Morphological and Molecular Evidences Among Four Heteroforms of *Avicennia marina* (Forssk) Vierh.

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Abstract: Morphological characteristics and random amplified polymorphic DNA (RAPD) marker were used to assess inter-specific relationships among four heteroforms of gray mangrove (*Avicennia marina* (Forssk) Vierh.) grown in Al-Sharm Al-Bahari site, 33Km south Al-Qussier, Red Sea Coast, Egypt. The four heteroforms viz. I, II, III and IV were detected in two distinct habitats (marine and desert). The morphological and molecular data indicated high variation between form I&III and II&IV. On the other hand, low variation between form I&II and III&IV. Dendrogram based on morphological, anatomical and genetic data supported the segregation of the four heteroforms of *Avicennia marina* into two groups; one includes form 1 & III and the second include form II & IV. The study concluded that the four heteroforms can be classified as two subspecies, *A. marina* subsp. *eucalyptifolia* (form I) and the *A. marina* subsp. *marina* (form II). In addition, forms III and IV considered as phenotypes from I and II, respectively.
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Keywords: Mangrove; *Avicennia marina*; Morphology, RAPD; Red Sea

1. Introduction:

Avicennia L. (F: Avicenneaceae) is a genus of mangrove woody trees or shrubs that grow in coastal habitats. It has the largest longitudinal and latitudinal distribution of all mangrove species, ranging from the east coast Africa from the Red Sea to South Africa to the west pacific from Japan to New Zealand (Le *et al*., 2003).

Moldenke (1975) and Tomlinson (1986) recorded that the genus *Avicennia* L. shows considerable morphological variation especially in leaves and flowers, and classified based on these attributes. *Avicennia* represents the largest polymorphic genus of the mangrove, where it well known ecologically, systematically, morphologically and genetically in comparison with other taxa (Duke, 1995).

Mabberly (1981) classified the genus *Avicennia* to 14 species according to morphological characters of leaves and flowers. While Tomlinson (1986) and Duke (1991, 1992) classified the *Avicennia* species to four major groups according to their morphological criteria; *A. marina* & *A. alba*, *A. officinalis* & *A. integra*, *A. rhumphiana*, and *A. germinans*, *A. schaueriana* & *A. bicolor*.

A. marina (Forssk.) Vierh is an important true mangrove species; it is a halophytic plant, grows as a shrub or tree to a height of three to ten meters. In addition, it has three subspecies; *A. marina australasica, A. marina eucalyptifolia* and *A. marina marina* (Schwarzbach and Mc Dade, 2002).

A. marina (Forssk.) Vierh represents the dominant mangrove species in Egypt, found along Red Sea Coast ling from Ras-Mohamed to Mersa-Halaib (Zahran, 1993).

Tomlinson (1986) recorded that *A. marina* species characterized by different heteroforms. Four heteroforms of *A. marina* were recorded in Al-Sharm Al-Bahary site, 33 km south Al-Qussier region in two different habitats. Two forms growing in the inundation area, while the other two are found in the desert closed to Red Sea shores. The two habitats have different physico-chemical properties as recorded by khalafallah (2002). Are those four heteroforms, subspecies of *A. marina* orecotypes/phenotypes? These four heteroforms need taxonomical study.

The previous taxonomical studies of genus *Avicennia* L. of *A. marina* species were achieved based on the morphological variations of the vegetative and reproductive organs. The advent of technology that directly examines genes and gene products, it has been found that the morphological characteristics not be completely reliable indicators for genetic variation or taxonomic differences, owing to their tendency to be highly influenced by environmental factors (Brown *et al*., 1997; Duke *et al*., 1998 and Bryars & Adam, 1999).

Recently, several studies have been carried out on mangrove species in order to assess genetic diversity using genetic markers, as studies the worldwide genetic diversity of *A. marina* using Allozyme and ALFP & microsatellite markers, RAPD, RLFP, ALFP DNA (Mguire *et al*., 2000 & 2002 and Le *et al*., 2003). Gallios & Burrus (1998) and Gauer & Cavalli-Molina (2000) reported that molecular genetic markers can provide a relatively unbiased method of quantifying genetic diversity in plants and their populations.

Said (2008) concluded that DNA based genetic markers have been recently integrated into the important of several plant systems and are expected to play a very important role in the future of molecular genetic and plant taxonomy.

The objectives of this study are: (1) investigation the morphological and anatomical characteristics of the four heteroforms, (b) study the genetic variation of the four heteroforms by application of the RAPD technology and (c) study the taxonomic relationship between the four heteroforms of *A. marina* in Al-Sharm Al-Bahari, Al-Qussier region, Red Sea Coast, Egypt.

2. Materials and methods

Study Site

Al-Sharm A-Bahari site was chosen as it contains the four heteroforms of *A. marina* plants in two different habitats. Forms I and II are growing in the inundation area, while Forms III and IV are found in the desert closed to Red Sea shores. The site is located at 33km south Al-Qussier city (25°52'04.58" N and 34°25'04.55"E, Fig. 1). Physico-chemical properties of the two habitats were analyzed by khallafallh (2002) and recorded in table (1&2). The four forms are photographed and showed in Fig. (2).

