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Full Length Article

Biochemical and molecular genetic characterization of some species of family Malvaceae, Egypt

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ABSTRACT

The aim of the present study intended mainly to investigate the interrelationships between the six studied taxa namely *Abutilon theophrasti*, *Lavatera cretica*, *Hibiscus trionum*, *Hibiscus sabdariffa*, *Malva parviflora* and *Sida alba* collected from ten different accessions in Egypt belonging to family Malvaceae. Biochemical studies include protein profile using SDS-PAGE technique and three isozymes (esterase, peroxidase and acid phosphatase). The electrophoretic analysis revealed the presence of eighteen bands of molecular weight ranging from 11.3 to 115.3 KD. The highest number of bands 15 was observed in *H. sabdariffa* (Hs1) collected from Menia el-Kamh district and the two accessions of *M. parviflora* where as the lowest number 11 bands were recorded in *H. trionum* collected from Talkha district. Four loci of peroxidase isozyme distinguished, three loci of acid phosphatase isozyme and two loci of esterase isozyme. In regarding to random amplified polymorphic DNA technique (RAPD), ten primers were used to differentiate between these accessions. Primer OPA-4 gave the highest percentage of polymorphism (100%), while primer OP-B6 produced the lowest percentage (50%).

1. Introduction

Malvaceae or the mallow family is the family of flowering plants containing over 200 genera with close to 2300 species. The largest genera *Hibiscus* (300 species), *Streculia* (250 species), *Dombeya* (225 species), *Pavonia* (200 species) and *Sida* (200 species). The principle economic use of Malvaceae plants is as a source of natural fibers, the family providing perhaps the

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Table 1 – Names and localities of ten accessions of the six studied taxa collected from Egypt.

No.	Taxa	Codes	Locality
1	*Abutilon Theophrasti* Medik.	At	El-Behera governorate (Abo homos district)
2	*Hibiscus sabdariffa* L.	Hs1	El-sharkyia governorate (Menia el-kamh district)
3	*Hibiscus sabdariffa* L.	Hs2	El-Dakahlyia governorate (Talkha district)
4	*Hibiscus trionum* L.	Ht1	Kafr el-sheikh governorate (El-Riyad district)
5	*Hibiscus trionum* L.	Ht2	El-Dakahlyia governorate (Talkha district)
6	*Lavatera cretica* L.	Lc	El-Behera governorate (Rashid district)
7	*Malva parviflora* L.	Mp1	El-sharkyia governorate (Menia el-kamh district)
8	*Malva parviflora* L.	Mp2	El-Dakahlyia governorate (Nabrooh district)
9	*Sida alba* L.	Sa1	El-sharkyia governorate (Menia el-kamh district)
10	*Sida alba* L.	Sa2	El-Dakahlyia governorate (Nabrooh district)

worlds three most important fiber crops plants of the family are also used for food, beverages, timber, in traditional medicine and in horticulture [1].

Many researches have been published on the ecology, taxonomy, genetic, cytology, chemotaxonomy, physiology, seed germination and economic uses of family Malvaceae such as [2] in ecology; in taxonomy [3], in chemotaxonomy [4] and in genetic researches [5] studied the pollen.

Aerial parts of many species belong to family Malvaceae have Betaines, Glycine betaines were obtained in high yield (0.5–4.6% dry weight). Also, trigonelline was recorded, but the yield was low (0.005–0.07% dry weight) [4].

Isozymes have been widely used as a molecular markers for the identification the genetic relationships among genera, species and varieties. The phylogenetic relationships in many genera have been studied by isozymes electrophoresis [6] and [7].

Seed protein electrophoresis has been successfully used in define species relationships in various groups of plants [8].

The technology of the molecular biology has been developed over the 20 years and provided new methods for observing the genetic differences among species. These techniques offer and give many advantages over the conventional methods [9].

Therefore, the present study was designed to clarify the genetic relationships among six taxa belong to family Malvaceae collected from ten different accessions from Egypt. This work is very important to document in gene banks for sustainable conservation of plant genetic resources.

2. Materials and methods

2.1. Accessions selection

Ten accessions of the six studied taxa Table 1 subjected to analysis using available characterization methods. Viable seeds of the studied taxa were collected from 50 mature individuals. Identification and nomenclature of studied species were according to [10] and [11].

2.2. Protein analysis

Electrophoresis analysis of seed proteins followed the method for discontinuous SDS-PAGE technique of [12].

2.3. Native PAGE for isozymes

Isozymes variations identified using native polyacrylamide gel electrophoresis. Three isozymes (esterase, peroxidase and acid phosphatase) studied. These isozymes were separated on polyacrylamide gel according to [13].

