

STUDIES ON THE SEEDS ORNAMENTATION OF SOME VERBENACEAE AND RELATED FAMILIES SPECIES AND THEIR TAXONOMIC SIGNIFICANCE

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ABSTRACT

Seed morphological characters of 7 species of the families Verbenaceae, Lamiaceae and Acanthaceae distributed in 6 genera were examined by SEM and LM. Macro- and micromorphological characters, including seed shape, color, size, weight, surface texture, stomata presence, aril presence, anticlinal and periclinal walls are presented. Two patterns were recognized based on the surface sculpturing pattern, reticulate (with six subtypes) and tuberculate. Descriptions, SEM micrographs and a key to the studied species are included. The results of the present study are found to be taxonomically supportive to the morphological evidence. Moreover, numerical analysis on the basis of the Unpaired Group Method of Average (UPGMA) clustering and factor loadings to seed data led us to recognize four major clades. One of them has only one species and the other three major clades include two species for each one.

Keywords: *Verbenaceae, Lamiaceae, Acanthaceae, Sculpture, SEM, Micromorphology, Numerical Taxonomy*

INTRODUCTION

Recent phylogenetic studies, Cantino *et al.*, (1992) have shown that numerous genera traditionally classified in Verbenaceae belong instead in Lamiaceae. *Avicennia*, a genus of mangrove trees, usually placed in Verbenaceae (Grandtner, 2005) or in its own family, Avicenniaceae (Boulos, 2009), has been placed in the Acanthaceae on the basis of molecular phylogenetic studies (<http://www.mobot.org/mobot/research/APweb/orders/lamialesweb.htm#Lamiales>). Many taxonomists have proposed different systems of classification and delimitation for various genera, species and subspecies in the family, thus, indicating problems in taxonomy (Caro, 1981; Cronquist, 1981; Howard 1989; Cantino, 1990; Salimena, 2000; Sanders, 2001).

The testa (seed coat) is the outer covering of every mature seed. The morphology of seed coat is usually stable and is less influenced by external environmental conditions while the seeds develop and ripen within the fruit (Heywood, 1971; Cole & Behnke, 1975; Barthlott and Ziegler, 1981). Most seed coats exhibit complex and highly diverse morphology and anatomy, providing valuable taxonomic characters (Barthlott and Ziegler, 1981; Barthlott, 1990). These characters, especially the macro- and microstructures of fruits and seeds are very significant for the classification of Angiosperm species (Kaya *et al.*, 2011) and can play an important role in the identification of species (Vaughan, 1968) and have traditionally been used to solve systematic and phylogenetic problems (Hufford, 1995; Karcz *et al.*, 2000; Gabr, 2014). Recently, the application of SEM to the study of the seed coat has become widespread (Heywood, 1971). Seed characteristics, particularly exomorphic features revealed by means of scanning electron microscopy (SEM), have been used in resolving problems of systematics of species (Tobe *et al.*, 1987; Karihaloo & Malik, 1994; Koul *et al.*, 2000; Yoshizaki, 2003; Javadi and Yamaguchi, 2004) and evolutionary relationships (Heywood, 1971; Barthlott, 1984; Segarra & Mateu 2001).

The present work deals with the macro- and micromorphological (LM & SEM) characters of seeds of 6 genera (7 species) of the families Verbenaceae, Lamiaceae and Acanthaceae in Egypt, to decide on the

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importance of seed characters as a criterion for separating genera and species in these families. The terminology used here follows authors such as Barthlott (1981; 1984) and Stearn (1992).

MATERIALS AND METHODS

Seed Materials

The present study is based on fresh plant samples, representing the seeds of six genera (7 species) of Verbenaceae, Lamiaceae and Acanthaceae in the flora of Egypt (Table 1). The plant specimens identified according to Täckholm (1974), El Hadidi & Fayed (1994/95), Boulos (2002; 2009) and <http://www.theplantlist.org/tpl/record/kew-42945>, and deposited in the Herbarium of Agriculture Museum, Flora and Phytotaxonomy Research Department (CAIM) and the Department of Botany, Faculty of Science, South Valley University Herbarium (QNA). Mature seeds were collected, then cleaned with alcohol and kept for drying.