Plant samples collection

Thirteen healthy samples (third internodes, third leaves, flowers and fruits) of the four forms of *A. marina* were collected at May 2008 from Al- Sharm Al- Bahary site, Al-Qusseir Red Sea Coast for the morphological, anatomical and molecular investigations. Voucher specimens are deposited in the herbarium of Botany Department, Women's College for Art, Science and Education, Ain Shams University

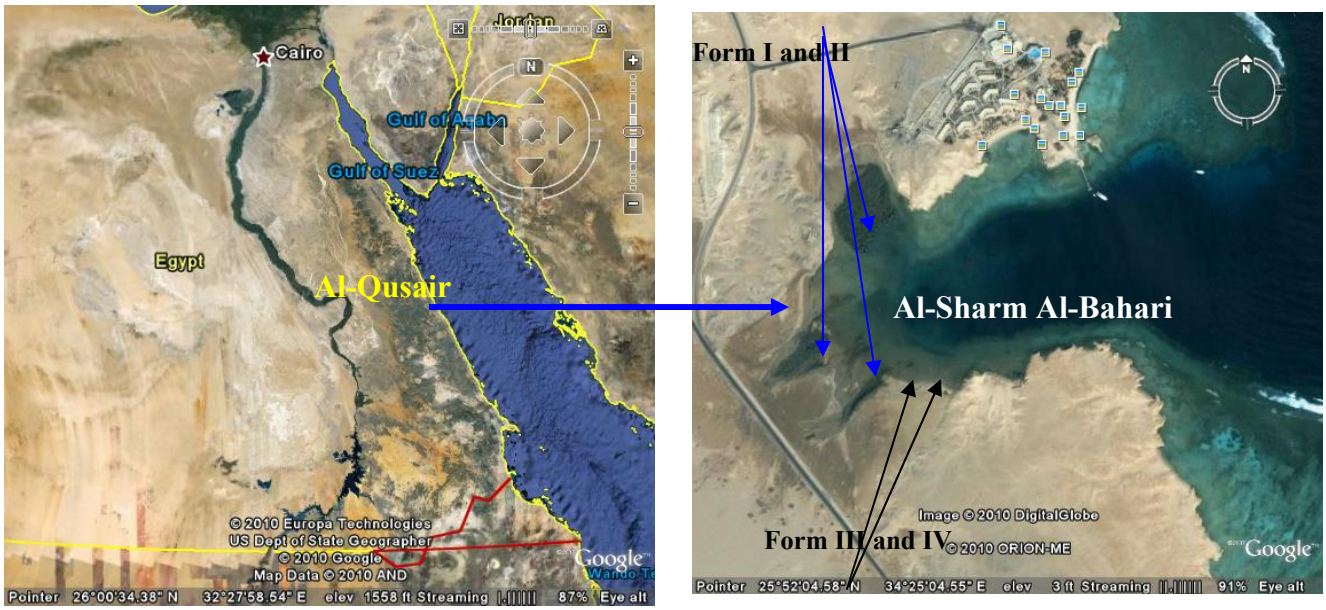

Fig. 1: Location of Al-Sharm Al-Bahari at Al-Qussair region, Red Sea coast, Egypt (Google earth program).

Photo. 1: Form I found in the inundated are

Photo. 2: Form II found in the inundated area

Photo. 3: Form III found in the desert

Photo. 4: Form IV found in the desert

Fig. 2: The four heteroforms of *A. marina*.

Table 1: Physico-chemical properties of the two habitats soils; A: soil of the inundation area where forms I and II are found and B: soil of the desert where forms III and IV are found

		A	B			A	B
Granulation %	Gravel	13.8	2.2	Anions (meq/100 g soil)	Cl^{-1}	27.9	8.5
	Coarse sand	20.8	7.4		HCO_3^{-1}	4.35	4.35
	Medium sand	40.8	61.8		CO_3^{-2}	0.00	0.20
	Fine sand	17.8	27.8		SO_4^{-2}	32.3	100.4
	Silt	1.2	0.4	Soluble cations (meq/100 g soil)	Na^{+1}	14.78	47.80
	Clay	5.4	0.4		K^{+1}	1.05	2.90
pH		7.8	8.3		Ca^{+2}	1.90	1.90
EC (dS / m)		18.93	4.18		Mg^{+2}	46.7	65.8
Total soluble salts (g / 100 g)		1.6	Waterlogged	Exchangeable cations (meq / 100 g soil)	Na^{+1}	7.83	8.70
Water content %		21.6	7.2		K^{+1}	0.53	050
NaCl (%)		1.63	0.50		Ca^{+2}	18.50	15.60
$CaCO_3$ (%)		30.0	19.0		Mg^{+2}	245.0	151.7
Organic mater (g/ 100 g)		0.50	2.77				

Table 2: Physico-chemical properties of water of aquatic habitat of Al-Sharm Al-Bahari site where forms I and II are found

pH	7.8	Cl^- (meq/L)	609.6	Na^+ (meq/L)	1635
EC (dS / m)	75.1	HCO^{3-} (meq/L)	7.30	K^+ (meq/L)	47.9
Total soluble salts (g / L)	47.4	$CO3^{2-}$ (meq/L)	70	Ca^{++} (meq/L)	23.6
Salinity (G/L)	38.1	$SO4^{2-}$ (meq/L)	1206	Mg^{++} (meq/L)	117.6
NaCl (g / L)	35.7				

Botanical investigation

Plant heights of the four heteroforms were measured in the field. The macro-morphology of the investigated forms was described directly from fresh specimens. Measurements included both numeric attributes (internode length, leaf length, flower length and number of flowers/inflorescence) and coefficient attributes (leaf narrowness and leaf area) as modified from Duke (1990). The micromorphology of stems, petioles and leaves of each form was carried out through hand-microtome cross sections at 10-15µm, stained with safranin and light green according to the methods described by Johanson (1940). The sections were photographed by using light microscope (Olympus) with digital camera (Canon Power Shot S80) connected to computer; the photographs were taken by Zoom Browser Ex program. The dimensions of sections were measured by using Corel Draw program ver. 11.

Molecular investigation

Total genomic DNA was extracted from 0.5g fresh young leaves of the four *A. marina* heteroforms according to the Dellaporta *et al.* (1983). The extraction examined by RAPD-PCR marker at Genetic Engineering and Biotechnology Center, Ain Shams University to determine inter and intra specific variations.

