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Molecular Techniques to ascertain the Genetic Strategies in some Malvaceae *s.l.* species

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FAMILY Malvaceae has been traditionally subjected to several taxonomic approaches regarding their taxon relationships and divisions. With the rapid advancement of PCR-based methods and DNA sequence information, taxonomists now have the opportunity to shift from traditional systems of classification to more recent systems. Molecular studies of Malvaceous taxa have provided new opinions about the grouping and phylogeny of these taxa. This work involved molecular analyses of twenty-three Malvaceae species. Three molecular techniques were used, namely, inter simple sequence repeat (ISSR), sequence-related amplified polymorphism (SRAP), and internal transcribed spacer (ITS), to investigate the relationships between the studied taxa. Phylogenetic relationships were constructed using Molecular Evolutionary Genetics Analysis and Phylogenetic Analysis Using Parsimony (MEGA and PAUP) software. The results of this investigation revealed 301 molecular characteristics (78 ISSR and 223 SRAP bands) between the taxa. These data support the taxonomic view of the Malvaceae *s.l.* subfamilies in the Angiosperm Phylogeny Group system of plant classification (APG systems).

Keywords: Classification, ISSR, ITS, Malvaceae *s.l.*, Phylogeny, SRAP.

Introduction

Malvaceae *s.l.* originated from the Latin word mallow. This name was first used by Pliny the Elder (Simpson, 2010). It is a worldwide, diverse, large, and economically important family with approximately 246 genera and 4225 species (Kew Science, 2021). The diversification centers are in tropical and temperate areas of both hemispheres (Cvetkovi′c et al., 2021). In Egypt, this family includes 10 genera and 32 species (Taeckholm, 1974; Boulos, 1995& 2009). The native range of this species is the Nile region, the Oases region, the Mediterranean coast, all deserts of Egypt, the Red Sea coast, Gebel Elba and the entire Sinai Peninsula (Boulos, 2009). The circumscription of the Malvaceae *s.l.* is controversial and faces many taxonomic suggestions. Previous studies (Cronquist, 1988; Thorne, 1992; Takhtajan, 1997; Kubitziki, 2003; Judd et al., 2008) considered the four families Bombacaceae, Sterculiaceae, Tiliaceae and Malvaceae as the "core Malvales". These four families were found to be closely related since the time of Linnaeus (1753) and are still affirmed by morphological, anatomical, chemical, and molecular studies (Chase et al., 1993; Judd & Manchester, 1997; Alverson et al., 1998; Bayer et al., 1999; Nyffeler et al., 2005; Péchon and Gigord, 2014). The vast development of PCR-based techniques and DNA sequence information has allowed taxonomists to break away from the classical systems of classification and given birth to the APG systems (Angiosperm Phylogeny Group system, I, II, III & IV, 1998-2016).

A previous study treated both Bombacoideae and Malvoideae as separate families rather than combined as subfamilies under Malvatheca. Additionally, these findings supported the identification of Sterculiaceae

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and Tiliaceae and pointed to the great difference in opinion given that Tiliaceae and Sterculiaceae are distinct families. Furthermore, these findings suggested that Dombeyaceae should be treated at the family level. In addition, *Abutilon* spp. with *Sida* spp. were placed in one tribe (Abutilae) (Shamso & Khattab, 2016).

In the present study, the position of the taxa within Malvaceae *s.l.* was unclear, and the grouping lacked a well-resolved framework using morphological characteristics only. Thus, Weising et al. (2006) recommended the use of specific molecular parameters for clear-cut Malvaceae *s.l.* to detect variability among closely related species. Therefore, this study aimed to assess the validity of the APG classification systems for Malvaceae *s.l.* through the use of different molecular markers; Inter Simple Sequence Repeat (ISSR), Sequence-Related Amplified Polymorphism (SRAP), and Internal Transcribed Spacer (ITS). This is done to evaluate the phylogenetic relationships among the studied species and obtain a more detailed phylogenetic classification.

Materials and Methods

Sample collections

A total of 23 species were used in this study as representative genera of Malvaceae in Egypt. Nineteen fresh specimens were collected from the Shehab Mazhar Botanical Garden in Baragil, Giza, Egypt, as well as from the botanical gardens of the Faculty of Science and Agriculture, Alexandria University. The remaining 4 dry specimens were obtained from the herbaria of Alexandria University, Tanta University, and Loutfy Boulos. Voucher specimens of the fresh samples were deposited in the Alex Herbarium, Alexandria, Egypt. The locations of the collected specimens and their related information are presented in Table 1. Unfortunately, Egyptian *Pavonia* arabica was not represented in the Egyptian herbaria, and to represent the genus in the study, the only available herbarium sample was collected from the United Arab Emirates.

The extraction of DNA from Malvaceae *s.l.* species was more problematic, especially for fresh specimens, than for herbarium specimens due to the presence of a large amount of polysaccharides and mucilage in their leaves. Therefore, molecular studies were conducted on the specimens by applying the modified extraction method of Dellaporta et al. (1983) to obtain good extraction and purification results.

ISSR analysis

A set of 10 primers obtained from Sigma was used to prescreen the species under investigation for polymorphisms. Only five primers produced clear scorable bands with good reproducibility and amplification patterns. The primers selected for PCR amplification were described by Celka et al. (2012) and Vanijajiva (2012) (Table 2). The nucleotide sequences with a GC content of 33-80% were selected to generate the DNA fingerprint profiles of all the genotypes. The selected primers had di-, tri- or pentanucleotide repeats anchored or not anchored at 3´. The annealing temperatures were optimized for those primers before performing the experiments.

SRAP analysis

Several combinations of 6 forward primers (Me1, Me2, Me3, Me4, Me7 and Me8) and 5 reverse primers (Em1, Em2, Em4, Em6 and Em10) were used. The abovementioned primers and their 11 combinations were selected according to Li & Quiros (2001) and Badrakhani et al. (2014) based on the maximum number of polymorphic bands obtained in their study (Table 3).

PCR amplification, cloning and sequencing of the ITS region

The entire ITS region (ITS1, 5.8S and ITS2) was sequenced following PCR amplification from the genomic DNA. The primers used were 5′ TCCGTAGGTGAACCTGCGG 3′ for ITS1 and 5′ TCCTCCGCTTATTGATATGC 3′ for ITS4. PCR amplification was carried out according to Tate et al. (2005). The DNA products obtained after electrophoresis were cut, weighed, recovered and purified according to the protocol of the MEG Aquick-spin total fragment DNA purification kit (iNtRON Biotechnology, Inc., South Korea).

Aliquots of each amplified product were run on a 2% agarose gel with ethidium bromide, visualized on a UV transilluminator, and photographed by a gel documentation system. A one kilo base pair DNA ladder (Sigma) was used as a DNA fragment size marker.

TABLE 1. Samples collected with their families, locations and dates of collection. (Families as proposed by the APG systems; 1998-2016).