2.4. DNA extraction

Genomic DNA of the ten accessions of six taxa was extracted from fresh young leaves according to [14].

2.5. Random amplified polymorphic DNA (RAPD-DNA)

Ten primers were used to generate RAPD markers according to [15] with some modifications. The sequence of these primers is given in Table 2. The percentage of polymorphism can be calculated according to this equation.

$$\%\ \text{of polymorphism} = \frac{\text{polymorphic bands}}{\text{total bands}} \times 100$$

2.6. Data analysis

All gels were photographed and analyzed using Bio-Rad video Documentation system Model Gel Doc 2000. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively). Data analyses were performed using SYSTAT version 7.0 program [16].

Table 2 – Primers and their composition used in RAPD analysis.

Primer names	Sequences
OP-AO1	CAGGCCCTTC
OP-AO4	AATCGGGCTG
OP-AO7	GAAACGGGTG
OP-A10	GTGATCGCAG
OP-A15	TTCCGAACCC
OP-BO1	GTTTCGCTCC
OP-BO4	GGACTGGAGT
OP-BO6	TGCTCTGCCC
OP-BO7	GGTGACGCAG
OP-B17	AGGGAACGAG

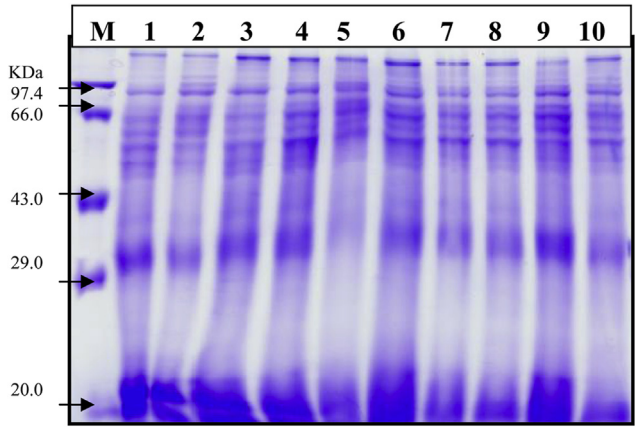

Fig. 1 – Polyacrylasmide gel illustrating seed proteins bands of some taxa belonging to family Malvaceae. (M) marker, 1 (At), 2 (Hs1), 3 (Hs2), 4 (Ht1), 5 (Ht2), 6 (Lc), 7 (MP1), 8 (Mp2), 9 (Sa1) and 10 (Sa2).

3. Results

3.1. *Protein analysis*

The electrophoretic banding patterns of extracted proteins have been studied in the ten accessions of the six taxa. These patterns were shown in Fig. 1. The distribution of protein bands in the different accessions of the six taxa based on their molecular weight range was shown in Table 3. The following is a brief description of the banding profiles for all accessions.

The electrophoretic protein profile of Mp1, Mp2 and Hs1 accessions consist 15 bands, 14 bands in At and Sa1, 13 bands in Hs2 and Ht1, 12 bands in Lc and Sa2, 11 bands in Ht2. the molecular weight bands for all accessions of the six studied taxa ranging from 11.3 to 115.3 KDa Table 3. The percentage of polymorphism was given in Table 3.

3.2. *Isozymes*

The electrophoretic analysis of peroxidase, acid phosphatase and esterase isozymes using native PAGE gel for the ten accessions of the six studied taxa recorded in Tables 4–6 and Plate 1.

Four loci of peroxidase isozyme distinguished which differ in their amount and relative migration distance. Locus 1 and locus 4 recorded in all taxa under study. Locus 2 recorded in all

Table 3 – Seed protein attributes of some species of family Malvaceae collected from different accessions, for accessions names see Table 1.

No.	Codes / (KDa)	At	Hs1	Hs2	Ht1	Ht2	Lc	Mp1	Mp2	Sa1	Sa2
1	115.3	+	+	+	+	+	+	+	+	+	+
2	109.2	+	+	+	+	+	+	+	+	+	+
3	106.8	-	+	+	-	+	-	+	+	-	-
4	99.7	+	+	+	+	+	-	+	+	+	+
5	98.2	-	+	-	-	-	-	+	+	+	+
6	91.3	+	+	+	+	+	+	+	+	+	+
7	80.3	-	+	-	+	+	+	+	+	+	+
8	71.6	-	+	-	-	-	-	-	-	-	-
9	68.5	+	+	-	+	+	+	+	+	+	-
10	62.4	+	+	+	+	-	+	+	+	+	+
11	58.3	+	-	+	-	-	-	-	-	-	-
12	50.1	+	-	+	-	-	-	-	-	-	-
13	35.8	+	+	+	+	-	+	+	+	+	+
14	26.7	+	+	-	+	+	+	+	+	+	-
15	22.6	+	+	+	+	-	+	+	+	+	+
16	17.5	+	-	+	+	+	+	+	+	+	+
17	12.4	+	+	+	+	+	+	+	+	+	+
18	11.3	+	+	+	+	+	+	+	+	+	+
19	Total bands	14	15	13	13	11	12	15	15	14	12
20	Polymorphic bands %	50	55.55	44.44	44.44	33.33	38.88	55.55	55.55	50	38.88

Table 4 – Bands of Acid phosphatase isozyme (ACPH.) for some species of family Malvaceae collected from Egypt.