Based on RAPD markers for amplification of unknown DNA sequences; single and short random oligonucleotide primers were used. Ten-mer random DNA oligonucleotide primers (UBC) were obtained from Operon kit (Operon Tech. Inc., USA). Their code and sequences were listed in table (3). After electrophoresis of the RAPD pattern, amplification was carried out according to Williams *et al.* (1990). Amplification products were size-fractionated on a 1% w/v agarose gel containing 10mg ml^{-1} of ethidium bromide. They were visualized under UV light and photographed. The gels were documented using gel documentation advanced software UVP-England Program.

Table (3): List of primers and their nucleotide sequences

GC %	Sequences(5̀ to 3̀)	Primer code	Number
1	Op- A19	CAA ACG TCG G	60%
2	Op- A3	AGT CAG CCA C	60%
3	Op-A7	GAA ACG GGT G	60%
4	Op- A18	AGG TGA CCG T	60%
5	Op – B17	AGG GAA CGA G	60%
6	Op- Z 7	CCA GGA GGA C	70%
7	Op- D3	GTC GCC GTC A	70%
8	Op- B15	GGA GGG TGT T	60%
9	Op – C2	GTG AGG CGT C	70%
10	Op- C5	GAT GAC CGC C	70%

Data analysis

Standard deviations of the morphologcal and anatomcal characters were calculated. The data were treated statistically using the one-way analysis of variance (ANOVA) as described by Snedecor and Cockran (1969), the means were compared by LSD using SPSS program version 15. The morphological, anatomical and genetic evidences were scored for presence (1) or absent (0) to the computation analysis under a program using similarity and dissimilarity assessment percentage method ver. 2.02 Rohlf (1998).

3. Results

Macromorphological investigations

Macromorphological characters of the four heteroforms *Avicennia marina* described or measured and recorded in tables 4&5. The recorded data indicated that from 59 studied characters, the four heteroforms shared in 51 (86%) characters but differed in 8 (14%) characters (habitat, stem surface, node shape, pnematophores presence, leaf base, leaf shape, leaf apex, upper surface colour of leaf). Form I&II growing in aquatic and have pnematophores, while forms III and IV growing in desert without pnematophors. The main difference focused between I&III as a group and II&IV as other group (table 4).

There is no significant difference between heights of forms I and II also forms III and IV, while it significantly differed between forms I&II as a group and forms III&IV as other group (Table 5). The other measured dimensions; internode length, petiole length, leaf length, leaf width, leaf narrowness, leaf area and no. of flowers/inflorescence significantly differed between forms I&III as a group and forms II&IV as other group.

Table 4: Macormorphological characters of the four heteroforms of *Avicenna marina*

			I	II	III	IV
Whole plant	**Habitat**	Aquatic	1	1	0	0
		Desertic	0	0	1	1
	Habit	Shrub	1	1	1	1
	Bark color	Grey	1	1	1	1
	Stem surface	Smooth	1	0	1	0
		Flaky	0	1	0	1
	Node shape	Normal	1	0	1	0
		Swollen	0	1	0	1
	Pnematophores		1	1	0	0
Petiole	**Base**	Narrow-grooved	1	1	0	1
		Circle	0	0	1	0
	Texture	Hairy	1	1	1	1
Leaf	**Type**	Simple	1	1	1	1
	Shape	Lanceolate	1	0	1	0
		Ovate	0	1	0	1
	Color (upper surface)	Dark green	1	0	1	0
		Light green	0	1	0	1
	Color (lower surface)	Grey	1	1	1	1
	Margin	Entire	1	1	1	1
	Apex	Acuminate	1	0	1	0
		Acute	0	1	0	1
	Arrangement	Opposite decussate	1	1	1	1
	Venation	Pinnate	1	1	1	1
	Texture (upper surface)	Hairless	1	1	1	1
	Texture (lower surface)	Hairy	1	1	1	1
Inflorescence	**Type**	Cymose	1	1	1	1
	Shape	Capitate	1	1	1	1
	Branching	Terminal Axillan on long stocks	1	1	1	1
	Position	Axillary on long staks	1	1	1	1
Flower	**Type**	Regular	1	1	1	1
	Sex	Bisexual	1	1	1	1
	Size	Small	1	1	1	1
Calyx	**Shape**	Bell-shaped	1	1	1	1
	Fusion	Gamosepalous	1	1	1	1
	Number of calyx lobe	5-lobed	1	1	1	1
	Texture	Hairy	1	1	1	1
	Nature	Persistent	1	1	1	1
	Color	Green	1	1	1	1
Corolla	**Shape**	Funnel shape	1	1	1	1
	Fusion	Gamopetalous	1	1	1	1
	Number of petals	Four	1	1	1	1
	Texture	Glabrous	1	1	1	1
	Nature	Deciduous	1	1	1	1
	Color	Yellow	1	1	1	1
Androecium	**Stamens type**	Epipetalous	1	1	1	1
	Number of stamens	Four	1	1	1	1

Table 4 cont.

