



Significance of Seed Storage Protein and Seed Morphological Characters in the Classification of Some Species of Malvaceae s.l.

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IN THIS study, we evaluated the storage proteins, macro- and micromorphological characters of seeds from 49 species belonging to 34 genera and seven subfamilies of the Malvaceae s.l. (i.e., Malvaceae s.s., Bombacaceae, Sterculiaceae, and Tiliaceae) along with one representative of the Elaeocarpaceae as an outgroup. We obtained microphotographs of the specimens using both light and scanning electron microscopes. The seed storage protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). We analyzed 73 recorded characters (with 157 characters-states) were analyzed using the upgraded WinClada and TNT cladistic programs. A cladogram was constructed which indicated a clear indication about the systematic position between the studied species within the Malvaceae s.l. The results showed specific synapomorphic diagnostic characters are quite significant in species delimitation within the Malvaceae s.l. viz., including seed shape, apex, base, colour, size, texture, hilum shape, position, seed coat sculpture patterns, and occurrence of strophiole. Our results also support the monophyly of the studied genera in Malvoideae except for *Gossypium* and *Thespesia*, while para-polyphyly in the other six subfamilies.

Keywords: Cladistic, Malvaceae s.l., Phylogeny, Seed Morphology, SDS-PAGE, SEM.

Introduction

Malvaceae Juss. are a cosmopolitan family that comprises 243 genera and 4225 species, distributed in tropical and subtropical regions (El-Naggar, 2001; Abid et al., 2016; APG, 2016). Most plants are of great economic, industrial, and medicinal importance. The Malvaceae s.l. (including: Malvaceae s.s., Bombacaceae, Sterculiaceae, and Tiliaceae) were divided into nine subfamilies viz; Grewioideae, Byttnerioideae, Tilioideae, Dombeyoideae, Malvoideae, Bombacoideae, Helicteroideae, Sterculioideae, and Brownlowioideae (APG, 2009; Christenhusz & Byng, 2016; Abdel Khalik et al., 2021). There are many contradictory hypotheses about the phylogenetic relationships between these families. During the last few decades, features of seed macro- and micromorphology have been recorded to be useful and quite significant to resolve many taxonomic problems in various overlapping taxonomic groups within the Malvaceae (Shavvon

et al., 2012; Bona, 2013; Patil et al., 2015a, 2015b; Abid et al., 2016; Azevedo & de Moraes, 2019; Tavakkoli & Assadi, 2019; Masullo et al., 2020).

The most useful features for seed recognition include the seed shape, colour, hilum shape, ornamentation, and seed weight (Ilakiya & Ramamoorthy, 2020; Karakish et al., 2020). Moreover, seed surface patterns have been used to solve taxonomic problems (Karaismailoğlu, 2015, 2019; Fawzi, 2018; Özbek & Uzunhisarcıklı, 2020).

Studies have shown that seed protein electrophoresis is a unique tool that helps resolve taxonomic and evolutionary issues (Ladizinsky & Hymowitz, 1979; Sofalian et al., 2015). Due to the stability, uniformity, and specificity of the protein profile (Robinson & Megarrity, 1975). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage protein is a reliable technique to examine the genetic differences and

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relationships among plant varieties (Payne & Lawrence, 1983; El-Sheekh & Abomohra, 2020; El-Sheekh et al., 2020; Elghamery et al., 2021) and to study the intra- and inter-species variations in plants (Sofalian et al., 2015). Molecular systematics is still considered the most accurate tool for clarifying the diversity among species (Wood & Ohimain, 2020).

The delimitation of the four Malvaceae s.l. families is still problematic (Sanyal, 2016). Moreover, the status of many genera and species is unresolved. Therefore, the aims of this study are: (i) to reveal the most useful seed macro- and micromorphological characters using stereomicroscope (LM) and scanning electron microscope (SEM), (ii) to clarify the taxonomic disposition of different taxa within the family using seed protein electrophoresis, and (iii) to analyze the data cladistically and construct a cladogram to clarify the phylogenetic relationships between the studied taxa within Malvaceae s.l.

Materials and Methods

Materials

We collected mature, dry seeds from 49 species belonging to 34 genera of Malvaceae s.l., along with a representative *Elaeocarpaceae* species as an outgroup, from different localities in Egypt, along with 16 imported species and two herbarium ones. A list of the studied species with their location is provided (Table.1).

The studied taxa were represented by seven subfamilies; Grewioideae, Byttnerioideae, Tilioideae, Dombeyoideae, Sterculioideae, Bombacoideae, and Malvoideae.

Methods

Macromorphological characters

The macromorphological characters include seed shape, color, and size were carried out from the fresh mature dried seeds. Five to ten seeds of each sample were selected to cover the range of variations. The specimens were examined using a Stereomicroscope and Digital Portable Microscope and then photographed by the aid of Nikon Coolpix p600 (60x) Digital Camera; the magnification power was expressed by (x).

Micromorphological characters

For micromorphological characters, the mature dried seeds were mounted on metallic stub using double-sided adhesive tape, then examined and

photographed by Scanning Electron Microscope with Energy-dispersive X-ray spectroscopy (SEM-EDX) QUANTA, FEG 250 at 10 kv using high vacuum mode at the Egypt Desalination Research Center of excellence - EDRC, Desert Research Center, Cairo, Egypt. The terminology adopted was according to Barthlott (1981), Stearn (1983), Cutler et al. (1998), El-Naggar (2001).

Preparation and solubilization of seed storage proteins

Total cellular proteins of seeds (50 samples) were analyzed by SDS-PAGE. The seeds were crushed using liquid nitrogen then collected in Eppendorf tubes and washed once with distilled water and then with 1mL of 1mM NaCl containing 5mM EDTA. Proteins were separated based on molecular weight by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970) and then heated in the presence of low molecular weight thiol (2-mercaptoethanol) and SDS denatured total cellular protein. One volume of the cell suspension was mixed with one volume of 2X treatment buffer (0.25M Tris-HCL pH =6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol) and boiled in a water bath for 2min then quickly transferred to ice water and kept until loading the gel.

Preparation and casting of the SDS-Polyacrylamide Gel

The gels were prepared from a monomer solution of 30% Acrylamide and 2.7% Bis-Acrylamide as separating gel containing 10% acrylamide monomer and stacking gel of 4%. Ammonium persulphate and TEMED were used as initiators for cross-linking and polymerization. The components of the separating gel solution were placed in a 125mL side arm vacuum flask, stoppered flask, and applied vacuum for several minutes. Ammonium persulphate and TEMED were added and the flask gently swirled to mix the solutions. The solution was pipetted into the assembled vertical slab gel unit in the casting mode to level 1.5cm from the top. N-butanol was layered on top of the solution. The gel was left to polymerize at room temperature for two hours. Before the addition of stacking gel, the N-butanol was poured from the surface of the gel and the surface was washed once with overlay buffer. A stacking solution was added to the top of the separating gel, combs were inserted, and the gel was allowed to polymerize for at least half an hour. After polymerization, the combs were removed slowly from the gel and each well was rinsed with tank buffer using a Hamilton syringe.

Protein electrophoresis

The gel apparatus was assembled, and the lower and upper chambers were filled with the tank buffer. A drop of Bromophenol blue was added as a tracking dye. A Hamilton syringe was

used to load equal amounts of proteins (25µL) in each well. A high-range molecular weight protein marker from Bio-Rad was used. Electrophoresis was carried out at about 100 volts (\approx 20-30mA) in 1x Tris/glycine- SDS-running buffer.

TABLE 1. List of the studied taxa and their localities

Families s.s.	No.	Taxa	Localities
Malvaceae	1	<i>Abelmoschus esculentus</i> (L.) Moench	1, 13
	2	<i>Abutilon hirtum</i> (Lam.) Sweet	1, 9
	3	* <i>Alcea rosea</i> L.	1, 2, 6, 9
	4	<i>Gossypium barbadense</i> L.	13
	5	<i>Hibiscus acetosella</i> Welw. ex Hiern	1
	6	<i>Hibiscus mutabilis</i> L.	1
	7	<i>Hibiscus rosa-sinensis</i> L.	Imported from France
	8	* <i>Hibiscus sabdariffa</i> L.	1, 13
	9	<i>Hibiscus syriacus</i> L.	Imported from Munich Germany
	10	<i>Hibiscus tiliaceus</i> L.	1, 3
	11	* <i>Hibiscus trionum</i> L.	Imported from Munich Germany
	12	<i>Lagunaria patersonii</i> (Andrews) G.Don	1
	13	* <i>Malva parviflora</i> L.	1, 9, 14
	14	* <i>Malva sylvestris</i> L.	Herbarium (Alexandria Desert Road)
	15	<i>Malvaviscus arboreus</i> Cav. var. <i>drummondii</i> (Torr. & A. Gray) Schery	Imported from Munich Germany
	16	<i>Pavonia spinifex</i> (L.) Cav.	1
	17	* <i>Sida spinosa</i> L.	Herbarium (Edfo in Cane Sugar Fields)
	18	<i>Thespesia populnea</i> (L.) Sol. ex Corrêa	1, 3, 15
	19	<i>Urena lobata</i> L.	Imported from Munich Germany
Bombacaceae	20	<i>Adansonia digitata</i> L.	Imported from France
	21	<i>Bombax ceiba</i> L.	1, 10, 11
	22	<i>Ceiba pentandra</i> (L.) Gaertn	1
	23	<i>Ceiba speciosa</i> (A.St.-Hil) Ravenna	1, 4, 9
	24	<i>Ochroma pyramidale</i> (Cav. ex Lam.) Urb.	Imported from Munich Germany
	25	<i>Pachira aquatica</i> Aubl.	Imported from Munich Germany
	26	<i>Pseudobombax ellipticum</i> (Kunth) Dugand	1, 5, 12

TABLE 1. Cont.