Codes / Bands	At	Hs1	Hs2	Ht1	Ht2	Lc	Mp1	Mp2	Sa1	Sa2
ACPH.1	1	1	1	1	1	1	1	1	1	1
ACPH.2	1	1	1	0	1	1	1	1	1	1
ACPH.3	0	0	0	0	0	0	0	0	1	0
Total bands	2	2	2	1	2	2	2	2	3	2

Table 5 – Bands of peroxidase isozyme (per.) for some species of family Malvaceae collected from Egypt.

Codes / Bands	At	Hs1	Hs2	Ht1	Ht2	Lc	Mp1	Mp2	Sa1	Sa2
Per.1	1	1	1	1	1	1	1	1	1	1
Per.2	1	1	0	1	1	1	1	1	1	1
Per.3	0	0	0	1	1	1	1	1	1	1
Per.4	1	1	1	1	1	1	1	1	1	1
Total bands	3	3	2	4	4	4	4	4	4	4

Table 6 – Bands of Esterase isozyme (ACPH.) for some species of family Malvaceae collected from Egypt.

Codes / Bands	At	Hs1	Hs2	Ht1	Ht2	Lc	Mp1	Mp2	Sa1	Sa2
Est.1	1	1	1	1	1	1	1	1	1	1
Est.2	1	1	1	1	1	1	1	1	1	1
Total bands	2	2	2	2	2	2	2	2	2	2

taxa under study except *H. sabdariffa* collected from Talka, El-Dakahlyia Governorate. Locus 3 recorded in all taxa under study except *Abutilon theophartis* and the two accessions of *Hibiscus sabdariffa*. A total of three loci of acid phosphatase isozyme distinguished which differ in their amount and relative migration distance. Locus 1 found in all taxa under study, locus 2 recorded in all taxa except *Hibiscus trionum* collected from El- Riyad- Kafr El-Sheikh. Locus 3 found only in *Sida alba* collected from Menia El-Kamh El-Sharkyia Table 5. Two loci of esterase isozyme were found in all studied taxa Table 6. Concerning all accessions of the six studied taxa, the highest percentage of polymorphism 66.6% recorded in acid phosphatase isozyme, 50% in peroxidase isozyme and 0% in esterase Table 7.

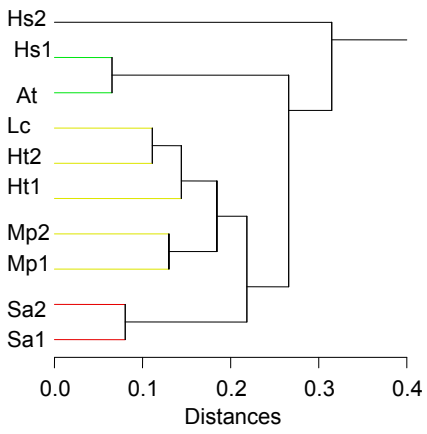

Fig. 2 – Phenogram showing the relationships among the six studied species of family Malvaceae from ten different accession in Egypt using distance metric 1 Gamma coefficient and average linkage method (for accessions name see Table 1).

3.3. *DNA fingerprint*

In the present study ten primers used to differentiate among the ten accessions of the six studied taxa as recorded in Table 8 and Plate 2. The percentages of polymorphisms were recorded in Table 9. The results were reported as follow:

3.4. *Primer OPA-1*

The results revealed that this primer produced a total number of 12 bands. Bands of molecular size 830 bp and 370 bp were recorded in all taxa under study. Bands of molecular size 600 bp and 680 bp were recorded only in *Lavatera cretica* so that this band could be used as a positive molecular marker for *L. cretica*. The band of a molecular size 700 bp recorded in all taxa