Family	Code	Taxa	Site	Date
Tiliaceae *s.s.*	1	***Corchorus olitorius* L.[6]	Cultivated area, Nile Delta	October 2019
	2	*Grewia pondoensis* Burret.[6]	Shehab Mazhar botanic garden Giza, Egypt	June 2019
	3*	*Triumfetta flavescens* Hochst. ex A. Rich[6]	Gebel Elba	March 1997
Sterculiaceae *s.s.*	4	***Brachychiton discolor* F. Muell.[3]	Shehab Mazhar botanic garden, Giza, Egypt	June 2019
	5	*Dombeya wallichii* (Lindl.) Benth. ex Baill.[4]	Shehab Mazhar botanical garden, Giza, Egypt	June 2019
	6	*Guazuma ulmifolia* Lam.[5]	Shehab Mazhar botanic garden, Giza, Egypt	June 2019
	7*	*Melhania denhamii* R. Br.[4]	Gebel Elba, Wadi Eikwan upstream, southeast of Halaieb. N. 22 00’ 00” E. 36 39’ 21”. Code 18750	March 1998
	8	*Pterospermum acerifolium* (L.) Willd.[4]	Shehab Mazhar botanic garden, Giza, Egypt	June 2019
Bombacaceae *s.s.*	9	***Bombax. ceiba* L.[1]	Faculty of Agriculture botanic garden, Alex. University	March 2020
	10	***Ceiba pentandra* (L.) Gaertn.[1]	Shehab Mazhar botanic garden, Giza, Egypt	June 2019
	11	*Ceiba speciosa* (A.St.-Hil., A.Juss. & Cambess.)[1] Ravenna	Faculty of Science botanic garden, Alex. University.	May 2018
	12	*Pseudobombax ellipticum* (Kunth) Dugand[1]	Shehab Mazhar Botanic garden, Giza, Egypt	June 2019
Malvaceae *s.s.*	13	**Abelmoschus esculentus (L.) Moench 2	*Shehab Mazhar botanic garden, Giza, Egypt.*	June 2019
	14	**Abutilon hirtum (Lam.) Sweet. 2	*Shehab Mazhar botanic garden, Giza, Egypt.*	June 2018
	15	Alcea rosea L. 2	*Faculty of science botanic garden, Alex. University*	May 2018
	16	** Gossypium herbaceum L. 2	*Shehab Mazhar botanic garden, Giza, Egypt.*	June 2019
	17	**Hibiscus syriacus L. 2	*Shehab Mazhar botanic garden, Giza, Egypt*	June 2019
	18	Lagunaria patersonia (Andrews) G.Don	*Faculty of science botanic garden, Alex. University*	May 2018
	19	**Malva parviflora L. 2	*Alexandria Burg El-Arab coastal road*	April 2019
	20	Malvaviscus arboreus Dill ex Cav. 2	*Shehab Mazhar botanic garden, Giza, Egypt*	June 2019
	21*	Pavonia arabica Hochst & Steud. 2 ex Boiss.	*Headland of beach north of oceanic hotel, Khor Fakkan, United Arab Emirates. Code 3696*	May 1998
	22*	Sida alba L. 2	*Wadi Feiran (St. 123) N. 28 45’ 48” E. 33 23’ 448” A. 242 m. Code 19123*	May 2005
	23	**Thespesia populnea (L.) Sol. ex Corrêa. 2	*Shehab Mazhar botanic garden, Giza, Egypt*	June 2019

[1] Bombacoideae [2] Malvoideae [3] Sterculioideae [4] Dombeyoideae [5] Byttnerioideae [6] Grewioideae

* Samples obtained from herbarium sheets, ** Samples used in sequence analysis.

TABLE 2. Primer sequences, Repeat motif, GC% and T_m used in the ISSR analysis.

Primer	Sequence 5′ ⟶ 3′	Repeat motif	GC%	T_m (ºC)
ISSR3	AG AG AG AG AG AG AG T	$(AG)_7$	47	44
IS810	GA GA GA GA GA GA GA GA T	$(GA)_8$	47	50
IS813	CT CT CT CT CT CT CT CT T	$(CT)_8$	47	50
IS834	ATG ATG ATG ATG AT G	$(ATG)_5$	33	40
IS846	GGGT GGGGT GGGGT G	$(GGGGT)_2$	80	54

TABLE 3. Primer sequences and their combinations employed in the SRAP analysis.

Forward Primers	Forward Sequence 5′ 3′ ⟶	Reverse Primers	Reverse Sequence 5′ 3′ ⟶
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em4	GACTGCGTACGAATTTGA
Me4	TGAGTCCAAACCGGACC	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGTTG	Em10	GACTGCGTACGAATTTAG
Me8	TGAGTCCAAACCGGTGT		
Primer Combinations			
Me1-Em2	Me3-Em1	Me4-Em2	Me8-Em1
Me2-Em1	Me3-Em2	Me4-Em6	Me8-Em2
Me2-Em6	Me3-Em4	Me7-Em10	

Statistical analyses

The ISSR and SRAP gels were analyzed through TotalLab image analysis software (version 1.1.4301, 26877). Only intensely stained unambiguous bands were used in the analysis. The bands were scored as binary characters: absent (0) or present (1). Six different parameters and indices were used to characterize the efficiency of each marker and primer to detect polymorphisms among the different species used. The percentage of polymorphisms (pb%) was calculated. The Polymorphic Information Content (PIC), Resolving Power (Rp) and Marker Index (MI) were subsequently evaluated according to De Riek et al. (2001), Sorkheh et al. (2007), and Prevost & Wilkinson (1999), respectively. The Effective Multiplex Ratio (EMR) and Multiplex Ratio (MR) were calculated according to Powell et al. (1996). The data were obtained by scoring the ISSR and SRAP profiles with different primers, individually and collectively, and subsequently constructing a similarity matrix using Jaccard's coefficients (Jaccard, 1908). The similarity values used for Cluster analysis were calculated by using the Unweighted Pair Group Method with theArithmetic means (UPGMA) algorithm, and dendrogram construction was performed with the PAST program v.3 (Hammer et al., 2001).

Phylogenetic data

Purified DNA from 10 out of the 23 studied species was sequenced by Macrogene Company (South Korea; Table 1). *Elaeocarpus nitentifolius* Merr. & Chun was chosen as an outgroup (Judd & Manchester, 1997). The sequences were subjected to pairwise and multiple sequence alignment using CLUSTAL W version 2 (Thompson et al., 1997). Phylogenetic relationships were constructed using MEGA version 11 (Tamura et al., 2021) and another software package, PAUP version 4 (Swofford, 2002), to assess the phylogenetic relationships. In MEGA software, aligned sequences were analyzed by *p*-distance and UPGMA methods of sorting. In PAUP software, aligned sequences were evaluated by the Wagner parsimony method using 'branch & bound', 'heuristic' and the parsimony method of likelihood. Moreover, the software generated a phylogenetic tree based on transition/transversion ratios, the consistency index (CI) and the homoplasy index (HI) Farris (1989 a, 1989 b).

Results

ISSR analysis

Five primers out of ten ISSR with di-, tri- and pentanucleotides were used to screen 23 species of Malvaceae *s.l.* These primers produced clear, reproducible bands of genomic DNA, as represented by ISSR34 (Figure 1 & S1). The total and specific number of bands as well as the percentage of polymorphisms are presented in Table 4. The generated bands were variable in size and number depending upon their sequence repeat motifs in different species. The polymorphic amplicon size ranged from 100 to 1000 bp, with 100% polymorphism.