Table 1: List of the Studied Species with Relevant Information about their Source

| No. | Species | Family | Location | Collector |
|-----|--|--------------------|-------------------------|----------------|
| 1 | <i>Avicennia marina</i> (Forssk.) Vierh. | <i>Acanthaceae</i> | The Red Sea coast | H. A. Mohammed |
| 2 | <i>Clerodendrum phlomidis</i> L.f. | <i>Lamiaceae</i> | Aswan Botanical Garden | H. A. Mohammed |
| 3 | <i>Lantana camara</i> L. | <i>Verbenaceae</i> | South Valley University | H. A. Mohammed |
| 4 | <i>Lantana montevidensis</i> (Spreng.) Briq. | <i>Verbenaceae</i> | Aswan Botanical Garden | H. A. Mohammed |
| 5 | <i>Phyla nodiflora</i> (L.) Greene | <i>Verbenaceae</i> | South Valley University | H. A. Mohammed |
| 6 | <i>Tectona grandis</i> L.f. | <i>Lamiaceae</i> | Aswan Botanical Garden | H. A. Mohammed |
| 7 | <i>Vitex agnus-castus</i> L. | <i>Lamiaceae</i> | Aswan Botanical Garden | H. A. Mohammed |

For Light Microscope (LM)

The macromorphological characters of the studied seeds were carried out using stereo-microscopy (Stemi2000-C) and the measurements, shapes, color, and the presence of aril was recorded.

For Scanning Electron Microscope (SEM)

The elected seeds were mounted on stubs using double face carbon tape, and coated with a thin layer of gold/palladium for 3 minutes using an EMITECH K550 sputter coater. SEM photographs were captured using the JSM T200 at the Electron Microscopy Unit, Assuit University, Assuit, Egypt.

Numerical Analysis

A total of fourteen characters was evaluated for each species, comprising eleven morphological (qualitative) and three morphometrical (quantitative) characters. The Seven species were clustered based on phenotypic traits. The scales portray a dissimilarity index calculated using the Euclidean distance coefficient, and the dendrogram was developed using Unweighted Pair Group Method using arithmetic Averages (UPGMA) clustering procedures according to Seberg *et al.*, (1991) and Sokal & Michener (1958). Factor loadings were also applied to recognize the most intrinsic characters which enhanced separation of the studied species. All calculations were made using the STATISTICA software (STATISTICA 5.0).

RESULTS AND DISCUSSION

Results

Seed characters are as important as making them effective for study taxonomical relationships among genera and species of Verbenaceae, Lamiaceae and Acanthaceae.

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Seeds Shape

Seeds are more or less similar in shape being cordate, obovate, ovate, rounded-ovate, broadly- elliptic, irregular ovate or spherical. Seeds are cordate in *Avicennia marina*, obovate in *Clerodendrum phlomidis*, ovate in *Lantana camara*, rounded-ovate in *Lantana montevidensis*, broadly- elliptic in *Phyla nodiflora*, irregular ovate in *Tectona grandis* and finally spherical in *Vitex agnus-castus*. The seeds shape contributes to differentiate *Avicennia marina*, *Clerodendrum phlomidis*, *Phyla nodiflora*, and *Vitex agnus-castus* seeds from the other investigated species (Table 2, Figures 1-7).

Seeds Length

Length of seeds ranges from 1.68 mm - 22.00 mm. The smallest seed is those of *Phyla nodiflora* (1.68 mm) and the largest one is those of *Avicennia marina* (22.00 mm). Seeds length of the other species ranged between (2.72 mm - 7.50 mm). Seeds length participates to differentiate between the related species of *Avicennia marina* (22.00 mm), *Clerodendrum phlomidis* (7.50 mm), *Phyla nodiflora* (1.68 mm), *Tectona grandis* (5.41 mm) and *Vitex agnus-castus* (2.72 mm) from the rest species (Table 2, Figures 1-7).

Seed Width

Seeds width varies greatly among the examined species, the largest seed found in *Avicennia marina* (width = 17.00 mm), the smallest seed found in *Phyla nodiflora* (width = 1.17 mm), while the rest species have seeds their width ranged from 2.23- 3.79 mm. The seeds' width was found useful to separate species of *Avicennia marina* and *Phyla nodiflora* from the other examined species (Table 2, Figures 1-7).

Seed Color

The seed color varies from grayish green, brown, light orange, light orange, pale yellow, gray, orange, light green, creamy to brown. It is grayish green in *Avicennia marina*, brown to light orange in *Clerodendrum phlomidis*, light orange to pale yellow in *Lantana camara*, gray in *Lantana montevidensis*, orange to light green in *Phyla nodiflora*, creamy in *Tectona grandis* and brown in *Vitex agnus-castus*. The seed color is used to distinguish between all the investigated species (Table 2, Figures 1-7).

Seeds Weight (Mean, mg)

Weight of seeds varies greatly among the examined species, the biggest seed weight is 2243.00 mg in *Avicennia marina* and the smaller weight is 0.40 mg in *Phyla nodiflora*, While the average weight in the rest species is between 4.30 – 52.00 mg. It is found that weight of seeds is very useful to distinguish between the studied species, where a large variation in weight of the studied species can be clearly seen (Table 2, Figures 1-7).

