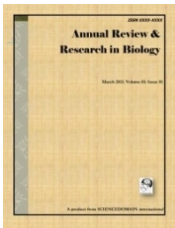

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Seed Morphology and Protein Patterns (SDS-PAGE) as a Mean in Classification of Some Taxa of the Subfamily Mimosoideae (Fabaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MET, EAK wrote the protocol and supervised the work in all its aspects. Author MMM collected the samples, worked in the practical part and written the draft. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study is developed to discuss whether the plant morphology and seed characters (macro and micromorphological and protein pattern) can provide an additional fundamental tool helping in explanation of the taxonomic trends at specific and infra-specific level within the 47 studied taxa belonging to subfamily Mimosoideae (Fabaceae) and to compare the proposed taxonomic treatment based on numerical analysis (dendrogram) with other previous and current systems of classification.
Methodology: The macro and micromorphological characters of the whole plant and seed as well as seed protein pattern of 47 taxa of subfamily Mimosoideae, family Fabaceae were investigated (using LM, SEM and Stereomicroscope and SDS-PAGE technique respectively). The taxa under investigation represent three tribes, seven genera and 46 species including three subspecies. The macro and micromorphological criteria (219 attributes) and seed protein pattern attributes (38 bands) extracted were numerically analyzed using NTsys-Pc program (version 2.02).
Results: The taxonomic treatment of the Mimosoideae taxa under investigation were based on the numerical analysis of 257 macro-, micromorphology of whole plant and seed

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protein attributes. The dendrogram interprets the similarities and dissimilarities between the investigated taxa. The dendrogram revealed that the taxa under investigation were split into two main series and 25 groups. The splitting Series I includes 12 groups which represented by 20 of the studied *Acacia* species. Series II includes 13 groups which represented by 15 sp. of *Acacia* and the species of other six studied genera, group 17 as well as group 21 contain species from different genera. The specific and infra-specific relationships were discussed and compared with some current systems of classification.
Conclusion: There is no difference of opinion about the phyletic position of the Acacieae which is always considered a link between Mimoseae and Ingeae. However different affinities of the genus *Acacia* taken as a natural unit have been suggested. The tribe Mimoseae represents the polymorphic and older core of Mimosoideae.

Keywords: Mimosoideae; plant morphology; seed anatomy; seed morphology; seed proteins.

1. INTRODUCTION

The Fabaceae comprises approximately 697 genera and 3200 species [1]. Fabaceae is the second largest family based on species diversity after Asteraceae [2]. Subfamily Mimosoideae comprises approximately 79 genera distributed throughout tropical, subtropical and warm temperate regions of the world. The distribution of species among the genera recognized by [3] is very uneven. Almost 2/3 of the known species belonging to *Acacia* with 1200, *Mimosa* with 300-400 and *Inga* with 350-400 [4].

Acacia Mill. is considered the second largest genus in the family Fabaceae after *Astragalus* [5,6, 7,8,9]. *Acacia* if treated in the broad sense, includes 1450 species [10] with species distributed in Africa, America, Asia and Australia. *Acacia* is represented in Egypt by ten species, of which two [*A. nilotica* (L.) Delile and *A. tortilis* (Forssk.) Hayne] are represented by two subspecies each [11]. The cultivated species of the Mimosoideae in Egypt belong to the following genera: *Acacia*, *Adenanthera*, *Albizia*, *Calliandra*, *Dichrostachys, Enterolobium*, *Leucaena***,** *Mimosa*, *Pithecellobium* and *Prosopis*. According to different authors, the tribal classification of the Mimosoideae can be summarized in Table 1.

Table 1. Tribal classification of the Mimosoideae. (+ = present, - = absent)

Author / Tribe	[12]	[13]	[3]	[14]	[15]	[16]
Acacieae	+	+	+	+	+	+
Adenanthereae	−	+	+	−	−	−
Eumimoseae	−	+	−	−	−	−
Ingeae	+	+	+	+	+	+
Mimoseae	+	−	+	+	+	+
Mimozygantheae	+	−	+	+	+	−
Parkieae	+	+	+	+	−	−
Piptadenieae	−	+	−	−	−	−

The recent classification of Mimosoideae presented by [10] was a stop-gap measure and recognized four rather than five tribes in Mimosoideae, mainly based on the results of [16, 17]. In other respects the [10] classification largely retains the scheme [14] in recognizing the four tribes Acacieae, Ingeae, Mimoseae and Mimozygantheae but not Parkieae, despite acknowledgement that these tribes are not monophyletic.

Seed coat anatomy was examined in 251 species from both Acacieae and Ingeae, including both pleugrammic and overgrown-like types [18]. The main target of [18] is to describe in detail the general features of seed coat and to discuss the systematic value, distribution and adaptive significance of this character. A Previous study has been completed by examining the seed coat in 54 species of non-pleurogrammic seeds in the tribe Ingeae [19]. He concludes that the overgrown-like seeds are likely to be an adaptive response to wet tropical climates. The macromorphological characters of seeds in Mimosoideae specifically *Acacia sp*. in Egypt have been used successfully in taxonomy [20]. Depending on seed morphology and anatomy [21] studied seed characters in five species of *Calliandra* to establish similarities and differences among taxa for taxonomic characterization. [22] discussed the electrophoretic seed protein profiles combined with morphological characters of 27 species of *Prosopis*, elucidated systematic relationships between the American sections of *this genus*.

The ultimate goals of the study are to assess the extent of character congruence between plant and seed morphological variations and current generic limits, and to discuss whether these characters (macro and micromorphological and protein pattern) can provide an additional fundamental tool which help in explanation of the taxonomic trends at specific and infra-specific level within the subfamily and. Also to compare the proposed taxonomic treatment based on numerical analysis (dendrogram) with other previous and current systems of classification.

2. MATERIALS AND METHODS

In the present study, 47 taxa of Mimosoideae were collected from different localities representing three tribes, seven genera, 46 species and three subspecies. Some of the studied taxa were collected from some Egyptian Botanic Gardens and phytogeographical regions as shown in Table 2. The identification of wild and cultivated taxa takes place with the help of [11,23,24,25].

Table 2. The collected taxa and their localities

No	Taxa	Tribe	Site of collection
1	*Acacia auriculiformis* A.Cunn. ex Benth.	Acacieae	G
2	*A. boliviana* Rusby	"	G
3	*A. caven* (Molina) Molina	"	G
4	*A. choriophylla* Benth.	"	B
5	*A. cornigera* (L.) Willd.	"	G
6	*A. cultriformis* A.Cunn. ex G. Don	"	G
7	*A. dealbata* Link	"	G
8	*A. decurrens* Willd.	"	G
9	*A. elongata* Sieber ex DC.	"	G
10	*A. falciformis* DC.	"	G
11	*A. farnesiana* (L.) Willd.	"	G
12	*A. glaucophylla* Steud. ex A. Rich.	"	B
13	*A. horrida* (L.) Willd.	"	G
14	*A. howittii* F. Muell.	"	G

15	*A. laeta* R. Br.ex Benth.	"	B
16	*A. leiocalyx* Domin	"	G
17	*A. leptoloba* Pedley	"	G
18	*A. longifolia* (Andrews) Willd.	"	B
19	*A. macradenia* Benth.	"	G
20	*A. mearnsii* De Wild.	"	G
21	*A. muelleriana* Maiden & R.T. Baker	"	G
22	*A. nilotica* (L.) Delile subsp. *nilotica*	"	B
23	*A. oerfota* (Forssk.) Schweinf.	"	B
24	*A. perangusta* (C.T. White) Pedley	"	G
25	*A. peuce (*F. Muell.) Pedley	"	G
26	*A. podalyriifolia* A.Cunn. ex G. Don	"	G
27	*A. retinodes* Schltdl.	"	G
28	*A. salicina* Lindl.	"	G
29	*A. saligna* (Labill.) H. L. Wendl.	"	E
30	*A. seyal* Delile	"	D
31	*A. sieberiana* DC.	"	B
32	*A. terminalis* (Salisb.)J.F. Macbr.	"	G
33	*A. tortilis* (Forssk.) Hayne subsp. *raddiana* (Savi) Brenan	"	F
34	*A. tortilis* (Forssk.) Hayne subsp. *tortilis* (Savi) Brenan	"	F
35	*A. verniciflua* A. Cunn.	"	G
36	*A. verticillata* (L'Hér.) Willd.	"	G
37	*Albizia amara (*Roxb.) Boivin	Ingeae	B
38	*Al. gamblei* Prain	"	H
39	*Al. julibrissin* Durazz.	"	B
40	*Al. lebbeck* (L.) Benth.	"	H
41	*Al. procera* (Roxb.) Benth.	"	H
42	*Calliandra haematocephala* Hassk.	"	C
43	*Dichrostachys cinera* (L.) Wight & Arn.	Mimoseae	C
44	*Enterolobium contortisiliquum* (Vell.) Morong	Ingeae	D
45	*E. timbouva* Mart.	"	B
46	*Faidherbia albida* (Delile) A. Chev.	"	B
47	*Leucaena leucocephala* (Lam.) de Wit	Mimoseae	A

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2.1 Macromorphological Investigation [Whole Plant]

The macromorphological characters of the whole plant *viz*. habit, stem, leaf, glands, flowers, inflorescence and pod were extracted directly from the fresh specimens of the available taxa. The macromorphological characters of the foreign and some of the local taxa were derived from the site [25].

