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Molecular Variations and Photosynthetic Pigment Content of *Avicennia marina* Growing in Subtropical Habitat Types

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MANGROVE habitats are important ecosystems for their ecological value, and goods and services they offer. These costal habitats are currently vulnerable due to excessive human activities. The black mangrove *Avicennia marina* (Forssk.) Vierh. (*Avicenniaceae*) populations growing in Nabq protected area represents the northernmost latitudinal limit of the Indo-Pacific-East African naturally growing mangrove forests. In this study, nine *A. marina* populations inhabiting Gulf of Aqaba were studied to assess the genetic relationship among the populations by Start Codon Targeted (SCoT) and Inter Simple Sequence Repeat (ISSR) as DNA Markers. Variations of photosynthetic pigment content were assessed in the populations growing in different habitat types. The numbers of polymorphic bands were 18 and 6 for SCoT and ISSR, respectively. The percentage of polymorphism showed wide range amounted 40.9 for SCoT and 25 for ISSR, while the number of amplicon bands ranged from 8–10 in SCoT and 2-9 in ISSR. The genetic relationships among populations using SCoT and ISSR analysis showed close relationship between the nearby but different habitat type populations such as sand mound and littoral populations, or between populations of same habitat types in the study region. The photosynthetic pigment content of chlorophyll a and b, and carotenoids in the littoral and intertidal populations were higher than that in the sand mound and salt plain populations. Populations inhabiting Nabq protected area have wide range of polymorphism among different habitat types. Further studies are required to investigate the genetic relationships among populations of *A. marina* inhabiting the eastern and western sides of the Red Sea.

Keywords: Genetic variation, Gulf of Aqaba, Nabq Protected Area, SCoT and ISSR markers, Photosynthetic pigments.

Introduction

Mangroves are a special habitat type occupying the intersection between aquatic and terrestrial environments in tropical and subtropical areas. Mangrove habitat performs a crucial function in the provision of multiple ecological and economic goods and services and is considered the second most effective marine habitat after coral reefs (Al-Mur, 2021). Besides the protection of the shoreline, they provide suitable habitats for the other associated marine species (Manson et al., 2005). Ecologically, mangrove habitats is the highest productive ecosystem (Jennerjahn & Ittekkot, 2002; Donato et al., 2011; Adame et al., 2017). They protect the ocean from acidity and heat stress (Camp et al., 2016). Also, they help in carbon sequestration, balancing the carbon cycle and reducing global warming (Almahasheer et al., 2017; Adame et al., 2018). Globally, about 210 million people depend on mangrove-associated fisheries and mangrove-associated tourism (Hutchison et al., 2014; Spalding & Parrett, 2019).

In Egypt, the area covered by mangrove forests is estimated at 525km^2, and two mangrove plant

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species are found; *Rhizophora mucronata* Lam. *(Rhizophoraceae)* (red mangrove) and *Avicennia marina* (Forssk.) vierh. *(Avicenniaceae)* (black mangrove). These species inhabit the Red Sea's coastline where *R. mucronata* inhabits the most southern section of the Red Sea; while *A. marina* is distributed along the coast from south to north reaching the Gulf of Aqaba at Nabq protected area (the only mangrove species inhabit the northern coast of the Red Sea) representing the farthest northerly latitude at which natural mangrove forests are recorded in the Indo-Pacific and East African regions (Hegazy & Amer, 2002; Hegazy & Lovett-Doust, 2016).

This study encompassed molecular variation and photosynthetic pigments of nine populations inhabiting different habitats distributed along the Egyptian southern Gulf of Aqaba. Variations of DNA are detected through DNA markers (Guo et al., 2022). SCoT and ISSR are regarded as the most trustworthy markers among other markers (Gorji et al., 2011). The SCoT approach is a contemporary DNA-marker technology that deals with conserved sequences surrounding the start codon (ATG), as a result, it uses only one primer for both forward and reverse primers (Collard & Mackill, 2009). The ISSR analysis is a technique for identifying plant taxa based on variation identified between microsatellites. As a result, we used SCoT-PCR as a gene-targeting marker system in conjunction with the ISSR-PCR technique to investigate the molecular diversity and connections among *Avicennia marina* populations.Photosynthetic pigments are considered an appropriate indicator for understanding population health and its response to environmental stresses and environmental aspects (Richardson et al., 2002; Croft et al., 2017). Chlorophyll is the most important photosynthetic pigment that directly influences the photosynthesis process and the overall population's net primary productivity and is necessary for sustaining the biosphere. Additionally, it serves as a bio-indicator of the nutrient status, ecological stress, senescence, and disturbances of the studied populations (Main et al., 2011; Korus, 2013). Carotenoid pigments are common tetraterpenes that play critical functions for mangrove plant species such as a photoreceptive for photosynthesis and protective function against oxidative damage (Bandaranayake, 2002).