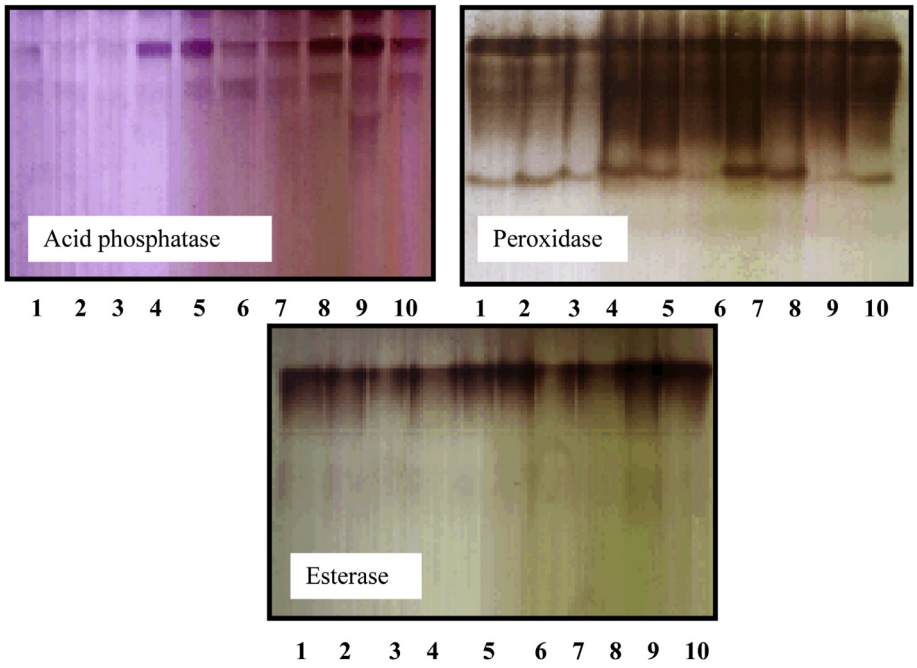

Plate 1 – The electrophoresis patterns of three isozymes of some taxa belonging to family Malvaceae. 1 (At), 2 (Hs1), 3 (Hs2), 4 (Ht1), 5 (Ht2), 6 (Lc), 7 (MP1), 8 (Mp2), 9 (Sa1) and 10 (Sa2).

under study except *L. cretica* so that this band could be used as a negative molecular marker for this species. The band of a molecular size 1260 bp recorded in *H. trionum*, *H. sabdariffa*, *Malva parviflora* and *S. alba*.

3.5. *Primer OPA-4*

The results revealed that this primer produced 12 bands among the ten accessions of the six studied taxa with a molecular size ranging from 310 to 2500 bp. The band of a molecular size 1500 bp recorded only in *M. parviflora* collected from Menia El-Kamh El-Sharkyia, it could be used as a positive molecular marker for this accession. The band of molecular weight 590 bp recorded in all taxa except two accessions of *S. alba*.

3.6. *Primer OPA-7*

The molecular size of the PCR products ranged from 230 bp to 850 bp. Bands of molecular size 680 bp and 420 bp recorded in all accessions of the studied taxa and missed in *A. theophartis* and the two accessions of *H. trionum*.

3.7. *Primer OPA-10*

The primer gave five polymorphic bands and two common bands with molecular size 420 bp and 680 bp. Band of a molecular size 330 bp recorded in all taxa under study except *H. sabdariffa* collected from Nabrooh –El-Dakahlyia Governorate. Band of a molecular size 550 bp recorded only in *M. parviflora* collected from Nabrooh, this band could be used as a molecular marker for *M. parviflora* collected from Nabrooh.

3.8. *Primer OPA-15*

The molecular sizes of PCR products ranged from 200 to 2900 bp. This primer gave seven polymorphic bands and four common bands with molecular sizes 200, 850, 990 and 2900 bp. Band with a molecular size 500 bp restricted to *M. parviflora* collected from Nabrooh and the band with molecular size 1680 bp restricted to *H. sabdariffa* collected from Menia El-Kamh El-Sharkyia Governorate. Band of a molecular size 620 bp recorded in all taxa under study except *H. sabdariffa* collected from Talkha- El-Dakahlyia Governorate. This band could be used as a negative molecular marker for *H. sabdariffa*

Table 7 – polymorphic bands of some species of family Malvaceae collected from Egypt.

Bands	Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
		Unique	Non-unique		
Isozymes					
Peroxidase	2	0	2	4	50%
Acid phosphatase	1	0	2	3	66.6%
Esterase	2	0	0	2	0%

Table 8 – Data matrix of RAPD- PCR for some species of family Malvaceae collected from Egypt.