Seeds Aril Presence

Aril is present only in the seeds of *Clerodendrum phlomidis* and absent in the remnant seeds. The existence of the seed aril is distinguished *Clerodendrum phlomidis* from the rest species (Table 2, Figures 1-7).

Seeds Surface Texture

The texture of seed surface is graded from reticulate to tuberculate. It is tuberculate, striate only in *Phyla nodiflora*.

There are several forms of reticulation, it may be reticulate-scaled (as in *Avicennia marina*); reticulate-undulate (as in *Clerodendrum phlomidis*); reticulate-scabrate, slightly granulate (as in *Lantana camara*); reticulate, smooth (as in *Lantana montevidensis*); reticulate, striate (as in *Tectona grandis*) and finally reticulate, scabrate- undulate (as in *Vitex agnus-castus*). The character of seed surface texture can be of considerable diagnostic and systematic value to distinguish clearly among all the investigated species (Table 3, Figures 1-7).

Reticulation Type

All reticulated seeds have simple reticulation. The reticulation of seeds was found useful to separate seeds of *phyla nodiflora* with tuberculate texture from the rest, reticulated seeds (Figures 1-7, Table 3).

Seed Coat Stomata Existence

Stomata of seed coat are present only in *Avicennia marina* and *Vitex agnus-castus* and absent in the remaining five species.

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Presence of seed coat stomata was useful to distinguish *Avicennia marina* and *Vitex agnus-castus* from the rest species (Figures 1-7, Table 3).

Seeds Anticlinal wall Shape

The seeds anticlinal wall shape is of high diagnostic and systematic interest among species. It varies from polygonal, elongated, round to variable in shape.

The polygonal wall shape may be regular as in *Avicennia marina* or irregular as in *Clerodendrum phlomidis* and *Lantana montevidensis*, elongated wall shape exists in both *Lantana camara* and *Tectona grandis*, rounded wall shape occurs only in *Phyla nodiflora* and variable wall shape represents only in *Vitex agnus-castus*.

The Seed anticlinal wall shape is used to distinguish of *Avicennia marina*, *Phyla nodiflora* and *Vitex agnus-castus* from another investigated species which are characterized by the existence of two species for each character (Plates 1-4, Table 3).

Seeds Anticlinal Wall Thickness

The anticlinal wall thickness of the seeds shows a somewhat disparity between the investigated species. It divides the examined species into two main sections, they are either thick as in *Clerodendrum phlomidis*, *Lantana montevidensis*, *Phyla nodiflora* and *Vitex agnus-castus* or thin as in *Avicennia marina*, *Lantana camara* and *Tectona grandis* (Figures 1-7, Table 3).

Seeds Anticlinal Wall Level

With the exception of *Avicennia marina* seeds which have grooved anticlinal wall level, the seeds of the remaining species have raised wall. By examining seeds anticlinal wall's level, we can distinguish *Avicennia marina* from the rest species (Figures 1-7, Table 3).

Seeds Periclinal Wall Level

Seeds periclinal wall level may be flat as in *Vitex agnus-castus*, convex as in *Avicennia marina* and *Phyla nodiflora* or concave as in *Clerodendrum phlomidis*, *Lantana camara*, *Lantana montevidensis* and *Tectona grandis*. The seeds periclinal wall level was found useful to separate seeds of *Vitex agnus-castus* with flat periclinal wall level, the seeds of *Avicennia marina* and *Phyla nodiflora* with convex periclinal wall level from the remnant, with concave periclinal wall level species (Figures 1-7, Table 3).

Seeds Periclinal Wall Texture

Seeds periclinal wall texture can serve as a good diagnostic character for the lowest taxonomic categories. There are five different forms of periclinal wall texture, it may be smooth, smooth to folds, slightly granulate to granulate, striate and coarse folds. The first smooth form appears in both *Avicennia marina* and *Lantana montevidensis*.

The second smooth to folds form shows in *Clerodendrum phlomidis*. The third slightly granulate to granulate form represents in *Lantana camara* and *Phyla nodiflora*. The fourth striate form exhibits in *Tectona grandis*. Finally the fifth coarse folds form seen in *Vitex agnus-castus*.