2.2 Macromorphological Investigation [Seed Whole Mount]

The investigated mature seeds (about 15- 20 seeds per taxa) were dried, cleaned and examined by Stereomicroscope to show the different exomorphic parameters *viz*. shape, dimensions, colour and surface. For SEM investigation, the seeds were dried and fixed to specimen stub, fixed to the specimen holder of Scanning Electron Microscope (Inspect S, version 3.1.2) maintained at accelerating potential voltage of 20-30 K.v. and photographed at different magnifications. The descriptive terminologies of seed surface sculpture used in the present study were based on the glossary of [26,27,28,29].

2.3 Micromorphological Investigation [Seed Coat Anatomy, LM]

Ten mature seeds for each taxa were softened in warm water (for 12-72h) and then dehydrated using a tertiary butyl alcohol series and sectioned at a thickness of 15-20 µm according to the traditional methods of [30]. The seed coat sections were permanently mounted in Canada balsam without any stain, investigated, described (LM), and photographed using Digital Camera. The magnification power was expressed by (×). The anatomical descriptive terms of seed coat in the present study were based on the terms of [19,31].

2.4 Seed Protein Pattern Analysis

Dry mature seeds were ground to meal using mortar. For protein extraction, 0.2 g of seed meal was homogenized with 0.2 ml of tris-HCl buffer containing 2% SDS at pH 6.8 and stored overnight at 4ºC. Centrifugation was performed at 9000 rpm for 6 minutes and the supernatant was collected for analysis. Protein samples were prepared by mixing 20 µl clear supernatant with 20 µl treatment buffer and denatured by heating at 90ºC on boiling water bath for 3 minutes; then a drop of bromophenol blue was added as tracking dye. Characterization of seed protein profiles in the present study were carried out using one dimensional sodium dodecyle sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) according to [32].

2.5 Numerical Analysis

The data obtained from macro and micromorphology and seed protein pattern of the investigated taxa were subjected to the numerical analysis using the NTsys-Pc program (version 2.02) [33]. The grouping of operational taxonomic units (OTU's) produced from the analysis were examined and compared with the previous and current taxonomic classifications of the Mimosoideae.

3. RESULTS AND DISCUSSION

The cumulative macromorphological features of the whole plant of the taxa under investigation were shown in Table 3. In the present study it was observed that the presence of mixed stipules is considered a unique character in *A. tortilis* subsp. *raddiana* and *A. tortilis* sub.sp. *tortilis*. This is in accordance with [34]. In *Dichrostachys cinera*, the bicoloured inflorescence is considered a key character to the taxon (the lower part of the inflorescence is pink while the upper is yellow). This is in agreement with the description view of [35].

The diversity of the seed exomorphic characters were collected and shown in Table 4 and plate I. As regards the seed surface sculpture pattern, aspect of anticlinal and periclinal wall variations (elevation and texture) can serve as good diagnostic parameters at the generic and specific level between the studied taxa but to certain limit. The seed coat anatomy features shown in Table 5 and plate II, the seed coat in all the studied taxa have a palisade malpighian cells whatever pleuro- or non-pleurogrammic, with mucilage stratum (40 taxa) or without mucilage stratum (7 taxa); with light line (44 taxa) and without light line in *Acacia laeta, A. leptoloba* and *A. seyal*. This conclusion is supported by the conclusion of [21,22,36].

Seed protein diversity as revealed by variation in SDS-PAGE has been used in the present investigation to re- assess the taxonomic relationships between 47 taxa. The produced protein banding patterns of the taxa studied are shown in plate III. A total number of 38 protein bands with approximately molecular weights ranging between 306 Kilodalton (KD) and 12 KD are recorded in the electropherograms of the samples studied. The highest molecular weight 306 KD was recorded in *Dichrostachys cinera* only. In individual sample the number of bands varied between 3 and 25 bands. The maximum number of bands (25) was recorded in *Acacia elongate* and *Acacia glaucophylla*; while the minimum number was observed in *Acacia farnesiana*. Some bands are common to the majority of the taxa e.g. bands numbered 16, 18, 22, 26, 27, 30 and 37; while other bands e.g. bands numbered 2, 3, 4, 5, 11,13, 14, 17, 21 and 36 are found in few species (Table 6). The bands produced by each sample were counted and their relative mobility was compared with those of the standard marker protein.

From morphological view point, the criteria from the seed surface sculpture pattern alone are not sufficient for delimitation between the studied taxa. In this connection more morphological and molecular studies with large number of taxa are required for sharp species delimitation. This is in accordance with the results and conclusion of [21,37]. In the present study seed macro-, and micromorphological criteria, in addition to seed protein bands facilitate to certain extend the separation of the studied taxa).

The classification produced from the dendrogram based on 257 macro and micromorphology of whole plant and seed as well as seed protein attributes of the investigated taxa of Mimosoideae (Fig. 1) was compared with some current system treatment of [14,15,16] of the Mimosoideae. The resulted dendrogram revealed that the taxa under investigation were split into two main series and 25 groups (Table 7). The application of such treatment could be discussed as in the following:

3.1 At the Series Level

The splitting into two main series; **Series I** includes 12 groups and **Series II** includes 13 groups. The former 12 groups are represented by 20 studied species of *Acacia* while the latter 13 groups represented by 15 species of *Acacia* and the species of other six genera studied, group 17 as well as group 21 contain species from different genera.

3.2 At the Group Level

The studied taxa are categorized into 25 groups. These groups are compared with the current taxonomic treatment of [10,14,16]. In addition, the proposed groups are discussed as in the following:

The group 17 appears as the nearest in taxonomic level of group 8 (Acacieae). [38,39] suggested that the relation between Acacieae, Ingeae and Mimoseae has always been considered as very close. In the present group *Albizia amara* (Ingeae) is categorized with *Leucaena leucocephala* (Mimoseae). In this connection it should be taken in the consideration that the old name of *Albizia amara* was *Albizia sericocephala* and *Mimosa amara* and the old name of *Leucaena leucocephala* was *Acacia glauca*, *Leucaena glabrata*, *Leucaena glauca* and *Mimosa leucocephala* [25].

The group 18 (Ingeae) appears as the nearest in taxonomic level of group 12 & 15 (Acacieae) and the group 19 (Ingeae) appears as the nearest in taxonomic level of group 6 (Acacieae). These results in the present study are in accordance with [39] who suggested that the relation between Acacieae and Ingeae has always been considered very close, the main distinction being the stamens are nearly always basally fused into a tube in the Ingeae. In group 21, it was noticed that *Dichrostachys cinera* (Mimoseae) and *Acacia cornigera* (Acacieae) are very close tacking in consideration the old names of each. The old names of the former were *Mimosa glomerata* and *Mimosa nutans* and the old names of the latter were *Mimosa cornigera* [25]. This result in the present study supported the idea of [40] that *Dichrostachys* may be considered a transitional stage between Mimoseae and Acacieae. The present work proved that this genus has characteristic features that differ from the other taxa of Mimosoideae. This conclusion was recorded before by [41,42]. The present relationship between the studied two species in this group was supported by [43] from another morphological view point (pollen morphology).

The very close relationship between the two species in group 22 is based on certain seed macro- and micromorphological characters (non-pleurogrammic, seed coat with two layers, hilum position and aspects of anticlinal & periclinal wall). The group 22 (Ingeae) appears as the nearest in taxonomic level of group 10 (Acacieae).

The group 23 (Ingeae) appears as the nearest in taxonomic level of group 16 (Acacieae). In the present work it was found that the genus *Calliandra* is very distinctive through mimosoid taxa. [44] supported affinities of some *Acacia* (Acacieae) with *Calliandra* (Ingeae). [45] referred to *Calliandra* as a very isolated genus within Mimosoideae. This is in agreement of [40].

[46] stated that the genus *Faidherbia* (group 24) raises however, the problem of the limits between the Ingeae and the Acacieae and the seed characters of this genus are very close to those of the Ingeae (*Albizia* and *Enterolobium*). This is in agreement with the present, and on the same lines workers as [14,43,47] suggested that the genus *Faidherbia* based on *Acacia albida* is distinct from *Acacia* in having the pollen grains organized into 30 – (32) to 16 – celled polyads and it is better transferred to the tribe Ingeae. The same authors assumed that this genus may link the Ingeae and Acacieae.

