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Macro & Micromorphological Characterizations and RAPD Analysis to Differentiate Four Forms of *Avicennia marina* (Forssk.) Vierh.

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Macro & micromorphological characteristics and random amplified polymorphic DNA (RAPD) marker were used to asses taxonomic relationships and genetic variation among four forms of gray mangrove (*Avicennia marina*) grown in two distinct habitats in Al-Sharm Al-Bahari, 33km south Al-Qussier, Red Sea Coast, Egypt. A close relationship was revealed between form I & III and form II & IV according to the macro-morphological and molecular data, on the other hand, the micro-morphological data showed high similarity between form I&II and form III&IV. Dendrogram based on macro-&micro-morphological and molecular data divided the four forms into two groups, the first one includes form I&III and the second group includes form II&IV. The present study suggests that *A. marina* in Al- Sharm Al-Bahary can be classified into two varieties; *A. marina* var. *eucalyptifolia* (Zipp.) (form 1) and *A. marina* var. *marina* (Moldenke) (form II) and two phenotypes (form III and IV) for forms I & II respectively.

Keywords: Mangrove; *Avicennia marina*; Macro-&micro-morphology; RAPD; Red Sea

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Introduction

Avicennia L. (Family: Avicenneaceae) is a pantropical mangrove genus of 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 of Africa from the Red Sea to South Africa to the west Pacific from Japan to New Zealand (Giang *et al.*, 2003). Moldenke (1975) and Tomlinson (1986) recorded that the genus *Avicennia* shows considerable morphological variation especially in leaves and flowers, and classified based on theses 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).

Tomlinson (1986) and Duke (1991 & 1992) classified the *Avicennia* species to four major groups according to their morphological criteria; *A. alba* & *A. marina*, *A. integra* & *A. officinalis*, *A. rhumphiana*, and *A. bicolor*, *A. germinans*, & *A. schaueriana*.

A. marina (Forssk.) Vierh. is an important true mangrove species, it is a halophytic plant that grows as a shrub or tree to a height of three to ten meters.

A number of botanists have proposed division of the species, but currently three subspecies are recognized viz. *A. marina* subspecies *marina* (Forssk. Vierh), subsp. *ecucalyptifolia* Zipp. and subsp. *Australasica* (Walp.) Moldenke (Schwarzback and McDade, 2002).

Several taxonomical studies of *A. marina* were achieved based on the morphological variations of the vegetative and reproductive organs. The advent of technology that directly examines genes and gene products, added to the morphological characteristics to completely reliable indicators for genetic variation or taxonomic differences, owing to their tendency to be highly influenced by environmental factors (Duck, 1995, Brown *et al.*, 1997; Duke *et al.*, 1998 and Bryars & Adam, 1999). However, 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.

In the last two decades a number of molecular markers have been integrated in biological research. These markers have contributed to the understanding of plant biodiversity at the taxonomic and genetic levels. Recently, many studies have been carried out to asses the genetic diversity of mangrove species using genetic markers such as allozymes, random amplified polymorphic DNA (RAPD), microsatellite analysis, AFLPs and SSRs (Mguire *et al.*, 2000 & 2002; and Giang *et al.*, 2003).

Giang *et al.* (2003) used the microsatellite and AFLP markers to examine the genetic variation of *A. marina* in the costal area of Vietnam, they found that both the techniques revealed large genetic differentiation indicating strong genetic structure among regional populations. While Zolgharnein *et al.* (2010) recorded low genetic differentiation among four populations of *A. marina* in Iran using the microsatellite analysis.

Parani *et al.* (1997) used RAPD and RLFP markers to estimate intra-and inter specific variation in 3 species of *Avicennia* (*A. alba, A. officinalis* and *A. marina*), they found that *A. marina* was more closely related to *A. alba* than to *A, oficinalis*.

Avicennia marina (Forssk.) represents the dominant mangrove species in Egypt, found along Red Sea Coast from Ras-Mohamed to Mersa Haliab (Zahran, 1993). Tomlinson (1986) recorded that *A. marina* species characterized by different heteroforms. Four forms of *A. marina* were recorded in Al-Sharm Al-Bahary site, 33 km south Al-Qussier region in two different habitats. Two forms are growing in the inundation area, while the other two forms are found in land close to the Red Sea Shores. The two habitats have different physico-chemical properties as recorded by khalafallah (2002). Are those four forms, varieties of *A. marina* or ecotypes/phenotypes? These four forms need taxonomical study.