Furthermore, Figure (5) exhibits the UPGMA cladistic tree of the seven species depending on 14 main seeds morphology characters which differentiate these species at four dissimilarity distance into four major clades. The first major clade comprises both *Vitex agnus-castus* and *Tectona grandis*. The second major clade contains only one species (*Phyla nodiflora*). The third major clade includes two species: *Lantana montevidensis* and *Lantana camara*. The fourth major clade involves also two species: *Clerodendrum phlomidis* and *Avicennia marina*. Factor loadings showed that the most intrinsic characters enhanced separation of the total species, which are seed shape, seed color, seed aril, surface texture, reticulation type, seed coat stomata and anticlinal wall thick as morphological characters and only seed weight as morphometrical character from the first factor. The second factor is represented by four characters, which are seed shape, seed aril, reticulation type and periclinal wall texture from morphological characters and three characters, which are seeds length, seed width and seed weight from morphometrical characters. The third factor is comprised of six characters as seed shape, seed color, surface texture, anticlinal wall shape, anticlinal wall thick and periclinal wall texture as morphological characters, two characters like seed width and seed weight as morphometrical characters (Table 4). The characters of separation are of high factor loadings ≥ 0.7 . They are represented by a percentage of the

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total variance equaling 57.14% for the first factor, 50.00% for the second factor and 57.14% for the third factor (Table 4).

Discussion and Conclusion

A clear cut distinction can be made among taxa based on the main external seed morphology (Abdel Khalik & Osman, 2007; Zorić *et al.*, 2010).

Seeds texture characters applied to seven species of Verbenaceae, Lamiaceae and Acanthaceae in Egypt proved to be useful in the distinction between two patterns, the first pattern was tuberculate, striate and represented only by *Phyla nodiflora*.

The second pattern was simple reticulate and observed in six forms, the first form was reticulate-scaled, discovered only in *Avicennia marina*, the second one was reticulate-undulate, shown only in *Clerodendrum phlomidis*, the third form was reticulate-scabrate, slightly granulate, observed only in *Lantana camara*, the fourth form was reticulate, smooth, recognized only in *Lantana montevidensis*, the fifth form was reticulate, striate, showed only in *Tectona grandis* and the latest form is reticulate, scabrate-undulate, appeared only in *Vitex agnus-castus* (Table 3).

Corner (1976) and Abdel Khalik & Osman (2007) demonstrated that seed measurements and characterizations could be taken as supplementary characteristics in plant taxonomy. Seeds were cordate, obovate, ovate, rounded-ovate, broadly-elliptic, irregular ovate or spherical in shape. In respect to size, the smallest seed was those of *Phyla nodiflora* (1.68×1.17 mm), the largest seed was those of *Avicennia marina* (22.00×17.00 mm) and size of the rest seeds was ranged between (2.72×2.23 - 7.50×3.79 mm) (Table 2).

In taxonomy consideration, seeds color usually is useless to provide an expected importance (Bhandari *et al.*, 1985), and might be highly diagnostic in the rank of tribes, families and orders (Barthlott & Ziegler, 1981; Dahlgren & Clifford, 1982). On contrary to Bhandari *et al.*, (1985) report, the color of mature seeds was found to be important in identification of the present species, where it was completely dissimilar in most seeds under investigation, it was grayish green in *Avicennia marina*, brown to light orange in *Clerodendrum phlomidis*, light orange to pale yellow in *Lantana camara*, gray in *Lantana montevidensis*, orange to light green in *Phyla nodiflora*, creamy in *Tectona grandis* and brown in *Vitex agnus-castus* (Table 2).

As well, there was considerable variation in weight of the studied seeds, which made the character of seed weight was very effective in distinguishing between examining species (Table 2). The existence of aril in seeds also contributed to differentiate *Clerodendrum phlomidis* from the remnant, without aril species (Table 2). The presence of seed coat stomata participated to differentiate *Avicennia marina* and *Vitex agnus-castus* from the rest, without stomata, investigated species (Table 3).

On the other hand, according to anticlinal wall shape, there were five forms, the first form was polygonal wall shape, represented only in *Avicennia marina*, the second form was irregular polygonal wall shape, showed in both *Clerodendrum phlomidis* and *Lantana montevidensis*, the third form was elongated wall shape, appeared in both *Lantana camara* and *Tectona grandis*, the fourth form was round wall shape, seen only in *Phyla nodiflora*, finally the fifth form was variable in shape wall and it was exhibited only in *Vitex agnus-castus* (Table 3). Anticlinal wall thickness as well contributed to distinguish among *Avicennia marina*, *Lantana camara* and *Tectona grandis* which had thin anticlinal walls from the rest, with thick anticlinal walls species. Regarding, the anticlinal wall's level, all the examined species possessed raised wall except *Avicennia marina* with grooved wall (Table 3).