The result in group 25 contradict the concept of [48] who proved that in the Mimosoideae only *Acacia* and *Pithecellobium* have seeds with aril.

Table 3. Macromorphological characters of the whole plant of the studied taxa. Taxa are arranged according to their numbers in Table 2. (+: Present, - : Absent)

Characters →

			Stipules		Petiole gland		Leaf (Leaflet)					Flower			Pod			
Taxa No.	Habit	Bark colour	Detection	Type	Detection	Position	Composition	Leaf / leaflet shape	No. of pair of leaflets	Texture	Average L × W (cm)	Colour	Infloresence	Dimensions diameter or length (cm)	Shape	Colour at maturation	Texture	Average L ×W (cm)
1	Tree	Grey-black	–	–	+	Gland at leaf base	Simple	Linear-elliptic	–	Glabrous	17 × 1.5	Yellow	Spike	5 – 8	Curved	Brown	Glabrous	11 × 0.8
2	Shrub	Dark brown	+	Spiny stipules	–	–	Bipinnate	Linear	10 – 20	"	0.6 × 0.2	White	Rounded heads	0.6 – 1	Linear-twisted	Reddish-brown	Pubescent	2.5 × 0.8
3	Tree	"	+	"	+	At base of each pair	"	"	"	"	0.15 × 0.05	White-yellow	"	0.8 – 1.4	Terete	Orange-brown	Glabrous	11 × 0.8
4	Shrub	"	+	"	–	–	"	Elliptic-obovate	2 – 7	"	0.6 × 0.2	White	"	3 – 6.5	Elliptic	Brown-black	"	9 × 2
5	"	Grey-brown	+	"	–	–	"	Linear	15 – 25	"	"	Yellow	Spike	2 – 4	Terete	Dark brown	"	6.5 × 0.6
6	"	Grey-green	–	–	–	–	Simple	Triangular	–	"	2.2 × 1.2	"	Rounded heads	0.6 – 1	Linear	Brown	"	11 × 0.8
7	Tree	"	–	–	–	–	Bipinnate	Linear-elliptic	15 – 25	"	5 × 2.5	"	"	1.5 – 2.5	"	"	"	4.5 × 1.2
8	"	Brown-black	–	–	+	At base of each pair	"	Linear	"	"	1× 0.06	"	"	0.6 – 1	"	"	"	6.5 × 0.6
9	Shrub	Grey-brown	–	–	–	–	Simple	"	–	"	9 × 0.4	"	"	0.3 – 0.6	"	Orange-brown	Pubescent	11 × 0.8
10	Tree	"	+	Spiny stipules	+	Gland at leaf base	"	Lanceolate	–	"	12 × 1.8	Creamy-yellow	"	0.6 – 1	Linear-twisted	Brown	Glabrous	9 × 2
11	"	Brown	+	"	–	–	Bipinnate	Oblanceolate	2 – 7	"	0.6 × 0.2	Yellow	"	0.8 – 1.4	Terete	Brown-black	"	4.5 × 1.2
12	"	"	–	–	+	Gland at leaf base	"	Linear	15 – 25	"	1 × 0.06	"	"	"	Linear	Brown	"	9 × 2
13	Shrub	Dark brown	+	Spiny stipules	–	–	"	"	2 – 7	"	0.15 × 0.05	White-yellow	"	0.6 – 1	"	Green-brown	Pubescent	4.5 × 1.2
14	Tree	Brown-black	–	–	–	–	Simple	Elliptic-lanceolate	–	Glabrous	1.5 × 0.6	Yellow	Rounded heads	0.8 – 1.4	Linear	Brown	Glabrous	6.5 × 0.6
15	"	Grey-green	+	Spiny stipules	–	–	Bipinnate	Elliptic-obovate	2 – 7	"	1 × 0.4	White-yellow	Raceme	3 – 6.5	"	Orange-brown	Leathery	"
16	"	Grey-brown	+	"	+	Gland at leaf base	Simple	Elliptic	–	"	12 × 1.8	Yellow	Spike	"	"	Dark brown	Glabrous	13 × 0.4
17	"	Dark brown	–	–	+	"	"	"	–	"	10 X 2.4	White	Raceme	0.6 – 1	"	Pale brown	"	9 × 2

18	"	"	–	–	–	–	"	Linear-lanceolate	–	"	"	Yellow	Spike	2 – 4	"	Brown	"	"
19	"	"	–	–	–	–	"	Elliptic	–	"	17 × 1.5	"	Rounded heads	0.8 – 1.4	Linear-twisted	"	"	11 × 0.8
20	"	Grey-black	–	–	+	At base of each pair	Bipinnate	Oblong-spatheolate	15 – 25	Pubescent	0.15 × 0.05	"	"	"	Linear	Brown-black	Scabrous	"
21	Shrub	Grey-brown	–	–	+	"	Pinnate	Linear	2 – 7	Glabrous	2.3 × 0.1	Creamy	"	0.3 – 0.6	"	Dark brown	Glabrous	6.5 × 0.6
22	"	Grey-black	+	Spiny stipules	+	"	Bipinnate	Elliptic	15 – 25	"	0.6 × 0.2	Yellow	"	0.8 – 1.4	"	Black	Tomentose	20 × 3
23	"	Grey-white	+	"	–	–	"	Linear	5 – 15	Pubescent	"	White	"	3 – 6.5	Elliptic	Pale yellow	Glabrous	11 × 0.8
24	Tree	Red-brown	–	–	+	Gland at leaf base	Simple	"	–	Glabrous	6 × 0.15	Yellow	"	0.6 – 1	Linear	Brown	"	"
25	"	Grey-black	–	–	+	"	"	Acicular	–	"	16 × 0.03	"	"	0.8 – 1.4	Spirally twisted	"	"	14 × 4
26	"	Grey-brown	–	–	+	"	"	Elliptic	–	"	3.5×1.5	"	"	0.6 – 1	Elliptic	Dark brown	"	11 × 0.8
27	"	Grey	–	–	+	"	"	Oblanceolate	–	"	12 × 1.8	"	"	"	Linear	Brown	"	13 × 0.4
28	"	Grey-brown	–	–	+	2-5 glands at leaf base	"	Elliptic	–	"	"	Creamy-yellow	"	"	Oblong	Grey-green	"	11 × 0.8
29	Shrub	Grey	–	–	+	Gland at leaf base	"	Linear	–	"	17 × 1.5	Yellow	Raceme	0.4 – 3	Linear	Brown	"	"
30	Tree	Red-brown	+	Spiny stipules	+	At base of terminal pair	Bipinnate	Linear-elliptic	10 – 20	"	0.8 × 0.15	"	Rounded heads	0.8 – 1.4	Curved	Pale brown	"	"
31	Shrub	Yellow-brown	+	"	–	–	"	Elliptic	30 – 40	Hairy	0.6 × 0.2	Creamy-yellow	"	"	Linear	Brown	"	6.5 × 0.6
32	"	Grey-brown	–	–	+	At base of each pair	"	Oblong-elliptic	5 – 15	Pubescent	1 × 0.4	Yellow	"	0.6 – 1	"	Reddish-brown	"	6.5× 2.2
33	Tree	Dark brown	+	Mixed stipules	+	Gland at leaf base	Bipinnate	Linear-elliptic	10 – 20	Pubescent	1.5 × 0.6	White-yellow	Rounded heads	0.6 – 1	Curved	Orange-brown	Pubescent	14× 4
34	Shrub	Brown	+	"	+	"	"	"	"	"	"	"	"	"	Spirally twisted	Brown	"	"
35	"	Grey	–	–	+	Along leaf petiole	Simple	Elliptic	–	Glabrous	10 × 2.4	Yellow	"	"	Linear	"	Glabrous	6.5 × 0.6
36	Tree	Brown-black	+	Spiny stipules	+	Gland at leaf base	"	Linear-lanceolate	–	"	1.5 × 0.6	"	Spike	3 – 6.5	"	Orange-brown	"	0.6 × 0.4
37	"	Grey-black	–	–	+	"	Bipinnate	Oblong- linear	15 – 25	"	0.6 × 0.2	Creamy-white	Rounded heads	2 – 4	Oblong-linear	Brown	Pubescent	20 × 3
38	"	Grey-brown	+	Spiny stipules	–	–	"	"	30 – 40	"	1 × 0.4	Green	"	1.5 – 2.5	Linear	Brown-black	"	11 × 0.8