The objectives of this study are to asses the genetic variation among four forms of *Avicennia marina* in Al-Sharm Al-Bahary, 33km south Al-Qussier, Red Sea Coast based on incidences derived macro-morphological and micro-morphological characters as well as molecular markers generated by RAPD-PCR.

Materials and Methods

Study Site

Al-Sharm Al-Bahari site was chosen as it contains the four forms 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 land close 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). The four forms are photographed and showed in Fig. (2).

Plant samples collection

Thirteen healthy samples (3rd internodes, 3rd 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.

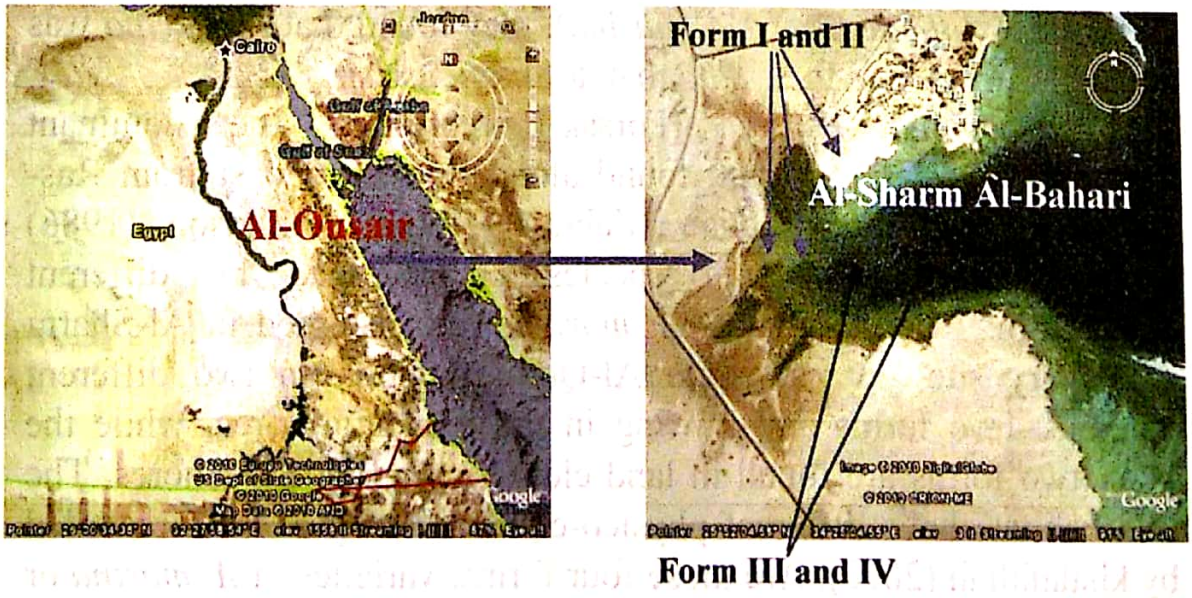


Fig. (1): Location of Al-Sharm Al-Bahari at Al-Qussair region, Red Sea coast, Egypt (Google earth program).

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 land close to Red Sea shores

Photo. 4: Form IV found in the land close to Red Sea shores

Fig. (2): The four forms of *A. marina*

Botanical investigation

Plant heights of the four forms 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 (1990a). The micro-morphology of stems, petioles and leaves of each form was examined in handmade microtome cross sections at 10-15µm, stained with safranin and light green according to the methods described by Johanson (1940). The sections were photographed under light microscope (Olympus) with digital camera (Canon Power Shot S80). The photographs were taken

by Zoom Browser Ex program. The dimensions of sections were measured using Corel Draw program version 11.

Molecular investigation

DNA isolation

DNA extraction and RAPD-PCR analysis were made in Genetic Engineering and Biotechnology center, Ain Shams University. Fresh leaves were collected and immediately ground in a mortar using liquid nitrogen (-196°C). Then about 0.5g of these leaves was used for DNA extraction (Dellaporta *et al.*, 1983). A volume of 2.5 ml extraction buffer was added immediately to the frozen plant tissues then transferred to a new eppendorf tube. A volume of 500 µl SDS (20%) was added, then incubated in water bath at 65°C for 20 min, followed by 15 min in ice, then centrifuged at 10,000 rpm, at 4°C for 10 min. The clear supernatant was removed and transferred to a new eppendorf tube. A volume of 5 ml phenol: chloroform: isoamyl alcohol (25:24:1). The clear supernatant of each sample was removed to a new eppendorf, then added 5 ml chloroform: isoamyl alcohol (24:1) followed by centrifugation at 10,000 rpm for 10 min at 4°C.