Furthermore, the periclinal wall level also showed the presence of three patterns, the first was convex, seen in both *Avicennia marina* and *Phyla nodiflora*, the second was concave, showed in *Clerodendrum phlomidis*, *Lantana camara*, *Lantana montevidensis* and *Tectona grandis*, the third was flat, appeared only in *Avicennia marina*. Finally, the periclinal wall texture also played a pivotal role in distinguishing between the investigated seeds, where five forms of textures were shown as follows: smooth in *Avicennia marina* and *Lantana montevidensis*, smooth to folded in *Clerodendrum phlomidis*, slightly granulate to granulate in *Lantana camara* and *Phyla nodiflora*, striate in *Tectona grandis* and coarse folds in *Vitex agnus-castus* (Table 3).

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Table 2: The Macro-Morphological Characters of the Studied Taxa Seeds

| Taxa | Shape | Length (Mean) mm | Width (Mean) mm | Colour | Weight mg | Aril Presence |
|-------------------------------|-------------------|---------------------|--------------------|-----------------------------|--------------|---------------|
| <i>Avicennia marina</i> | cordate | 22.00 | 17.0 | grayish green | 2243.00 | absent |
| <i>Clerodendrum phlomidis</i> | obovate | 7.50 | 3.79 | brown to light orange | 52.00 | present |
| <i>Lantana camara</i> | ovate | 4.95 | 3.24 | light orange to pale yellow | 16.00 | absent |
| <i>Lantana montevidensis</i> | rounded-ovate | 4.42 | 2.96 | Gray | 22.00 | absent |
| <i>Phyla nodiflora</i> | broadly- elliptic | 1.68 | 1.17 | orange to light green | 0.40 | absent |
| <i>Tectona grandis</i> | irregular ovate | 5.41 | 2.82 | Creamy | 13.60 | absent |
| <i>Vitex agnus-castus</i> | spherical | 2.72 | 2.23 | Brown | 4.30 | absent |

Table 3: The Micro-Morphological Characters of the Studied Taxa Seeds

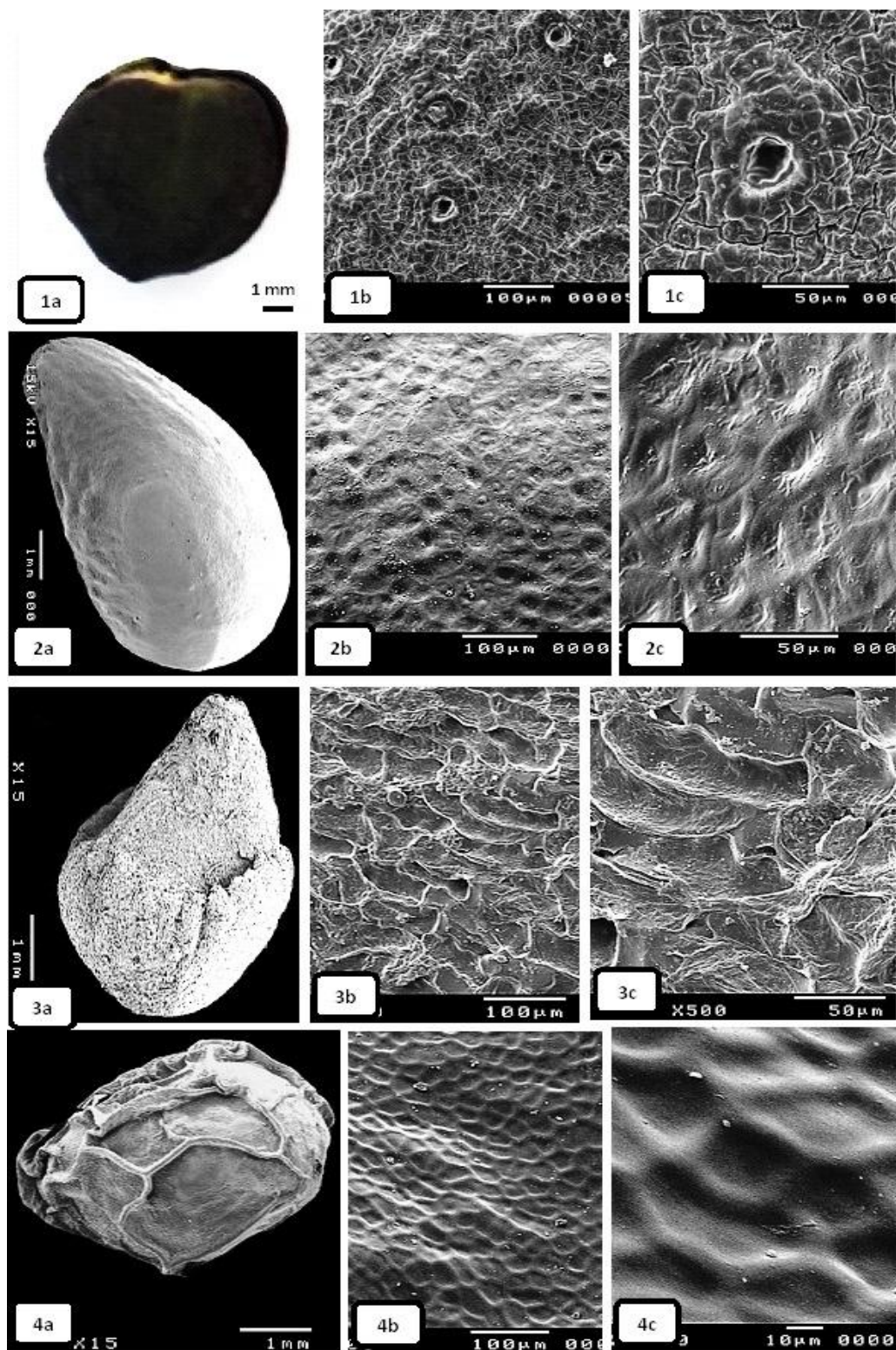
| Taxa | Surface Texture | Reticulation Type | Seed Coat Stomata | Anticlinal Wall Shape | Anticlinal Wall Thickness | Anticlinal Walls Level | Periclinal Wall Level | Periclinal Wall Texture |
|-------------------------------|---|----------------------|-------------------------|--------------------------|---------------------------------|---------------------------|--------------------------|-------------------------------|
| <i>Avicennia marina</i> | reticulate-scaled | simple reticulate | present | polygonal | Thin | grooved | convex | smooth |
| <i>Clerodendrum phlomidis</i> | reticulate-undulate | simple reticulate | Absent | irregular, polygonal | Thick | raised | concave | smooth to folds |
| <i>Lantana camara</i> | reticulate-scabrate, slightly granulate | simple reticulate | Absent | elongated | Thin | raised | concave | slightly granulate |
| <i>Lantana montevidensis</i> | reticulate, smooth | simple reticulate | Absent | irregular, polygonal | Thick | raised | concave | Smooth |
| <i>Phyla nodiflora</i> | tuberculate, striate | absent | Absent | round cells | Thick | raised | convex | Granulate |
| <i>Tectona grandis</i> | reticulate, striate | simple reticulate | Absent | elongated | Thin | raised | concave | Striate |
| <i>Vitex agnus-castus</i> | reticulate, scabrate-undulate | simple reticulate | present | variable cells | Thick | raised | flat | coarse folds |