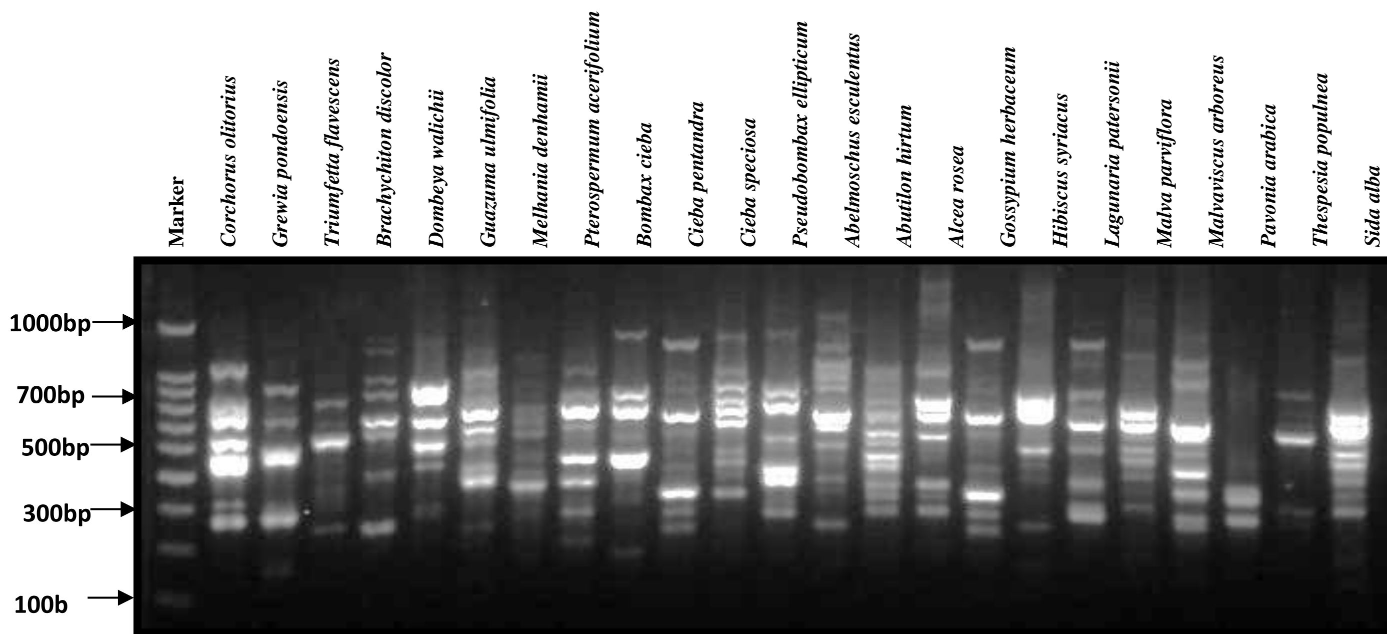

Fig. 1. Bands produced by using ISSR34 primer.

TABLE 4. Indices amplified with the 5 ISSR primers for the examined species of Malvaceae *s.l.*

Primer Combination	TB[1]	PB[2]	MB[3]	% PB[4]	SB[5]	PIC[6]	MI[7]	RP[8]	EMR[9]
ISSR3	18	18	0		2	0.75	55.44	8.22	74
ISSR10	16	17	0		1	0.59	74.60	15.75	126
ISSR13	14	15	0	100%	2	0.55	48.66	12.57	88
ISSR34	19	19	0		1	0.72	99.76	14.63	139
ISSR46	11	11	0		0	0.39	54.08	17.64	139
Total	78	78	0		7	3.00	332.55	68.81	566.00
Mean	-	-	-	-	-	0.60	66.51	13.76	113.20

[1]Total number of bands, [2]Polymorphic bands, [3]Monomorphic bands, [4]Percentage of polymorphism, [5]Specific bands, [6]Polymorphic information content, [7]Marker index, [8]Resolution power, [9]Effective multiplex ratio.

The use of ISSR primers was highly productive and polymorphic, with a total of 78 bands and seven specific bands within the examined species. The number of amplified amplicons ranged from 11 to 19, with a mean of 15.6 amplicons per primer. *Abutilon hirtum* (Lam.) Sweet produced the greatest number of bands and the highest percentage of polymorphisms (33 and 42.37%, respectively). However, *Pavonia arabica* Hochst. & Steud. ex Boiss. amplified the least number of bands and percentage polymorphism (4 and 5.17%, respectively). For each primer, 3 specific bands were from ISSR3, 2 from ISSR13, and 1 from both ISSR10 and ISSR34; however, no specific bands were from ISSR46. *A. hirtum* and *Ceiba speciosa* (A.St.-Hil., A.Juss. & Cambess.) Ravenna gave the maximum number of specific bands (2 bands). While each of *Lagunaria patersonia* (Andrews) G. Don, *Malva parviflora* L. and *Malvaviscus arboreus* Dill. ex Cav. produced the minimum values (1 band) (Table 4, 5).

TABLE 5. Band measurement of Malvaceae *s.l.* species amplified by the 5 ISSR primers.

Primers	bands	*Corchorus olitorius*	*Grewia pondoensis*	*Triumfetta flavescens*	*Brachychiton discolor*	*Dombeya walichii*	*Guazuma ulmifolia*	*Melhania denhamii*	*Pterospermum acerifolium*	*Bombax ceiba*	*Ceiba pentandra*	*Ceiba speciosa*	*Pseudobombax ellipticum*	*Abelmoschus esculentus*	*Abutilon hirtum*	*Alcea rosea*	*Gossypium herbaceum*	*Hibiscus syriacus*	*Lagunaria patersonii*	*Malva parviflora*	*Malvaviscus arboreus*	*Pavonia arabica*	*Thespesia populnea*	*Sida alba*
ISSR3	**TAB[1]**	0	0	0	0	0	4	1	2	3	0	4	2	7	6	2	6	7	8	7	6	0	0	**9**
	SB[2]	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	PB%[3]	0	0	0	0	0	22	6	11	17	0	22	11	39	33	11	33	39	44	39	33	0	0	**50**
ISSR10	**TAB[1]**	7	6	4	3	6	6	6	**10**	8	5	6	6	3	8	6	5	7	5	6	6	1	0	6
	SB[2]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	PB%[3]	44	38	25	19	38	38	38	**63**	50	31	38	38	19	50	38	31	44	31	38	38	6	0	38
ISSR13	**TAB[1]**	**7**	5	3	2	4	4	2	6	4	3	4	3	2	5	5	3	4	**7**	4	**7**	0	2	2
	SB[2]	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	PB%[3]	**50**	36	21	14	29	29	14	43	29	21	29	21	14	36	36	21	29	**50**	29	**50**	0	14	14
ISSR34	**TAB[1]**	7	5	3	7	5	6	5	7	6	6	**10**	7	7	9	6	7	4	6	6	7	2	4	7
	SB[2]	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	PB%[3]	37	26	16	37	26	32	26	37	32	32	**53**	37	37	47	32	37	21	32	32	37	11	21	37
ISSR46	**TAB[1]**	4	5	2	3	5	4	4	5	4	6	6	5	5	5	3	4	**7**	4	4	4	1	3	4
	SB[2]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PB%[3]	36	45	18	27	45	36	36	45	36	55	55	45	45	45	27	36	**64**	36	36	36	9	27	36
Mean	**TAB[1]**	**25**	**21**	**12**	**15**	**20**	**24**	**18**	**30**	**25**	**20**	**30**	**23**	**24**	**33**	**22**	**25**	**29**	**30**	**27**	**30**	**4**	**9**	**28**
	SB[2]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**1**	**2**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**
	PB%[3]	**33.4**	**29.0**	**16.1**	**19.4**	**27.6**	**31.2**	**24.0**	**39.8**	**32.6**	**27.7**	**39.1**	**30.5**	**30.8**	**42.4**	**28.6**	**31.8**	**39.2**	**38.7**	**34.6**	**38.8**	**5.2**	**12.5**	**35.0**

[1]Total number of bands, [2]Specific bands, [3]Percentage of polymorphism.

The efficiency of the ISSR primers slightly differed, as shown in Table 4. The PIC ranged from 0.39 (ISSR46) to 0.75 (ISSR3), with an average of 0. 60, and the MI varied from 48.66 (ISSR13) to 74.60 (ISSR10), with an average of 66.51. The average RP values were 13.76, which ranged from 8.22 (ISSR3) to 17.64 (ISSR46), with an average of 13.76. Additionally, the primer ISSR46 appeared to be the most efficient for assessing genetic diversity, as indicated by the high rate of RP. All primers showed 100% polymorphism with a high effective multiplex ratio (EMR), which varied from 88 (ISSR 13) to 139 (ISSR 34, ISSR 46), with a mean value of 113**.**

SRAP analysis

Using 11 primer combinations, clear, reproducible bands were produced, as represented by SRAP Me4-Em6 (Fig. 2 & S1). The total and specific bands as well as the percentages of polymorphisms are shown in Table 6.