To precipitate DNA, ice cold isopropanol was added to supernatant and kept at -20°C for one hour and centrifuged at 10,000rpm for 10 min at 4°C. The supernatant was discarded, the volume of 80% ethyl alcohol was added to the pellets and centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was removed and the pellets was left to air dry for 20 min, then re-suspended in 20 ml distilled water at 4°C. A volume of 0.5 µl RNase was added and incubated to 37°C for one hour.

RAPD profiling was preformed as recommended by William et al. (1990). It was carried out using oligonucleotide sequences often 10-mer random primers; the sequence of these primers is illustrated in Table (1). A volume of 10 µl of the RAPD products were electrophoresis in 1.4 % agarose gel containing 10mg ml^{-1} of ethidium bromide. The gels were visualized and photographed by gel documentation system (Gel Doc Bio Rad 2000) under UV transillumination and using a Polaroid (MP4 camera).

Table (1): List of primers and their nucleotide sequences

No.	Primer code	Sequences(5 to 3)	GC %
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

The macro-&micro-morphological 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 RAPD attributes were scored as presence (1) or absent (0). For the numerical analysis the NTSTYS, version 2.02 program (Rohlf, 1998) was used.

Results

Macro-morphological observations

Macro-morphological characters of the four forms of *A. marina* described or measured and recorded in Tables (2 and 3). The recorded data indicated that from 59 studied characters, the four forms shared 51 (86%) characters and differed in 8 (14%) characters (habitat, stem surface, node shape, pnematophores presence, leaf base, leaf shape, leaf apex, upper surface color of leaf). Forms I & II growing in the aquatic conditions have pnematophores (aerial roots), while forms III and IV growing off shores lack pnematophores (Table 2).

There is no significant difference in height 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 another group (Table 3). The other measured dimensions; internode length, petiole length, leaf length, leaf width, leaf narrowness, leaf area and number of

flowers/inflorescence significantly differed between forms I & III as a group and forms II&IV as another group.

Micro-morphological investigations

From thirty one anatomical characters, the four forms of *A. marina* differed in six characters (Table 4 and figs. 3, 4 & 5). The forms I and III have circular outline while forms II and IV have angular outline. The other different characters are thickness of cuticle layer of stem, petiole and leaf, number of mid rib vascular bundles, bundle sheath growth and sclerenchyma cells presence. These micro-morphological data of the four forms of *A. marina*; stems, petioles, leaves, showed a significant difference between forms I & II as a group and forms III & IV as another group in 44% of the studied characters (Table 5).

Table 2: Macormorphological character states of the four forms of *Avicenna marina* and their coding for analysis

			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_Tlobed	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 2 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 land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

Table 3: Macromorphological attributes of four forms of *A. marina*

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 land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

Table (4): Micro-morphological character states of the four forms of *Avicenna marina* and their coding for analysis

			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 land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

Table (5): Micro-morphological features of 3rd internode, leaf and petiole of 4 forms 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

	I	II	III	IV
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 land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