Table 4: Factor Loadings Showed the most Intrinsic Characters Enhanced Separations of the Studied Species

| Characters | Factor Loadings (Unrotated) | | |
|---|----------------------------------|-----------------|-----------------|
| | Extraction: Principal Components | | |
| Case / Factors | (Marked Loadings are > 0.700000) | | |
| | Factor 1 | Factor 2 | Factor 3 |
| 1 Seed Shape | 1.38576 | .75283 | .74456 |
| 2 Seed color | 1.38576 | .17907 | .74456 |
| 3 Seed aril | -1.39579 | .75283 | .17150 |
| 4 Surface texture | 1.38576 | .56631 | .74456 |
| 5 Reticulation type | -1.33371 | -.71436 | .27259 |
| 6 Seed coat stomata | -.71436 | .10409 | .33919 |
| 7 Anticlinal wall shape | .31052 | .55898 | -1.01972 |
| 8 Anticlinal wall thick | -1.00410 | .29053 | 1.24919 |
| 9 Anticlinal walls level | -.61598 | .17590 | .23346 |
| 10 Periclinal wall level | -.63923 | .10655 | .08783 |
| 11 Periclinal wall texture | .51287 | .80701 | -2.50315 |
| 12 Seeds length | .26636 | -1.69679 | -.61642 |
| 13 Seeds width | -.35115 | -.85203 | -1.14857 |
| 14 Seeds weight | .80728 | -2.49813 | .70045 |
| Percentage % | 57.14% | 50.00% | 57.14% |
| Percentage of total variation of the three factors extracted 164.28% | | | |