Families s.s.	No.	Taxa	Localities
Sterculiaceae	27	<i>Abroma augusta</i> (L.) L.f.	Imported from Munich Germany
	28	<i>Brachychiton acerifolius</i> (A.Cunn. ex G. Don) Macarthur	2, 6, 7
	29	<i>Brachychiton australis</i> (Schott & Endl.) A. Terracc.	1, 7
	30	<i>Brachychiton discolor</i> F. Muell.	1, 5
	31	<i>Brachychiton populneus</i> (Schott & Endl.) R.Br.	1
	32	<i>Brachychiton rupestris</i> (T.Mitch. ex Lindl.) K.Schum.	1, 2, 11
	33	<i>Cola acuminata</i> (P. Beauv.) Schott & Endl.	Imported from Munich Germany
	34	<i>Dombeya burgessiae</i> Gerrard ex Harv.	1, 2, 9
	35	<i>Dombeya tiliacea</i> (Endl.) Planch.	2
	36	<i>Dombeya wallichii</i> (Lindl.) Benth. ex Baill.	1
	37	<i>Firmiana simplex</i> (L.) W. Wight	1
	38	<i>Guazuma ulmifolia</i> Lam.	1
	39	<i>Hermannia flammea</i> Jacq.	Imported from Munich Germany
	40	<i>Pterospermum acerifolium</i> (L.) Willd.	1, 7
Tiliaceae	41	<i>Pterygota alata</i> (Roxb.) R. Br.	6, 8
	42	<i>Sterculia foetida</i> L.	5, 6
	43	<i>Sterculia subpeltata</i> Blume	Imported from Munich Germany
	44	<i>Theobroma cacao</i> L.	Imported from Thailand
	45	* <i>Corchorus olitorius</i> L.	1, 13
Tiliaceae	46	<i>Grewia asiatica</i> L.	1
	47	<i>Grewia occidentalis</i> L.	1, 2
	48	<i>Sparmannia ricinocarpa</i> (Eckl. & Zeyh.) Kuntze	Imported from Munich Germany
	49	<i>Tilia americana</i> L.	Imported from United States of America
Elaeocarpaceae (as outgroup)	50	<i>Elaeocarpus angustifolius</i> Blume	Imported from Netherland Holland

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2) Orman Botanical Garden, Giza, Egypt. 10) El-Kalifa El-Maamoun Street, Abbassia, Egypt.
3) Royal Botanical Garden, Palm Hills, 6th of October City, Giza, Egypt. 11) Garden of Faculty of Science, Helwan University, Egypt.
4) Six District, 6th of October City, Giza, Egypt. 12) El-Shams Club Garden, Egypt.
5) Giza Zoo, Giza, Egypt. 13) Kafr El-Sheikh, Egypt.
6) El-Zohria Botanical Garden, Doki, Egypt. 14) Borg El-Arab, International Matrouh El-Alameen Road, Egypt.
7) Agricultural Museum, Doki, Egypt. 15) Kasr El Kobba Palace Botanical Garden, Egypt.
8) Mohamed Ali Palace Garden, El-Manial, Egypt. * (Wild Specimen)

Staining and destaining of the protein gel

After electrophoresis, the gel was stained in 50mL of staining solution (0.125% Coomassie blue R-250, 50% methanol, and 10% acetic acid) for 30-45min with shaking at room temperature and destained in destaining solution (40% methanol and 7% acetic acid). The gel was placed between two sheets of cellophane membrane and dried on a gel drier for 2h and photographed.

Phylogenetic analysis

The seed storage protein, macro- and micromorphological data matrix of the studied taxa of Malvaceae *s.l.* were assembled for 73 characters and 157 seed character states (Tables 2, 3), including one outgroup was subjected to cladistic analysis to elucidate the interrelationships between the studied taxa of Malvaceae *s.l.*

Matrix was constructed using WinClada 1.00.08 (Nixon, 1999, 2002), maximum parsimony (MP) was conducted in TNT (Goloboff et al., 2000, 2008), implementing the parsimony ratchet (Nixon, 1999, 2002).

The most parsimonious trees were estimated using WinClada 1.00.08 (Nixon, 1999, 2002), keeping a maximum of 10000 trees, 1000 replicates, and 10 starting trees per replicate. The shortest tree was condensed and summarized by a strict consensus tree. The data were conducted by using heuristic searches and a tree bisection recognition (TBR) branch swapping algorithm. Characters with inapplicable states were coded using the reductive coding strategy as demonstrated by Strong & Lipscomb (1999). Multistate characters were treated as unordered to avoid false statements on character polarity.

The character states changes were optimized on the cladogram by making ACCTRAN test (ACCELERATES the TRANSformation of characters on the tree) (Farris optimization).

The reliability of clades was assessed by bootstrapping (using 100 replicates addition) (Felsenstein, 1985). Bootstrapping values (close to 100%) mean uniform support, i.e., if boot strapping value for a certain clade is close to 100%, nearly all the characters are informative for that this is a group (Berry &

Gaseuel, 1996).

Results*Seed macro- and micromorphological characters (Tables. 2, 3; Plates I - IV)*

The obtained 10 macro- and micromorphological characters (65 character states) of the studied taxa were summarized as follows:

Seed shapes: varied from globose to sub-globose, ovoid, cuneate ovoid, oblong ovoid, flattened ovoid, ellipsoid, oblong ellipsoid, fusiform, rhomboidal, pyriform, angular, crescent, boat-shaped, reniform to broad reniform. **Seed apex:** obtuse in most taxa except nine taxa are acute and three of acuminate apex. **Seed base:** obtuse in almost taxa except two having acute base and one truncate base. **Seed beak:** present only in eight chocolate brown, reddish brown, scarlet red, dusty to dark or blackish brown, blackish green, dusty black to black. **Trichomes:** present in 14 taxa only while absent in all the 36 remaining taxa. **Seed size:** ranging from very large (more than 10mm), large (6-10mm), medium (3-6mm), small (1-3mm), and very small (0.5-1mm). **Seed coat sculpture pattern:** varied from lineolate, alveolate, areolate, undulate, pusticulate, ribbed, striate, scalariform, colliculate, rugose, ruminant, tuberculate, verrucate, falsifoveate, to foveate and, reticulate. **Hilum:** distinct in all the studied taxa except *Corchorus*, its shape either rounded, elliptic, or slit-like, and varied from apical to sub-apical, basal to sub-basal, or lateral in position. **Strophiole:** present only in all the taxa of sub-family Malvoideae (19 taxa), while absent in the 31 remaining studied taxa.

Seed storage protein (SDS-Polyacrylamide Gel Electrophoresis) (Tables. 2, 3; Plates. V & VI)

The resulted 18 main seed storage protein bands (36 character states) of the studied taxa ranging in their molecular weight from (116kDa to 15kDa) were summarized as follows:

Band of M.W. (116) present in only eight taxa while absent in all the 42 remaining taxa. **Band of M.W. (101)** appeared in 10 taxa and absent in the 40 remaining taxa. **Band of M.W. (93)** present in 21 taxa and absent in the 29 remaining taxa. **Band of M.W. (83)** present in 23 taxa and absent in the 27 remaining taxa. **Band of M.W. (75)** present in 19 taxa while absent in the 31 remaining taxa.

Band of M.W. (70) appeared in 31 taxa while absent in the 19 remaining taxa. **Band of M.W. (62)** present in 20 taxa while absent in the 30 remaining taxa. **Band of M.W. (54)** present in all the studied taxa except outgroup *Elaeocarpus*. **Band of M.W. (47)** present in 31 taxa while absent in the 19 remaining taxa. **Band of M.W. (42)** appeared in 17 taxa while absent in all the 33 remaining taxa. **Band of M.W. (38)** present in 43 taxa while absent in the seven remaining taxa. **Band of M.W. (34)** present in 30 taxa while absent in the 20 remaining taxa. **Band of M.W. (29)** appeared in 26 taxa while absent in the 24 remaining taxa. **Band of M.W. (24)** present in 32 taxa while absent in the 18 remaining taxa. **Band of M.W. (21)** present in 33 taxa while absent in the 17 remaining taxa. **Band of M.W. (19)** was present in 26 taxa while absent in the 24 remaining taxa. **Band of M.W. (17)** present in 21 taxa while absent in the 29 remaining taxa. **Band of M.W. (15)** present in all the studied taxa except outgroup *Elaeocarpus*.

Mostly all the bands were polymorphic and recorded as absent (0) or present (1) without considering intensity.

Discussion

Phylogenetic analysis based on seed storage protein, seed macro- and micromorphological characters:

The cladistic analysis was performed by using (TNT) program to the obtained data of seed storage protein, macro- and micromorphology of (73 characters with 157 character states) (Tables. 2, 3). The equally weighted parsimony analysis of the obtained data yield (150) trees (cladograms) of length (L) = 307. They had a consistency index (CI) = 0.28 and a retention index (RI) = 0.64, while homoplasy index (HI) = 0.72. To choose the most parsimonious tree we made a strict consensus tree that had (32) nodes collapsed.

The resulted cladogram (Figs. 1, 2) revealed that it had a root common ancestor which is the outgroup (*Elaeocarpus angustifolius*), rooted in a separated clade and weakly supported by having oblong-ellipsoid to fusiform seed with obtuse base, black colour, large size, lineolate sculpture, distinct sub-apical hilum. On the other hand, the other four families studied were connected in a large strongly supported clade (BS = 95) sharing the presence of seed storage protein band with

M.W. 54 and 15.

In this respect, Cronquist (1988) noted that Elaeocarpaceae stand apart from the rest of the order Malvales, but even so, the relationship is so close that they had often been included in Tiliaceae. He considered Elaeocarpaceae to be the most “archaic” family in the order due to the absence of all the diagnostic characters shared by the Core Malvacean families. Our results are in agreement with this finding. Furthermore, the results support the view of APG IV (2016) in transferring Elaeocarpaceae to order Oxalidales instead of Malvales.

There is a slight graduation of the studied taxa among sub-familial levels in relation to the outgroup (root of the tree). It was clearly observed that taxa belonging to sub-families (Tilioideae and Sterculioideae) except some subclades are directly attached to the rooted tree with some taxa of Bombacoideae and Byttnerioideae (consider as ancestor and more primitive) while the two taxa of Grewioideae, most taxa of Dombeyoideae and nearly all taxa of Malvoideae (except *Gossypium* and *Thespesia*) were found far away from the root of the tree.

This indicates that Sterculiaceae is the most primitive family among our studied families followed by Tiliaceae, then Bombacaceae, and ending with the most recent (advanced) family Malvaceae this results in accordance with (Cronquist, 1988; Thorne, 1992; La Duke & Doebley, 1995; Judd & Manchester, 1997; Takhtajan, 1997).

From the cladogram (Fig. 1), two large main clades and small clades and sub-clades were obtained representing seven sub-families (Grewioideae, Tilioideae, Dombeyoideae, Sterculioideae, Byttnerioideae, Bombacoideae and Malvoideae).

With respect to the first large main clade, it was strongly supported (BS = 82) by having a seed storage protein band with M.W. of 47 containing the following taxa (all *Brachychiton* spp., *Firmiana*, *Cola*, *Sterculia foetida*, and *Pterygota*) from Sterculioideae, *Guazuma* from Byttnerioideae, *Pterospermum* from Dombeyoideae, *Tilia* from Tilioideae, all the Bombacoideae taxa except *Adansonia*, in addition to *Gossypium* and *Thespesia* from Malvoideae.

TABLE 2. List of seed storage protein, seed macro-, and micromorphological characters and character states with their codes used in cladistics analysis of the studied taxa of the family Malvaceae s.l.