39	"	"	–	–	–	–	"	Oblong-elliptic	15 – 25	"	"	Pink	Rounded heads	"	"	Brown	"	14 × 4
40	"	Grey	–	–	–	–	"	"	5 – 15	"	2.3 × 0.1	White-yellow	"	5 – 8	Oblong	"	Stiff	13 × 0.4
41	"	Grey-brown	–	–	+	Gland at leaf base	"	"	10 – 20	Pubescent	3.5 × 1.5	White	"	2 – 4	Elliptic	Reddish-brown	Glabrous	14 × 4
42	Shrub	"	–	–	–	–	"	Oblong- linear	2 – 7	Glabrous	2.2 × 1.2	Pink	Clustered heads	"	Linear	Brown	"	11 × 0.8
43	"	"	–	–	+	At base of each pair	"	Linear	15 – 25	Hairy	0.6 × 0.2	Pink / yellow	Spike	5 – 8	Linear-twisted	Dark brown	"	6.5 × 2.2
44	Tree	"	–	–	–	–	"	Lanceolate	10 – 20	Glabrous	1.5 × 0.6	White	Rounded heads	1.5 – 2.5	Kidney	Brown-black	Wrinkled	9 × 2
45	"	"	–	–	–	–	"	"	5 – 15	"	1 × 0.4	"	"	2 – 4	"	"	Glabrous	6.5 × 2.2
46	"	Grey-white	+	Spiny stipules	–	–	"	Oblong-elliptic	"	"	3.5 × 1.5	Yellow	Spike	1.5 – 2.5	Curved	Reddish-brown	Wrinkled	20 × 3
47	Shrub	Grey	–	–	+	Gland at leaf base	"	Oblong- linear	10 – 20	"	1 × 0.06	White	Rounded heads	2 – 4	Linear	Brown	Glabrous	"

Table 4. Macromorphological characters of the seed of the studied taxa using steriomicroscope, digital camera & SEM. Taxa are arranged according to their numbers in Table 2. (+ : present; - : absent)

Character →

Taxa No.	Seed (sterio &digital camera)					Aril	SEM								
								Anticlinal Wall			Periclinal Wall		Hilum		
	Compression	Colour	Shape	Texture	L × W (mm)		Seed Surface Sculpture	Level	Surface Sculpture	Margin	Level	Surface Sculpture	Position	level	Shape
1	Not compressed	Black	Elliptic	Smooth	5 × 3.5	+	Reticulate	Elevated	Reticulate	Straight	Depressed	Reticulate	Terminal	Elevated	Globose
2	Slightly compressed	Pale green	Globose	"	4.5 × 2.5	–	Rugose	"	Smooth	Undulate	"	Wrinkled	"	"	Oval
3	"	Brown	"	"	7 × 4	+	Sulcate	"	"	"	"	Smooth	"	Slightly depressed	"
4	"	"	Oval	"	3.5 × 2.5	–	Reticulate / rugose	"	"	"	"	"	"	Elevated	Globose
5	Not compressed	"	Elliptic	"	4.5 × 2.5	–	Pusticulate	Depressed	"	"	Elevated	Striate-lineolate	"	"	Oval
6	"	Brown-black	"	"	"	+	Reticulate / ruminate	"	"	"	"	Ruminate	Sub-terminal	Depressed	Globose
7	Slightly compressed	"	Oval	"	3.5 × 2.5	+	"	"	"	"	"	"	"	Slightly depressed	"

8	Not compressed	"	Elliptic	"	4.5 × 2.5	+	Reticulate / foveate	Elevated	Falsifoveate	Straight	Depressed	Smooth	Terminal	Depressed	Elliptic
9	Slightly compressed	"	"	"	6 × 2	+	Reticulate / favulariate	Depressed	Smooth	Undulate	Elevated	"	Sub-terminal	Slightly depressed	Globose
10	"	"	Globose	Wrinkled	5 × 3.5	+	Tuberculate	"	"	Straight	"	"	"	"	"
11	"	Brown	Elliptic	Smooth	6.5 × 4.5	–	Reticulate / rugose	Elevated	"	Undulate	Depressed	Wrinkled	Terminal	Depressed	Oval
12	"	"	Oval	Wrinkled	"	+	Reticulate / foveate	"	Ruminate	Straight	"	Ruminate	Sub-terminal	Slightly depressed	Globose
13	Compressed	"	"	Smooth	7 × 4	+	Ocellate	"	Smooth	"	"	Smooth	Terminal	"	Oval
14	Slightly compressed	Brown-black	Elliptic	Smooth	4.5 × 2.5	+	Reticulate	Depressed	Smooth	Straight	Elevated	Wrinkled	Terminal	Slightly depressed	Oval
15	Compressed	Brown	Globose	"	8 × 8	–	Reticulate / favulariate	"	"	Undulate	"	Favulariate	"	Slightly elevated	"
16	Slightly compressed	Black	Elliptic	"	6 × 2	+	Reticulate	"	"	Straight	"	Falsifoveate	"	Elevated	Globose
17	"	"	"	"	"	+	Reticulate / ruminate	"	"	Undulate	"	Ruminate	"	Slightly depressed	"
18	"	Brown-black	"	Wrinkled	4.5 × 2.5	+	Falsifoveate	Elevated	"	Straight	Depressed	Smooth	Sub-terminal	Slightly elevated	Elliptic
19	"	"	"	Smooth	"	+	Reticulate / ruminate	Depressed	"	"	Elevated	Wrinkled	"	Slightly depressed	Globose
20	"	Black	Oval	Wrinkled	3.5 × 2.5	+	Ruminate	"	"	Undulate	"	"	Terminal	"	"
21	"	"	Elliptic	Smooth	6 × 2	+	Reticulate / ruminate	"	"	"	"	"	Sub-terminal	"	Oval
22	Not compressed	Brown	Oval	"	8.5 × 5	–	Reticulate / rugose	Elevated	"	"	Depressed	Striate	Terminal	"	Globose
23	"	Olive-brown	"	Wrinkled	7.5 × 5.5	+	Reticulate	"	"	Straight	"	Wrinkled	"	Elevated	"
24	Slightly compressed	Black	Elliptic	Smooth	3.5 × 2.5	+	Reticulate / ruminate	Depressed	Wrinkled	"	Elevated	"	Sub-terminal	"	"
25	"	Brown-black	Globose	Wrinkled	8 × 8	–	Reticulate / favulariate	"	"	Undulate	"	"	Terminal	Slightly depressed	Elliptic
26	"	"	Elliptic	Smooth	6 × 2	+	Reticulate / ruminate	"	"	"	"	"	Sub-terminal	"	Globose
27	Not compressed	"	"	"	4.5 × 2.5	+	Ruminate	"	Smooth	Straight	"	"	"	"	Elliptic
28	"	Black	Oval	"	6 × 2	+	Pusticulate	"	Wrinkled	Undulate	"	"	"	Slightly elevated	Globose
29	"	Brown	Elliptic	"	4.5 × 2.5	+	Falsifoveate /colleculate	Elevated	Granulate	"	Depressed	Smooth	"	"	Oval
30	Compressed	Olive-brown	Oval	"	5 × 3.5	+	Rugose	"	Smooth	"	"	"	"	Elevated	Globose
31	"	Brown	"	"	7.5 × 5.5	+	Reticulate / rugose	"	"	"	"	"	"	Leveled	"
32	Slightly compressed	Black	Oval	Smooth	5 × 3.5	+	Falsifoveate	Elevated	Smooth	Undulate	Depresse	Wrinkled	Sub-	Leveled	Elliptic

										d		terminal		
33	"	Brown	Elliptic	"	0.6 × 0.3	–	Reticulate / foveate	"	Wrinkled	"	"	"	Terminal	"	"
34	"	"	"	"	"	–	Sulcate	"	Smooth	"	"	Smooth	"	Slightly elevated	Globose
35	Not compressed	"	Oval	"	7 × 4	+	Reticulate	"	"	"	"	Wrinkled	"	Elevated	"
36	Slightly compressed	"	Elliptic	"	3.5 × 2.5	+	Reticulate / ruminate	Depressed	"	"	Elevated	Ruminate	Sub-terminal	Depressed	Oval
37	Compressed	"	Oval	"	7 × 4	–	Reticulate / foveate	Elevated	Ruminate	Straight	Depressed	Smooth	Terminal	"	"
38	"	Yellow	Globose	"	7 × 7	–	Reticulate / ruminate	Depressed	Smooth	Undulate	Elevated	Ruminate	"	Leveled	Fusiform
39	"	Brown	Elliptic	"	8.5 × 5	–	Falsifoveate	Elevated	Wrinkled	Straight	Depressed	Wrinkled	Sub-terminal	Elevated	Globose
40	"	"	Globose	"	"	–	Verrucate	Depressed	Smooth	Undulate	Elevated	Smooth	Terminal	"	Elliptic
41	Slightly compressed	"	Oval	"	5 × 3.5	–	Scalariform	Elevated	Wrinkled	Straight	Depressed	Wrinkled	"	"	Oval
42	Compressed	Olive-brown	Elliptic	"	8.5 × 5	–	Ruminate	Depressed	Smooth	Undulate	Elevated	Ruminate	"	Depressed	Fusiform
43	"	Brown	Oval	"	4.5 × 2.5	–	Verrucate	"	"	"	"	Smooth	Sub-terminal	Slightly depressed	Globose
44	Slightly compressed	"	Elliptic	"	13 × 5.5	+	Reticulate	Elevated	"	"	Depressed	Wrinkled	Terminal	Leveled	"
45	"	"	Oval	"	9 × 6	+	"	"	"	Straight	"	Smooth	Sub-terminal	"	"
46	"	"	"	"	7 × 4	–	Tuberculate	Depressed	"	"	Elevated	"	Terminal	Depressed	Oval
47	Compressed	"	"	"	"	–	Pusticulate	"	Lineate	Undulate	"	Lineate	"	"	Elliptic

Table 5. Micromorphological characters of the seed coat of the studied taxa using lm. taxa are arranged according to their numbers in Table 2. (+: present; - : absent).