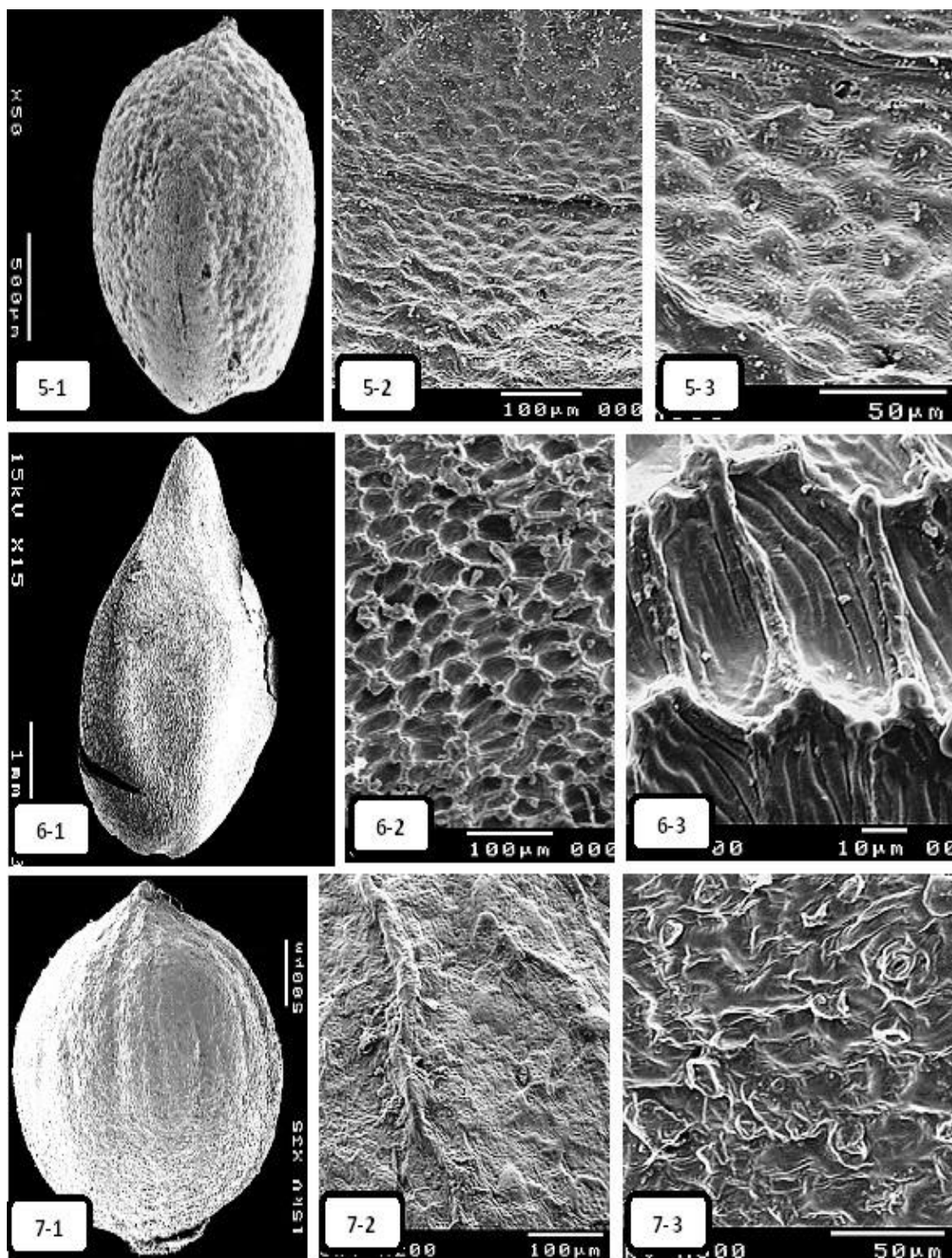
The figures in red color and bold highlight the significant characters.

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Figs 1-4: Scanning electron microscopy photographs of seeds 1 - *Avicennia marina* (Forssk.) Vierh. ; 2 - *Clerodendrum phlomidis* L.f. ; 3 - *Lantana camara* L. ; 4 - *Lantana montevidensis* (Spreng.) Briq. a – entire seed; b – Part of seed coat; c – enlargement Part of seed coat.

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Figs 5-7: Scanning electron microscopy photographs of seeds 5 - *Phylla nodiflora* (L.) Greene ; 6 - *Tectona grandis* L.f. ; 7 - *Vitex agnus-castus* L.

a – entire seed; b – Part of seed coat; c – enlargement Part of seed coat.