			I	II	III	IV
	Number of anther locules	Four	1	1	1	1
	Anther fertility	Fertile	1	1	1	1
	Anther Dehiscence	Longitudinal slits	1	1	1	1
Gynoecium	**Type**	2-Carpelled	1	1	1	1
	Fusion	Syncarpous	1	1	1	1
	Ovary type	Superior	1	1	1	1
	Ovary texture	Hairy above &glabrous beneath	1	1	1	1
	No. of locules	4-locular	1	1	1	1
	No. of ovules/locuoli	One	1	1	1	1
	Placenta Type	Axile	1	1	1	1
	Style texture	Hairy	1	1	1	1
	Stigma Shape	Bilobed	1	1	1	1
	Stigma texture	Glabrous	1	1	1	1
Fruit	**Type**	Fleshy	1	1	1	1
	Capsule dehiscence	Bivalved	1	1	1	1
Seeds	**Type**	Endospermic	1	1	1	1
	Texture	Glabrous	1	1	1	1
	Color	Green	1	1	1	1
	Germination	Epicotyle	1	1	1	1
	Embryo type	Crypto-viviparous	1	1	1	1

I: Form I found in the inundated area II: Form II found in the inundated area
III: Form III found in the desert IV: Form IV found in the desert

Table 5: Morphological attributes of four heteroforms of *A. marina* plants

Attributes	I	II	III	IV
Plant height (m)	2.90a ±0.52	2.85a ± 0.48	1.80b ±0.32	1.65b ±0.25
Internode length (cm)	6.6a ± 0.23	2.6b ± 0.08	5.0a ± 0.20	3.0b ± 0.15
Petiole length (cm)	1.5a ± 0.24	1.2b ± 0.13	1.5a ± 0.24	1.1b ± 0.09
Leaf length (cm)	7.3a ± 0.46	6.5b ± 0.87	7.5a ± 0.87	6.5b ± 0.8
Leaf width (cm)	2.1b ± 0.16	3.5a ± 0.46	2.3b ± 0.24	3.6a ± 0.68
No. of flowers/inflorescence	21b ± 1.07	27a ± 1.18	24b ± 1.28	27a ± 1.01
Flower Overall length (m. m)	3.0b ±.04	5.0a ± 0.06	3.5b ± 0.06	6.0a ± 0.24
Leaf narrowness 4w (cm)	3.6a ± 0.07	2.0c ± 0.06	3.2a ± 0.12	2.6b ± 0.08
Leaf area LxW/2 (cm^2)	7.7b ± 0.22	11.4a ± 0.54	8.6b ± 0.38	11.7a ± 43

Values have the same latter in the same raw is not significant at *P>0.05*
I: Form I found in the inundated area II: Form II found in the inundated area
III: Form III found in the desert IV: Form IV found in the desert

Micromorphological investigations

From thirty one anatomical characters, the four heteroforms of *Avicenna marina* differed in six characters (table 6 and figs. 3, 4&5). The main differed character is focused on stem outline. Forms I and III have circular outline while forms II and IV have angular outline. The other differed characters are thickness of cuticle layer of stem, petiole and leaf, no. of mid rib vascular bundles, bundle sheath growth and sclerenchyma cells presence.

The anatomical data of the four heteroforms of *Avicennia marina*; stems, petioles, leaves, showed a significant difference between forms I&II as a group and Forms III&IV as other group in more than 44% of the studied characters (Table 7).

Table (6): Micromorphological characters of the four heteroforms of *Avicenna marina*

			I	II	III	IV
Stem	**Outline**	Circular	1	0	1	0
		Angular	0	1	0	1
	Cuticle layer	Thick	1	1	0	1
		Thin	0	0	1	0
	Epidermal cells	Radiall	1	1	1	1
	Epidermal hairs types	glandularnon glandular hairs	1	1	1	1
	Gortical cells types	Parenchyma, Callenchyma, Sclerenchyma & air spaces	1	1	1	1
	Pericycle	Continuous ring of sclerenclyma	1	1	1	1
	Cambium origin	Inner most Cortical layers & pericycle	1	1	1	1
	V. B. shape	Angular	1	1	1	1
	phloem	Continuous layer of phloem & conjunctive Parenchyma	1	1	1	1
	Hypoderms	2-6 row	1	1	1	1
	Xylem	Continuous cylinder interfascular rays	1	1	1	1
	Secondary Thickening	Anomalous	1	1	1	1
	Pith cells Type	Parenchyma & Sclerenchyma	1	1	1	1
Petiole	**Out line shape**	Crescent with narrow groove	1	1	1	1
	Cuticle	Thick	1	0	0	1
		Thin	0	1	1	0
	Epidermal cells	Radial	1	1	1	1
	Epidermal hairs	Salt glands & non glandular septate uni seriate hairs	1	1	1	1
	Ground tissue	Collenchyma, sclerenchymas & airspaces	1	1	1	1
	Main V. B. shape	Arcshaped	1	1	1	1
	No of additional small V. B.	One pair	1	1	1	1
	Small V. B. type	Concentric & amphicribral	1	1	1	1
Leaf	**Cuticle layer**	Thick	1	0	1	1
		Thin	0	1	0	0
	Epidermal cells	Elongated	1	1	1	1
	Epidermal hairs (U.S.)	Salt glands	1	1	1	1
	Epidermal hairs (L.S.)	Salt glands & non glandular hairs	1	1	1	1
	Hypodermal layer	Several layers (6-8 rows)	1	1	1	1
	Mesophyll type	Dorsiventral	1	1	1	1
	Mid rib V. B. No.	One	1	1	1	0
		Two	0	0	0	1
	Mid rib V. B. type	Concentric & mphicribral	1	1	1	1
	Bundle sheath	Completed	0	1	1	1
		Uncompleted	1	0	0	0
	Sclerenchyma cells presence		1	1	0	0

U.S.: Upper surface L.S.: Lower surface V. B.: Vascular bundle
I: Form I found in the inundated area II: Form II found in the inundated area
III: Form III found in the desert IV: Form IV found in the desert

Table 7: Anatomical features of 3rd internode, leaf and petiole of 4 heteroforms of *A. marina* (± SD)