No.	Characters	Character states and their codes	
1	Seed storage proteins	116	Absent (0) Present (1)
2		101	Absent (0) Present (1)
3		93	Absent (0) Present (1)
4		83	Absent (0) Present (1)
5		75	Absent (0) Present (1)
6		70	Absent (0) Present (1)
7		62	Absent (0) Present (1)
8		54	Absent (0) Present (1)
9		47	Absent (0) Present (1)
10		42	Absent (0) Present (1)
11		38	Absent (0) Present (1)
12		34	Absent (0) Present (1)
13		29	Absent (0) Present (1)
14		24	Absent (0) Present (1)
15		21	Absent (0) Present (1)
16		19	Absent (0) Present (1)
17		17	Absent (0) Present (1)
18		15	Absent (0) Present (1)
19	Seed	Globose	Absent (0) Present (1)
20		Sub-globose	Absent (0) Present (1)
21		Ovoid	Absent (0) Present (1)
22		Cuneate-ovoid	Absent (0) Present (1)
23		Oblong-ovoid	Absent (0) Present (1)
24		Flattened-ovoid	Absent (0) Present (1)
25		Ellipsoid	Absent (0) Present (1)
26		Oblong-ellipsoid	Absent (0) Present (1)
27		Fusiform	Absent (0) Present (1)
28		Rhomboidal	Absent (0) Present (1)
29		Pyriform	Absent (0) Present (1)
30		Angular	Absent (0) Present (1)
31		Crescent-shaped	Absent (0) Present (1)
32		Boat-shaped	Absent (0) Present (1)
33		Reniform	Absent (0) Present (1)
34		Broadly reniform	Absent (0) Present (1)
35		Apex	Obtuse (0) Acute (1) Acuminate (2)
36		Base	Truncate (0) Obtuse (1) Acute (2)
37		Beak	Absent (0) Present (1)

TABLE 2. Cont.

No.	Characters	Character states and their codes	
38		Light beige Absent (0) Present (1)	
39		Yellowish brown Absent (0) Present (1)	
40		Golden brown Absent (0) Present (1)	
41		Chocolate brown Absent (0) Present (1)	
42	Colour	Reddish brown Absent (0) Present (1)	
43		Scarlet red Absent (0) Present (1)	
44		Dusty brown Absent (0) Present (1)	
45		Dark brown Absent (0) Present (1)	
46		Blackish brown Absent (0) Present (1)	
47		Blackish green Absent (0) Present (1)	
48		Dusty black Absent (0) Present (1)	
49		Black Absent (0) Present (1)	
50		Trichomes	Occurance Absent (0) Present (1)
51			Types Unicellular, unfused (0) Unicellular, stellate (1) Tufts of (3-6) multicellular, fused (Fan-shaped) (2) Glandular (3)
52	Persistence Deciduous (0) Persistent (1)		
53	Size	Very large (more than 10 mm) (0) Large (6-10 mm) (1) Medium (3-6 mm) (2) Small (1-3 mm) (3) Very small (0.5-1 mm) (4)	
54	Seed Epidermal Coat Sculpture Pattern	Lineolate Absent (0) Present (1)	
55		Alveolate Absent (0) Present (1)	
56		Areolate Absent (0) Present (1)	
57		Undulate Absent (0) Present (1)	
58		Pusticulate Absent (0) Present (1)	
59		Ribbed Absent (0) Present (1)	
60		Striate Absent (0) Present (1)	
61		Scalariform Absent (0) Present (1)	
62		Colliculate Absent (0) Present (1)	
63		Rugose Absent (0) Present (1)	
64		Ruminate Absent (0) Present (1)	
65		Tuberculate Absent (0) Present (1)	
66		Verrucate Absent (0) Present (1)	
67		Falsifoveate Absent (0) Present (1)	
68		Foveate Absent (0) Present (1)	
69		Reticulate Absent (0) Present (1)	
70		Visibility Indistinct (0) Distinct (1)	
71	Hilum	Shape of aperature Rounded (0) Elliptic (1) Slit-like (2)	
72		Position Apical (0) Sub-apical (1) Basal (2) Sub-basal (3) Lateral (4)	
73	Strophiole	Absent (0) Present (1)	

TABLE 3. Data matrix of the seed storage protein, macro-, and micromorphological character states values of the studied taxa of Malvaceae s.l. used in the cladistic analysis, (S) scored for the polymorphic characters, and (-) for inapplicable character states

No.	Taxa	No. of characters																																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37						
0	<i>Elaeocarpus angustifolius</i> (Outgroup)	0	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0				
1	<i>Abelmoschus esculentus</i>	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0		
2	<i>Abutilon hirtum</i>	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	
3	<i>Alcea rosea</i>	1	0	1	1	1	0	1	0	1	1	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	
4	<i>Gossypium barbadense</i>	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1		
5	<i>Hibiscus acetosella</i>	0	0	0	0	0	1	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	
6	<i>Hibiscus mutabilis</i>	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	
7	<i>Hibiscus rosa-sinensis</i>	0	0	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
8	<i>Hibiscus sabdariffa</i>	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	
9	<i>Hibiscus syriacus</i>	1	0	1	1	1	0	1	1	1	1	1	0	0	1	1	0	1	1	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
10	<i>Hibiscus tiliaceus</i>	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
11	<i>Hibiscus trionum</i>	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	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
12	<i>Lagunaria patersonii</i>	0	1	1	0	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
13	<i>Malva parviflora</i>	0	1	1	0	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	
14	<i>Malva sylvestris</i>	0	1	1	0	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	
15	<i>Malva viscosa</i> var. <i>drummondii</i>	0	1	1	0	1	1	1	1	1	1	1	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	0	1	0	0	1	1	
16	<i>Pavonia spinifex</i>	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	
17	<i>Sida spinosa</i>	0	0	0	0	0	1	1	1	1	1	1	0	0	1	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	1	0	
18	<i>Thespesia populnea</i>	0	1	1	1	1	1	1	1	1	1	1	0	1	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	1	1	1	
19	<i>Urena lobata</i>	1	0	1	1	1	1	1	0	1	1	1	0	1	0	1	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	
20	<i>Adansonia digitata</i>	0	0	0	0	1	1	0	0	1	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	0	0	0	1	0	0	0	1	
21	<i>Bombax ceiba</i>	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	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	2	1	
22	<i>Ceiba pentandra</i>	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	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	
23	<i>Ceiba speciosa</i>	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	

TABLE 3. Cont.

No.	Taxa	No. of characters																																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37									
24	<i>Ochroma pyramidale</i>	1	1	1	1	1	1	1	1	1	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	0	0	2	1	1							
25	<i>Pachira aquatica</i>	1	1	1	1	1	1	1	1	1	1	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	0	0	0	1	0						
26	<i>Pseudobombax ellipticum</i>	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0					
27	<i>Abroma augusta</i>	0	0	0	0	0	0	1	0	0	1	1	1	1	1	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	1	0				
28	<i>Brachychiton acerifolius</i>	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
29	<i>Brachychiton australis</i>	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
30	<i>Brachychiton discolor</i>	0	0	0	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
31	<i>Brachychiton populneus</i>	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
32	<i>Brachychiton rupestris</i>	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
33	<i>Cola acuminata</i>	0	0	1	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0				
34	<i>Dombeva burgessiae</i>	0	0	0	0	0	0	1	0	0	1	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	0	0			
35	<i>Dombeva tiliacea</i>	0	0	0	0	1	0	1	0	0	1	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	1	0			
36	<i>Dombeva wallichii</i>	0	0	0	0	1	0	1	0	0	1	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	1	0			
37	<i>Firmiana simplex</i>	0	0	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0			
38	<i>Guazuma ulmifolia</i>	0	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	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	1	0		
39	<i>Hermannia flammea</i>	0	0	0	0	1	0	1	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	0	1	0		
40	<i>Pterospermum acerifolium</i>	0	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	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	1	0	
41	<i>Pterygota alata</i>	0	0	1	1	1	1	0	1	1	0	1	1	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	0	0	0	0	0	0	1	0	
42	<i>Sterculia foetida</i>	0	0	1	1	1	1	0	1	1	0	1	1	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	0	0	0	0	0	0	1	0	
43	<i>Sterculia subpelata</i>	0	0	0	1	1	0	0	1	0	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	0	0	0	0	0	0	0	1	0
44	<i>Theobroma cacao</i>	0	0	0	0	0	0	1	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	0	0	0	1	0
45	<i>Corchorus olitorius</i>	1	0	0	1	1	0	1	1	0	1	1	1	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	0	0	0	0	0	0	2	1	
46	<i>Grewia asiatica</i>	0	0	0	0	1	0	1	0	0	1	1	1	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	0	0	0	0	0	0	0	1	0
47	<i>Grewia occidentalis</i>	0	0	0	0	1	0	1	0	0	1	1	1	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	0	0	0	0	0	0	0	1	0
48	<i>Sparmannia ricinocarpa</i>	1	0	0	0	1	0	1	1	0	1	1	1	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	0	0	0	0	0	0	1	0	
49	<i>Tilia americana</i>	0	0	0	0	0	0	1	1	0	1	1	1	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	0	0	0	0	0	0	0	1	0

TABLE 3. Cont.

No.	Taxa	No. of characters																																								
		38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73					
0	<i>Elaeocarpus angustifolius</i> (Outgroup)	0	0	0	0	0	0	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	0	0	0	0	0	0	0		
1	<i>Abelmoschus esculentus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	4	1	
2	<i>Abutilon hirtum</i>	0	0	0	0	1	0	0	0	0	0	0	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	2	4	1			
3	<i>Alecea rosea</i>	0	0	0	0	1	0	0	0	0	0	0	1	\$	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	2	4	1				
4	<i>Gossypium barbadense</i>	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	2	1		
5	<i>Hibiscus acetosella</i>	0	0	0	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	4	1			
6	<i>Hibiscus mutabilis</i>	0	1	0	0	0	0	0	0	0	0	0	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	4	1				
7	<i>Hibiscus rosa-sinensis</i>	0	0	0	0	0	0	0	0	0	0	1	1	3	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	2	4	1			
8	<i>Hibiscus sabdariffa</i>	0	1	0	0	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	4	1				
9	<i>Hibiscus syriacus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	4	1			
10	<i>Hibiscus tiliaceus</i>	0	0	1	0	0	0	0	0	0	0	0	1	\$	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	2	4	1				
11	<i>Hibiscus trionum</i>	0	0	0	0	0	0	0	0	0	0	0	1	\$	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	2	4	1			
12	<i>Lagunaria patersonii</i>	0	0	0	0	0	1	0	0	0	0	0	0	—	—	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	4	1		
13	<i>Mahva parviflora</i>	0	0	0	0	0	0	0	0	0	0	0	0	—	—	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	2	4	1	
14	<i>Mahva sylvestris</i>	0	0	0	0	0	0	0	0	0	0	0	0	—	—	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	2	4	1	
15	<i>Mahavisicus arboreus</i> var. <i>drummondii</i>	0	0	0	0	1	0	0	0	0	0	0	1	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	2	4	1	
16	<i>Pavonia spinifex</i>	0	0	0	0	1	0	0	0	0	0	0	0	—	—	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	4	1	
17	<i>Sida spinosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	—	—	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1		
18	<i>Thespesia populnea</i>	0	0	1	0	0	0	0	0	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	0	0	1	1	0	2	1	
19	<i>Urena lobata</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	4	1		
20	<i>Adansonia digitata</i>	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	0	0	0	0	0	0	1	0	1	0		
21	<i>Bombax ceiba</i>	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	1	1	0	0		
22	<i>Ceiba pentandra</i>	0	0	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	0	0	0	0	0	0	1	1	0	0	
23	<i>Ceiba speciosa</i>	0	0	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	0	0	0	0	0	0	1	1	0	0	

TABLE 3. Cont.