Character →

Taxa	Pleurogramic or non-pleurogrammic	No. of Seed Coat Layers	Malpighian Cells		External hourglass cells	Mesophyll					Internal hourglass cells
						Parenchyma		Parenchyma + Resinoid Tissue			
			Mucilage Stratum	Light Line		Thickness	No. of Mesophyll layer	Detection	Aspect of Resinoid Tissue	No. of layers	
1	Pleurogramic	Four	+	+	+	Thick	9 – 11	–	–	–	+
2	"	Two	+	+	–	"	11 – 14	–	–	–	–
3	Non-pleurogramic	"	+	+	–	"	15 – 18	–	–	–	–
4	Pleurogramic	Three	–	+	+	"	26 – 30	–	–	–	–
5	"	"	+	+	+	"	"	–	–	–	–
6	Non-pleurogramic	Two	+	+	–	"	11 – 14	–	–	–	–

7	"	"	+	+	–	"	9 – 11	–	–	–	–
8	Pleurogramic	"	+	+	–	Thin	7 – 9	+	inner complete ring of mesophyll	4 – 6	–
9	Non-pleurogramic	Four	–	+	+	Thick	9 – 11	–	–	–	+
10	Pleurogramic	Three	+	+	+	"	7 – 9	–	–	–	–
11	"	Two	+	+	–	"	30 – 38	–	–	–	–
12	Non-pleurogramic	Three	+	+	+	"	11 – 14	–	–	–	–
13	"	"	+	+	+	"	"	–	–	–	–
14	Pleurogramic	Four	–	+	+	"	"	–	–	–	+
15	Non-pleurogramic	Two	+	–	–	"	"	–	–	–	–
16	Pleurogramic	Four	+	+	+	Thick	11 – 14	–	–	–	+
17	Non-pleurogramic	Three	+	–	+	"	9 – 11	–	–	–	–
18	Pleurogramic	Two	+	+	–	"	4 – 6	+	inner complete ring of mesophyll	4 – 6	–
19	Non-pleurogramic	Four	+	+	+	"	9 – 11	–	–	–	+
20	Pleurogramic	"	+	+	+	Thin	"	–	–	–	+
21	"	Two	–	+	–	Thick	11 – 14	–	–	–	–
22	"	"	+	+	–	"	38 – 44	–	–	–	–
23	Non-pleurogramic	"	–	+	–	"	30 – 38	–	–	–	–
24	"	"	+	+	–	"	9 – 11	–	–	–	–
25	"	"	+	+	–	"	18 – 22	–	–	–	–
26	Pleurogramic	Four	+	+	+	"	9 – 11	–	–	–	+
27	"	"	+	+	+	"	"	–	–	–	+
28	"	Two	+	+	–	"	22 – 26	–	–	–	–
29	"	"	+	+	–	"	9 – 11	–	–	–	–
30	"	"	+	–	–	"	7 – 9	–	–	–	–
31	Non-pleurogramic	Three	+	+	+	"	15 – 18	–	–	–	–
32	Pleurogramic	Four	+	+	+	"	7 – 9	–	–	–	+
33	Non-pleurogramic	Two	+	+	–	"	15 – 18	–	–	–	–
34	"	"	–	+	–	"	"	–	–	–	–
35	"	Four	–	+	+	"	"	–	–	–	+
36	"	"	+	+	+	"	11 – 14	–	–	–	+
37	Non-pleurogramic	Two	+	+	–	Thick	15 – 18	+	intermediate ring inside mesophyll	4 – 6	–
38	"	"	+	+	–	Thin	9 – 11	+	"	2 – 4	–
39	Pleurogramic	Three	+	+	+	Thick	11 – 14	+	patches inside mesophyll	4 – 6	–
40	Non-pleurogramic	Two	+	+	–	"	22 – 26	+	"	2 – 4	–
41	"	"	+	+	–	"	"	–	–	–	–
42	Pleurogramic	four	+	+	+	"	18 – 22	–	–	–	+
43	Non-pleurogramic	Two	+	+	–	"	7 – 9	–	–	–	–
44	Pleurogramic	"	+	+	–	"	38 – 44	+	intermediate ring inside mesophyll	7 – 10	–
45	"	Three	+	+	+	"	30 – 38	+	patches inside mesophyll	2 – 4	–
46	Non-pleurogramic	Two	+	+	–	"	18 – 22	–	–	–	–
47	Non-pleurogramic	Three	+	+	+	Thin	4 – 6	+	inner complete ring of mesophyll	7 – 10	–

Table 6. Molecular weight of the recorded seed protein bands of the studied taxa. Taxa are arranged according to their numbers in table 2. The presence or absence of each band were treated as a binary character in a data matrix (code 1 and 0 respectively)

Band No.	MW KD	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
01	306	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
02	205	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
03	202	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
04	144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
05	120	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
06	115	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
07	108	1	0	0	0	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	1	0	0	0	0
08	102	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
09	98	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0
10	90	1	0	1	0	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0
11	80	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	78	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0
13	64	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	58	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
15	52	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
16	48	1	0	0	0	1	1	1	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
17	47	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
18	45	0	0	0	0	1	1	1	1	1	1	1	0	0	1	0	0	1	1	0	0	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
19	42	0	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	1	1	1	0	0	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
20	38	1	0	1	0	0	0	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
21	37	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	35	1	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
24	34	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1
25	33	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1	1	0	0	1	1	1	1	0	0	0	1	1	0	1	0	0	1	1	0	1	1	0	0	0	0	0	0	1	0	1	1
26	32	1	1	1	0	1	1	1	0	1	1	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0	0	0	1	1	0	1
27	31	1	1	1	1	0	0	0	0	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	1	0	1
28	30	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
29	29	1	0	1	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0
30	27	1	0	1	1	1	1	0	0	1	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	1
31	25	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0
32	24	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
33	23	0	0	0	1	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0
34	22	1	1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0
35	20	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0
36	18	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
37	15	0	0	1	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	1
38	12	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0

Table 7. Proposed treatment of mimosoideae based on numerical analysis of 257 macro-, micromorphology of whole plant and seed protein attributes,

Series	Group	Taxa under investigation
	Gr. 1	01- *Acacia auriculiformis*
	Gr. 2	06- *Acacia cultriformis*
		07- *A. dealbata*
	Gr. 3	36- *Acacia verticillata*
	Gr. 4	16- *Acacia leiocalyx*
	Gr. 5	17- *Acacia leptoloba*
		19- *A. macradenia*
	Gr. 6	24- *Acacia perangusta*
		26- *A. podalyriifolia*
S1		27- *A. retinodes*
	Gr. 7	28- *Acacia salicina*
	Gr. 8	10- *Acacia falciformis*
	Gr. 9	25- *Acacia peuce*
	Gr. 10	09- *Acacia elongata*
		14- *A. howittii*
		21- *A. muelleriana*
	Gr. 11	12- *Acacia glaucophylla*
		20- *A. mearnsii*
	Gr. 12	08- *Acacia decurrens*
		18- *A. longifolia*
	Gr. 13	02- *Acacia boliviana*
		03- *A. caven*
		13- *A. horrid*
	Gr. 14	33- *Acacia tortilis* subsp. *raddiana*
		34- *A. tortilis* subsp. *Tortilis*
	Gr. 15	04- *Acacia choriophylla*
		22- *A. nilotica* subsp. *nilotica*
		11- *A. farnesiana*
	Gr. 16	29- *Acacia saligna*
		30- *A. seyal*
		32- *A. terminalis*
		31- *A. sieberiana*
		35- *A. verniciflua*
S2	**Gr. 17**	37- *Albizia amara*
		47- *Leucaena leucocephala*
	Gr. 18	39- *Albizia julibrissin*
		41- *Al. procera*
	Gr. 19	44- *Enterolobium contortisiliquum*
		45- *E. timbouva*
	Gr. 20	23- *Acacia oerfota*
	Gr. 21	05- *Acacia cornigera*
		43- *Dichrostachys cinera*
	Gr. 22	38- *Albizia gamblei*
		40- *Al. lebbeck*
	Gr. 23	42- *Calliandra haematocephala*
	Gr. 24	46- *Faidherbia albida*
	Gr. 25	15- *Acacia laeta*