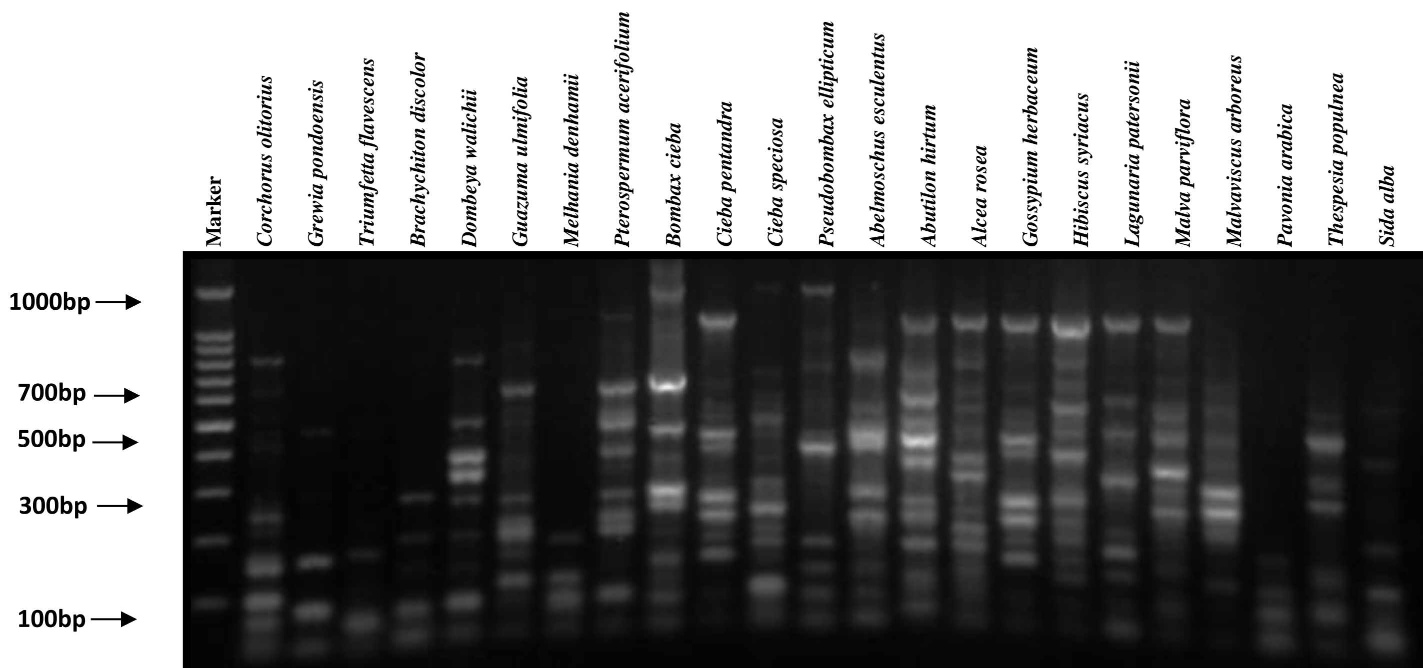

Fig. 2. SRAP of the primer combination Me4-Em6.

TABLE 6. Indices amplified with the examined species of Malvaceae *s.l.* by the 11 SRAP primer combinations.

Primer Combinations	TB[1]	PB[2]	MB[3]	% PB[4]	SB[5]	PIC[6]	MI[7]	RP[8]	EMR[9]
Me1-Em2	21	20	1	95%	2	0.763	127.38	16.00	166.99
Me2-Em1	21	21	0	100%	5	0.786	113.20	13.71	144.00
Me2-Em6	17	16	1	94%	2	0.65	66.33	12.12	102.00
Me3-Em1	17	16	1	94%	0	0.647	70.51	12.94	109.00
Me3-Em2	18	18	0	100%	0	0.669	95.73	15.89	143.00
Me3-Em4	22	22	0	100%	2	0.841	126.97	13.73	151.00
Me4-Em2	17	16	1	94%	0	0.61	85.45	16.59	140.00
Me4-Em6	25	25	0	100%	1	0.957	167.51	14.00	175.00
Me7-Em10	20	19	1	95%	5	0.776	87.71	13.40	113.00
Me8-Em1	23	23	0	100%	5	0.91	110.13	10.52	121.00
Me8-Em2	22	21	1	95.5%	1	0.809	137.47	15.55	170.00
Total	**223**	**217**	**6**	**97.3%**	**23**	**8.42**	**1188.40**	**154.44**	**1534.99**
Mean	-	-	-	-	-	**0.77**	**108.04**	**14.04**	**139.54**

[1]Total number of bands, [2]Polymorphic bands, [3]Monomorphic bands, [4]Percentage of polymorphism, [5]Specific bands, [6]Polymorphic information content, [7]Marker index, [8]Resolution power, [9]Effective multiplex ratio.

Two hundred and seventeen out of 223 bands were polymorphic, with a few common bands (6), as shown in Table 6. *Hibiscus syriacus* L. achieved the maximum number of bands (90), while *P. arabica* produced the minimum number (25). Consequently, the percentage of polymorphisms ranged from 38.11% to 8.15 %, respectively. The studied taxa revealed a total of 23 specific bands were detected: 5 in *Bombax ceiba* L., 4 in *Pterospermum acerifolium* (L.) Willd, 3 in *Ceiba pentandra* (L.) Gaertn., 2 in both *Corchorus olitorius* L. and *Grewia pondoensis* Burret, and 1 in *Triumfetta flavescens* Hochst ex A.Rich, *Guazuma ulmifolia* Lam., *C. speciosa*, *Pseudobombax ellipticum* (Kunth) Dugand, *Gossypium herbaceum* L., *H. syriacus* and *Sida alba* L. The detected bands ranged from 1000 to 50bp with a high percentage of polymorphism ranging from 94% (the Me2-Em6, Me3-Em1 and Me4-Em2 primer combinations) to 100% (the Me2-Em1, Me3-Em2, Me3-Em4, Me4-Em6 and Me8-Em1 primer combinations). There were 5 specific bands corresponding to Me2-Em1, Me7-Em10 and Me8-Em1; 2 corresponding to Me1-Em2, Me2-Em6 and Me3-Em4; and 1 corresponding to Me4-Em6 and Me8-Em2 (Table 7).

The efficacy of the 11 primer combinations based on the SRAP-PCR analysis (Table 6) was high, which was also reflected in the MR data (20.2). The PIC was 0.77, with values ranging from 0.61 (primer Me4-Em2) to 0.91 (primer Me8-Em1). The mean MI was 137.47, which varied from 66.3 (primer Me2-Em6) to 167.51 (primer Me4-Em6). The RP values ranged from 10.52 (primer Me8-Em1) to 16.95 (primer Me4-Em2), with an average of 14.04. The mean EMR value was 170, which varied from 102 to 175 for the primers Me2-Em6 and Me4-Em6, respectively.

The dendrogram based on the banding patterns for each of theISSR and SRAP analyses did not show good clustering, and therefore, a combined dendrogram was constructed according to Ward's method using the Jaccard coefficient (Fig. 3). It is distinguished into two major clades; I and II. Clade 'I' included five species of (Malvaceae *s.s.* which are *Abelmoschus esculentus* L.Moench, *P. arabica, H. syriacus, M. arboreus* and *Alcea rosea* L.), while Clade 'II' included the remaining eighteen species with intermingled families (Tiliaceae s.s., Sterculiaceae s.s. and Bombacaceae s.s.) in several subclades.

ITS polymorphisms

In the present study, ten species were selected for the ITS sequence technique: *C. olitorius*, *Brachychiton discolor* F. Muell, *B. ceiba*, *C. pentandra*, *A. esculentus*, *Abutilon hirtum*, *G. herbaceum*, *H. syriacus*, *M. parviflora* and *Thespesia populnea* (L.) Sol.êx Correa. These species represented the distinct subclades of the combined dendrogram, in addition to the problematic Malvaceae *s.s* species. The ITS sequences produced a single band at a size of 700 bp (Fig. 4). The sequence data of the ten species were aligned pairwise using CLUSTAL W software (S2), and the alignment results are summarized in Table (8). The length of the ITS region varied from 677 bp in *A. hirtum* to 798 bp in *C. olitorious*, with a mean of 727 bp. The 'G+C' content for the entire spacer region ranged from 54.06% (*A. hirtum*) to 67.23% (*B. ceiba*), with a mean of 60.95%. The numbers of conserved and variable sites within the sequences were 287 and 511, respectively. The sequencing data for all the examined ten species have been deposited in GenBank/NCBI under the accession numbers OQ302173-OQ302182.