In general, the growth of A. marina populations ranges from small shrubs in dry habitats to large trees [up to 40m] in humid tropical ecosystems (Duke et al., 1998; Tomlinson, 2016). Moreover, the phenotypic variation in different populations is depending on the mutual action of the environment and the genotype (Peloso et al., 2017; Santos et al., 2024). Four main habitat types are inhabited by A. marina populations along the South Sinai coast, sea-side habitats are the intertidal and shoreline while, salt plains and sand mounds are landward (Hegazy, 1998; Mashaly et al., 2016), characterized by different environmental factors. Previous studies were carried out on A. marina populations along South Sinai coast habitats including morphology (Teraminami et al., 2014), rehabilitation (Saenger, 2002; Kairo & Hegazy, 2003), phytochemistry (Ibrahim et al., 2022), carbon contents (Mashaly et al., 2016), CO_2 Sequestration (El-Hussieny & Ismail, 2015), pollution (Dicks, 1986; Naim, 2004) and inhabited soil chemical characters (Madkour & Mohammed, 2008; Madkour et al., 2014), soil organic carbon (Eid & Shaltout, 2016). These studies did not cover the molecular variation among different populations. The objective of this study is to evaluate the molecular variation and pigment content of Avicennia marina in different populations inhabiting four habitat types in the South Sinai region which represent the farthest northmost latitudinal natural mangrove distribution in the East African Indo-Pacific area.

Materials and Methods

Plant materials

Mature fresh leaves and growing branch tips from nine *A. marina* populations were collected in April 2020 from four different locations in the Nabq protected area, Gulf of Aqaba, South Sinai, Egypt (Table 1 & Fig.1).

DNA extraction

Using a DNAeasy Plant Mini Kit [QIAGEN-Santa Clarita- CA], DNA extract was obtained from 100 mg plant materials collected from the nine *A. marina* populations. Fresh plant materials were frozen after collection using liquid nitrogen and milled using a prechilled mortar and pestle. The milled material was used for genomic DNA extraction. The purity of the extracted DNA was assessed using the A260/280 ratio as an indication, and the DNA samples were electrophoresed using a 1% agarose gel to investigate the integrity of the DNA and measure purity and concentration. The DNA marker [100bp DNA ladder, H3 RTU] was also loaded.

TABLE 1. Location of the study populations of *A. marina* growing in the four study sites and their habitat types of Nabq protected area

Population #	Location	GPS	Habitat type
1	Monquatea'a	N 28.19795°, E 34.42973°	Intertidal
2	Monquatea'a	N 28.19553°, E 34.43128°	Intertidal
3	Rowaisseya	N 28.17200°, E 34.44930°	Intertidal
4	Rowaisseya	N 28.16617°, E 34.44776°	Salt plain
5	Abu-Zabad	N 28.14680°, E 34.44243°	Sand mound
6	Abu-Zabad	N 28.15142°, E 34.44784°	Littoral
7	Abu-Zabad	N 28.15118°, E 34.44673°	Salt plain
8	Gharquana	N 28.13127°, E 34.44140°	Intertidal
9	Gharquana	N 28.12985°, E 34.44012°	Littoral