No.	Taxa	No. of characters	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73				
24	<i>Ochroma pyramidale</i>		0	0	1	0	0	0	0	0	0	0	0	0	0	0	—	—	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0				
25	<i>Pachira aquatica</i>		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	1	0	1	0	0	0	0	0		
26	<i>Pseudobombax ellipticum</i>		0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0		
27	<i>Abronia augusta</i>		0	0	0	0	0	0	0	0	0	0	1	0	0	0	—	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	2	0	0		
28	<i>Brachychiton acerifolius</i>		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	1	1	0	0	1	0	1	0	2	0	0		
29	<i>Brachychiton australis</i>		0	0	0	0	0	0	0	0	0	1	0	0	0	0	—	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	2	0	0	
30	<i>Brachychiton discolor</i>		0	0	0	0	0	0	0	0	0	0	1	0	0	0	—	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0	
31	<i>Brachychiton populneus</i>		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	1	1	0	0	1	0	1	0	2	0	0	
32	<i>Brachychiton rupestris</i>		1	0	0	0	0	0	0	0	0	0	0	0	0	0	—	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	2	0	0	
33	<i>Cola acuminata</i>		0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	2	0	0
34	<i>Dombeya burgesiae</i>		0	0	0	0	1	0	0	0	0	0	0	0	0	0	—	3	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0
35	<i>Dombeya tiliacea</i>		0	0	0	0	1	0	0	0	0	0	0	0	0	0	—	3	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	
36	<i>Dombeya wallichii</i>		0	0	0	0	1	0	0	0	0	0	0	0	0	0	—	3	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0
37	<i>Firmiana simplex</i>		0	0	1	0	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	1	0	1	1	1	1	3	0	0	
38	<i>Guazuma ulmifolia</i>		0	0	1	0	0	0	0	0	0	0	0	0	0	0	—	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	
39	<i>Hermannia flammea</i>		0	0	1	0	0	0	0	0	0	0	0	0	0	0	—	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	4	0	0	0	0	
40	<i>Pterospermum acerifolium</i>		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	1	1	0	2	0	0	0	
41	<i>Pterygota alata</i>		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	1	1	0	2	0	0	0	
42	<i>Sterculia foetida</i>		0	0	0	0	0	0	0	0	0	1	0	0	0	0	—	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2	0	0	0	0	
43	<i>Sterculia subpeltata</i>		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	0	0	1	0	0	0	1	0	2	0	0	0	0	
44	<i>Theobroma cacao</i>		0	0	0	1	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	1	0	2	0	0	0	0	
45	<i>Corchorus olitorius</i>		0	0	0	0	0	0	0	0	1	0	0	0	0	0	—	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
46	<i>Grewia asiatica</i>		1	0	0	0	0	0	0	0	0	0	0	0	0	0	—	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	0	0	0	
47	<i>Grewia occidentalis</i>		1	0	0	0	0	0	0	0	0	0	0	0	0	0	—	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	
48	<i>Sparmannia ricinocarpa</i>		0	0	0	0	0	0	0	1	0	0	0	0	0	0	—	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	3	0	0	0	
49	<i>Tilia americana</i>		0	0	0	0	0	0	0	1	0	0	0	0	0	0	—	3	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	

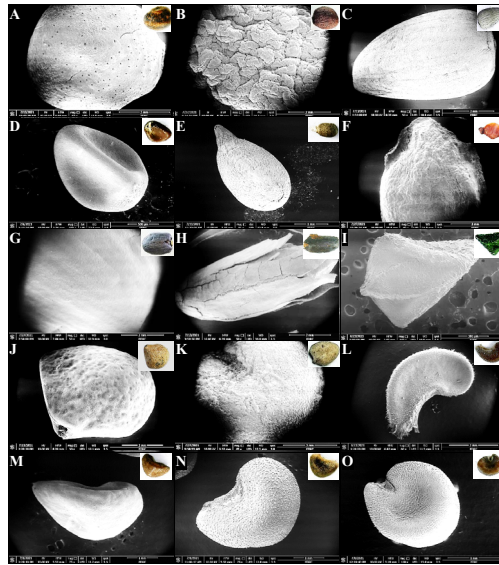


Plate I. Scanning electron micrographs for different seed shapes, A. globose in *Ceiba pentandra*, B. sub-globose in *Cola acuminata*, C. ovoid in *Brachychiton rupestris*, D. cuneate-ovoid in *Sida spinosa*, E. oblong-ovoid in *Ochroma pyramidale*, F. flattened-ovoid in *Pterospermum acerifolium*, G. ellipsoid in *Sterculia foetida*, H. oblong-ellipsoid to fusiform in *Elaeocarpus angustifolius*, I. rhomboidal in *Corchorus olitorius*, J. pyriform in *Grewia occidentalis*, K. angular in *Adansonia digitata*, L. crescent-shaped in *Alcea rosea*, M. boat-shaped in *Pavonia spinifex*, N. reniform in *Hibiscus tiliaceus*, O. broadly reniform in *Malva parviflora*

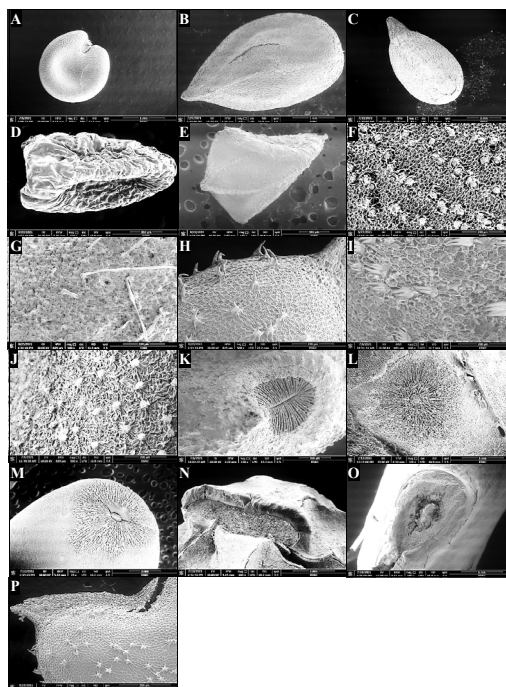


Plate II. Scanning electron micrographs for different seed apex, base, trichomes, hilum shape, position, and strophiole, A. obtuse apex and base in *Malva sylvestris*, B. acute apex in *Malvaviscus arboreus* Cav. var. *drummondii*, C. acuminate beaked apex in *Ochroma pyramidale*, D. truncate base in *Dombeya burgessiae*, E. acute base in *Corchorus olitorius*, F. deciduous trichomes in *Abelmoschus esculentus*, G. unicellular unfused trichomes in *Gossypium barbadense*, H. unicellular stellate trichomes in *Abutilon hirtum*, I. tufts of (3-6) multicellular fused trichomes in *Hibiscus acetosella*, J. glandular trichomes in *Hibiscus tiliaceus*, K. slit-like lateral hilum in *Hibiscus rosa-sinensis*, L. rounded apical hilum in *Ceiba speciosa*, M. rounded basal hilum in *Brachychiton australis*, N. elliptic sub-basal hilum in *Firmiana simplex*, O. rounded sub apical in *Elaeocarpus angustifolius*, P. strophiole in *Abutilon hirtum*

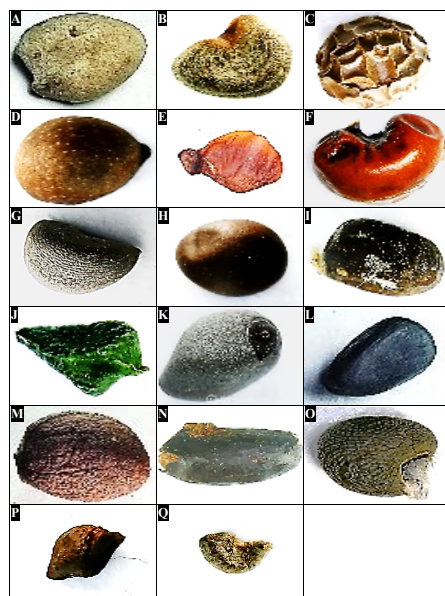


Plate III. Scanning electron micrographs for different seeds colour and sizes, A. light beige in *Adansonia digitata*, B. yellowish beige in *Hibiscus sabdariffa*, C. golden brown in *Firmiana simplex*, D. chocolate brown in *Bombax ceiba*, E. reddish brown in *Pterospermum acerifolium*, F. scarlet red in *Lagunaria patersonii*, G. dusty brown in *Urena lobata*, H. dark brown in *Pseudobombax ellipticum*, I. blackish brown in *Gossypium barbadense*, J. blackish green in *Corchorus olitorius*, K. dusty black in *Brachychiton australis*, L. black in *Brachychiton discolor*, M. very large in *Cola acuminata*, N. large in *Elaeocarpus angustifolius*, O. medium in *Abelmoschus esculentus*, P. small in *Dombeya tiliacea*, Q. very small in *Hermannia flammea*

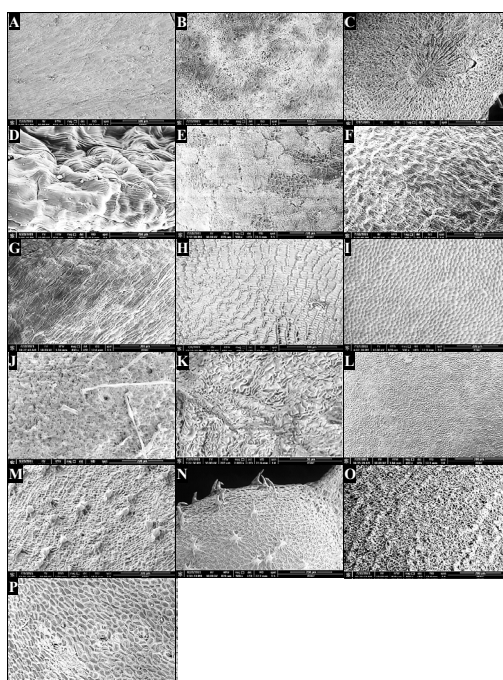


Plate IV. Scanning electron micrographs for different seed coat sculpture patterns, A. lineolate in *Elaeocarpus angustifolius*, B. alveolate in *Grewia occidentalis*, C. areolate in *Hibiscus mutabilis*, D. undulate in *Dombeya burgessiae*, E. pusticulate in *Guazuma ulmifolia*, F. ribbed in *Tilia americana*, G. striate in *Dombeya tiliacea*, H. scalariform in *Malva parviflora*, I. colliculate in *Sterculia foetida*, J. rugose in *Gossypium barbadense*, K. ruminant in *Corchorus olitorius*, L. tuberculate in *Sterculia subpeltata*, M. verrucate in *Hibiscus trionum*, N. falsifoveate in *Abutilon hirtum*, O. foveate in *Urena lobata*, P. reticulate in *Ceiba speciosa*