ITS phylogenetic analysis

The phylogenetic relationship was constructed using MEGA and PAUP software, and the simulation analysis was carried out by choosing the ITS sequence of *E. nitentifolius* as an outgroup from the GenBank/NCBI sequence database (GenBank: KP093062.1). The constructed phylogenetic trees achieved the same topology. In this study, the ten Malvaceae *s.l.* species were classified as subfamilies or tribes rather than at the family level. With the PAUP software, the ITS dataset was analyzed via the parsimony method (using the likelihood and pairwise comparisons of nucleotide substitutions), which revealed that transversions were more common than transitions (Table 9, Fig. 5). In the dendrogram and phylogenetic tree, two major clades were distinguished: 'I' and 'II'. The major clade 'I' contained the outgroup (*E. nitentifolius*), and the major clade 'II' contained *C. olitorius* (Grewioideae) in clade 'A' from the rest of the species in clade 'B'. Clade 'B' is distinguished into two groups: 'a' and 'b'. Bombacoideae (*B. ceiba* and *C. pentandra*) were assembled in group 'a', and both Sterculioideae and Malvoideae were assembled in group 'b', with 2 branches, '1' and '2'. The four tribes were distinct in Malvoideae, Abutilae (*A. hirtum*), Gossypie (*G. herbaceum* and *Thespesia populnea*), Hibiscie (*A. esculentus* and *H. syriacus*) and Malvie (*M. parviflora*). The transition/transversion (Ti/Tv) ratios ranged from 0.82 in *C. olitorius* (Grewioideae) to 1.158 in *T. populnea* (Malvoideae, tribe Gossypie).

Parsimony analysis with accelerated transformation character state optimization (ACCTRAN) yielded a tree length of 1132 steps. After excluding the uninformative characters, the consistency index (CI) was 0.671, and the homoplasy index (HI) was 0.329.

TABLE 7. Genetic diversity and relationship of Malvaceae *s.l.* species revealed by SRAP marker and their means.

Primer combinations	bands	*Corchorus olitorius*	*Grewia pondoensis*	*Triumfetta flavescens*	*Brachychiton discolor*	*Dombeya walichii*	*Guazuma ulmifolia*	*Melhania denhamii*	*Pterospermum acerifolium*	*Bombax ceiba*	*Ceiba pentandra*	*Ceiba speciosa*	*Pseudobombax ellipticum*	*Abelmoschus esculentus*	*Abutilon hirtum*	*Alcea rosea*	*Gossypium herbaceum*	*Hibiscus syriacus*	*Lagunaria patersonii*	*Malva parviflora*	*Malvaviscus arboreus*	*Pavonia arabica*	*Thespesia populnea*	*Sida alba*
Me1-Em2	**[1]TAB**	**10**	8	4	3	9	**10**	4	9	**10**	8	8	6	9	9	7	7	8	7	7	8	3	6	8
	[2]SB	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	**42.86**	33.33	14.29	9.52	38.10	**42.86**	14.29	38.10	**42.86**	33.33	33.33	23.81	38.10	38.10	28.57	28.57	33.33	28.57	28.57	33.33	9.52	23.81	33.33
Me2-Em1	**[1]TAB**	7	8	2	5	4	**10**	4	5	9	7	7	6	8	7	6	5	9	7	8	7	3	3	7
	[2]SB	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	[3]PB %	33.33	38.10	9.52	23.81	19.05	**47.62**	19.05	23.81	42.86	33.33	33.33	28.57	38.10	33.33	28.57	23.81	42.86	33.33	38.10	33.33	14.29	14.29	33.33
Me2-Em6	**[1]TAB**	5	6	2	1	3	6	2	**9**	6	6	3	4	7	5	4	5	8	4	3	5	2	3	4
	[2]SB	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	[3]PB %	23.53	29.41	5.88	0.00	11.76	29.41	5.88	**47.06**	29.41	29.41	11.76	17.65	35.29	23.53	17.65	23.53	41.18	17.65	11.76	23.53	5.88	11.76	17.65
Me3-Em1	**[1]TAB**	5	5	2	2	6	7	2	5	3	5	8	2	7	6	6	4	**9**	6	7	3	1	5	4
	[2]SB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	23.53	23.53	5.88	5.88	29.41	35.29	5.88	23.53	11.76	23.53	41.18	5.88	35.29	29.41	29.41	17.65	**47.06**	29.41	35.29	11.76	0.00	23.53	17.65
Me3-Em2	**[1]TAB**	**9**	7	6	3	8	6	3	**9**	6	2	8	**9**	7	8	4	5	6	6	8	8	3	3	**9**
	[2]SB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	**50.00**	38.89	33.33	16.67	44.44	33.33	16.67	**50.00**	33.33	11.11	44.44	**50.00**	38.89	44.44	22.22	27.78	33.33	33.33	44.44	44.44	16.67	16.67	**50.00**

[1]Total number of bands, [2]Specific bands, [3]Percentage of polymorphism.

TABLE 7. (Continuous)

Primer combinations	bands	*Corchorus olitorius*	*Grewia pondoensis*	*Triumfetta flavescens*	*Brachychiton discolor*	*Dombeya walichii*	*Guazuma ulmifolia*	*Melhania denhamii*	*Pterospermum acerifolium*	*Bombax ceiba*	*Ceiba pentandra*	*Ceiba speciosa*	*Pseudobombax ellipticum*	*Abelmoschus esculentus*	*Abutilon hirtum*	*Alcea rosea*	*Gossypium herbaceum*	*Hibiscus syriacus*	*Lagunaria patersonii*	*Malva parviflora*	*Malvaviscus arboreus*	*Pavonia arabica*	*Thespesia populnea*	*Sida alba*
Me3-Em4	[1]TA	**10**	3	1	6	7	7	6	**10**	**10**	0	**10**	7	7	9	8	5	**10**	9	8	7	2	1	8
	[2]SB	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	**45.45**	13.64	4.55	27.27	31.82	31.82	27.27	**45.45**	**45.45**	0.00	**45.45**	31.82	31.82	40.91	36.36	22.73	**45.45**	40.91	36.36	31.82	9.09	4.55	36.36
Me4-Em2	[1]TA	5	5	5	1	8	**10**	5	6	6	1	6	6	7	6	8	8	**10**	7	8	8	1	6	8
	[2]SB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	23.53	23.53	23.53	0.00	41.18	**52.94**	23.53	29.41	29.41	0.00	29.41	29.41	35.29	29.41	41.18	41.18	**52.94**	35.29	41.18	41.18	0.00	29.41	41.18
Me4-Em6	[1]TA	8	5	3	5	9	9	4	8	**12**	9	9	8	8	10	**12**	7	11	8	8	7	4	6	5
	[2]SB	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	32.00	20.00	12.00	20.00	36.00	36.00	16.00	32.00	**48.00**	36.00	36.00	32.00	32.00	40.00	**48.00**	28.00	44.00	32.00	32.00	28.00	16.00	24.00	20.00
Me7-Em10	[1]TA	7	3	2	1	8	4	1	7	7	**10**	4	3	3	6	7	8	7	5	6	5	2	2	6
	[2]SB	0	1	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	30.00	10.00	5.00	0.00	35.00	15.00	0.00	30.00	30.00	**45.00**	15.00	10.00	10.00	25.00	30.00	35.00	30.00	20.00	25.00	20.00	5.00	5.00	25.00
Me8-Em1	[1]TA	5	8	3	4	7	7	2	6	6	7	6	5	0	9	9	**11**	5	6	5	0	2	1	7
	[2]SB	0	1	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
	[3]PB %	21.74	34.78	13.04	17.39	30.43	30.43	8.70	26.09	26.09	30.43	26.09	21.74	0.00	39.13	39.13	**47.83**	21.74	26.09	21.74	0.00	8.70	4.35	30.43
Me8-Em2	[1]TA	10	6	3	6	7	8	4	8	10	9	9	**11**	6	9	8	8	7	6	**11**	7	2	7	9
	[2]SB	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	[3]PB %	40.91	22.73	9.09	22.73	27.27	31.82	13.64	31.82	40.91	36.36	36.36	**45.45**	22.73	36.36	31.82	31.82	27.27	22.73	**45.45**	27.27	4.55	27.27	36.36
Mean	[1]TA	**81**	**64**	**33**	**37**	**76**	**84**	**37**	**82**	**85**	**64**	**78**	**67**	**69**	**84**	**79**	**73**	**90**	**71**	**79**	**65**	**25**	**43**	**75**
	[2]SB	**2**	**2**	**1**	**0**	**0**	**1**	**0**	**4**	**5**	**3**	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**
	[3]PB %	**33.35**	**26.18**	**12.37**	**13.02**	**31.32**	**35.14**	**13.72**	**34.30**	**34.55**	**25.32**	**32.03**	**26.94**	**28.86**	**34.51**	**32.08**	**29.81**	**38.11**	**29.03**	**32.72**	**26.79**	**8.15**	**16.78**	**31.03**

