orbicular: absent(0), present(1)
	Colour
8.	Light brown: absent(0), present(1)
9.	Brown-black: absent(0), present(1)
10.	Dark brown: absent(0), present(1)
	Surface
11.	Lineate: absent(0), present(1)
12.	Lineate-lineolate: absent(0), present(1)
13.	Lineate & reticulate: absent(0), present(1)

14.	Lineolate & reticulate: absent(0), present(1)
15.	Reticulate: absent(0), present(1)
16.	Sclariform: absent(0), present(1)
17.	Ruminate & lineate: absent(0), present(1)
18.	Crenatus & ridged: absent(0), present(1)
19.	Hilum visibility: indistinct(0), distinct(1)

Table 3. Data matrix of Nyctaginaceae scored for 19 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**
Boerhavia diandra	1.85	0.7	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1
B. diffusa	1.15	0.95	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1
B. procumbens	1.7	0.7	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
B. repens	1.4	0.55	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1
B. rubiunda	1.95	0.9	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
Commicarpus boissieri	2.9	1.15	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1
C. helenae	3.15	1.15	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	1
C. stenocarpus	3.25	1.15	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1
Mirabilis jalapa	4.35	4.5	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0

Within the first group the species *B.procumbens* and *B.repens* are closely related with each other on the basis of non-angular seeds, both the species can be further separated on the basis of axillary panical inflorescence (Nasir, 1977; Bittrich and Kuhn, 1993) and elliptic seeds in *B. procumbens*, while *B. repens* have oblanceolate seeds and flowers in axillary cymes (Nasir, 1977; Bittrich and Kuhn, 1993). The remaining 3 species of the genus *Boerhavia* viz., *B. diandra*, *B. diffusa* and *B. rubicunda* have angular seeds. Among them *B. diandra* and *B. rubicunda* are closely related as they form a sister group by having more or less similar seed characters, but they can be further separated from each other by having diffuse branches with sessile or sub sessile flowers (Nasir, 1977) and seeds with reticulate surface (Plate I) in *B. diandra*. While *B.rubicunda* have erect branches with pedicellate flowers (Nasir, 1977) and sclariform seeds (Plate I). Similarly *B. diffusa* shows close affinity with *B. diandra* as both the species also shares common seed characters, the only difference is found in their surface patterns. These findings are also supported by the presence of slender branches in *B. diandra* and alternate branches in *B. diffusa* (Nasir, 1977). The second group comprises the species of the genus *Commicarpus* viz., *C. boissieri, C. helenae* and *C.stenocarpus*. All the species arises from the common point and clearly indicates their strong affinity with each other on the basis of seed characters, they can only be separated on the basis of anthocarp structure (Nasir, 1977; Bittrich and Kuhn, 1993) and seed surface patterns (Plate II).

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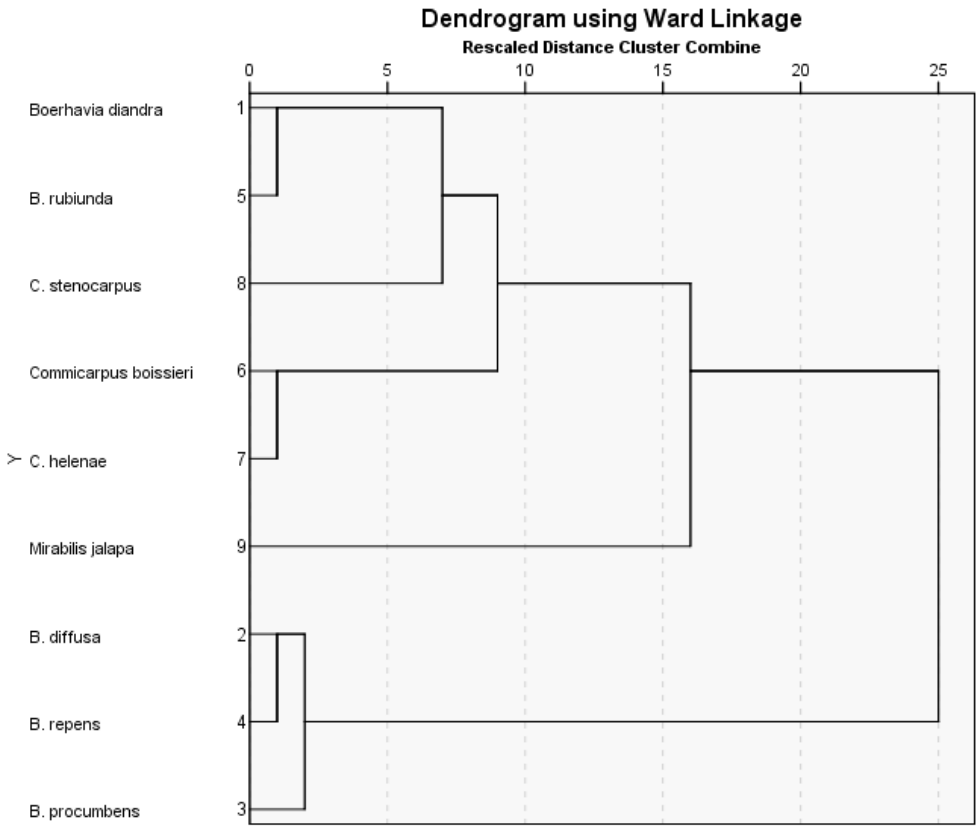

Fig. 4. Dengrogram showing the relationship within the taxa of the family Nyctaginaceae.

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