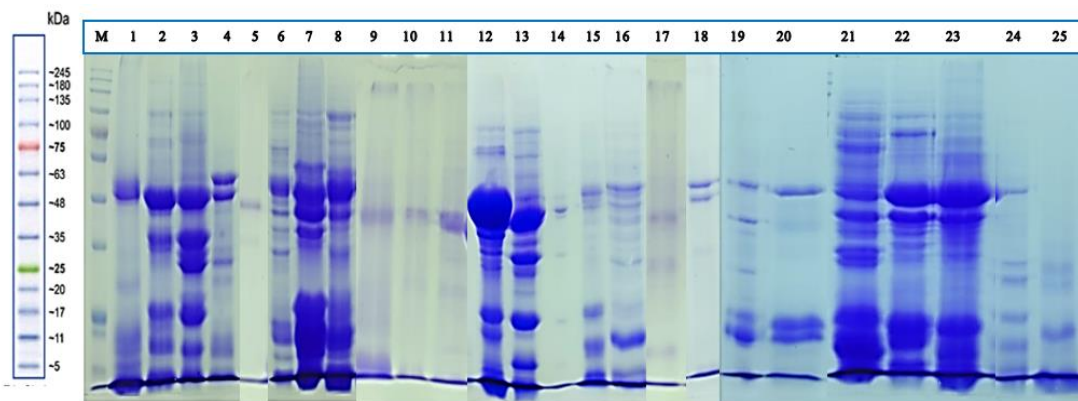


Plate V. Seed storage protein profile bands using (SDS-polyacrylamide gel electrophoresis) of the specimens from (1) to (25) as arranged in the materials table, supported with the marker

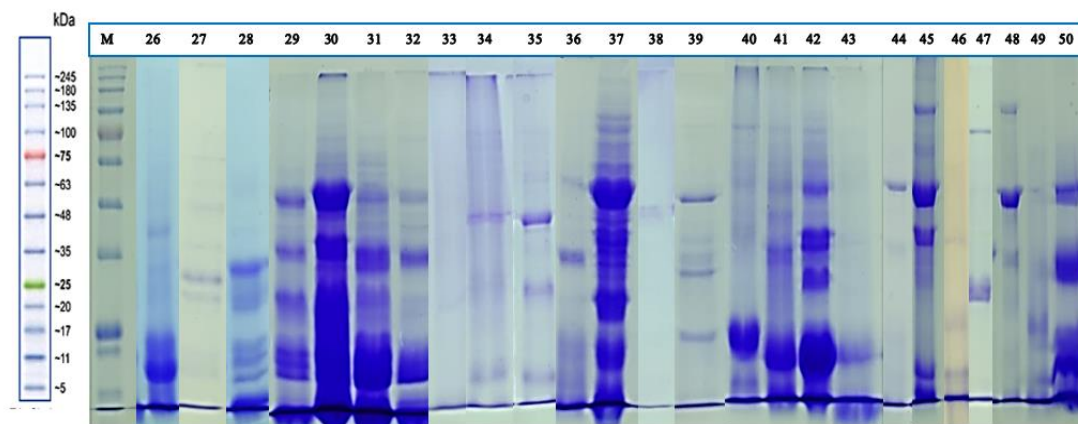


Plate VI. Seed storage protein profile bands using (SDS-polyacrylamide gel electrophoresis) of the specimens from (26) to (50) as arranged in the materials table, supported with the marker

We could clearly see that *Brachychiton discolor* was separated alone in one clade moderately supported (BS= 64) by having a colliculate seed coat sculpture pattern. Then we had two medium long clades, the first one moderately supported clade (BS= 55) carrying most of the Bombacoideae taxa (*Bombax*, *Pseudobombax*, two *Ceiba* spp.) and the rest of *Brachychiton* spp. from Sterculioideae, in addition to *Tilia* from Tilioideae. While the second clade represents (*Firmiana*, *Cola*, *Sterculia foetida*, and *Pterygota*) from Sterculioideae, *Guazuma* from Byttnerioideae, *Pterospermum* from Dombeyoideae, *Ochroma*, and *Pachira* from Bombacoideae, in addition to *Gossypium* and *Thespesia* from Malvoideae.

We could see *Bombax* and *Pseudobombax* appeared alone in a clade parallel to each other. So, it clearly appeared that *Pseudobombax* is closely related to *Bombax* while far away from *Pachira* in another strongly supported clade (BS=96). This result supports the parphyly of subfamily

Bombacoideae. Our result in accordance with Carvalho-Sobrinho & Queiroz (2011).

After that, we had the two *Ceiba* spp. clade strongly supported (BS= 94) sharing the absence of seed storage protein band with M.W. of 34 and 29, globose to angular seed with blackish brown colour. *Ceiba speciosa* was separated from *C. pentandra* by the presence of a seed storage protein band with M.W. of 116, 101, 93, 83, 75, 70, 62, and verrucate seed coat sculpture pattern. Concerning the second small clade, we could see clearly that *Tilia* spp. was separated alone in one clade strongly supported (BS= 86) by having a dark brown seed, small size, with ribbed to scalariform sculpture pattern.

The rest of the clade carries out the other four *Brachychiton* spp. moderately supported (BS= 60) by having verrucate to falsifoveate seed coat sculpture pattern.

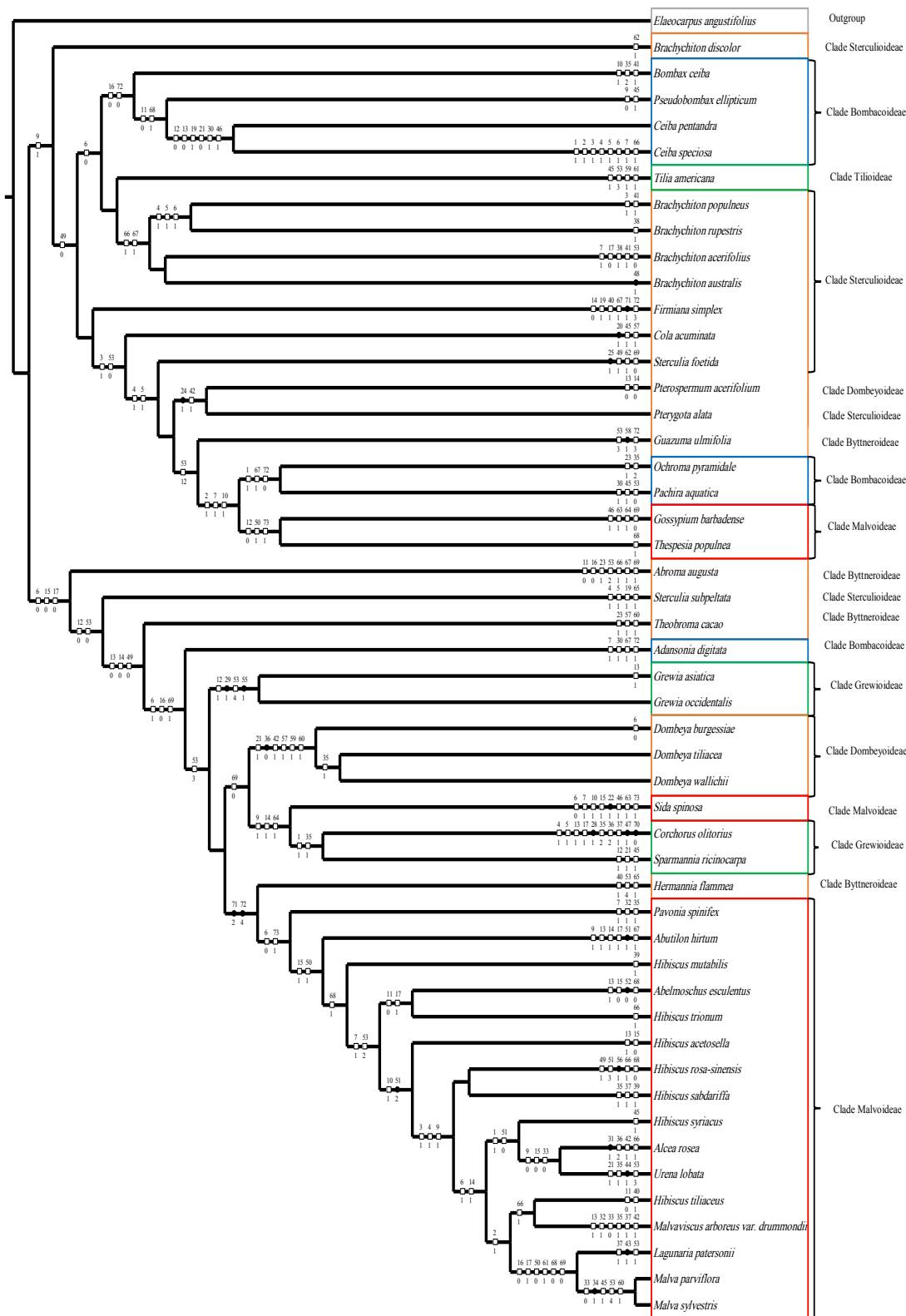


Fig. 1. A representative cladogram based on 73 seed storage proteins, macro- and micromorphological characters, and 157 character states of the studied taxa of the family Malvaceae s.l. Numbers above the branches represent character number, and those below the branch indicates the number of character states; black circle shows the homologous character, while the white rectangular shows the homoplasious character