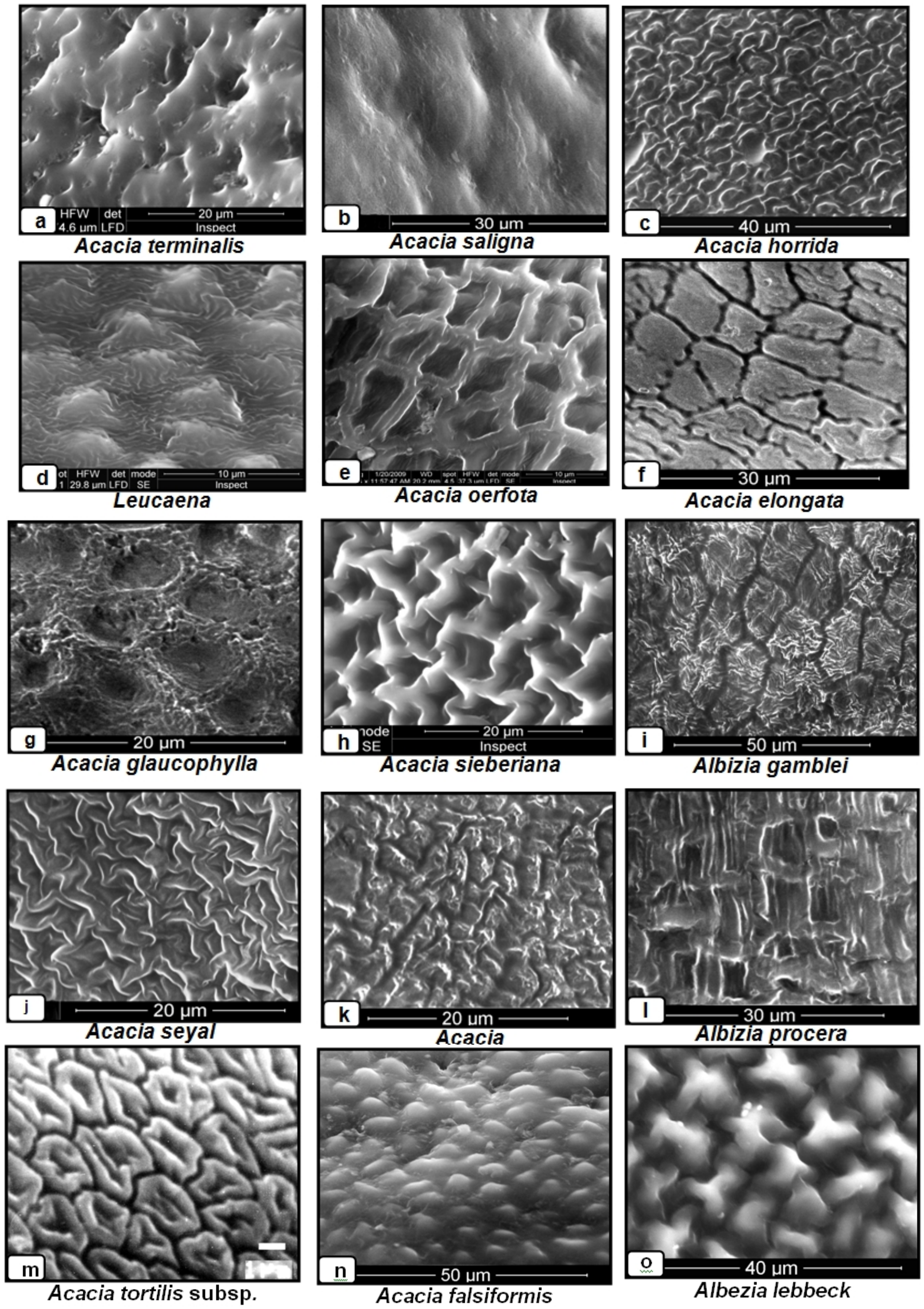

Plate I. Text Figs. a-o. Microphotographs of the major seed surface sculpture (SEM). a: Falsifoveate, b: Falsifoveate-Colliculate, c: Ocellate, d: Pusticulate, e: Reticulate, f: Reticulate-Favulariate, g: Reticulate-Foveate, h: Reticulate-Rugose. i: Reticulate-Ruminate, j: Rugose, k: Ruminate, l: Scalariform, m: Sulcate, n: Tuberculate, o: Verrucat

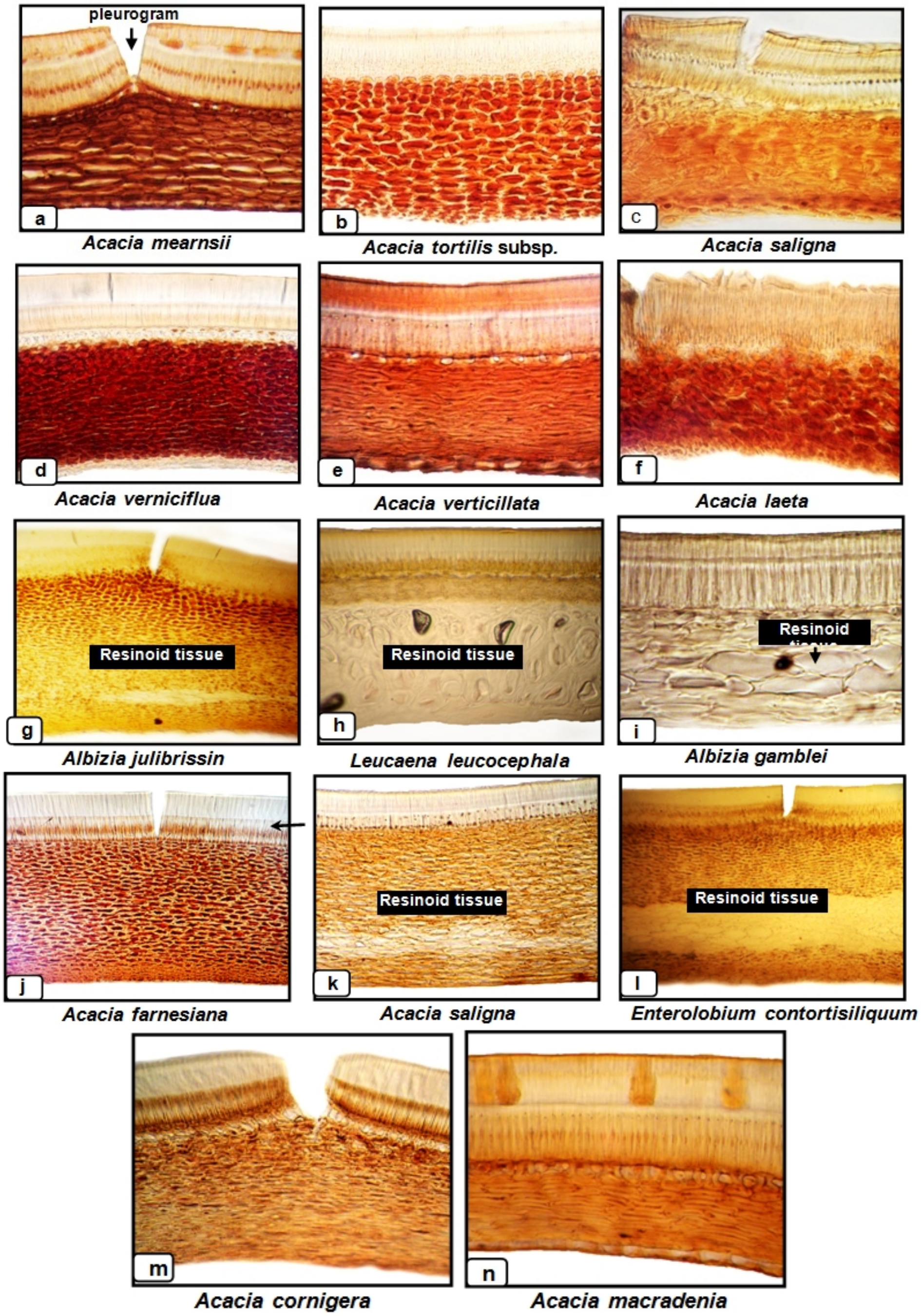

Plate II. Text Figs. a-h. Microphotographs of seed anatomy. a: Pleurogrammic seed; b: Non-pleurogrammic seed; c: Mucilage stratum present; d: Mucilage stratum absent; e: Malpighian cells with light line; f: Malpighian cells without light line; g: Resinoid tissue (patches); h: Resinoid tissue (inner ring of mesophyll). i: Resinoid tissue (Patches); j-l: Seed coat with two layers; m: Seed coat with three layers; n: Seed coat with four layers