DNA marker	Size (bp)	accessions									
		1	2	3	4	5	6	7	8	9	10
OPA-1											
AF01	1850	0	1	0	0	0	0	0	0	0	1
AF02	1370	0	0	1	0	0	0	1	1	0	0
AF03	1260	0	1	1	1	1	0	1	1	1	1
AF04	950	0	0	1	0	0	0	1	0	0	0
AF05	830	1	1	1	1	1	1	1	1	1	1
AF06	700	1	1	1	1	1	0	1	1	1	1
AF07	680	0	0	0	0	0	1	0	0	0	0
AF08	600	0	0	0	0	0	1	0	0	0	0
AF09	570	0	1	1	1	0	0	1	0	1	1
AF10	500	0	1	1	1	1	1	1	0	1	1
AF11	410	1	1	1	1	1	1	1	0	0	0
AF12	370	1	1	1	1	1	1	1	1	1	1
Total	**12**	**4**	**8**	**9**	**7**	**6**	**6**	**9**	**5**	**6**	**7**
OPA-4											
AF13	2500	0	0	0	0	1	1	0	0	0	0
AF14	2000	0	0	0	0	0	1	1	0	0	0
AF15	1750	0	0	1	0	1	1	0	0	0	0
AF16	1500	0	0	0	0	0	0	1	0	0	0
AF17	1325	1	1	1	1	1	1	0	0	0	0
AF18	1050	0	0	1	1	1	1	1	1	1	1
AF19	850	0	0	0	0	0	1	1	0	0	0
AF20	760	1	1	0	0	0	0	0	0	0	0
AF21	700	1	1	1	0	1	1	1	1	1	1
AF22	590	1	1	1	1	1	1	1	1	0	0
AF23	470	1	1	1	0	1	1	1	1	0	0
AF24	310	1	1	1	0	1	0	1	1	0	0
Total	**12**	**5**	**5**	**7**	**3**	**8**	**9**	**7**	**6**	**2**	**2**
OPA-7											
AF25	850	0	1	1	0	0	1	1	1	1	1
AF26	680	1	1	1	1	1	1	1	1	1	1
AF27	600	0	0	0	0	0	0	1	0	1	0
AF28	420	1	1	1	1	1	1	1	1	1	1
AF29	330	1	0	1	0	0	1	1	1	1	1
AF30	230	0	0	1	0	0	1	1	0	0	1
Total	**6**	**3**	**3**	**5**	**2**	**2**	**5**	**6**	**4**	**5**	**5**
OPA-10											
AF31	850	0	0	1	1	1	1	1	0	0	1
AF32	680	1	1	1	1	1	1	1	1	1	1
AF33	600	1	0	1	0	0	0	0	0	0	1
AF34	550	0	0	0	0	0	0	0	1	0	0
AF35	420	1	1	1	1	1	1	1	1	1	1
AF36	330	1	1	0	1	1	1	1	1	1	1
AF37	230	1	1	0	0	1	1	0	1	1	1
Total	**7**	**5**	**4**	**4**	**4**	**5**	**5**	**4**	**5**	**4**	**6**
OPA-15											
AF38	2900	1	1	1	1	1	1	1	1	1	1
AF39	1900	1	1	0	1	1	0	0	1	1	0
AF40	1680	0	1	0	0	0	0	0	0	0	0
AF41	1380	1	1	1	1	1	1	0	1	1	1
AF42	1200	0	0	0	0	1	0	0	1	0	0
AF43	1100	0	0	0	1	0	0	0	0	1	0
AF44	990	1	1	1	1	1	1	1	1	1	1
AF45	850	1	1	1	1	1	1	1	1	1	1
AF46	620	1	1	0	1	1	1	1	1	1	1
AF47	500	0	0	0	0	0	0	0	1	0	0
AF48	200	1	1	1	1	1	1	1	1	1	1
Total	**11**	**7**	**8**	**5**	**8**	**8**	**6**	**5**	**9**	**8**	**6**

Table 8 – (*Continued*).