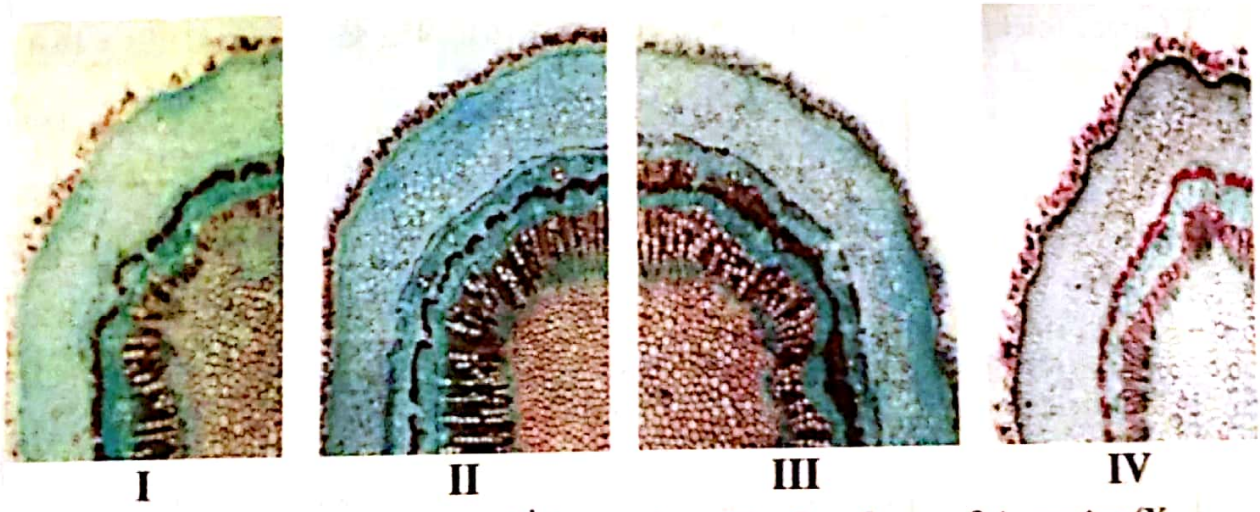


Fig. 3: Transverse sections of the 3rd internodes of the four forms of *A. marina* (X 160).

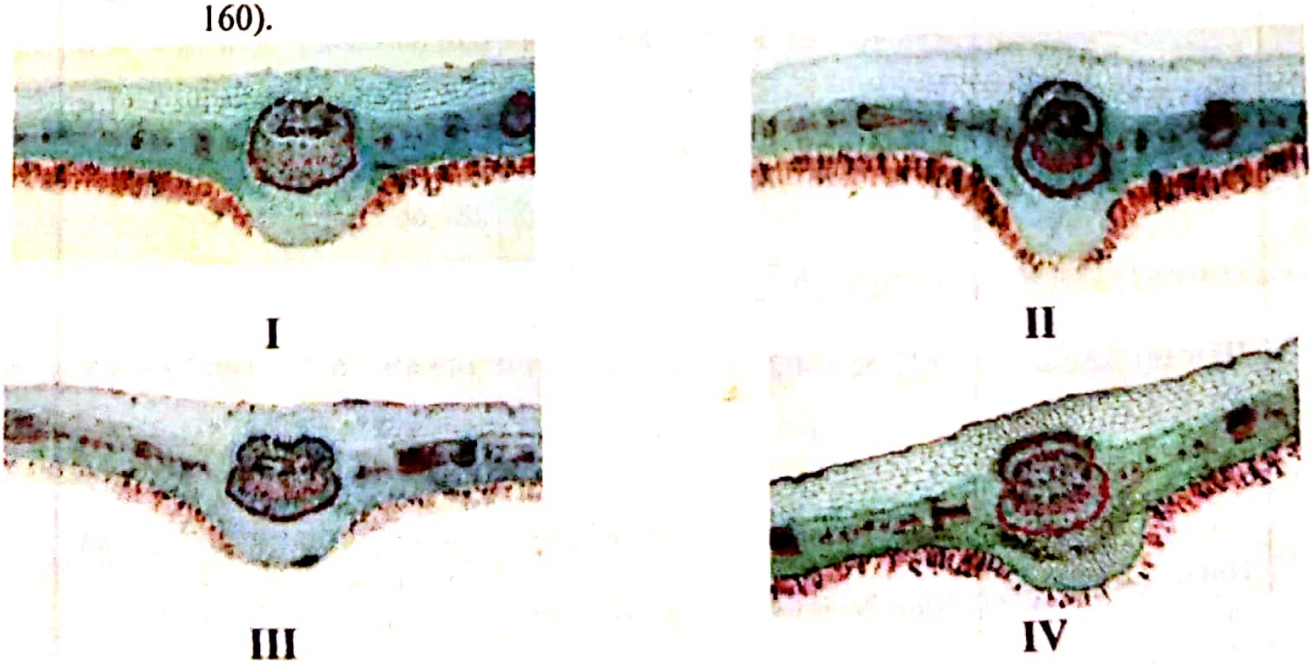
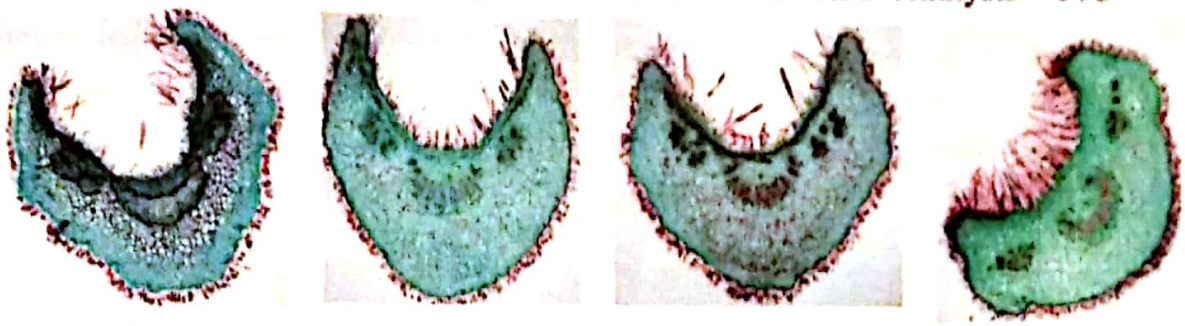