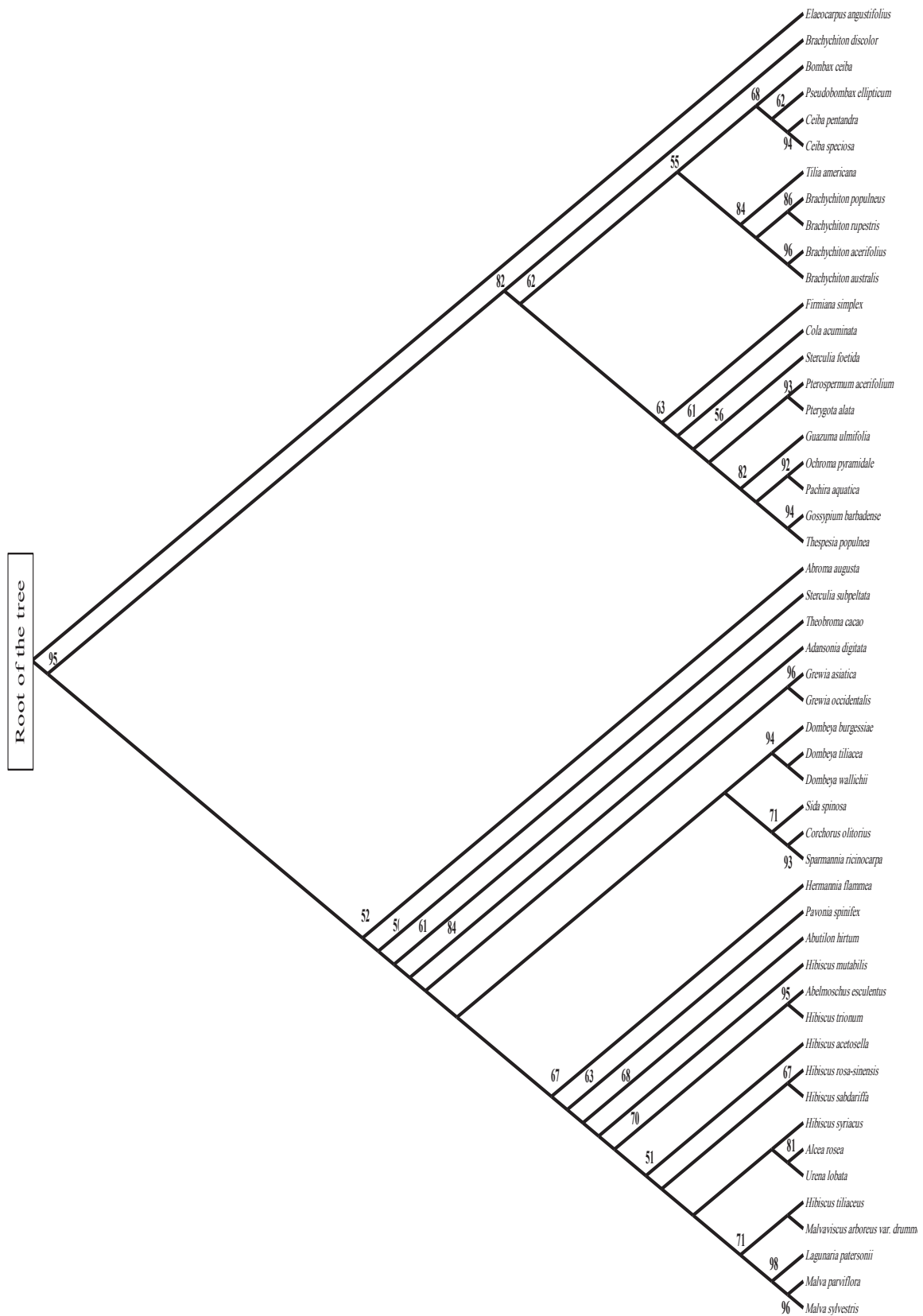


Fig. 2. The rooted shape of one of the resulting trees (cladogram) shows Boot Strapping values supporting each branch of the tree [Values < 50 weak support, values = 50 moderate support, values > 50 strong support]

Then we had *B. acerifolius* with *B. australis* in a strongly supported clade (BS = 96). *Brachychiton acerifolius* was separated by the presence of seed storage protein band with M.W. of 62, absence of that with M.W. of 17, light beige to chocolate brown very large seed, while *B. australis* is dusty black. Özbek & Uzunhisarcıklı (2020) demonstrated that dimension and seed pattern is important morphological characters for the separation of the studied taxa.

With respect to family Sterculiaceae, the cladogram revealed the presence of small clear clades (*Dombeya spp.* and *Brachychiton spp.*), the other taxa: *Guazuma*, *Pterospermum*, *Firmiana*, *Pterygota*, *Cola*, *Abroma*, *Theobroma*, *Hermannia*, and *Sterculia* separated each in parallel clades supported by a few characters. This clearly indicates the paraphyly of this family (Shamsi & Khattab, 2016).

In the case of the second medium long clade, we had *Firmiana*, *Cola*, and *Sterculia foetida* all in three parallel moderately supported (BS = 63, 61, and 56) clades. *Firmiana* was separated by the absence of seed storage protein band with M.W. of 24, globose golden brown seed, falsifoveate sculpture pattern, and elliptic sub-basal hilum. While *Cola* was separated by having a sub-globose dark brown seed, and undulate sculpture pattern. *Sterculia foetida* was separated by having an ellipsoid black seed, colliculate to reticulate sculpture pattern.

For the rest of the clade, we had *Pterospermum* from Dombeyoideae and *Pterygota* from Sterculioideae linked together in small strongly supported (BS= 93) clade sharing flattened-ovoid reddish brown winged seed. *Pterospermum* was separated from *Pterygota* by the absence of seed storage protein band with M.W. of 29 and 24. The winged seed of the genus *Pterospermum* (sub-family Dombeyoideae) aids in the dispersal with the wind (Ganesan et al., 2020), and our findings are congruent with these results. After that we had *Guazuma* separated alone in one clade strongly supported (BS= 82) by having a small size, light beige seed, with sub-basal hilum.

Furthermore, we could see two of the Bombacoideae taxa (*Ochroma* and *Pachira*) linked with two of the Malvoideae taxa (*Gossypium* and *Thespesia*) sharing the presence of seed storage protein band with M.W. of 101, 62 and 42. With

respect to the Bombacoideae clade, both *Ochroma* and *Pachira* were linked together in strongly supported (BS= 92) clade sharing the presence of seed storage protein band with M.W. of 116, 62 and having apical hilum. *Ochroma* could be distinguished from *Pachira* by having an oblong ovoid seed with an acuminate apex, while *Pachira* was separated by having angular, dark brown, and very large seed.

Concerning the Malvoideae clade, both *Gossypium* and *Thespesia* were linked together in strongly supported (BS = 94) clade sharing the absence of seed storage protein band with M.W. of 34, presence of seed trichomes and strophiole. Seed colour is a good reliable constant character and is used for taxa delimitation (Ahmed & Qaiser, 1989). The seed sculpture pattern is effective as a diagnostic parameter in taxa delimitation (Perveen & Qaiser, 2012). These findings are in agreement with our results. Also, the strophiolate seed is one important feature of the sub-family Malvoideae (Abid et al., 2016). Our findings agree with their results.

Gossypium could be distinguished from *Thespesia* by having blackish brown seed, and rugose to ruminant-reticulate sculpture pattern, while *Thespesia* was foveate sculpture pattern. So, the distinctive tribe Gossypieae has been considered transitional between Bombacaceae and Malvaceae (Cronquist, 1981; Ibrahim et al., 2018). The present analysis also supports this view.

The other large clade has the rest 29 taxa, sharing the absence of seed storage protein band with M.W. of 75, 21, 17. At first, we had two Byttnerioideae taxa (*Abroma* and *Theobroma*) in addition to one Sterculioideae taxon (*Sterculia subpeltata*) appeared in three parallel moderately supported (BS= 52, 50, and 61) clades. *Abroma* could be distinguished from others by the absence of seed storage protein band with M.W. of 38, 19, oblong ovoid medium seed, with verrucate, falsifoveate to reticulate sculpture pattern. *Sterculia subpeltata* was separated by having a seed storage protein band with M.W. of 83, 75, globose seed, and tuberculate sculpture pattern. While *Theobroma* was separated by having oblong-ovoid seed, undulate to striate sculpture pattern.

After that we had the last studied taxon of sub-family Bombacoideae *Adansonia* appeared alone

in separated parallel clade strongly supported (BS= 84) by having seed storage protein band with M.W. of 62, angular seed, falsifoveate sculpture pattern, and sub-apical hilum.

Then we had eight small clades in addition to eight small parallel sub-clades. At first, we had two *Grewia spp.* (sub-family Grewioideae) linked together in one strongly supported (BS= 96) clade sharing the presence of seed storage protein band with M.W. of 34, pyriform very small seed, alreolate sculpture pattern.

Grewia asiatica was separated from *G. occidentalis* by having a seed storage protein band with M.W. of 29. After that we had a medium clade divided into two small clades, the first one carrying three *Dombeya spp.* and the other one having *Sida* from Malvoideae alone in a separated clade in addition to *Corchorus* and *Sparmannia* (sub-family Grewioideae) linked to each other. Sub-family Grewioideae has quite diverse and stable seed morphological characters which could be used to delimit the taxa at generic and specific levels (Ya et al., 2007). In our work, the genus *Corchorus* (sub-family Grewioideae) was separated by having angular seeds while the genus *Grewia* has a non angular seed.

With respect to *Dombeya spp.* clade, it was strongly supported (BS= 94) and all taxa sharing the ovoid seed with truncate base, reddish brown colour, undulate, ribbed, and striate sculpture patterns. *Dombeya burgessiae* was separated by the absence of seed storage protein band with M.W. of 70, while both *D. tiliacea* and *D. wallichii* share acute seed apex. These taxa of *Dombeya* moved between the two families, Sterculiaceae and Tiliaceae. So, the results revealed that tribe Dombeyeae from family Sterculiaceae is nested together within Tiliaceae. This indicates that Dombeyeae is more closely related to sub-family Dombyoideae than sub-family Sterculioideae.

For the sub-family Dombyoideae, our results support that *D. burgessiae* consider a monophyletic clade while *D. tiliacea* and *D. wallichii* are paraphyletic due to the broad morphological variation within the genus. Our findings partly agree with Skema (2012) result. The results demonstrated that *Sida* (sub-family Malvoideae) was separated with a single taxonomic distance from other species. Our finding is in agreement with that of Said et al. (2018).

After this we had *Sida* from Malvoideae appeared alone in separated strongly supported (BS= 71) clade sharing the absence seed storage protein band with M.W. of 70, presence of that with M.W. of 62, 42, 21, cuneate ovoid blackish brown seed, with rugose sculpture pattern and presence of strophiole.

Then we had two Grewioideae *spp. Corchorus* in a strongly supported clade (BS= 93) separated from *Sparmannia* by having seed storage protein band with M.W. of 83, 75, 29, 17, rhomboidal seed with acuminate apex, acute base, presence of beak, blackish green colour, and indistinct hilum. While *Sparmannia* has seed storage protein band with M.W. of 34, ovoid dark brown seed. So, it is clear that clade of *Grewia* is monophyletic while *Corchorus* clade is paraphyletic.

After that, we had one large clade strongly supported (BS= 82) by having a slit-like lateral hilum which carried out the rest five small clades in addition to the rest seven parallel sub-clades. All taxa in this clade represented under sub-family Malvoideae except *Hermannia* from sub-family Byttnerioideae which had been appeared alone in separated clade moderately supported (BS= 67) by having golden brown, very small seed, and tuberculate sculpture pattern. The darkness of some *Hermannia* species has been found to relate to the tannin content. The tannins often remain white until the last stage of development (Dickie & Stuppy, 2003). This phenomenon leads to increased wall strength and resistance to microorganisms.

After that, we could see three malvacean taxa (*Pavonia*, *Abutilon*, and *Hibiscus mutabilis*) appeared in small parallel moderately supported sub-clades (BS= 63, 68, and 70). Also, it must be noticed that almost all Malvoideae taxa share the presence of strophiole in their seeds and have different types of seed trichomes except *Lagunaria*, two *Malva spp.*, *Pavonia*, and *Sida*, with glabrous seeds. Also, all have slit-like lateral hilum except *Gossypium*, *Thespesia*, and *Sida* having rounded basal hilum. Most Malvoideae taxa have reniform seed shape except *Alcea* crescent-shaped, *Gossypium*, *Thespesia*, and *Urena* ovoid, both two *Malva spp.* broad reniform, *Pavonia*, and *Malvaviscus* boat shaped, *Sida* cuneate ovoid.