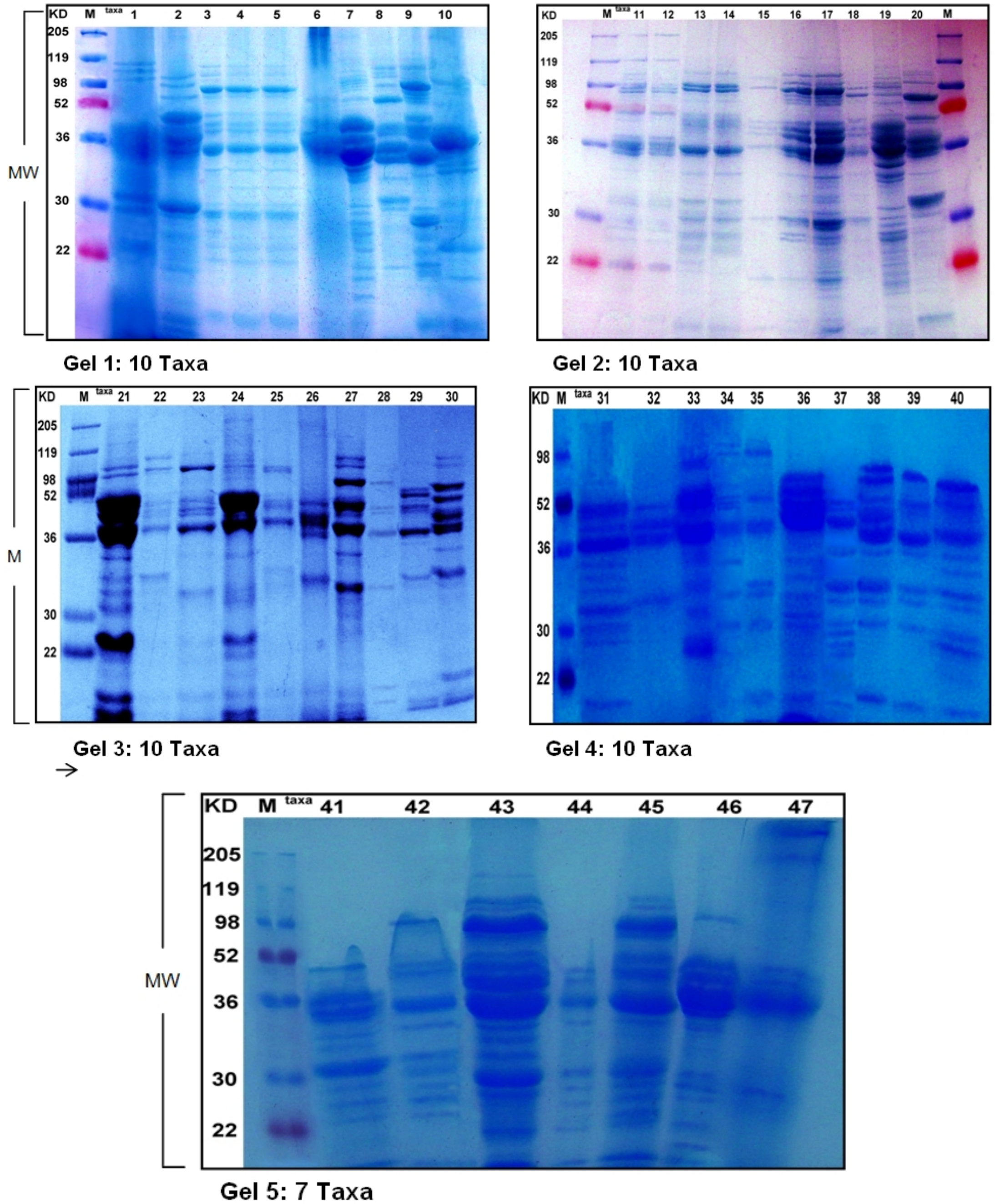

Plate III. Photographs of polyacrylamide gel illustrating electrophoretic band profiles of seed proteins of the studied taxa. KD: kilodalton; M: Marker; MW: Molecular weight.

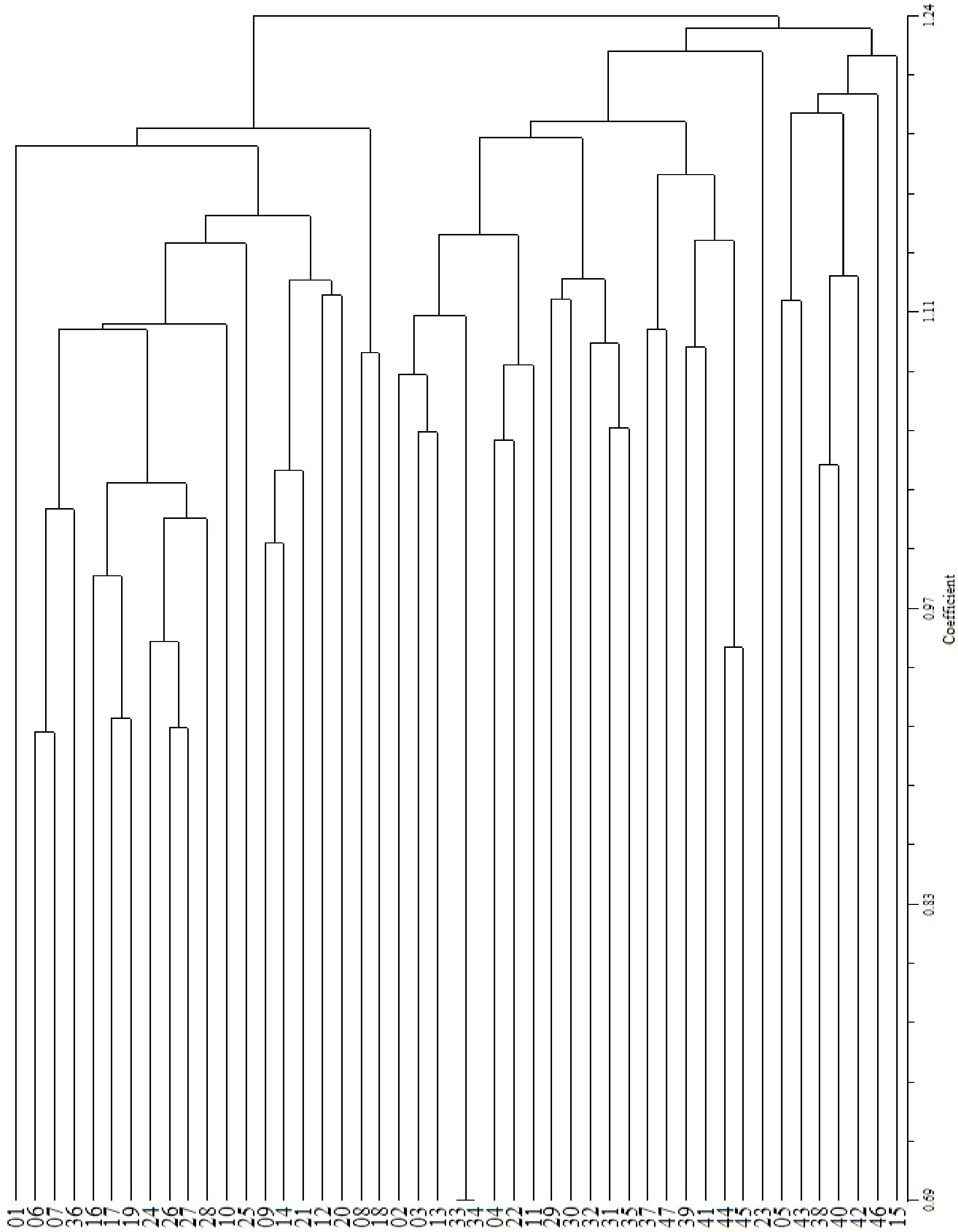

Fig. 1. Dendrogram based on 256 attributes of macro and micromorphology (whole plant and seeds) as a mean of seed protein pattern, illustrating average of taxonomic distance between the studied taxa of Mimosoideae