[1]Total number of bands, [2]Specific bands, [3]Percentage of polymorphism.

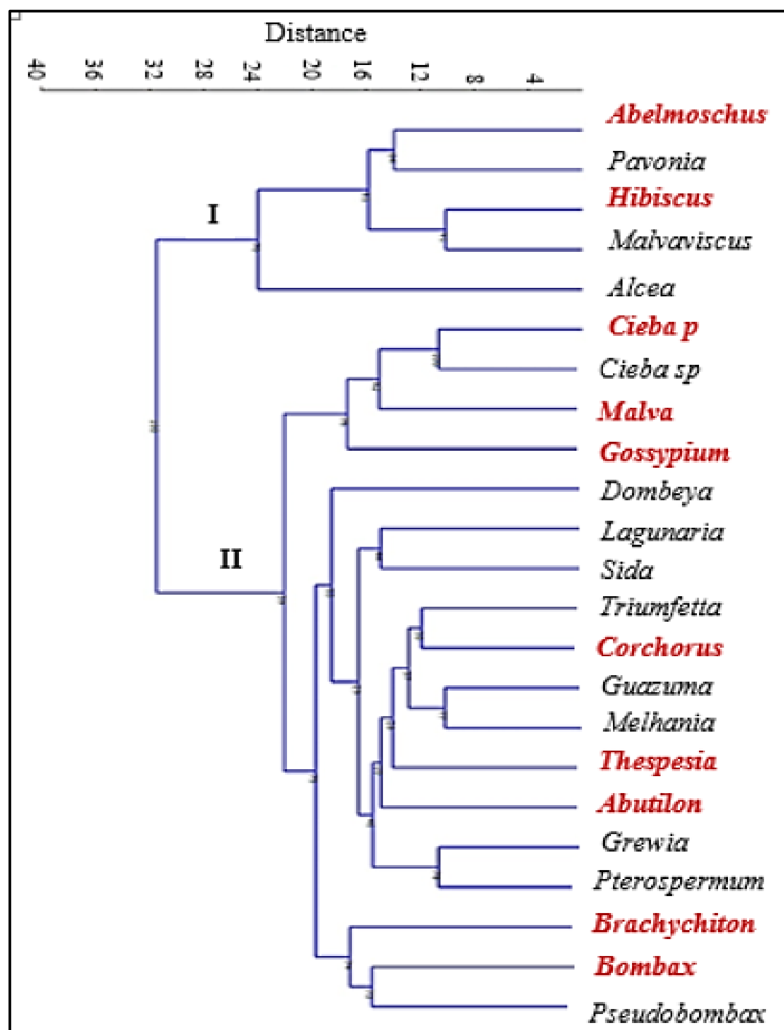

Fig. 3. Ward's dendrogram using Jaccard coefficient of Malvaceae *s.l.* species based on combined molecular markers (ISSR and SRAP).

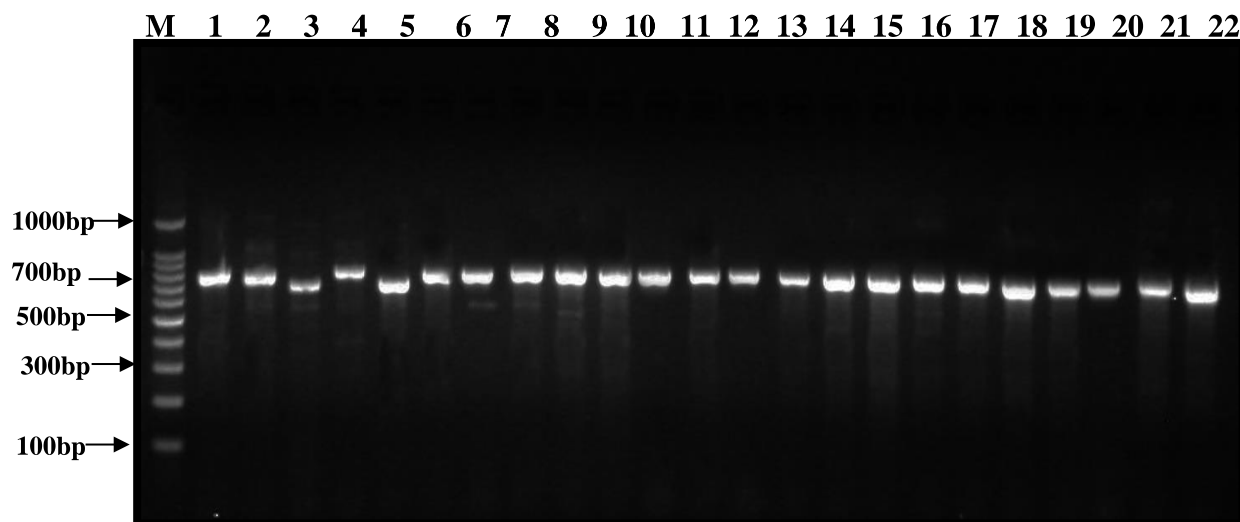

Fig. 4. The amplified product of the ITS region (approx. 700 bp) for 23 Malvaceae *s.l.* *M: 1 kbp marker/ladder.*

TABLE 8. The number of private segregating sites and the number of shared polymorphisms among different species of the 10 selected Malvaceae *s.l.* species.