There is a correlation between seed morphology

and plant form in which smaller seeds predict the herbaceous nature (Malvaceae and Tiliaceae) while larger seeds are associated with primitive shrubs and trees habit (Bombacaceae and Sterculiaceae) agreed with Gómez et al. (2019). Also, hairy seeds are regarded to be more advanced than glabrous seeds. Trichomes serve as physical barriers for defense against insects (Kariyat et al., 2017). Useful in phylogenetic verification by the production of chemicals. Also, the density, length, and distribution pattern of trichomes on the seed coat correspond to physiological roles in resisting natural stress (Xiao et al., 2017). The type and shape of hilum are considered as a fingerprint of the attachment of ovules to the ovary during the ovary maturation period.

Firstly, *Pavonia* was separated alone in a small sub-clade by having a seed storage protein band with M.W. of 62, boat-shaped seed, with an acute apex. Then we had *Abutilon* appeared alone in another small parallel sub-clade sharing the presence of seed storage protein band with M.W. of 47, 29, 24, 17, seed with unicellular stellate trichomes and falsifoveate sculpture pattern. This result is in accordance with that of Bano & Deora (2017).

Hibiscus mutabilis also separated alone in small parallel sub-clade by having a yellowish brown seed. After that, we could see *Abelmoschus* and *Hibiscus trionum* in one small strongly supported (BS= 95) clade sharing absence of seed storage protein band with M.W. of 38, presence of that with M.W. of 17.

Abelmoschus could be distinguished from *H. trionum* by the presence of seed storage protein band with M.W. of 29, absence of that with M.W. of 15, deciduous seed trichomes, foveate sculpture pattern, while *H. trionum* was separated by having verrucate sculpture pattern. This result agreed to some extent with that of Patil et al. (2020).

Then we had the (11) rest of malvlean taxa in a large moderately supported clade (BS = 52) sharing the presence of seed storage protein band with M.W. of 42, and tufts of (3-6) multicellular, fused trichomes (fan-shaped). *H. acetosella* appeared alone in a small parallel sub-clade by having a seed storage protein band with M.W. of 29, and an absence of that with M.W. of 21.

After that both *H. rosa-sinensis* and *H.*

sabdariffa were linked together in a moderately supported (BS= 67) small clade, *H. rosa-sinensis* could be distinguished from *H. sabdariffa* by having black seed, glandular trichomes, and areolate to verrucate sculpture pattern, while *Hibiscus sabdariffa* was separated by having an acute beaked apex, and yellowish brown seed. Our results are in accordance to some extent with little deviation for *Hibiscus spp.* of Mostafa et al. (2020).

Then we had *H. syriacus* in another small parallel sub-clade sharing unicellular unfused trichomes and the presence of seed storage protein band with M.W. of 116, with a small strongly supported (BS= 81) small clade carrying *Alcea* and *Urena*. *Hibiscus syriacus* was separated alone by having a dark brown seed. *Alcea* was separated by having a crescent-shaped seed with an acuminate apex, reddish brown colour, and verrucate sculpture pattern. While *Urena* could be distinguished by having an ovoid shape with acute apex, and a small size seed with dusty brown colour. We had another small strongly supported (BS= 71) clade carrying out both *H. tiliaceus* and *Malvaviscus*. *Hibiscus tiliaceus* could be distinguished from *Malvaviscus* by the absence of seed storage protein band with M.W. of 38, and golden brown seed. While *Malvaviscus* was separated by the presence of seed storage protein band with M.W. of 29, boat-shaped seed with acute apex and obtuse base, reddish brown colour.

After that we had *Lagunaria* appeared alone in one small parallel sub-clade strongly supported (BS= 98) by the presence of a beak, large seed, with scarlet red colour. Finally, we had the two *Malva spp.* (*parviflora* and *sylvestris*) in the last small strongly supported (BS= 96) clade sharing broadly reniform dark brown seed, very small size, striate to scalariform sculpture pattern.

The guiding model used when delimiting the four traditional families have been one of a progression from the primitive Tiliaceae through the intermediate Sterculiaceae and Bombacaceae to the most advanced Malvaceae (Cronquist, 1988; Judd & Manchester, 1997; Takhtajan, 1997). While Rao (1953) deviated from this model by considering the Sterculiaceae to be the basal group. Our results agreed with the second opinion, and also indicated that Sterculiaceae is para or polyphyletic.

From our cladistics results, it was clear that family Malvaceae *s.s.* is homogenous and mainly monophyletic except *Gossypium*, and *Thespesia* had a sister relationship to Bombacaceae, and this result agreed with the other previous results (Judd & Manchester, 1997; Alverson et al., 1999; Bayer, 1999; Bayer et al., 1999; Shams & Khattab, 2016; Ibrahim et al., 2018) who confirmed the monophyly of the traditional Malvaceae. While these results revealed that, Tiliaceae, Sterculiaceae, and Bombacaceae were paraphyletic. In this respect, Hinsley (2006) had kept all four families as a single family (Malvaceae *s.l.*) and our results in accordance with these results also.

Conclusion

In conclusion, the cladistic results of this study revealed that sub-family Malvoideae except for some taxa refer to the most advanced and recent common ancestor of the studied sub-families. The cladogram based on seed storage protein, macro- and micromorphological seed characters showed the root common ancestor was the outgroup (*Elaeocarpus angustifolius*) followed by *Brachychiton discolor* (sub-family Sterculioideae). There was a gradual arrangement of the studied taxa among sub-familial level in relation to the outgroup (root of the tree). This result indicates that sub-family Sterculioideae followed by Bombacoideae, Tilioideae are the most primitive sub-families among the other studied ones, followed by Dombeyoideae, Byttnerioideae, Grewioideae ending recently by the most advanced sub-family Malvoideae. This study validates the utility of some morphological seed characters viz. seed shape, colour, size, sculpture patterns, hilum, presence of strophiole and trichomes, besides seed storage protein which can serve as a reliable approach for classification of the studied taxa of family Malvaceae *s.l.* Also, the results of this study support the monophyly of the most taxa of sub-family Malvoideae except some species and para-polyphyly of the other studied six sub-families. Some genera scattered between the different seven sub-families so, our results support to keep all the four families as the single family (Malvaceae *s.l.*). Finally, this study presents additional criteria for the phylogenetic relationships between the four traditional families but not enough to resolve the problem of many genera. There are still more relationships between some of the studied taxa that need to be clarified in the future.

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References

- Abdel Khalik, K.A., Al-Ruzayza, S., Farid, A. (2021) Taxonomic significances of seed morphology in some tribes of subfamily Malvoideae (Malvaceae) in Saudi Arabia. *Australian Journal of Crop Science*, **15**(8), 1204-1216.
- Abid, R., Ather, A., Qaiser, M. (2016) Seed morphology and its taxonomic significance in the family Malvaceae. *Pakistan Journal of Botany*, **48**(6), 2307-2341.
- Ahmed, R., Qaiser, M. (1989) Seed morphological studies of some common plants of Karachi. *Pakistan Journal of Botany*, **21**(2), 218-246.
- Alverson, W.S., Whitelock, B.A., Nyffeler, R., Bayer, C., Baum, D.A. (1999) Phylogeny of the core Malvales: evidence from *ndhF* sequence data. *American Journal of Botany*, **86**, 1474-1486.
- APG III. (2009) Angiosperms Phylogeny Group: "An update of the Angiosperm Phylogeny Group

- classification for the orders and families of flowering plants: APG III", *Botanical Journal of the Linnean Society* **161**(2), 105-121.
- APG IV. (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, **181**, 1–20.
- Azevedo, I.H., de Moraes, P.L. (2019) Seed Morphology of *Ruelliae* Species (Acanthaceae) in Brazil and Its Taxonomic Implications. *Systematic Botany*, **44**(3), 631-651.
- Bano, I., Deora, G.S. (2017) Studies on micromorphological taxonomic variations in *Abutilon* species of Indian Thar Desert. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, **12**, 60-68.
- Barthlott, W. (1981) Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic Journal of Botany*, **1**, 345–355.
- Bayer, C. (1999) The bicolor unit: homology and transformation of an inflorescence structure unique to core Malvales. *Plant Systematics and Evolution*, **214**, 187–198.
- Bayer, C., Fay, M.F, Bruijn, A.Y.D.E., Savolainen, V., Morton, C.M., Kubitzki, K., Alverson, W.S., Chase, M.W. (1999) Support for an expanded family concept of Malvaceae within a recircumscribed Order Malvales: a combined analysis of plastid *atpB* and *rbcL* DNA sequences. *Botanical Journal of the Linnean Society* **129**, 267-303.
- Berry, V., Gascuel, O. (1996) On the interpretation of bootstrap trees: Appropriate threshold of clade selection and induced gain. *Molecular Biology and Evolution*, **13**(7), 999-1011.
- Bona, M. (2013) Seed-coat micro-sculpturing of Turkish *Lepidium* (Brassicaceae) and its systematic application. *Turkish Journal of Botany*, **37**(4), 662–668.
- Carvalho-Sobrinho, J.G.D., Queiroz, L.P.D. (2011) Morphological cladistic analysis of *Pseudobombax* Dugand (Malvaceae, Bombacoideae) and allied genera. *Brazilian Journal of Botany*, **34**, 197-209.
- Christenhusz, M.J., Byng, J.W. (2016) The number of known plants species in the world and its annual increase. *Phytotaxa*, **261**(3), 201-217.
- Cronquist, A. (1981) An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.
- Cronquist, A. (1988) "*The Evolution and Classification of Flowering Plants*". edn. 2. The New York Botanical Garden, Bronx.
- Cutler, D.F., Gregory, M., Wilkinson, H.P., Gornall, R.J., Al-Shammery, K.I.A. (1998) "*Anatomy of the Dicotyledons. Volume IV: Saxifragales (sensu Armen Takhtajan 1983)*". edn. 2. Clarendon Press.
- Dickie, J.B., Stuppy, W.H. (2003) Seed and Fruit Structure: significance in seed conservation operations. In: "*Seed Conservation: Turning Science Into Practice*". London: The Royal Botanic Gardens, Kew, pp. 252-279.
- El-Naggar, S.M. (2001) Systematic implications of seed coat sculpture in Malvaceae. *Pakistan Journal of Biological Sciences*, **4**(7), 822-828.
- Elghamery, A., Shaban, A., Farrag, R., El-basuoni, M. (2021) Genetic diversity of Egyptian cowpea (*Vigna unguiculata* (L.) Walp.) landraces and their genetic relationships based on seed storage proteins and isozymes. *Egyptian Journal of Botany*, **61**(2), 361-374.
- El-Sheekh, M., Abomohra, A.E.F. (2020) The therapeutic potential of spirulina to combat COVID-19 infection. *Egyptian Journal of Botany*, **60**(3), 605-609.
- El-Sheekh, M., Morsy, H., Hassan, L. (2020) Assessment of the optimum growth medium and the effect of different light intensities on growth and photosynthetic pigments of *Chlorella vulgaris* and *Scenedesmus arvernensis*. *Egyptian Journal of Botany*, **60**(2), 395-404.
- Fawzi, N.M. (2018) Seed morphology and its implication in classification of some selected species of genus *Corchorus* L. (Malvaceae). *Middle East Journal of Agriculture Research*, **7**, 1-11.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