OPB-1											
AF49	1750	1	1	1	1	1	1	1	1	1	1
AF50	1660	0	0	1	1	0	1	1	0	1	0
AF51	1550	0	0	0	0	0	1	0	0	1	0
AF52	1320	0	0	1	0	0	0	0	0	0	0
AF53	990	0	0	1	0	0	1	1	0	1	0
AF54	900	0	0	0	0	0	0	0	0	1	1
AF55	780	1	1	1	1	1	1	1	1	1	1
AF56	660	1	1	1	1	1	1	1	1	1	1
AF57	620	0	0	0	0	0	0	1	0	0	0
AF58	530	1	1	1	1	1	1	1	1	1	1
AF59	420	0	0	1	1	1	1	1	1	1	1
AF60	350	0	0	1	1	1	1	0	1	1	1
Total	**12**	**4**	**4**	**9**	**7**	**6**	**9**	**8**	**6**	**1**	**7**
OPB-4											
AF61	1650	0	0	0	1	0	0	1	0	0	0
AF62	1450	0	0	0	0	0	0	1	0	0	1
AF63	1360	0	1	0	1	0	1	0	1	1	0
AF64	1120	0	0	0	1	1	1	0	0	1	0
AF65	900	0	0	1	1	1	1	1	1	0	1
AF66	840	0	0	0	0	1	1	1	1	0	0
AF67	680	0	0	1	0	0	0	0	0	1	0
AF68	620	1	1	1	1	1	1	1	1	1	1
AF69	590	0	0	0	1	0	0	0	0	0	0
AF70	490	1	1	1	1	1	1	1	1	1	1
AF71	315	1	1	1	1	1	1	1	1	1	1
Total	**11**	**3**	**4**	**5**	**8**	**6**	**7**	**7**	**6**	**6**	**5**
OPB-6											
AF72	3100	0	0	0	0	0	0	0	0	1	0
AF73	2000	0	0	0	0	1	1	1	1	0	1
AF74	1620	0	0	0	0	0	0	1	1	1	1
AF75	1400	0	0	0	0	0	0	0	0	1	1
AF76	1340	1	0	0	0	1	1	1	1	0	0
AF77	1100	1	1	1	1	1	1	1	1	1	1
AF78	950	1	1	1	1	1	1	1	1	1	1
AF80	830	1	1	1	1	1	1	1	1	1	1
AF81	800	0	0	0	0	0	0	0	1	1	1
AF82	500	1	1	1	1	1	1	1	1	1	1
AF83	340	1	1	1	1	1	1	1	1	1	1
AF84	250	1	1	1	1	1	1	1	1	1	1
Total	**12**	**7**	**6**	**6**	**6**	**8**	**8**	**9**	**9**	**9**	**10**
OPB-7											
AF85	1250	1	1	1	1	1	1	1	1	1	1
AF86	990	1	1	1	1	1	1	1	1	1	1
AF87	830	1	1	1	1	1	1	1	1	1	1
AF88	740	0	0	0	0	0	0	0	0	1	0
AF89	700	0	0	1	0	0	0	0	0	0	0
AF90	680	1	1	0	1	0	0	0	1	0	0
AF91	580	0	0	1	0	1	0	0	0	1	1
AF92	525	1	1	0	0	0	0	0	0	0	0
AF93	415	1	1	1	0	0	0	0	0	0	0
AF94	210	1	1	0	0	1	0	0	0	1	0
Total	**10**	**7**	**7**	**6**	**4**	**5**	**3**	**3**	**4**	**6**	**4**
OPB-17											
AF95	1500	0	0	1	0	1	1	0	0	0	0
AF96	1230	1	1	1	1	1	1	1	1	1	1
AF97	940	1	1	1	1	1	1	1	1	1	1
AF98	820	1	1	1	1	1	1	1	1	1	1
AF99	750	0	0	1	0	1	0	0	0	1	0
AF100	670	1	1	1	1	1	1	1	1	1	1
AF101	560	1	0	1	1	0	1	0	0	0	0
AF102	530	0	1	0	0	0	0	0	1	0	0
AF103	420	0	1	0	0	0	0	0	0	0	0
AF104	340	0	1	0	0	0	0	0	0	0	0
AF105	220	1	1	1	1	0	0	1	1	1	1
Total	**11**	**6**	**8**	**8**	**6**	**6**	**6**	**5**	**6**	**6**	**5**

collected from Talkha- El- Dakahlyia. Band of a molecular size 1380 bp recorded in all taxa except *M. parviflora* collected from Menia El-Kamh. This band could be used as a negative molecular marker for *M. parviflora* collected from Menia El-Kamh.

3.9. *Primer OPB-1*

The results revealed that this primer produced a total number of 12 bands. Band of a molecular size 620 bp recorded only in *M. parviflora* collected from Menia El-Kamh. It could be used as a positive molecular marker for *M. parviflora* collected from this accession. Band of a molecular size 1320 bp recorded only in *H. sabdariffa* collected from Talkha- El-Dakahlyia Governorate. This band could be used as a molecular marker for *H. sabdariffa* collected from this accession. This primer gave four common bands with molecular sizes 530 bp, 660 bp, 780 bp and 1750 bp.

3.10. *Primer OPB-4*

The molecular size of the PCR products ranged from 315 bp to 1650 bp. This primer gave three common bands with molecular sizes 315 bp, 490 bp, 620 bp and eight polymorphic bands. Band of a molecular size 590 bp recorded only in *H. trionum* collected from El- Riyad- Kafr El-Sheikh Governorate. It could be used as a positive marker for *H. trionum* collected from this accession.