Species	Length (bp)	A	T	C	G	G+C content (%)
Corchorus olitorius	798	163 (20%)	162 (21%)	233 (29%)	240 (30%)	59.27
Brachychiton discolor	745	155 (20%)	134 (20%)	208 (27%)	248 (33%)	61.21
Bombax ceiba	711	117 (16%)	116 (17%)	235 (33%)	243 (34%)	67.23
Ceiba pentandra	706	128 (18%)	117 (18%)	221 (31%)	240 (33%)	65.30
Abelmoschus esculentus	735	152 (20%)	126 (19%)	218 (29%)	239 (32%)	62.18
Abutilon hirtum	677	155 (22%)	156 (25%)	181 (26%)	185 (27%)	54.06
Gossypium herbaceum	705	143 (20%)	137 (21%)	203 (28%)	222 (31%)	60.28
Hibiscus syriacus	727	151 (20%)	153 (23%)	209 (28%)	214 (29%)	58.18
Malva parviflora	714	140 (19%)	134 (20%)	218 (30%)	222 (31%)	61.62
Thespesia populnea	755	153 (20%)	148 (20%)	219 (29%)	235 (31%)	60.13
Mean	**727**	**145 (19.5%)**	**138.8 (20.4%)**	**214 (29%)**	**228 (31.1%)**	**60.95%**
Number of Variable Site	**511**					

TABLE 9. Transition/transversion (Ti/Tv) ratios obtained by PAUP software according to the aligned sequence of ITS for the ten selected species of Malvaceae *s.l.*

Species	*Corchorus olitorius*	*Brachychiton discolor*	*Bombax ceiba*	*Ceiba pentandra*	*Abelmoschus esculentus*	*Abutilon hirtum*	*Gossypium herbaceum*	*Hibiscus syriacus*	*Malva parviflora*	*Thespesia populnea*	Mean
Corchorus olitorius		0.86	0.78	0.80	0.90	0.91	0.79	0.86	0.73	0.75	**0.82**
Brachychiton discolor	80/93		0.91	0.87	0.74	0.91	0.79	0.81	0.67	0.86	**0.824**
Bombax ceiba	77/99	92/101		0.83	1.07	1.90	0.88	1.21	0.96	0.86	**1.04**
Ceiba pentandra	80/99	90/103	40/48		1.04	1.29	0.75	1.01	0.84	0.79	**0.913**
Abelmoschus esculentus	71/79	64/87	76/71	70/76		1.11	0.99	1.30	0.94	1.00	**1.01**
Abutilon hirtum	79/87	86/95	106/56	90/70	70/63		0.83	0.90	1.10	0.82	**1.086**
Gossypium herbaceum	79/100	78/99	91/104	83/111	71/72	71/86		0.84	0.74	3.69	**1.144**
Hibiscus syriacus	71/83	79/97	95/78	83/82	51/39	64/71	72/86		0.89	0.89	**0.968**
Malva parviflora	72/99	69/103	87/91	83/99	66/70	77/70	67/90	71/80		0.76	**0.848**
Thespesia populnea	77/103	81/94	89/104	84/107	72/72	70/85	48/13	72/84	68/89		**1.158**

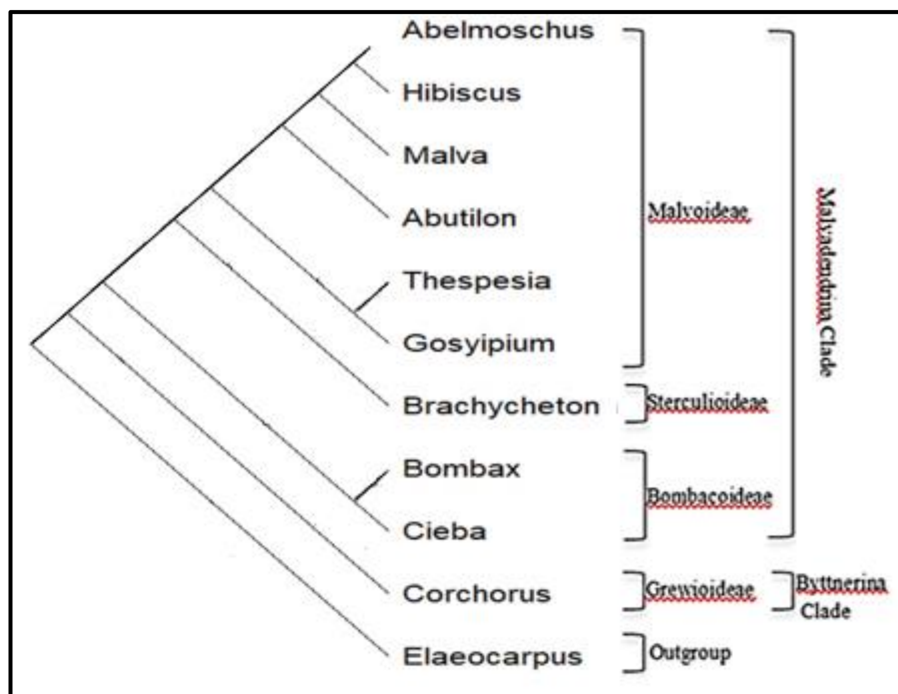

Fig. 5. UPGMA phylogenetic tree based on ITS sequencing of 10 Malvaceae *s.l.* species generated by MEGA software with the outgroup.

Discussion

Molecular data, improvements in DNA sequencing and computational power allowed the construction of phylogenetic relationships and provided an enhanced understanding of evolutionary processes (Besse, 2014, El-Sherif et al., 2020). Based on molecular analyses, Malvaceae was placed in the subclass Dilleniidae. According to Bayer et al. (1999), Malvaceae *s.l.* was divided into nine subfamilies: Bombacoideae, Brownlowioideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicteroideae, Malvoideae, Sterculioideae and Tilioideae. Baum et al. (1998), Kubitzki & Chase (2003), Baum et al. (2004) and Wilkie et al. (2006) claimed that groups within Malvaceae *s.l.* require extensive review to provide accommodation for its molecular-based subfamilies. Weising et al. (2006) supported the use of molecular markers to detect variability among closely related taxa. The present study aimed to clarify the debate between classical and modern circumscription of Malvaceae *s.l.* through molecular analyses. Molecular analyses were performed by using 301 molecular characteristics (78 ISSR and 223 SRAP bands) and highlighting the phylogenetic relationships among the studied taxa.

The inter-simple sequence repeats (ISSR) is a dominant marker that have been successfully used in genetic diversity and evolutionary studies in Malvaceae *s.l.* (Celka et al., 2012; Vanijajiva, 2012). Similarly, sequence-related amplified polymorphism (SRAP) is a codominant molecular marker introduced by Li & Queros (2001) that targets coding regions within the genome and is frequently used for gene tagging and genetic diversity (Badrakhani et al., 2014). The primer ISSR34 had the maximum number of amplified bands (19), while ISSR3 had the greatest number of specific bands (3). From an efficiency point of view, ISSR34 attained the maximum values for the PIC, MI, RP and EMR indices, followed by the ISSR3 primer. The present study recommends the use of these two primers, especially ISSR34, for the distinctiveness among taxa of Malvaceae *s.l.* *A. hirtum* amplified the maximum number of bands (33) and consequently the largest polymorphism percentage (42.37%). While *P. arabica* produced the lowest band and polymorphism percentage values (4 and 5.17%, respectively). Both *A. hirtum* and *C. speciosa* share the maximum number of specific bands (2). Using the SRAP technique, the percentage of polymorphisms revealed by the six primer combinations ranged from 94-95.5%, with one common band per primer. Therefore, the study suggested the use of these primer combinations for the identification of Malvaceae *s.l.* taxa. Five primer combinations generated 100% polymorphism without any common bands. These primers can be used as distinctive markers among taxa. The primer combination Me4-Em6 achieved the maximum number of bands (25) and the highest values for the PIC, MI, RP and EMR indices. The combinations of Me2-Em1, Me7-Em10 and Me8-Em1 generated the

most specific bands (5 bands each). *H. syriacus* was characterized by the highest number of bands (90) and percentage polymorphism (38.11%); on the other hand, *P. arabica* had the lowest number of bands (25) and percentage polymorphism (8.15%). A comparison of the taxa revealed one (*T. flavescens*, *G. ulmifolia*, *C. speciosa*, *P. elipticum*, *G. herbaceum*, *H. syriacus* and *S. alba*), two (*C. olitorius* and *G. pondoensis*), three (*C. pentandra*), four (*P. acerifloium*) and five (*B. ceiba*) specific bands, while the remaining examined taxa lacked specific bands. These findings are in agreement with those of Badrakhani et al. (2014), who reported that the SRAP technique is powerful for identifying genetic distance among species of Malvaceae *s.l.* and could be explained by the detection of polymorphisms in coding regions that are conserved among closely related taxa.

