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Research Article

EVOLUTION OF SOME MORPHOLOGICAL CHARACTERS IN MALVOIDEAE

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ABSTRACT

Phylogenetic analysis of sixteen taxa of Malvoideae and three outgroup taxa is done using forty-one morphological characters from vegetative and reproductive organs. The most parsimonious trees are searched in PAUP by keeping the members of a tribe together. The most recent molecular phylogeny-based tribe concepts of Malvoideae are considered. Among the three most parsimonious trees obtained by heuristic search, apomorphic changes in the lineages of one tree are discussed. The basal lineages have several unambiguous apomorphies with high bootstrap support. But most of the terminal lineages have ambiguous apomorphic support. The phylogenetically useful morphological characters can be useful for the identification of the taxa. This study suggests that a thorough morphological characterization of the subfamily Malvoideae is needed to explain all the molecular phylogeny-based lineages of Malvoideae morphologically.

Keywords: *Malvoideae, Morphology, Phylogenetic Analysis, Apomorphy*

INTRODUCTION

The subfamily Malvoideae is one among nine subfamilies of the broadly circumscribed Malvaceae (Bayer *et al.*, 1999). To establish monophyly of several families of traditionally recognized Malvales, eight previously recognized families were merged with Malvaceae s.str., though the later family was monophyletic. Thus the presently recognized Malvoideae is equivalent with Malvaceae *s.str.* Judd and Manchester (1997) for the first time established monophyly of Malvaceae *s.str.* based on morphological and phytochemical data. In the same study Bombacaceae, Sterculiaceae and Tiliaceae of core-Malvales were designated as paraphyletic. Later, Fay *et al.*, (1998), Alverson *et al.*, (1998), Bayer *et al.* (1999) and Soltis *et al.* (2000) supported the finding of Judd and Manchester (1997) by molecular data and established newly circumscribed Malvaceae *sensu lato*. Bayer and Kubitzki (2003) recognized four tribes of Malvoideae, viz. Gossypieae, Hibisceae, Kydieae and Malveae. Tate *et al.* (2005) discussed the phylogenetic relationship based on ITS sequences within the genera of the tribe Malveae but could not establish the monophyly of 14 generic alliances. They recommended that analysis by chloroplast DNA sequences could be effective for better phylogenetic resolution. The monophyly of the tribe Hibiscieae is also not proven at all based on molecular data (Pfeil *et al.*, 2002). Reichenbach (1828) initially distinguished the tribe Hibiscieae from Malveae based on capsule fruit rather than schizocarpic fruit. Alefeld (1861) created a new tribe Gossypieae from some genera of Hibiscieae. The tribe Gossypieae possesses some unique features such as gossypol glands, conduplicate folding of an embryo, involucral nectarines etc. (Pfeil *et al.*, 2002). Since most of the phylogenetic studies on Malvoideae have been done by molecular data, the evolution of morphological characters with the genera of the subfamily is poorly discussed. Discussion on morphological character evolution within Malvoideae can help us to understand the morphological evolutionary process within the subfamily. It can also help to identify taxa based on phylogenetically significant morphological features.

MATERIALS AND METHODS

Plant samples

To study the morphological phylogeny within Malvoideae, sixteen species from the tribes Malveae, Hibiscieae and Gossypieae were collected and studied. Two species from Grewioideae and one species from Sterculioideae were also included in this study. These three taxa served as outgroups in phylogenetic analysis. A list of studies taxa is given in table 1.

Table 1: List of studies taxa under Malvoideae. In the phylogenetic study serial no. 4, 7, and 8 have served as outgroups.