3.11. *Primer OPB-6*

This primer produced a total number of 12 bands. Six bands are common and six polymorphic bands. Band of a molecular size 3100 bp recorded only in *S. alba* collected from Menia El-Kamh. This band could be used as a positive marker for *S. alba* collected from Menia El-Kamh.

3.12. *Primer OPB-7*

This primer produced a total number of ten bands, three of which common bands and seven polymorphic bands. The band of a molecular size 740 bp recorded only in *S. alba* collected from Menia El-Kamh so that, this band could be used as a molecular marker for *S. alba* collected from Menia El-Kamh. The band of a molecular size 700 bp recorded only in *H. sabdariffa* collected from Talkha- El- Dakahlyia. This band could be used as a positive marker for *H. sabdariffa* collected from Talkha – El-Dakahlyia.

3.13. *Primer OPB-17*

The molecular size of the PCR products from 220 bp to 1500 bp. This primer OPB-17 gave seven polymorphic bands and four common bands. Bands of molecular sizes 420, 340 bp recorded only in *H. sabdariffa* collected from Menia El-Kamh El-Sharkyia. These bands could be used as positive molecular markers for *H. sabdariffa* collected from Menia El-Kamh El-Sharkyia.

Regarding the polymorphism of all accessions of the six studied taxa, the maximum value of polymorphism 100% recorded in primer OPA-4. However, the remaining percentages of polymorphism took specific trends where 83.3% in primer OPA-1, 72.7% in primer OPB-4, 71.4 in primer OPA-10, 70% in primer OPB-7, 66.6% in primers (OPA-7, OPB-1), 63.3% in primers (OPB-17, OPA-15) and 50% in primer OPB-6.

4. Discussion

Biochemical and molecular techniques are provided approaches for evaluating genetic diversity in plants. These methods are favored because they are independent of the developmental stage of the plant [17]. Biochemical evidences such as seed storage protein electrophoresis and isozyme polymorphisms are convenient evidences for assessing genetic relationships [18] and [19].

The variation in SDS-PAGE of seed protein profiles have successfully been used to differentiate between species [20] and provide a valid source of taxonomic evidence for addressing the relationships at the different taxonomic levels [21].

The electro-phenogram of the examined ten accessions of the six studied taxa revealed a total number of eighteen bands. The highest number of bands 15 was observed in *H. sabdariffa* collected from Menia El-Kamh, El-Sharkyia and the two accessions of *M. parviflora*. The molecular weight of these bands ranging from 11.3 to 115.3 KDa, where as the lowest number 11 bands were recorded in *H. trionum* collected from Talkha- El-Dakahlyia Governorate the molecular weight of these eleven bands ranging from 11.3 KDa to 115.3 KDa.

Isozymes polymorphisms are used effectively to assess genetic relationships among individuals, populations and closely related species [22] and [23]. The applications of isozymes polymorphism are still important for population genetic studies and in addressing infra-specific relationships [24].

There are three isozymes (esterase, peroxidase and acid phosphatase) were used to differentiate among the ten accessions of the six studied taxa belonging to family Malvaceae. It was found that acid phosphatase and peroxidase isozymes are more effectively in differentiation among these accessions of the studied taxa, while esterase isozyme was not able to differentiate.

Proteins and isozymes electrophoretic markers have been used in many crops to some extent. The major limitation of these two procedures is the lack of enough polymorphism among closely related cultivars [25]. For this reason, DNA based genetic markers have been integrated into several plant systems and are playing a very important role in molecular genetics and plant breeding [25] and [26].

Randomly amplified polymorphic DNA (RAPD) technique has been used in many different applications involving the detection of DNA sequence polymorphisms [27] to identify varieties [28] and to assess the genetic diversity [29] and [30]. In this study ten primers used to differentiate among the ten accessions of the six studied taxa belonging to family Malvaceae. The primers gave reproducible results but the best primer used to differentiate was primer OPA-4 and gave the highest percentage of polymorphism. Primers OPA-7 and OPB-1 gave the same percentages of polymorphism, primers OPB-