Fig. 4: Transverse sections of the 3rd leaf of the four *A. marina* forms (X 160).



I II III IV

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

I: Form I found in the inundated area
II: Form II found in the inundated area
III: Form III found in the land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

Genetic characters based on RAPD analysis

Ten-mer arbitrary oliganuleotide primers were initially used to establish RAPD-PCR fingerprint of the four forms of *A. marina*, the results were demonstrated in Table (6) and Fig. (6). A total of 95 RAPD bands have been revealed across the examined four forms of *A. marina*, 36 were monomorphic and the other 59 bands were polymorphic. The number of bands was changed in the intensity of amplification of fragments with the same length. The number of bands for each primer ranged from 5 (OP-CO2 and OP-CO5) to 17 (OP-D-3) and the bands varied in size between 100-1000 bp. The polymorphism percentage revealed by the different primers ranged from 42.857% (OPA-18) to 81.818% (OPB-17) with an average 61.73%. Table (7) and Fig. 6 illustrated the distribution and molecular weight of unique bands revealed by RAPD among the examined four forms of *A. marina*. The maximum number of unique bands was revealed in form I (13 bands) by six primers OPA-7, OPA-18, OPA-19, OPA-15, OPA-17 and OPCO-5. On the other hand, the minimum number (1) was revealed in forms II & IV by primers OPD-3 and OP-Z-7 respectively.

The clustering analysis technique based on the morphological anatomical and genetic data illustrated that the average distance of the four forms were 46, at this level they divided into two groups (fig. 7). The first one includes form I & III at taxonomic distance 37, and the second includes form II & IV at level 15.