Sl. no.	Plant name	Abbreviation used	Tribe (Subfamily)
1	*Abelmoschus esculentus* (L.) Moench.	Abel_escu	Hibisceae (Malvoideae)
2	*Abutilon indicum* G. Don.	Abu_ind	Malveae (Malvoideae)
3	*Alcea rosea* L.	Alce_rose	Malveae (Malvoideae)
4	*Corchorus capsularis* L.	Corch_cap	——— (Grewioideae)
5	*Fioria vitifolia* L.	Fio_viti	Hibisceae (Malvoideae)
6	*Gossypium herbaceum* L.	Gossy_herb	Gossypieae (Malvoideae)
7	*Grewia asiatica* L.	Gre_asia	——— (Grewioideae)
8	*Heritiera fomes* Buch.-Ham. (Heri fome)	Heri_fome	——— (Sterculioideae)
9	*Hibiscus rosa-sinensis* L.	Hibis_rosa	Hibisceae (Malvoideae)
10	*Malachra capitata* L.	Mala_capi	Hibisceae (Malvoideae)
11	*Malvastrum coromandelianum* (L.) Garcke	Mal_coro	Malveae (Malvoideae)
12	*Malvaviscus arboreus* Cav.	Malva_arbo	Hibisceae (Malvoideae)
13	*Sida acuta* Burm.	Sida_acu	Malveae (Malvoideae)
14	*Sida cordata* Borssum Waalkes	Sida_cor	Malveae (Malvoideae)
15	*Sida rhombifolia* var. *rhomboidea* (Roxb.)	Sida_rho_r	Malveae (Malvoideae)
16	*Sida rhombifolia* var. *obovata* Wall. ex	Sida_rho_o	Malveae (Malvoideae)
17	*Sida rhombifolia* var. *typica* K. Schumann	Sida_rho_t	Malveae (Malvoideae)
18	*Thespesia populnea* Corr.	Thes_popul	Gossypieae (Malvoideae)
19	*Urena lobata* Linn.	Urena_loba	Hibisceae (Malvoideae)

Selection of morphological characters and their coding

Morphological characters were generated from fresh plant materials. Forty-one morphological characters were selected from habit, life forms, leaves, stipules, flowers, fruits and pollens. The number of non-reproductive characters was ten and the remaining characters were taken from flowers, fruits and pollens (table 2). The data matrix and nexus file for phylogenetic analysis were generated using Mesquite 2.75 (Maddison and Maddison, 2011) software.

Table 2: Morphological characters used in the cladistic analysis of Malvoideae

Character		Character states
1.	Habit	[0] tree; [1] Herb/ shrub
2.	Plant body	[0] erect; [1] prostrate
3.	Stipule shape	[0] subulate-filiform, [1] lanceolate-ovate
4.	Stipule margin	[0] entire; [1] segmented
5.	Leaf type	[0] simple; [1] palmately lobed

Character		Character states
6.	Leaf lamina shape	[0] elliptic; [1] lanceolate-ovate; [2] rhombic-trullate; [3] cordate; [4] obovate; [5] orbicular
7.	Leaf lamina margin	[0] entire; [1] serrate; [2] lobed
8.	Leaf apex	[0] acute; [1] obtuse
9.	Flower type	[0] perfect; [1] imperfect
10.	Flower size	[0] >25 mm; [1] <20 mm
11.	Epicalyx	[0] lacking; [1] poorly developed, [2] clearly developed
12.	Epcalyx union	[0] free; [1] united
13.	Shapes of episepals	[0] linear-lanceolate; [1] ovate; [2] cordate
14.	Number of nerves present in episepals	[0] 1; [1] 3-12
15.	Episepals margin	[0] entire; [1] segmented
16.	Number of episepals	[0] 3; [1] >4
17.	Epicalyx size in respect to calyx	[0] smaller than calyx; [1] same/ larger than calyx
18.	Calyx union	[0] free; [1] united
.19.	Corolla	[0] present; [1] absent
20.	Staminal tube	[0] absent; [1] present
21.	Staminal tube surface	[0] glabrous; [1] pubescent
22.	Length of staminal tube in respect to corolla	[0] smaller than corolla, [1] larger than corolla
23.	Number of stamens	[0] >35; [1] 10-30
24.	Number of theca in anther	[0] monothecal; [1] bithecal
25.	Shape of anther	[0] reniform; [1] others
26.	Style branching	[0] branched; [1] unbranched
27.	Number of ovary chamber	[0] >15; [1] 5-15; [2] 4
28.	Stigma tip	[0] >15; [1] 10-15; [2] 10; [3] 5-10; [4] 5; [5] 1 or lobed
29.	Fruit type	[0] schizocarp; [1] others
30.	Number of mericarp	[0] 15-40; [1] 5-10
31.	Gossypol gland	[0] absent; [1] present
32.	Trichome	[0] stellate; [1] stellate with simple; [2] indumentum
33.	Pollen aperture type	[0] colpate/ colporate; [1] porate
34.	Pollen shape	[0] prolate; [1] globose
35.	Pore diameter	[0] 8-12 µm; [1] 2-6 µm
36.	Number of pores per pollen	[0] 3; [1] 5-30; [2] >40
37.	Spine on pollen wall	[0] absent; [1] present
38.	Spine type	[0] solid; [1] hollow
39.	Spine length	[0] 6-8 µm; [1] 14-28 µm
40.	Apex of spine	[0] acute; [1] curved
41.	Endopore	[0] absent; [1] present