	I	II	III	IV
Internode				
Diameter	2753.4b ±158	2941.2b ±164	3670.2a ±171	3738.0a ±176
Hair thickness	145.49a ± 6.2	104.8b ± 4.7	112.0b ± 5.1	141.8a ± 6.9
Cortex thickness	401.52b ± 15.3	402.2b ± 16.0	422.5a ± 17.6	411.9a ± 16.4
Cylindrical vascular diameter	1985.8b ± 141	2015.4b ± 150	2376.3a ± 164	2305.9a ± 157
Fiber layer thickness	54.3b ± 4.2	54.1b ± 4.2	102.3a ± 7.6	98.5a ± 6.8
Phloem thickness	136.4ab ± 7.5	119.4b ± 6.6	132.5a ± 8.2	142.4a ± 8.9
Xylem thickness	216.2b ± 7.6	242.5a ± 12.7	250.4a ± 12.5	253.0a ± 11.9
Pith diameter	786.5d ± 36.1	1011.8c ± 47.6	1755.2a ± 51.4	1354.1b ± 49.2
Leaf				
Mid rib thickness	1200.2b ± 96.8	1225.4b± 102.4	1298.6a ± 110.3	1362.1a ± 112.5
Mid rib V. B. length	498.4c ± 21.5	589.8b ± 26.4	656.6a ± 28.2	678.1a ± 31.0
Mid rib V. B. width	895.7a ± 54.3	786.4b ± 43.2	912.2a ± 63.2	817.4b ± 57.6
Wing thickness	574c ± 30.5	647.8b ± 33.2	668.6b ± 36.4	773.0a ± 38.5
Hypodermis thickness	261.1c ± 10.1	274.2bc ± 10.8	282.6b ± 11.4	309.81a ± 13.5
Palisade thickness	223.5c ± 8.7	241.0b ± 12.4	231.2c ± 9.6	254.8a ± 12.6
Spongy thickness	142.9d ± 5.3	177.5c ± 6.4	193.4b ± 8.2	207.0a ± 8.7
Hair thickness	122.5c ± 4.2	140.5b ± 7.6	145.8b ± 6.2	195.2a ± 8.8
No of Xylem arches	45b ± 6	43b ± 7	55a ± 8	45b ± 6
Petiole				
Vertical thickness	1219.0b ± 86	1231.0b ± 95	1249.6a ± 99	1262.7a ± 83
Horizontal thickness	2066.2c ± 133	2280.4b ± 142	2291.1b ± 140	2382.0a ± 135
Hair thickness (outer)	112.8a ± 5.7	97.4b ± 6.2	90.7b ± 4.5	123.5a ± 5.8
Hair thickness (inter)	350.1c ± 4.9	426.7a ± 9.3	318.3b ± 7.5	445.0a ± 10.2
Xylem thickness	147.1d ± 5.4	184.0b ± 4.3	165.2c ± 6.3	216.1a ± 4.8
Phloem thickness	53.8d ± 3.4	76.6c ± 3.6	101.6b ± 5.3	135.4a ± 5.6
No of Xylem arches	52c ± 5	68a ± 4	62b ± 4	76a ± 5
No. of V. B.	3b ± 0	3b ± 0	5a ± 0	5a ± 0

Values have the same latter in the same raw is not significant at *P>0.05*

I: Form I found in the inundated area II: Form II found in the inundated area

III: Form III found in the desert IV: Form IV found in the desert

Genetic characters based on RAPD analysis

Ten-mer arbitrary oliganucleotide primers were initially used to establish RAPD- PCR fingerprints of the four forms *A. marina* plants, the results were demonstrated in table (8) and Fig (6).

A total number of 95 fragments were visualized across the four heteroforms. The number of bands was variable in each form as present or absent with a particular length in the RAPD patterns and change in the intensity of amplification of fragments with the same length. The primers produced band numbers ranging from 5 (primer OP-CO5) to 17 (primer OP-D-3) with size ranges between 100-1000 bp.

DNA polymorphism recorded 59 polymorphic bands with an average 5.9 polymorphic fragments per primer, and the polymorphism percentage ranged from 42.857% (primer OPA-18) to 81.818% (primer OPB-17) with an average 61.73 %. On the other hand the highest monomorphic bands were 8 bands at primer OPD-3.

Table (9) and Fig (6) represent the distribution of molecular weight of unique bands. The maximum no. of the unique bands was observed in primer OP-A-7 and OP-Z-7 (6 and 7, respectively). The maximum no. of unique bands were revealed in form I (13 bands) and form III (10 bands) at primers OP-A3, OP A-7, OPA-18, OPA-19, OPB-15, OP B-17, OPCO-5, OP-D-3 and OP-Z-7. On the other hand, the minimum no. was revealed in forms II and IV (1 band for each form) at primers OP-D-3 and OP-Z-7, respectively.

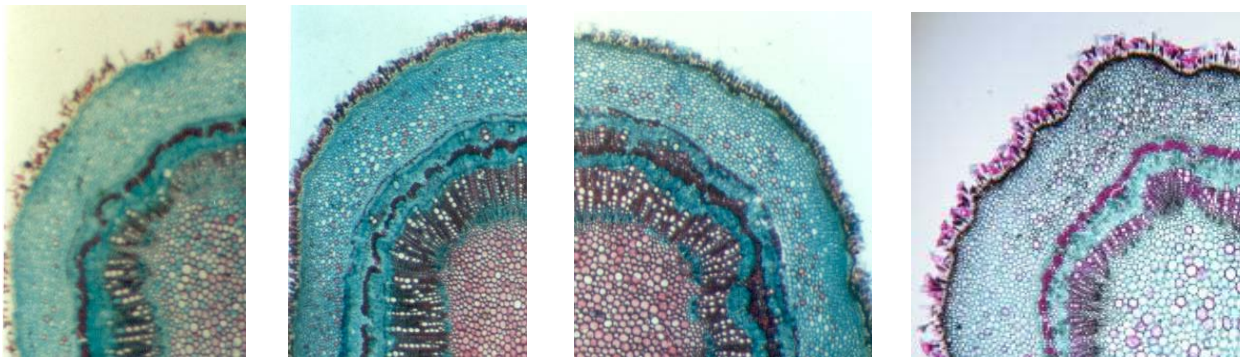

Photo. 1 Photo. 2 Photo. 3 Photo. 4

Fig. 3: Transverse sections of the 3rd internodes of the four *A. marina* (X 160).