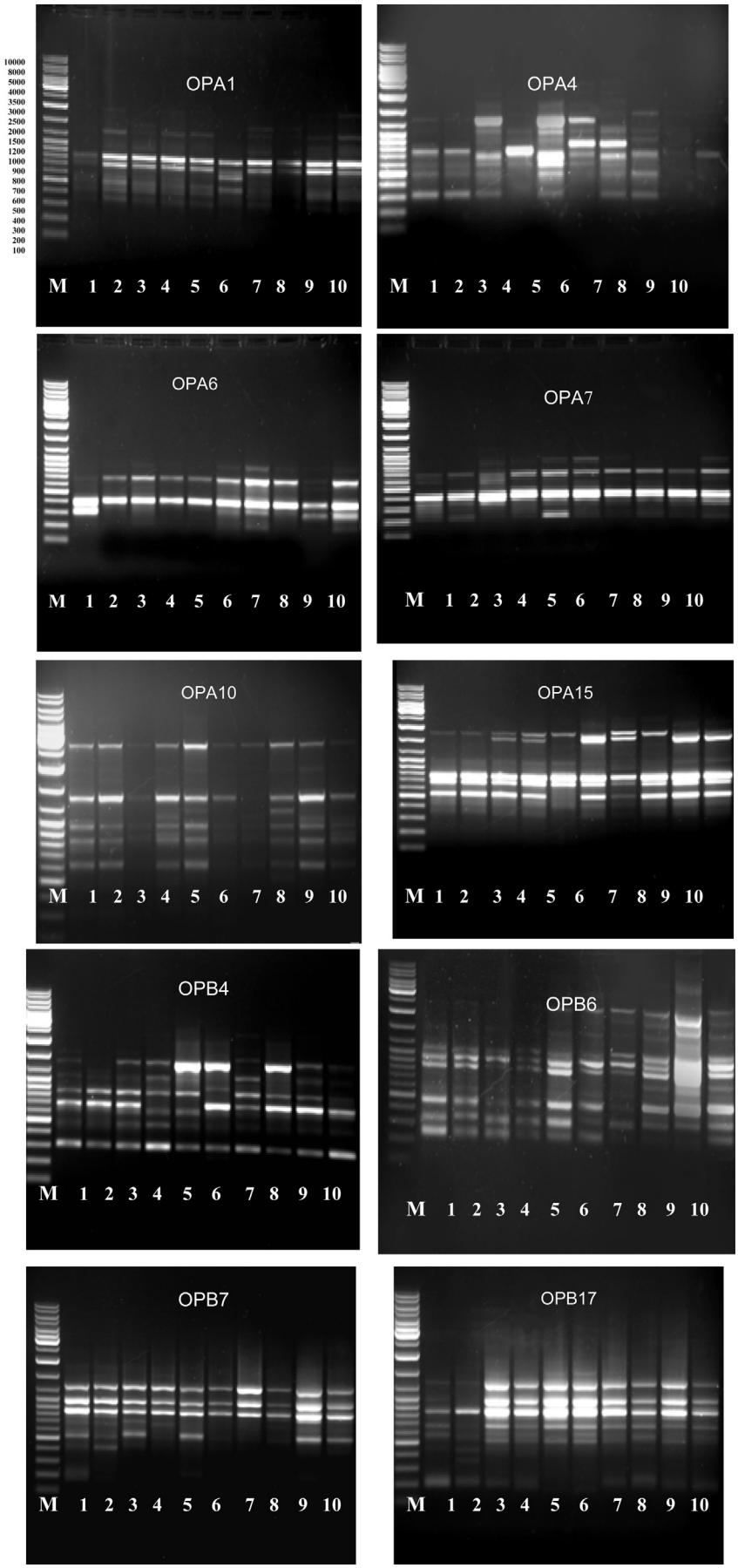

Plate 2 – DNA polymorphism based on RAPD- PCR analysis of some species of family Malvaceae, Egypt. (M) marker, 1 (At), 2 (Hs1), 3 (Hs2), 4 (Ht1), 5 (Ht2), 6 (Lc), 7 (MP1), 8 (Mp2), 9 (Sa1) and 10 (Sa2).

Table 9 – Polymorphic bands of some species of family Malvaceae collected from Egypt.

Bands	Monomorphic bands	Polymorphic		Total bands	Polymorphic %
		Unique	Non-unique		
Primers					
OP-A1	2	3	7	12	83.3%
OP-A4	–	1	11	12	100%
OP-A7	2	–	4	6	66.6%
OP-A10	2	2	3	7	71.4%
OP-A15	4	4	3	11	63.6%
OP-B1	4	2	6	12	66.6%
OP-B4	3	1	7	11	72.7%
OP-B6	6	1	5	12	50%
OP-B7	3	1	6	10	70%
OP-B17	4	2	5	11	63.6%

17 and OPA-15 also gave the same percentages of polymorphism.

Cluster analysis was conducted to generate a dendrogram Fig. 2 clustering possible relationships among the ten studied accessions of six species of family Malvaceae in Egypt based on compiled all matrix data. Investigated accessions were divided into two groups at 0.315, the first group includes Hs2 collected from Talkha district, and the second group is divided into two subgroups at a distance of 0.266. The first subgroup includes Hs1 and At, while the second subgroup includes Lc, Ht1, Ht2, Mp1, Mp2, Sa1 and Sa2. In the second subgroup Sa1 and Sa2 were separated from the rest at a distance 0.218.

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