Phylogenetic analysis

Morphological phylogenetic analysis was conducted in PAUP version 4.0a for Windows (Swofford, 2022). The Maximum Parsimonious Trees (MPTs) were obtained by employing CONSTRAINTS command to keep the members of a tribe together. Among the total 19 taxa, three were considered as outgroups. All multistate characters were unordered. SIMPLE data addition sequence, TBR (tree-bisection-reconnection) branch swapping algorithm, and 100 initial "MAXTREES" setting options were used keeping branch collapse (creating polytomies) and MULPARS option in effect. The number of bootstrap replicates was fixed to 100 during bootstrap analysis.

Tree drawing

One of the three most parsimonious trees was drawn in FigTree version 1.4.4 software (Rambaut, 2012). The tree was further modified using image editing software.

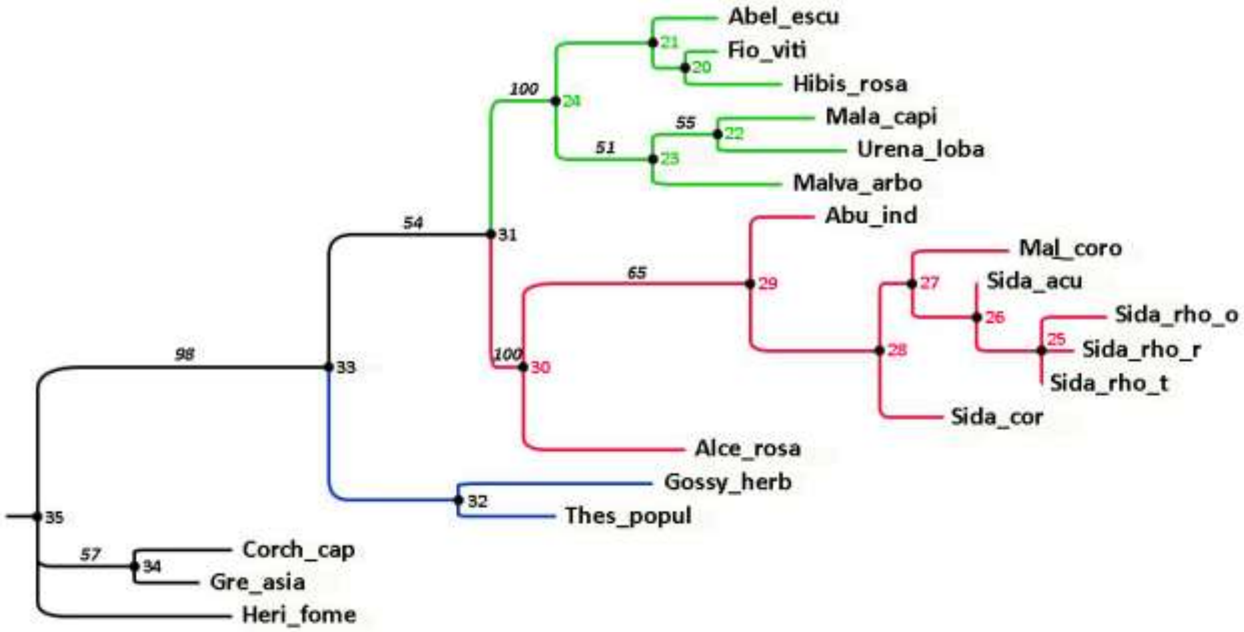

Figure 1. One most parsimonious tree of Malvoideae was obtained by a heuristic search for 19 taxa and 41 morphological characters. Phylogenetic tree [Tree length = 101, Consistency index (CI) = 0.5347, Homoplasy index (HI) = 0.4653, Retention index (RI) = 0.5983] was constructed by keeping members of a tribe together. Green clade- tribe Hibisceae; red clade- tribe Malveae and blue clade-tribe Gossypieae. Numbers aside the node (black circles) indicate branch numbers. Italicized numbers on some clades indicate bootstrap values. Clades without any numbers indicate bootstrap support is less than 50.

RESULTS AND DISCUSSION

Heuristic search gives three most parsimonious trees with length= 101 and CI (consistency index) value= 0.5347. One of the most parsimonious trees is given in Fig. 1. The basal lineages of the three tribes have high bootstrap support and agree with the tribal classification by Bayer & Kubitzki (2003). Within the tribe Hibisceae, three genera (*Malachra*, *Urena* and *Malvaviscus*) form a sub-clade with more than 50 bootstrap values. All genera of the tribe Malveae also form a robust clade with high bootstrap value at basal lineage. The character evolution in terminal lineages shows several homoplasious changes which may raise questions on the reliability of these lineages on the purely morphological ground. Since apomorphic changes are considered to be the evolutionary novelty (Simpson, 2010), the unambiguous apomorphic changes in the lineages are to be discussed with importance.