Photo. 1: Form I found in the inundated area
Photo. 2: Form II found in the inundated area
Photo. 3: Form III found in the desert
Photo. 4: Form IV found in the desert

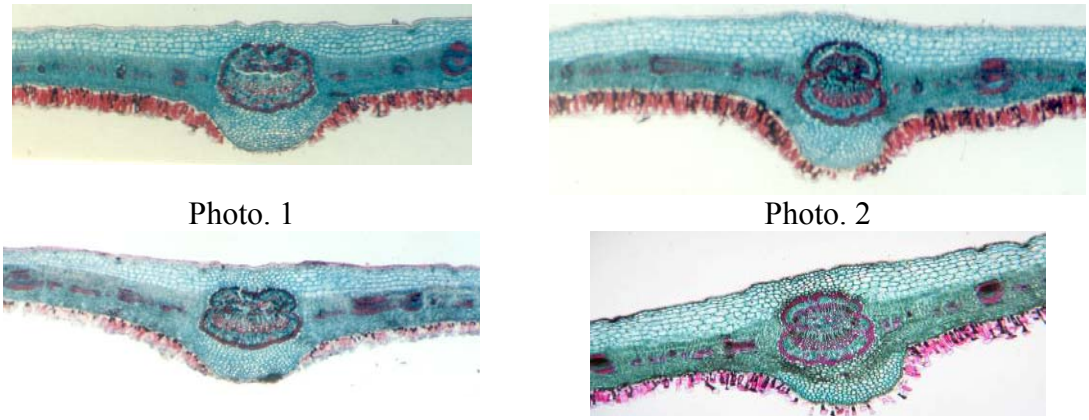

Photo. 1 Photo. 2

Photo. 3 Photo. 4

Fig. 4: Transverse sections of the 3rd leaf of the four *A. marina* heteroforms (X 160).

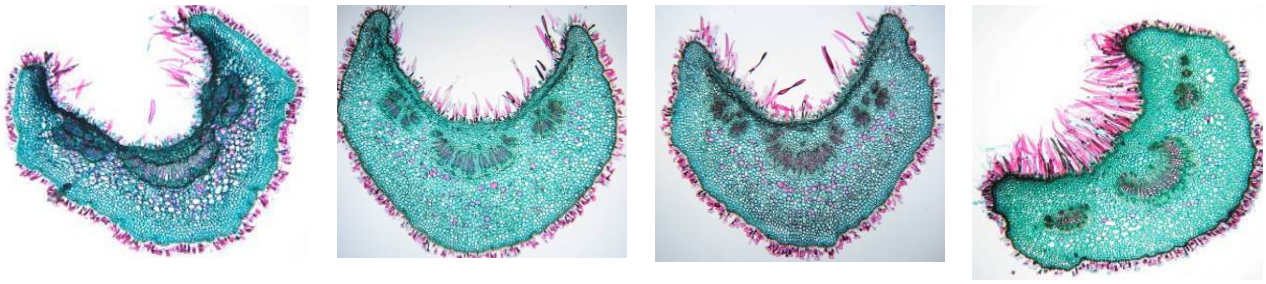

Photo. 1 Photo. 2 Photo. 3 Photo. 4

Fig. 5: Transverse sections of the leaf petiole of the four *A. marina* heteroforms (X 160).

Photo. 1: Form I found in the inundated area
Photo. 2: Form II found in the inundated area
Photo. 3: Form III found in the desert
Photo. 4: Form IV found in the desert

Clustering technique analysis based on morphological, anatomical and genetic evidences resulted a dendrogram, classified the four heteroforms of *Avicennia marina* to two groups. The first group contains form I and from III separated at distance 37, while the second group contain form II and form IV separated at distance 15 (fig. 7).