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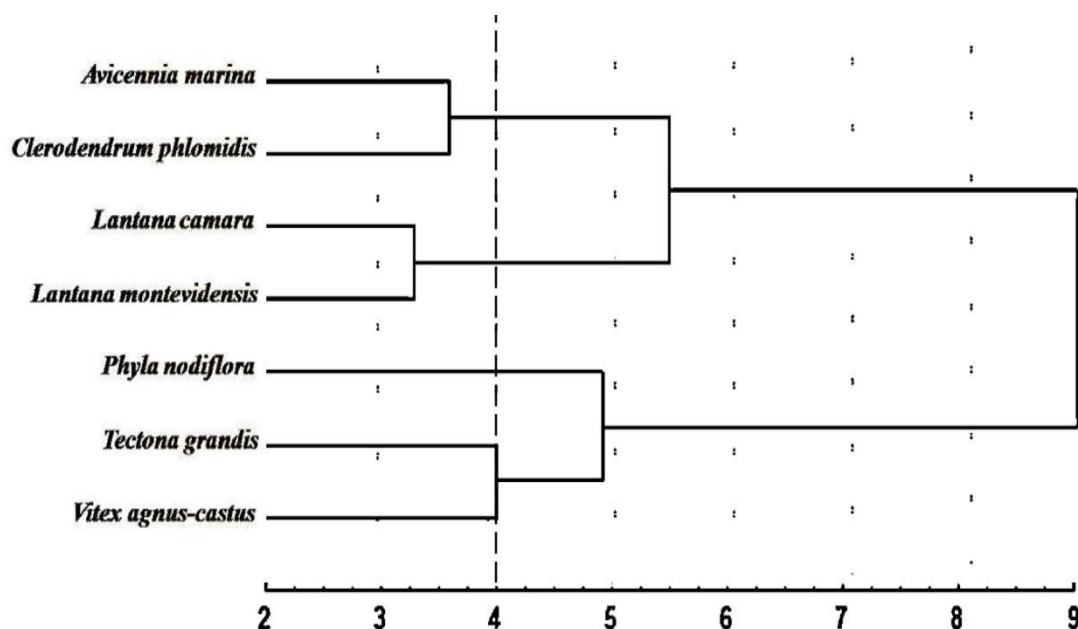


Figure 8. Phenogram of the 7 studied species, clustering with the Unweighted Pair Group Method using arithmetic Averages (UPGMA method).

Moreover, a number of seeds morphology attributes were recorded, and numerical methods (UPGMA and factor loadings) were used to examine the correlations among 7 Verbenaceae, Lamiaceae and Acanthaceae species and evaluate the level of variation within and among these species. Generally, our results with the UPGMA clustering and principal component analysis (PCA) show congruence and indicate that there are four main clades, which included the distribution of 7 studied species (Figure 8). Our UPGMA results showed that both *Vitex agnus-castus* and *Tectona grandis* are separated in the first major clade of the cladistic tree, *Phyla nodiflora* is separated in the second major clade, both *Lantana montevidensis* and *Lantana camara* are separated in the third major clade and in the end both *Clerodendrum phlomidis* and *Avicennia marina* are separated in the fourth major clade. Our results revealed a clear separation between *Phyla nodiflora* and all other investigated species. Moreover, a closer relationship between both *Vitex agnus-castus* and *Tectona grandis* occurred in the same first major clade, also between both *Lantana montevidensis* and *Lantana camara* found in the same third major clade, and between both *Clerodendrum phlomidis* and *Avicennia marina* showed in the same fourth major clade (Figure 8). Then, the applied methods of UPGMA and PCA may be used to study the variation among the species to determine the relationship among different species.

Further, In some classifications, the genus *Avicennia* has been placed in the family Verbenaceae. Moldenke (1960; 1967; 1968) placed *Avicennia* apart from the Verbenaceae in the separate family Avicenniaceae, as was first proposed by Allan (1961). Recently, phylogenetic studies have supported the view of placing *Avicennia* under Acanthaceae (Das et al., 2015). In conclusion, the present study supports the use of seed morphological characters as a parameter for species identification. The results suggest a close relationship between different investigated species of Verbenaceae, Lamiaceae and Acanthaceae. Based on the above mentioned characters, the following diagnostic key was constructed for identification of the studied species.

- 1.a. Surface texture tuberculate-striate *Phyla nodiflora*
- b. Main surface texture with reticulate texture 2
- 2.a. Anticlinal walls level grooved *Avicennia marina*

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| | |
|---|-------------------------------|
| b. Anticlinal walls level raised | 3 |
| 3.a. Anticlinal wall shape irregular with polygonal cells | 4 |
| b. Anticlinal wall shape otherwise | 5 |
| 4.a. Surface texture reticulate, undulate | <i>Clerodendron phlomidis</i> |
| b. Surface texture reticulate, smooth | <i>Lantana montevidensis</i> |
| 5.a. Anticlinal wall shape with variable cells | <i>Vitex agnus-castus</i> |
| b. Anticlinal wall shape with elongated cells | 6 |
| 6.a. Periclinal walls texture slightly granulate | <i>Lantana camara</i> |
| b. Periclinal walls texture striate | <i>Tectona grandis</i> |

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