The lineage from the hypothetical ancestor (node- 35 of Fig. 1) of Malvoideae, Grewioideae, Sterculioideae to node- 33 shows several unambiguous synapomorphies. This linage is robust as well by having 98 bootstrap support values. The bithecal and oblong anthers were evolved to monothecal and reniform anthers in Malvoideae. These features are synapomorphic to the members of Malvoideae. Unique changes in some features of pollen were also happed in this lineage. The colpate or colporate, prolate, non-echinate and few numbers of aperture containing pollens of the ancestor were evolved to porate, globular, echinate and numerous aperture containing pollens in Malvoideae. Members of Malvoideae are easily recognizable by these anther and pollen features and therefore very useful for identification purposes.

Table 2: Change of character states in the lineages (internodes) of a most parsimonious tree (shown in fig. 1). Bold arrows and characters indicate that the evolutionary changes are unambiguous whereas normal arrows indicate ambiguous evolutionary events.

Lineage	Character	CI	Change
Node_35 → Node_33	1 (Habit)	0.333	0 → 1
	10 (Flower size)	0.333	1 **➔** 0
	11 (Epicalyx)	0.400	0 **➔** 2
	24 (No. of theca in anther)	**1.000**	1 **➔** 0
	25 (Shape of anther)	**1.000**	1 **➔** 0
	33 (Pollen aperture type)	**1.000**	0 **➔** 1
	34 (Pollen shape)	**1.000**	0 **➔** 1
	36 (No. of pores per pollen)	**1.000**	0 **➔** 1
	37 (Spine on pollen wall)	**1.000**	0 **➔**1
node_33 → node_31	7 (lamina margin)	0.500	0 → 1
	16 (No. of episepals)	0.500	0 → 1
	26 (Style)	0.500	1 **➔** 0
	28 (Number of stigma)	0.714	5 → 0
	29 (Fruit type)	0.500	1 → 0
node_31 → node_24	28 (Number of stigma)	0.714	0 → 2
	30 (Mericarp no.)	0.500	0 → 1
node_24 → node_21	28 (Number of stigma)	0.714	2 → 4
	29 (Fruit type)	0.500	0 → 1
	35 (pore diameter)	0.333	1 **➔** 0
node_21 → Abel escu	**5 (Leaf type)**	**1.000**	0 **➔** 1
	26 (Style)	0.500	0 **➔** 1
node_21 → node_20	6 (leaf lamina)	0.500	5 **➔** 1
node_20 → Fio viti	32 (Trichome)	0.333	1 **➔** 0
node_20 → Hibis rosa	14 (No. of nerves present in episepals)	0.333	1 **➔** 0
	21 (Stminal tube surface)	0.333	0 **➔** 1
	22 (Length of Staminal tube in respect to corol)	0.500	0 **➔** 1
node_24 → node_23	8 (leaf apex)	0.333	0 **➔** 1
	12 (Episepals)	0.500	0 **➔** 1
	23 (no . of stamen)	0.250	0 **➔** 1
node_23 → node_22	10 (Flower size)	0.333	0 **➔** 1
	17 (epicalyx size in respect to calyx)	0.333	0 → 1