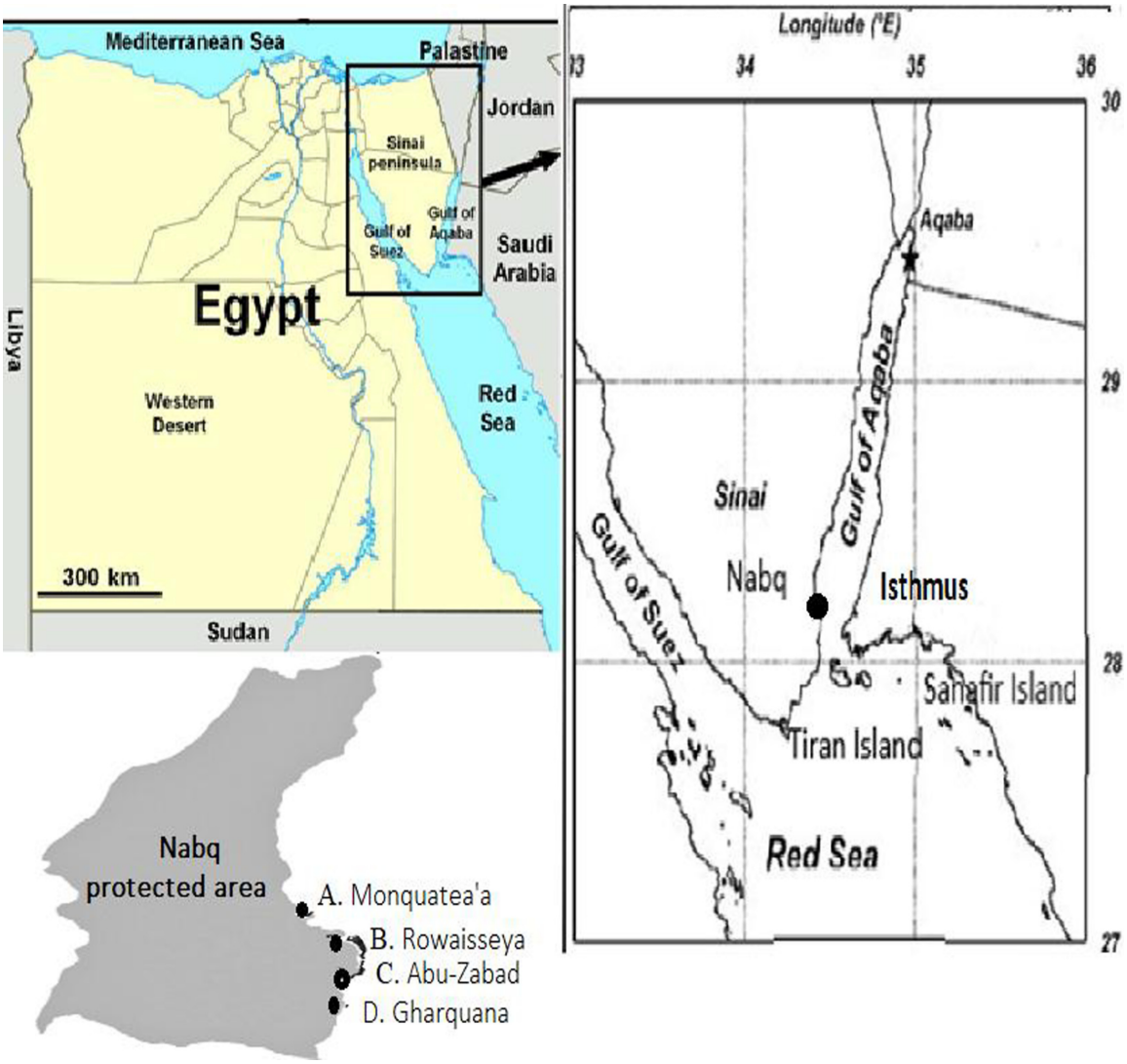

Fig. 1. Location of the study area and study sites (A, B, C & D) in Nabq protected area

SCoT and ISSRPCR analysis

For the SCoT and ISSR analyses, an array of 5 primers was employed for each molecular marker to amplify the earned DNA and determine the polymorphism (Table 2). In a 25- µL reaction volume, the PCR amplification was done using 12.5µL (2X) Master Mix "Thermo Scientific™" 3µL genomic DNA (10ng), 2.5µL primer, and 7µL nuclease-free water. The SCoT and ISSR cycling parameters were amplified via PCR as represented in (Table 2). the PCR reactions were amplified with the following program, the preliminary denaturation was at 95°C for 5min; then 40 cycles of denaturation at 95°C for 1min, the annealing was at 39.6 - 52.2°C for 1min (depending on the melting temperature of the primers) and the extension at 72°C for 2min followed by a final extension at 72°C for 10min for ISSR and SCoT Marker. For separation, the PCR products were run for 60-90min. at 80V on a 1.5% agarose gel with ethidium bromide (0.5g/mL) in TBE buffer. To figure out the size of the bands in the SCoT and ISSR profiles, a molecular marker of 100bp was also loaded. The bands were detected using a UV transilluminator and photographed by a gel documentation system [BIO-RAD 2000]. Scores were assigned to the bands generated by the SCoT and ISSR marker amplifications, the existence of the clear bands was scored as (1), and their absence as (0). Bands with the same mobility were regarded as similar and given the same scores. The binary statistic matrix was created. The unweighted pair group technique with arithmetic averages [UPGMA] was used to determine the Jaccard›s similarity coefficient between genotypes. The heatmap was created using the [ClustVis] program (Metsalu & Vilo, 2015). The maximum and minimum measurements were mapped with a color gradient utilizing the SCoT and ISSR analysis data matrix.