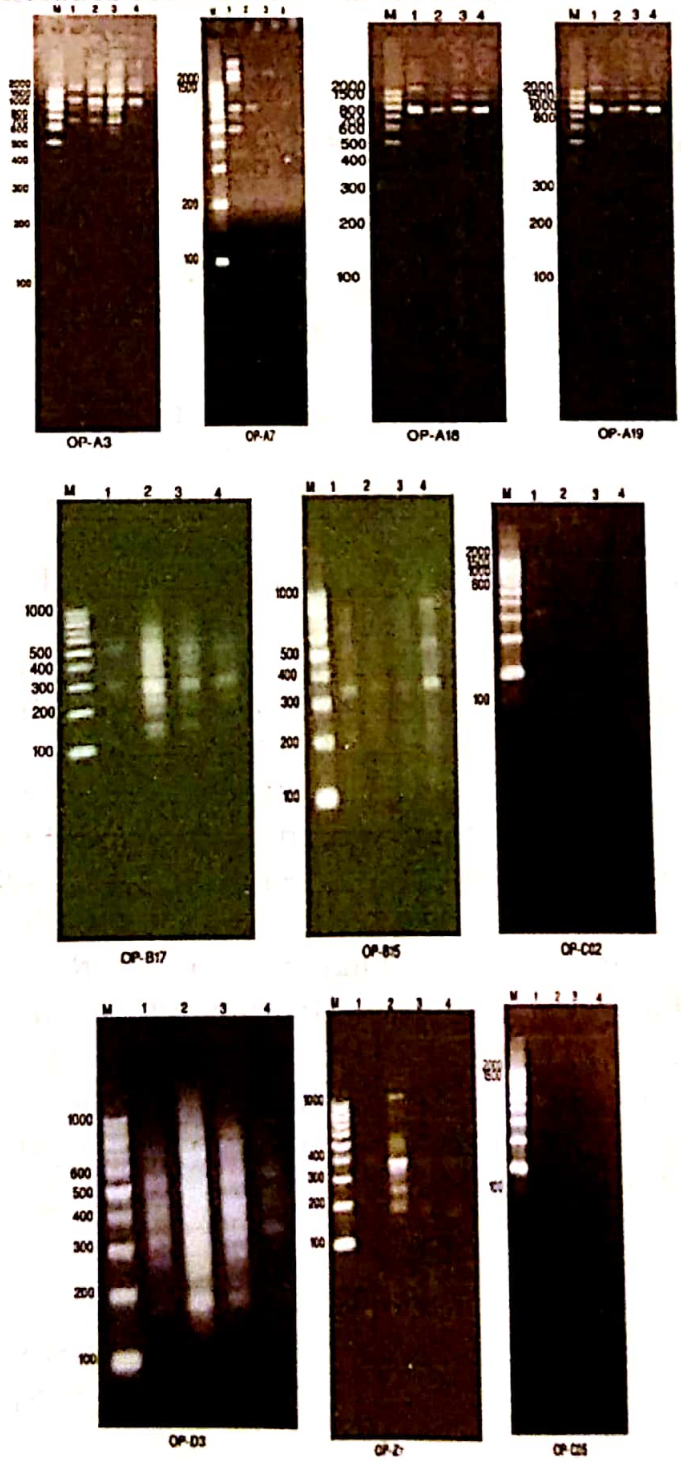


Fig 6: RAPD polymorphism of four forms of *Avicennia marina* with ten random primers.

M: Marker

I: Form I found in the inundated area
II: Form II found in the inundated area
III: Form III found in the land close to Red Sea shores
IV: Form IV found in the land close to Red Sea shores

Table (6): Number of total bands, monomorphic (common) bands and polymorphic bands percentage of polymorphism revealed by the ten 10-mer primers in the four forms 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 (7): The distribution and molecular weight of unique bands (markers) revealed by RAPD among the examined samples of four forms of *A. marina*.

Primers	Samples		No. of unique band	MW (bp)
	Unique band number	Form		
OP-A3	(7)	Form (III)	1	856.962
OP-A-7	(1,3,5,6,9,10)	Form (I)	6	2080.949-1838.408-1495.348-1252.729-723.624-662.324
OP-A-18	(1,7)	Form (I)	2	1885.503-798.693
OP-A-19	(2,5) (4,9)	Form (I) Form (III)	2 2	2013.551-1702.76 1417.485-879.969
OP-B-15	(1)	Form (I)	1	1034.664

Primers	Samples		No. of unique band	MW (bp)
	Unique band number	Form		
OP-B-17	(10)	Form (I)	1	166.516
OP-Co-2	-	-	-	-
OP-Co-5	(5)	Form (I)	1	601.872
OP-D-3	(9)	Form (II)	1	455.051
	(10)	Form (III)	1	450.179
OP-Z-7	(1,2,4,5,7,8)	Form (III)	6	1144.343-900.085-612.982-482.142-349.262-306.582 247.851
	(10)	Form (IV)	1	