Lineage	Character	CI	Change
node_22 → Mala capi	11 (Epicalyx)	0.400	2 ➔ 0
	32 (Trichome)	0.333	1 ➔ 0
	41 (Endopore)	0.250	0 ➔ 1
node_22 → Urena loba	6 (leaf lamina)	0.500	5 ➔ 3
	7 (lamina margin)	0.500	1 ➔ 2
	35 (pore diameter)	0.333	1 ➔ 0
	40 (Apex of spine)	**1.000**	0 ➔ 1
node_23 → Malva arbo	6 (leaf lamina)	0.500	5 ➔ 1
	14 (No. of nerves present in episepals)	0.333	1 ➔ 0
	21 (Stminal tube surface)	0.333	0 ➔ 1
	22 (Length of Staminal tube in respect to corol)	0.500	0 ➔ 1
node_31 → node_30	27 (No. of chamber)	0.667	1 → 0
node_30 → node_29	6 (leaf lamina)	0.500	5 ➔ 3
	10 (Flower size)	0.333	0 ➔ 1
	11 (Epicalyx)	0.400	2 ➔ 0
	14 (No. of nerves present in episepals)	0.333	1 → 0
	16 (No. of episepals)	0.500	1 → 0
	38 (Spine type)	**1.000**	1 ➔ 0
	39 (Spine length)	0.500	1 ➔ 0
node_29 → Abu ind	32 (Trichome)	0.333	1 ➔ 0
	41 (Endopore)	0.250	0 ➔ 1
node_29 → node_28	23 (no . of stamen)	0.250	0 ➔ 1
	27 (No. of chamber)	0.667	0 → 1
	28 (Number of stigma)	0.714	0 → 1
	30 (Mericarp no.)	0.500	0 → 1
node_28 → node_27	6 (leaf lamina)	0.500	3 ➔ 1
node_27 → Malvastrum coro	3 (Stipule shape)	0.250	0 ➔ 1
	11 (Epicalyx)	0.400	0 ➔ 1
	41 (Endopore)	0.250	0 ➔ 1
node_27 → node_26	28 (Number of stigma)	0.714	1 → 3
	32 (Trichome)	0.333	1 ➔ 0
node_26 → Sida acuta	28 (Number of stigma)	0.714	3 ➔ 2
node_26 → S rho v. ob	6 (leaf lamina)	0.500	1 ➔ 4
	8 (leaf apex)	0.333	0 ➔ 1
node_26 → node_25	6 (leaf lamina)	0.500	1 ➔ 2
node_25 → S rho v rh	21 (Stminal tube surface)	0.333	0 ➔ 1
node_28 → Sida cord	**2 (Plant body)**	**1.000**	0 ➔ 1
	28 (Number of stigma)	0.714	1 → 4
node_30 → Alc ros	3 (Stipule shape)	0.250	0 ➔ 1
	4 (Stipule margin)	**1.000**	0 ➔ 1
	12 (Episepals)	0.500	0 ➔ 1
	17 (epicalyx size in respect to calyx)	0.333	0 ➔ 1
	36 (No. of pores per pollen)	**1.000**	1 ➔ 2
node_33 → node_32	3 (Stipule shape)	0.250	0 ➔ 1
	6 (leaf lamina)	0.500	5 ➔ 3
	31 (Gossypol gland)	**1.000**	0 ➔ 1
	41 (Endopore)	0.250	0 ➔ 1
node_32 → Gossy herb	7 (lamina margin)	0.500	0 → 2