4. CONCLUSION

The present study confirmed the view of [39] whereas there is no difference of opinion about the phyletic position of the Acacieae is always considered a link between Mimoseae and Ingeae. However different affinities of the genus *Acacia* taken as a natural unit have been suggested [39,40]. The tribe Mimoseae represents the polymorphic and older core of the subfamily and has significantly higher seed diversity, sometimes even with a single genus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Polhill RM, Raven PH, Stirton CH. ′Evolution and systematic of the Leguminosae′. In Polhill RM, Raven PH. Edetors. Advances in Legume Systematics, Part 1. Royal Botanic Gardens: Kew; 1981.
2. Vural C, Ekici M, Akan H. Seed morphology and its systematic implications for genus Astragalus L. sections Onobrychoidei DC., Uliginosi Gray and Ornithopodium Bunge (Fabaceae). Pl. Syst. Evol. Suppl. 2008;274:255–263.
3. Hutchinson J. The Genera of Flowering Plants. Dicotyledones 1. Oxford: Clarendon Press; 1964.
4. Elias TS. The genera of Mimosoideae (Leguminosae) in the South Eastern United Stated. J. Arn. Arbor. 1974;55(1):67-118.
5. Maslin BR. Should Acacia be divided? Bulletin of the international Group for the Study of Mimosoideae. 1988;16:54-76.
6. Mabberley DJ. The Plant Book, a Portable Dictionary of the Vascular Plants 2nd ed. Cambridge: University Press Cambridge, UK; 1997.
7. Orchard AE, Maslin BR. Proposal to conserve the name *Acacia* (Leguminosae: mimosoideae) with a conserved type. Taxon. 2003;52:362-363.
8. Maslin BR. Generic and subgeneric names in *Acacia* following retypification of the genus. Muelleria. 2008;26(1):7-9.
9. Murphy DJ. A review of the classification of *Acacia* (Leguminosae, Mimosoideae). Muelleria. 2008;26(1):10-26.
10. Lewis G, Schrire B, Mackinder B, Lock M. Legumes of the World. Royal Botanic Gardens: Kew; 2005.
11. Boulos L. Flora of Egypt. Vol. (1) Al Hadara Publishing Company: Cairo, Egypt; 1999.
12. Bentham G. Revision of the Suborder Mimoseae. Transactions of the Linnean Society of London. 1875;30:335-670.
13. Taubert P. Mimosoideae in A. Engler and K. Prantl, Nat. Pflanzenfamiien III. 1891-1892;3:99-125.
14. Elias TS. Mimosoideae. In: Polhill RM, Raven PH, editors. Advances in Legume Systematic. Part 1. Royal Botanic Gardens: Kew; 1981.
15. Lewis GP. ′Tribe Acacieae′. In: Lewis GP, Schrire B, Mackinder B, Lock M editors. Legumes of the World. Royal Botanic Garden: Kew; 2005.
16. Luckow M. ′Tribe Mimoseae′. In: Lewis GP, Schrire B, Mackinder B, Lock M, editors. Legumes of the world. Royal Botanic Gardens: Kew; 2005.
17. Luckow M, White PJ. Bruneau A. Genetic Relationships among the Basal Genera of Mimosoid Legumes. In Herendeen PS. Bruneau A. Edetors. Advances in Legume Systematics, part 9. Royal Botanic Gardens: Kew; 2000.

18. Maumont S. Intérêt taxonomique de l'histologie des teguments séminaux chez les *Acacieae* et les Ingeae (Leguminosae-Mimosoideae). Ph.D. Thesis. Université P. Sabatier, France (summary in Bull. Groupe Int. Étude Mimosoideae). 1990;18:151-152. French
19. Maumont S. Seed coat anatomy of the non-pleurogrammic seeds in the tribe Ingeae (Leguminosae, Mimosoideae). Brittonia. 1993;45(3):249-259.
20. Al-Gohary IH, Mohamed AH. Seed morphology of *Acacia* in Egypt and its taxonomic significance. Int. J. Agric. & Biol. 2007;9(3):435-438.
21. Leython S. Jauregui D. Seed Morphology and Anatomy of the seed coat in five species of *Calliandra* (Leguminosae-Mimosoideae) from Venezuela. Revista de Biologia Tropical. 2008;56(3):1075-1086.
22. Burghardt AD, Espert SM. Phylogeny of *Prosopis* (Leguminosae) as shown by morphological and biochemical evidence. Australian Systematic Botany. 2007;20:332-339.
23. Montasir AH, Hassib, M. Illustrated Manual Flora of Egypt. Part I. Bull: Fac. Sci. Ain Shams. Univ; 1956.
24. Täckholm V. Students′ Flora of Egypt. 2nd ed. Cairo University Herbarium; 1974.
25. GRIN, (Germoplasm Resources Information Network) Online Database USDA, ARS, National Resource Lab. Beltsuille, Myryland. 2005. Accessed: September 2008. Available: http://www.ars-gin.gov/var/apache/ege-bin/npgs/html.
26. Barthlott, W. Epidermal and seed surface characters of plants: Systematic applicability and some evolutionary aspects. Nord. J. Bot. 1981;1:345-355.
27. Karam MF. Scanning electron microscope studies of seed characters in *Trifolium* L. (Fabaceae). Phytomorphology. 1997;47(1):51-58.
28. Javadi F, Yamaguchi H. A note on seed coat and plumule morphological variation in the genus *Cicer* L. (Fabaceae). Sci. Rep. Grad. Sch. Agric. & Biol. Sci. 2004;56:7-16
29. Salimpour, Mostafavi G, Sharifnia F. Micromorphologic study of the seed of the genus *Trifolium*, section *Lotoidea*, in Iran. Pak. J. Biol. Sci. 2007;10(3):378-382.
30. Johansen DA. Plant Microtechnique. New York Book Company; 1940.
31. Corner EJH. The Seeds of Dicotyledons, Vol. II. Cambridge: Cambridge University Press; 1976.
32. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-685.
33. Rohlf FJ. NTsys-pc, Numerical Taxonomy and Multivariate Analysis System. Exeter Software: New York; 2000.
34. Dommergues Y, Duhoux E, Diem HG. Les arbres fixateurs d'azote. Orstom, Fao, editions espaces 34: Montpellier; 1999.
35. Vogt K. A Field Guide to the Identification, Propagation and Uses of Common Trees and Shrubs of Dryland Sudan. SOS Sahel International: UK; 1995.
36. Cavanagh AK. The MMorphology and Anatomy of the Mimosoid seeds- towards a standardization of terminology. Bull. Groupe Int. Étude Mimosoideae. 1981;9:61-68.
37. Robbertse PJ. A Scanning electron microscopic investigation of the pollen of South African Acacia species. J. South African Bot. 1974;40:91-99.
38. Guinet P. Geographic Patterns of the Main Pollen Characters in Genus *Acacia* (Leguminosae) With a Particular Reference to Subgenus Phyllodineae. In: Blackmore, Ferguson IK, editors. Pollen and Spores: Form and Function. Academic press: London; 1986.
39. Guinet P. The genus *Acacia* (Leguminosae, Mimosoideae): its affinities as borne out by its pollen characters. Pl.Syst. Evol. Suppl. 1990;5:81-90.

40. Guinet P. Mimosoideae, the Characters of Their Pollen Grains. In: Polhill RM, Raven PH, editors. Advances in legume systematic, Part 2. Royal Botanic Gardens: Kew; 1981.
41. Dnyansagar V. Embryological studies in the Leguminosae, XI. Embryological features and formula and taxonomy of the Mimosaceae. J. Ind. Bot. Soc. 1954;34:362-374.
42. Brenan JP, Brummitt RK. The variation of *Dichrostachys cinera* (L.) Wight & Arn. Bol. Soc. Broteriana. 1965;39:61-115.
43. Tantawy ME, Khalifa SF, Hamed KA, Elazab HM. Palynological study on some taxa of Mimosoideae (Leguminosae). Int. J. Agric. & Biol. 2005;7(6):857-68.
44. Pedley L. Derivation and dispersal of *Acacia* (Leguminosae), with particular reference to Australia, and the recognition of *Senegalia* and *Racosperma*. Bot. J. Linn. Soc. 1986;92:219-254.
45. Guinet P, Hernandez, HM. Pollen characters in the genera *Zapoteca* and *Calliandra* (Leguminoseae, Mimosoideae) their systematic and phylogenetic relevance. Pollen et spores. 1990;31:5-22.
46. Guinet P, Lugardon B. Diverité des structures de l'exine dans le genre *Acacia* (Mimosoideae). Pollen et spores. 1976;18:483-511.
47. El-Azab H. The Pollen Morphology of Certain Leguminosae (Subfamily: Mimosoideae) and Its Sharing in The Taxonomy of The Studied Taxa. Ph.D. Thesis. Botany Department, Faculty of Science, Ain Shams University; 2005.
48. Gunn CR. Fruit and seeds of genera in the subfamily Mimosoideae (Fabaceae). U.S. Department of Agriculture, Agricultural Research Service. Tech. Bull. 1984;1-194.

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