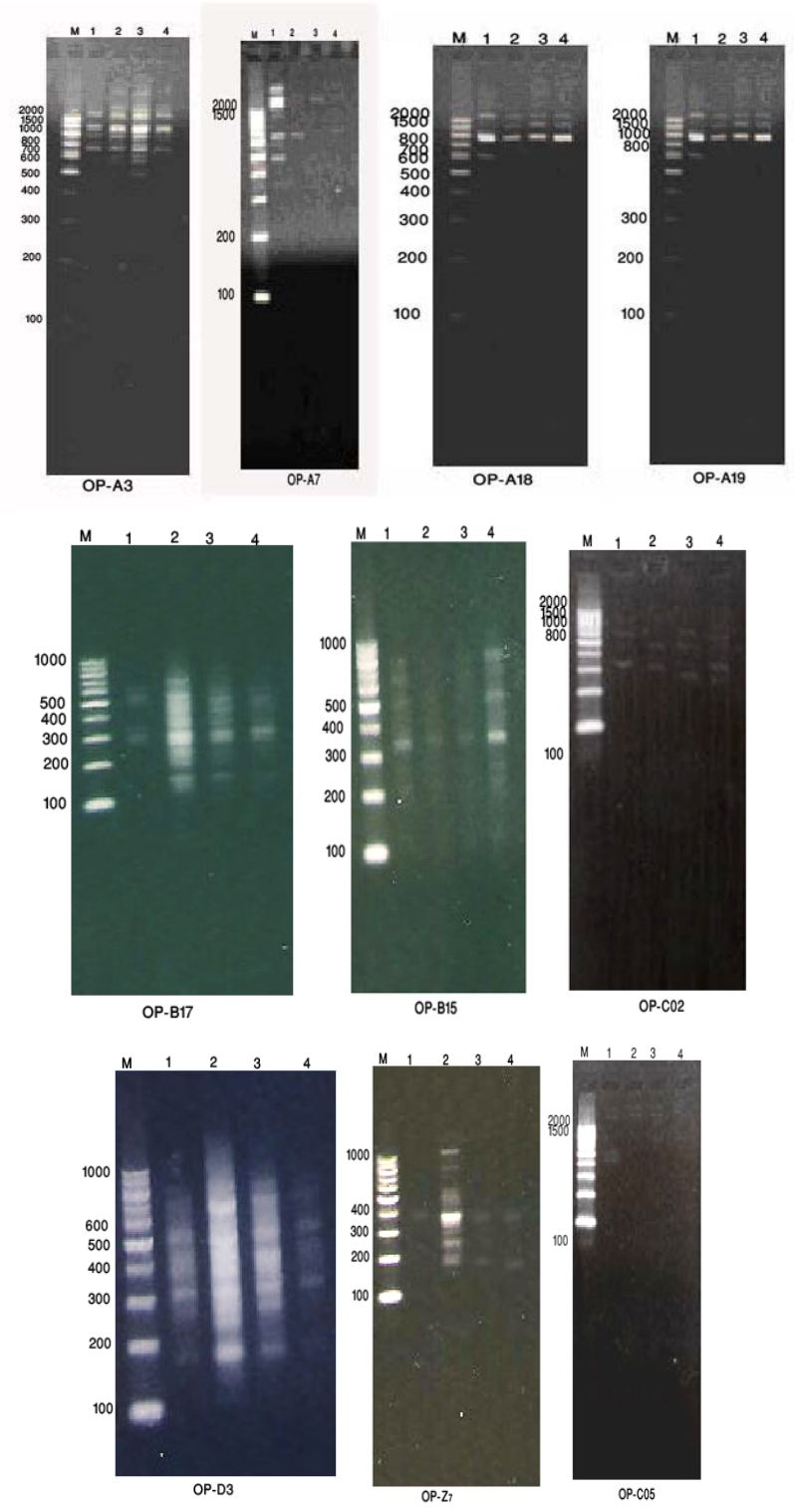

Fig 6: RAPD polymorphism of four heteroforms of *Avicennia marina* with ten random primers.
M: Marker 1: Form I found in the inundated area 2: Form II found in the inundated area
3: Form III found in the desert 4: Form IV found in the desert

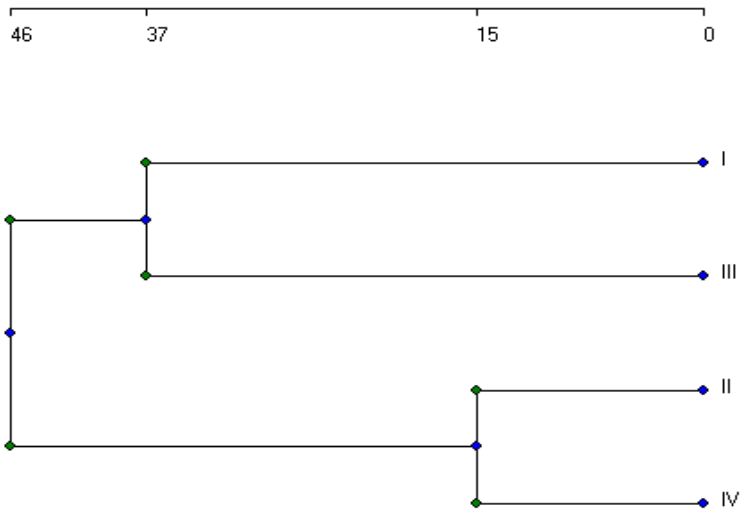

Fig. 7: Dendrogram of the four heteroforms of *A. marina* based on their morphological, anatomical and genetic evidences.

Table 8 Number of total bands, monomorphic (common) bands and polymorphic bands percentage of polymorphism revealed by the ten 10-mer primers in the four heteroforms of *Avicennia marina* by RAPD marker

Primers	Total no. of bands	Monomorphic bands	Polymorphic bands		Polymorphism %
			Unique	Non unique	
OP-A3	10	5	4	1	50%
OP-A-7	11	4	1	6	63.636%
OP-A-18	7	4	1	2	42.857%
OP-A-19	9	2	3	4	77.778%
OP-B-15	9	4	4	1	55.556%
OP-B-17	11	2	8	1	81.818%
OP-Co-2	5	2	3	--	60%
OP-Co-5	5	2	2	1	60%
OP-D-3	17	8	7	2	52.941%
OP-Z-7	11	3	1	7	72.727%
Total	95	36	59		Average 61.73%

Table 9: The distribution and molecular weight of unique bands (markers) revealed by RAPD among the examined samples of four heteroforms of *A. marina*.