Lineage	**Character**	**CI**	**Change**
	13 (Shape of episepals)	**1.000**	0 ➔ 2
	15 (Margin of episepals)	**1.000**	0 ➔ 1
	17 (epicalyx size in respect to calyx)	0.333	0 ➔ 1
	35 (pore diameter)	0.333	1 ➔ 0
	39 (Spine length)	0.500	1 ➔ 0
node_32 → Thes popul	1 (Habit)	0.333	1 → 0
	11 (Epicalyx)	0.400	2 ➔ 1
	32 (Trichome)	0.333	1 ➔ 2
node_35 → node_34	7 (lamina margin)	0.500	0 → 1
	18 (Calyx union)	**1.000**	1 ➔ 0
	20 (Staminal tube)	**1.000**	1 ➔ 0
node_34 → Corchorus	1 (Habit)	0.333	0 → 1
	6 (leaf lamina)	0.500	5 ➔ 1
	23 (no . of stamen)	0.250	0 → 1
node_34 → Grewia	3 (Stipule shape)	0.250	0 ➔ 1
	27 (No. of chamber)	0.667	1 ➔ 2
node_35 → Heritiera	6 (leaf lamina)	0.500	5 ➔ 0
	8 (leaf apex)	0.333	0 ➔ 1
	9 (Flower type)	**1.000**	0 ➔ 1
	19 (Corolla)	**1.000**	0 ➔ 1
	23 (no . of stamen)	0.250	0 → 1
	32 (Trichome)	0.333	1 ➔ 2

The lineages for the outgroup taxa also show some apomorphies and synapomorphies. The imperfect flower in *Heritiera* (Sterculioideae) was evolved from the perfect flower of its ancestor (node- 35). This change is unambiguous and therefore an important apomorphic change. The lineage between node-35 and -34 shows two synapomorphies. In this lineage, the sepals became free and the staminal tube was lost. Node- 34 is the hypothetical ancestor of *Corchorus* and *Grewia* of Grewioideae.

The development of the gossypol gland in Gossypieae is an important evolutionary event to segregate the tribe from other members of Malvoideae (Fryxell, 1988). The present study also supports this view. The development of the gossypol gland in Gossypieae is the only synapomorphy in this study. Interestingly, the lineage between node-33 and -31lacks any unambiguous synapomorphies though bootstrap support of this lineage is 54. Node- 31 is the hypothetical ancestor of both Malveae and Hibiscieae. Like the previous lineage, the lineages for the ancestors of the tribe Malveae and Hibisceae are devoid of any unambiguous morphological synapomorphy. It suggests that more critical study on morphology is needed to explain the lineages morphologically. *Alcea* is unique under Malveae due to its development of segmented stipule margin and more than 40 apertures per pollen. The development of solid spine on pollen wall differentiates the genera *Abutilon*, *Malachra* and *Sida* from *Alcea*. This change is unambiguous and thus phylogenetically informative within the sub-clade of Malveae. The prostrate plant body of *S. cordata* isolates it from other species of *Sida*. The evolution of the prostate plant body in *S. cordata* is an apomorphic event.

Within the clade of Hibisceae, only two apomorphic changes were observed. The first one is the evolution of palmately lobed leaf in *Abelmoschus*. The second one is the evolution of the curved apex of pollen spine in *Urena*. Besides, all other lineages are defined by ambiguous apomorphies.

CONCLUSION

Since plant identification is an important component of angiosperm taxonomy, dependency on morphological features is always to be considered in utmost importance even though the molecular phylogenetic relationship is more acceptable to modern-day taxonomists. If arbitrarily selected characters are used for identification it will be incongruent with the molecular phylogenetic concept of the taxa. If a phylogenetic tree generated by molecular data can be fully explained by morphological character evolution then it can reduce the conflict between traditional versus molecular taxonomy. But it needs a thorough examination of morphological features.

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