- Ganesan, S.K., Middleton, D.J., Wilkie, P. (2020) A revision of *Pterospermum* (Malvaceae: Dombeyoideae) in Malasia. *Edinburgh Journal of Botany*, **77**(2), 161-241.
- Goloboff, P., Farris, J.S., Nixon, K.C. (2000) TNT (Tree analysis using New Technology) (BETA) ver. 1.1 Tucumán, Argentina: Published by the authors.
- Goloboff, P.A., Farris, J.S., Nixon, K.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774-786.
- Gómez, J.J.M., del Pozo, D.G., Cervantes, E. (2019) Seed Shape Quantification in the Malvaceae Reveals Cardioid-Shaped Seeds Predominantly in Herbs. *Botanica*, **25**(1), 21-31.
- Hinsley, S.R. (2006) Classification of Malvaceae: Overview, composition: Position: Division. Malvaceae Info (Home). Net Page.
- Ibrahim, Z.M., Hassan, S.A., El-Azab, H.M., Badawi, A. (2018) Cladistic analysis of some taxa in Malvaceae *s.l.* "Core Malvales" based on anatomical characteristics. *The Egyptian Journal of Experimental Biology (Botany)*, **14**(1), 87-105.
- Ilakiya, J., Ramamoorthy, D. (2021) SEM and light microscopic studies in seeds of *Hibiscus surattensis* L. and phylogenetic attributes in Puducherry region, India. *Geology, Ecology, and Landscapes*, **5**(4), 269-279.
- Judd, W.S., Manchester, S.R. (1997) Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological, and chemical characters. *Brittonia*, **49**, 384-405.
- Karaismailoğlu, M.C. (2015) Morphological and anatomical features of seeds of Turkish *Romulea* taxa (Iridaceae) and their taxonomic significance. *Acta Botanica Croatica*, **74**(1), 31-41.
- Karaismailoğlu, M.C. (2019) Comparative morphology and anatomy of seeds of some Aethionema WT Aiton (Brassicaceae) taxa from Turkey. *Bangladesh Journal of Plant Taxonomy*, **26**(1), 1-12.
- Karakish, E., Al-Ruzayza, S., Khalik, K. A. (2020) Comparative anatomical studies of some species of family Malvaceae from Saudi Arabia and its systematic significance. *The Egyptian Journal of Experimental Biology (Botany)*, **16**(2), 203-203.
- Kariyat, R.R., Smith, J.D., Stephenson, A.G., De Moraes, C.M., Mescher, M.C. (2017) Non-glandular trichomes of *Solanum carolinense* deter feeding by *Manduca sexta* caterpillars and cause damage to the gut peritrophic matrix. *Proceedings of the Royal Society B: Biological Sciences*, **284** (1849).
- La Duke, J.C. Doebley, J. (1995) A chloroplast DNA based phylogeny of the Malvaceae. *Systematic Botany*, **20**, 259-271.
- Ladizinsky, G., Hymowitz, T. (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. *Theoretical and Applied Genetics*, **54**(4), 145-151.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature*. **227**(5259), 680-685.
- Masullo, F.D.A., Siqueira, S.F.H., Barros, C.F., Bovini, M.G., De Toni, K.L. (2020) Fruits of neotropical species of the tribe Malveae (Malvoideae-Malvaceae): macro- and micromorphology. *Acta Botanica Brasílica*, **34**, 301-311.
- Mostafa, A., Eisa, S.S., Mohamed, A.A., Al-Shamey, I. (2020) Taxonomic Revision and Numerical Analysis of *Hibiscus* L. in Egypt. *Arab Universities Journal of Agricultural Sciences*, **28**(1), 117-130.
- Nixon, K.C. (1999) WinClada. Software and documentation by the author. Ithaca, New York: Cornell University; Windows OS.
- Nixon, K.C. (2002) WinClada computer program, version 1.00.18-OptCodeFix (Beta). K.C. Nixon, Ithaca, New York.
- Özbek, F., Uzunhisarcıklı, E. (2020) Mericarp and Seed Morphology of the genus *Althaea* L. (Malvaceae) in Turkey. *Gazi University Journal of Science*, **33**(3), 622-628.
- Patil, P., Malik, S.K., Sutar, S., Yadav, S., John, J., Bhat, K.V. (2015a) Taxonomic importance of seed macro- and micro-morphology in *Abelmoschus* (Malvaceae). *Nordic Journal of Botany*, **33**(6), 696-707.

- Patil, P., Sutar, S., Joseph, J.K., Malik, S., Rao, S., Yadav, S., Bhat, K.V. (2015b) A systematic review of the genus *Abelmoschus* (Malvaceae). *Rheedea*, **25**(1), 14-30.
- Patil, P., Malik, S.K., Bhat, K.V. (2020) Significance of seed trichome micro-morphology in systematic treatment of *Abelmoschus-Hibiscus* complex. *The Indian Society of Genetics and Plant Breeding*, **80**(01), 77-83.
- Payne, P.I., Lawrence, G.J. (1983) Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Research Communications*, **11**(1), 29-35.
- Perveen, A.N.J.U.M., Qaiser, M.U.H.A.M.M.A.D. (2012) Pollen flora of Pakistan-LXIX. Poaceae. *Pakistan Journal of Botany*, **44**(2), 747-756.
- Rao, N.S., Datta, R.J.I. (1953) Chromosomes of the genus *Corchorus*. *Nature, London*, **171**, 754.
- Robinson, P.J., Megarrity, R.G. (1975) Characterization of *Stylosanthes* introductions by using seed protein patterns. *Australian Journal of Agricultural Research*, **26**(3), 467-479.
- Said, W.M., Ahmed, Z.M., Mohamed, T.R. (2018) Morphological and Anatomical Studies on Some Taxa of Sub Family Malvoideae (Malvaceae). *Journal of Scientific Research in Science*, **35**(part 1), 371-389.
- Sanyal, S. (2016) Morpho-Taxonomic Studies of Some Members of Malvales (Cronquist, 1981). Occuring in Saltlake City, North 24 Parganas, West Bengal, *International Journal of Innovative Research in Science, Engineering and Technology*, **5**(5), 8564-8572.
- Shamso, E., Khattab, A. (2016) Phenetic relationship between Malvaceae and its related families. *Taekholmia*, **36**(1), 115-135.
- Shavvon, R.S., Mehrvarz, S.S., Golmohammadi, N. (2012) Evidence from micromorphology and gross morphology of the genus *Loranthus* (Loranthaceae) in Iran. *Turkish Journal of Botany*, **36**(6), 655-666.
- Skema, C. (2012) Toward a new circumscription of *Dombeya* (Malvales: Dombeyaceae): A molecular phylogenetic and morphological study of *Dombeya* of Madagascar and a new segregate genus, *Andringitra*. *Taxon*, **61**(3), 612-628.
- Sofalian, O., Badarian, P., Asghari, A., Sedghi, M., Firoozi, B. (2015) Identification of seed storage protein polymorphism in some soybean (*Glycine max* Merrill) genotypes using SDS-PAGE technique. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, **25**(2), 127-133.
- Stearn, W.T. (1983) *Botanical Latin*, 3rd. rev. David and Charles Inc., Vermont.
- Strong, E.E., Lipscomb, D. (1999) Character coding and inapplicable data. *Cladistics*, **15**, 363-371.
- Takhtajan, A.L. (1997) "*Diversity and Classification of Flowering Plants*". Columbia University Press, New York.
- Tavakkoli, Z., Assadi, M. (2019) Micro-morphological characteristics of seed and ovary trichomes of Iranian *Glaucium* Mill. species (Papaveraceae) and their taxonomic importance. *Nordic Journal of Botany*, **37**(12). <https://doi.org/10.1111/njb.02593>
- Thorne, R.F. (1992) Classification and Geography of the Flowering Plants. *Botanical Review* (Lancaster), **58**, 225-348.
- Wood, T.T., Ohimain, E.I. (2020) Molecular and Morphological Diversity Studies of Five *Cola* (Schott and Endl.) Species in Ibadan, Nigeria. *Annual Research and Review in Biology*, **35**(9), 1-13.
- Xiao, K., Mao, X., Lin, Y., Xu, H., Zhu, Y., Cai, Q., Zhang, J. (2017) Trichome, a functional diversity phenotype in plant. *Journal of Molecular Biology*, **6**(1), 183.
- Ya, T., Gilbert, M.G. Dorr, L.J. (2007) "*Flora of China*", Tiliaceae. vol 12 pp. 240-249. Science Press, Beijing, China.

أهمية صفات البروتين المخترن في البذور وكذا الصفات الظاهرية للبذور في تصنيف بعض أنواع من الفصيلة الخبازية بمفهومها الواسع

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أجريت هذه الدراسة على 49 نوعا نباتيا تنتمي إلى 34 جنسا من الفصيلة الخبازية بمفهومها الواسع تقع ضمن سبع تحت فصائل بالإضافة إلى نوعا واحدا (*Elaeocarpus angustifolius*) إستخدم ك (out group). وذلك بهدف إستنباط دلائل تصنيفية يمكن من خلالها تقييم العلاقات التطورية بين الأنواع قيد الدراسة.

ولتحقيق هذا الهدف تم تجميع العينات النباتية من أماكن متفرقة بمصر (fresh, herbarium) إلى جانب إستيراد بعض العينات من الخارج (16 نوعا) وتم تعريفها طبقا للمراجع الخاصة بذلك. أيضا تم فحص وتسجيل صفات الشكل الظاهري للبذور بإستخدام المجهر الضوئي وكذلك الإلكتروني الماسح وتم تصوير العينات. بالإضافة إلى تسجيل صفات البروتين المخترن في البذور بواسطة تقنية التفريد الكهربائي على هلام الأكريلاميد SDS-Polyacrylamide Gel Electrophoresis technique

تم تجميع الصفات وحالاتها (73 صفة، 157 حالة صفة) في جدول مجمع وإخضاعها للتحليل التفريري بإستخدام برامج إحصائية متطورة مثل برنامج WinClada وTNT.

أيضا أظهرت شجرة التفرع التطوري أهمية بعض الصفات الشكلية للبذور في الفصل بين الأنواع قيد الدراسة ومنها: شكل البذور - القمة - القاعدة - اللون - الحجم - الملمس - شكل السرة وموقعها - الزركشة الموجودة علي قصرة البذرة و وجود أو غياب ال strophoile.

وقد إتفقت نتائج هذه الدراسة مع بعض الدراسات السابقة و أكدت علي أن الأنواع المدروسة لتحت الفصيلة الخبازية جميعها وحيدة الأصل السلفي monophyly ماعدا جنسي القطن *Gossypium, Thespesia* ومتعددة أو متناظرة الأصل السلفي para-polyphyly في باقي تحت الفصائل المدروسة.