Generally, both ISSR and SRAP are valuable as distinctive techniques for identifying Malvaceae *s.l.* taxa (Ghafoor & Hamarashid, 2022; Meerza et al., 2023). Both techniques achieve high PIC values (> 0.5), which is attributed to their ability to serve as a distinctive identification marker (Badrakhani et al., 2014). In comparison to the two DNA techniques, SRAP analysis yielded more MR than ISSR which indicated the efficiency of SRAP because of its greater ratio of the total number of bands. The dendrograms obtained by ISSR and SRAP are noninformative and unclear enough to show the positioning of Malvaceae *s.l.* species. The species are mixed in different clades that do not follow any of the proposed systems of classification of Malvaceae.

Several factors make the ITS region valuable for use in phylogenetic analyses (Álvarez, & Wendel, 2003). First, the ITS region is highly repeated in plant nuclear genomes, along with other components of the nrDNA multigene family, including a highly variable region between the ribosomal repeats, the intergenic spacer. The high copy number of the nrDNA repeat facilitates the amplification and sequencing of the nrDNA. Second, the nrDNA multigene family has undergone rapid concerted evolution (Baldwin et al., 1995). This property of the ITS region is most important from a phylogenetic standpoint and promotes the accurate reconstruction of species relationships by sequencing (Slotta, 2000). ITS sequencing clearly distinguished three tribes: Gossypie (705- 755 bp), Hibiscie (727-735 bp) and Malvie (677-714 bp). This conclusion is supported by Takhtjan (2009), Reveal (2012) and APG (2016). In phylogenetic analyses based on the sequencing of the ITS region, *E. nitentifolius* (Elaeocarpaceae) was used as an outgroup. It was considered to be closely related to the core Malvales (Cronquist, 1988; Judd & Manchester, 1997).

The obtained phylogenetic tree based on ITS sequencing using PAUP software attained a relatively high consistency index (CI) of 0.671 as an assessment of the strength of the phylogenetic signal. Farris (1989a) determines the value of '1' for a perfect fit to the value '0' for the poorest fit. Sanderson and Hufford (1996) mentioned CI as a parameter of the goodness of fit of a dataset to a hierarchical tree structure. Moreover, the homoplasy index (HI) is relatively low (0.329), which indicates low homoplasy; i.e., the similarity between species is attributed to common ancestry and is a result of divergent evolution (Cvetković et al., 2021). For a more reliable construction of the phylogenetic tree, the ratio of transversions to transitions (Ti/Tv) was determined, which ranged from 0.67 to 3.64 in the studied taxa. Taxa with a greater number of transversions (lower Ti/Tv values) would be older, diverging early in the history of evolution (Saha et al., 2013). The obtained phylogenetic tree distinguished three clades (A, B and C). Clade A represents the outgroup (*E. nitentifolius*), while Clades B and C represent Byttnerina and Malvadendrina, respectively. The subfamily Grewioideae (Tiliaceae), which is represented by *C. olitorious*, is considered the most primitive taxon (Edlin, 1935; Cronquist, 1981& 1988; Takhtajan, 1980& 1997; Thorne, 1992& 2000) and treats Tiliaceae as the most primitive family in core Malvales. This is supported by the low mean value of Ti/Tv (0.82), which implies the primitiveness of this subfamily. Cvetković et al. (2021) placed Grewioideae in a separate clade based on the plastome dataset. On the other hand, Warming (1895) and Rao (1952) noted Sterculiaceae as the most primitive group. Jones and Good (2016), Johnson et al. (2019) and Stijik et al. (2020) mentioned that the ranking and phylogenetic relationships of a clade are crucial steps in evolutionary analyses of this complex group. Clade C assorts the Malvadendrina clade with three subfamilies: 1, 2 and 3 of Bombacoideae, Sterculioideae and Malvoideae, respectively. Bombacoideae and Malvoideae together form a well-supported clade, Malvatheca, as supported previously by many authors by molecular analysis (Alverson et al. 1998; Bayer et al. 1999; Nyffeler et al. 2005). The sequencing data treat Bombacoideae as a more primitive subfamily than Sterculiodeae, with an average Tv/Ti of 0.976. This finding contradicts the findings of Edlin (1935), Cronquist (1981& 1988), Takhtajan (1980& 1997), and Thorne (1992& 2000), who considered Sterculioideae (Sterculiaceae) the primitive subfamily. The phylogenetic data suggest the inclusion of Sterculioideae within Malvadendrina,

this conclusion was previously remarked as an unclear opinion described by Wilkie et al. (2006) and Hernández-Gutiérrez & Magallon (2019). Malvoideae (Malvaceae s.s.) is by far the largest subfamily in Malvaceae *s.l.* (c. 1800 species). It acquired the highest average Tv/Ti (1.036), which was attributed to the advanced placement in the tree. This study was well supported by all the previous authors. The dendrogram of the ITS sequencing data revealed three tribes within Malvoideae: the Gossypie, Hibiscie and Malvie tribes. However, the phylogenetic tree discriminates four tribes: Abutilae, Gossypie, Hibiscie and Malvie. This finding agrees with the classical tribal system of Hutchinson (1967) and contradicts those of Shultz-Motel (1974), Takhtjan (2009) and the APG (2016). The tribe Abutilae (*A. hirtum*) was distinguished by the highest number of total and specific bands produced in ISSR, while in SRAP, it had a high percentage of polymorphisms. Phylogenetically, the present study suggested the tribal level of Abutilae.

The study indicated that the use of ISSR and SRAP as two powerful distinctive molecular techniques especially SRAP was proven more efficient. Both techniques were more successful at accessing a natural classification, with special referrals to sequencing techniques. The present study disagrees with the view of the traditional four families included in the classical systems of classification, and it agrees with the APG IV system of the nine subfamilies classification within two clades. However, the infrastructures of those clades were unclear, especially with the limits between Sterculioideae and Dombeyoideae, as well as between Malvoideae and Bombacoideae. Additionally, most of the tribal systems for both Malvoideae and Bombacoideae were confirmed.

Dedication: To Professor El-Sadek Laila, who passed away before we edited this work; God Bless you and thank you for all you have done.

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Ethics Approval: Not applicable.

Supplementary Information:

Supplementary file 1 (S1): ISSR and SRAP Amplifications.

Supplementary file 2 (S2): Sequence alignment of the ITS region.

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التقنيات الجزيئية للتأكد من الاستراتيجيات الوراثية في بعض أصناف Malvaceae s.l.

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لقد تعرضت عائلة Malvaceae تقليديًا للعديد من الأساليب التصنيفية فيما يتعلق بعلاقاتها وأقسامها التصنيفية. ومع التقدم السريع في الأساليب المعتمدة على تفاعل البوليميراز المتسلسل ومعلومات تسلسل الحمض النووي، أصبح لدى علماء التصنيف الآن فرصة التحول من أنظمة التصنيف التقليدية إلى أنظمة أحدث. قدمت الدراسات الجزيئية للأصناف Malvaceous آراء جديدة حول تجمع هذه الأصناف وتطورها. تضمن هذا العمل تحليلات جزيئية لثلاثة وعشرين نوعًا من أنواع Malvaceae. تم استخدام ثلاث تقنيات جزيئية، وهي تكرار التسلسل البسيط (ISSR)، وتعدد الأشكال المضخم المرتبط بالتسلسل (SRAP)، والمباعد الداخلي المكتوب (ITS)، لدراسة العلاقات بين الأصناف المدروسة. تم بناء علاقات التطور الوراثي باستخدام تحليل الوراثة التطورية الجزيئية والتحليل الوراثي باستخدام برنامج Parsimony (MEGA وPAUP). كشفت نتائج هذا البحث عن 301 خاصية جزيئية (78 ISSR و223 نطاق SRAP) بين الأصناف. تدعم هذه البيانات وجهة النظر التصنيفية لعائلات Malvaceae s.l.s الفرعية في نظام Angiosperm Phylogeny Group لتصنيف النباتات (أنظمة APG).