Primers	Samples		No. of unique band	MW (bp)
	Unique band number	Form		
OP-A3	(7)	Form (3)	1	856.962
OP-A-7	(1,3,5,6,9,10)	Form (1)	6	2080.949-1838.408-1495.348-1252.729-723.624-662.324
OP-A-18	(1,7)	Form (1)	2	1885.503-798.693
OP-A-19	(2,5) (4,9)	Form (1) Form (3)	2 2	2013.551-1702.76 1417.485-879.969
OP-B-15	(1)	Form (1)	1	1034.664
OP-B-17	(10)	Form (1)	1	166.516
OP-Co-2	-	-	-	-
OP-Co-5	(5)	Form (1)	1	601.872
OP-D-3	(9) (10)	Form (2) Form (3)	1 1	455.051 450.179
OP-Z-7	(1,2,4,5,7,8) (10)	Form (3) Form (4)	6 1	1144.343-900.085-612.982-482.142-349.262-306.582 247.851

4. Discussion

Morphological, anatomical and genetic evidences of *Avicennia marina* four heteroforms in two different habitats at Al-Sharm Al-Bahari site, Al-Qussier region, Red Sea Coast, Egypt, were studied to reveal the taxonomic inter-specific relationships among them. Two forms of *Avicennia marina* (named form I and form II) are grown in marine aquatic habitat in the inundation area. The other two forms (named form III and form IV) are grown in the desert habitat. Significant difference recorded between the physico-chemical properties of the habitat's soil (texture, pH, EC, salt content and minerals content). The main difference between the two habitats focused on soil water content, the first habitat is waterlogged, while the second one have low water content and high aeration. The environmental differences in the two habitats according to many studies (Tomlinson, 1986; Duke, 1990; lakshmi e*t al.,* 2000; Melville and Burchett, 2002; Melville, *et al.*, 2004; Chen, *et al.*, 2008; Deng *et al.*, 2009; Salas-Leiva *et al.*, 2009) have effects on the morphological, anatomical and genetic evidences of the four heteroforms.

Present study recorded high variations in the morphological characters between forms I & II (growing in aquatic habitat) and between forms III & IV (growing in the desert habitat). On the other hand, low variations between forms I & III and also between forms II & IV were recorded. Negative correlation has been recorded between habitat properties and morphological characters of stem, leaf and petiole. These results indicated that form I and form II in spite of they are growing in the same habitat, but they have low similarity index. On the other hand form I and form III while they are growing in two different habitats, but they have high similarity index.

Tomlinson (1979) reported that some morphological characters are stable in different habitats and they are genetically controlled as leaf apex, leaf shape, and stem surface.

Duke (1990) and Duke *et al.* (1998) found negative correlation between morphological criteria of *Avicennia marina* leaves and environmental conditions. In this respect, Melville and Burchett (2002) reported that, leaf morphology may be used as a genetic marker of population differentiation. The measurements included leaf area, length, average width, apex, thickness and succulence or water content.

According to Tomlinson (1986) and Melville and Burchett (2002), the morphological characters separated the four heteroforms to two grouped, the first group contains form I and form III, while the 2nd group contains form II and form IV.

The anatomical features of *Avicennia marina* four heteroforms in the present study are in agreement with description of Fahn and Shimony (1977), Metcalfe and Chalk (1979) and Tomlinson (1986). Anatomical sections of the four heteroforms have the same structure but with differed in the section layers thickness (cuticle, cortex, phloem, xylem, etc). The difference in layers thickness can be attributed to the environmental variations. This hypothesis is supported by the present findings, where as form I and form II found in the same environmental conditions (waterlogged habitat) and have significant differences in the morphological characters, but they differed in their stem, petiole and leaf dimensions. Stem outline of form I and III is circular while that of forms II and IV is angular. Tomlinson (1986) concluded that the inundated plants are more frequently by the saline tidal water than those of the ridge plants, the former group has to maintain large number of narrow vessels overcoming cultivation problem, density and diameter of vessels are influenced by environmental fluctuation. Recent anatomical data of the four heteroforms of *Avicennia marina* didn't show clear trend in forms classification.

Genetic diversity has been recorded in populations of mangrove species. Nettel and Dodd (2007) and Nettel *et al.* (2008) observed genetic diversity for *A. germinans* population along the Pacific coast of Central America. Results of Melville and Burchett (2002) indicate that the genetic differences among three estuaries populated by Australian *Avicennia marina* were not greatly influenced by sediment characteristics, but rather by geographic distance.

Random amplified polymorphic DNA (RAPD) marker was used to assess genetic diversity and inter-specific relationships among the four heteroforms of Egyptian *Avicennia marina*. The present results obtained from RAPD analysis revealed that low genetic variation between forms I &III and forms II & IV but high genetic variation were detected between forms I&II and forms III&IV. These results beside it can used as indicators in species taxonomy, Allphin *et al.* (1998), Hartl (1988) and Mitton, (1989) considered that the greater levels of genetic variation within species and populations are an advantage in the face of the environmental and anthropogenic challenges.

The data obtained from morphological, anatomical criteria and RAPD analysis suggested segregation of the four heteroforms of *Avicennia marina* into two groups, the first group contains Form I and form III, while form II and form IV represent the 2nd group.

Results of the present study on the four heteroforms provide evidence for one species, *A.*

marina, comprising two subspecies. According to Tomlinson (1986), Duke (1990), Duke *et al.* (1998) and Moldenke (1960 and 1967), forms I & III are *A. marina* (Forsk.) Vierh. variety *eucalyptifolia* and forms II & IV are *A. marina* (Forsk.)Vierh. variety *marina*.

According to the familiar formula of Stace (1980); Genotype + Environment → phenotype, it can be considered form III is a phenotype to form I and form IV is a phenotype to form II.

In summary, from the morphological, anatomical and molecular evidences, *Avicennia marina* population in Al-Sharm Al-Bahari site, Al-Qussier region, Red Sea Coast, Egypt, contain two subspecies; *A. marina* (Forsk.) Vierh. variety *eucalyptifolia* and *A. marina* (Forsk.)Vierh. variety *marina*. The two subspecies have distinct morphological characters and they grow in aquatic habitat. The environmental factors play a part in modifying the genotype to produce the phenotype. Both subspecies has phenotype grows in desert habitat.

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