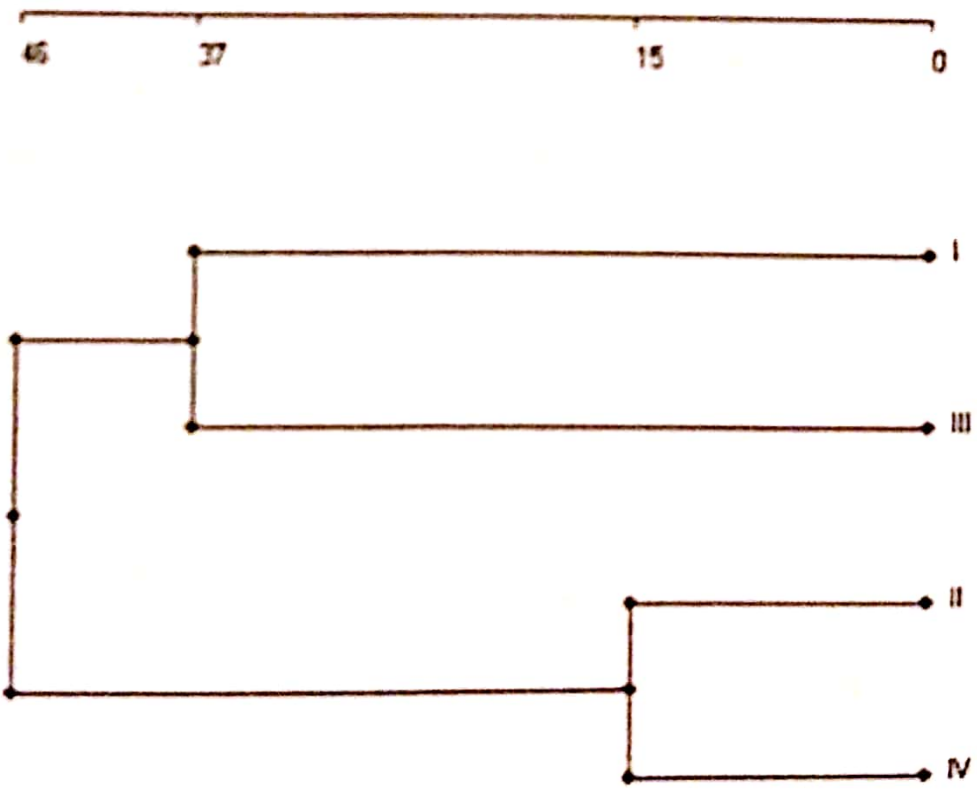


Fig. 7: Dendrogram of the four forms of *A. marina* based on their morphological, anatomical and genetic evidences.

Discussion

Morphological, anatomical and genetic evidences of *A. marina* four forms in Al-Sharm Al-Bahari site, Al- Qussier region, Red Sea Coast Egypt were studied to reveal the taxonomic relationships and genetic diversity among them. Two forms of *A. marina* (form I and II) are growing in marine aquatic habitat in the inundation area and the another two (form III and IV) are growing in the land close to Red Sea shore habitat. Significant differences have been recorded in the physico-chemical properties of the two habitats soils (texture, pH, Electric conductivity and water, salt & minerals contents) by Khalafallah (2002). According to (Tomlinson, 1986; Duke, 1990a; lakshmi *et al.*, 2000; Melville and Burchett, 2002; Melville, *et al.*, 2004; Chen, *et al.*, 2008; Deng *et al.*, 2009; Salas-Leiva *et al.*, 2009) the environmental differences have effects on the morphological, anatomical and genetic evidences of the species.

Some morphological characters are stable in different habitats and they are genetically controlled (Tomlinson, 1979). In addition, Duke (1990a) and Duke *et al.* (1998) found negative correlation between morphological criteria of *Avicennia marina* leaves and environmental conditions.

The present study recorded high variations in the morphological characters between forms I & II and between forms III & IV. On the other hand, low variations between forms I & III and 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 their growing in the same habitat, have low similarity index, while form I and form III while are growing in different habitats, but they have high similarity index.

The anatomical features of *A. marina* four forms 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 forms have the same structure but 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 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 forms of *A. marina* didn't show clear trend in forms differentiation.

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) indicated 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 intra-specific relationships among the four forms of Egyptian *A. marina*. The 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 can be 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. Duke (1992) found that *Avicennia* species were isolated by natural barriers of both land and sea presenting exchange of genetic material.

Results of the present study on the four forms provide an evidence for dividing species, *A. marina* into two varieties. According to Tomlinson (1986), Duke (1990a), Duke *et al.* (1998) and Moldenke (1960 and 1967), forms I & III are *A. marina* var. *eucalyptifolia* (Zipp.) and forms II & IV are *A. marina* var. *marina* (Moldenke).

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 varieties; *A. marina* var. *eucalyptifolia* (Zipp.) (form 1) and *A. marina* var. *marina* (Moldenke). The two varieties have distinct morphological characters and they grow in aquatic habitat. The environmental factors play a part in modifying the genotype to produce the phenotype.

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