Photosynthetic pigments

Chlorophyll a, b, and carotenoid pigment contents were investigated using the Hiscox and Israelstam method (Hiscox & Israelstam, 1979). Five replicates for each population, leaf disks (1.77cm^2; approx. were randomly sampled and added to 15mL stoppered dark tubes containing dimethyl sulfoxide [DMSO]. The samples were kept at 4°C until all the pigments had been extracted. In dim light, the residual plant material was filtered, and the filtrate volume was then brought to 10mL volume using [DMSO]. A UV-visible spectrophotometer [Shimadzu UV-1208 model; Canby, OR, USA] was used to measure the optical density at wavelengths of 622, 436, 440, 480, 649, and 665nm, the blank was pure [DMSO]. There are typically 5 replicates of each sample in the data, with values expressed as average ± SE. The chlorophyll (a, b, and total) and carotenoid contents were expressed as mg/g (fresh weight) and estimated according to the following equations:

$$\text{Chl-a} = 12.19 * A665 - 3.45 * A649 \quad (\text{mg/g})$$

$$\text{Chl-b} = 21.99 * A649 - 5.32 * A665 \quad (\text{mg/g})$$

$$\text{Caro} = (1000 * A480 - 2.14 * \text{Chla} - 70.16 * \text{Chlb})/220 \quad (\text{mg/g})$$

where, Chl-a= chlorophyll a, Chl-b = chlorophyll b, total chlorophyll= Chl-a + Chl-b, Caro= carotenoids, and Ax= absorbance at x nm.

Data analysis

The amplified bands were manually scored before being used for SCoT and ISSR data processing using Gel Analyzer 3 © "The first Arabic Bioinformatic software for gel analysis" (Ahmed, 2008). The binary data matrix was designed by scoring the bands as either absent [0] or present [1]. The Jaccard similarity coefficient was used to create a similarity matrix (Maguire & Sedgley, 1997). After that, the unweighted pair group technique with arithmetic mean [UPGMA] was used to calculate the Dice's similarity matrix coefficients among the populations. The PAST program version 1.91 (Hammer, 2001) was utilized to perform Principal Coordinate Analysis (PCA) based on Euclidean similarity index, and this matrix was used to create a phylogenetic tree, or dendrogram for the SCoT and ISSR markers. For the chlorophyll and carotenoid pigments data, the results were expressed as average ± SE. The Statistical Package for Social Sciences [SPSS 22.0 for Windows] was used for the statistical analysis. One way ANOVA and Duncan›s multiple range test [P 0.05] were used for population comparisons.

Results

SCoT analysis

The five studied primers created reproducible bands in the studied populations, the total number of scorable bands was 44 [average 8.8 /primer] (Table 2 & Fig. 2A). The polymorphic bands were 18 [average 3.6 /primer], polymorphism

percentage (≈40.91). Each primer includes amplicons varying from 8 bands for [primer SCoT-13, SCOT-14, and SCOT-71] to 10 bands for [primers SCoT-31 and SCoT-66]. The number of polymorphic bands per primer varies from 0 for [primer SCoT-13] to 8 for [primers SCoT-66].

TABLE 2. List of primers, codes, annealing temperature, total bands, polymorphic bands, and % polymorphism for SCoT-PCR and ISSR-PCR analysis [A = Adenine, T= Thymine, G= Guanine, C= Cytosine]

Primer code	Primer code Sequence (5′– 3′)	Annealing temperature	Total bands	Polymorphic bands	% polymorphism
SCOT-13	5′ACGACATGGCGACCATCG3′	54	8	0	0
SCOT-14	5′ACGACATGGCGACCACGC3′	52	8	2	25
SCOT-31	5′CCATGGCTACCACCGCCT3′	52	10	7	70
SCOT-66	5′ACCATGGCTACCAGCGAG3′	51	10	8	80
SCOT-71	5′CCATGGCTACCACCGCCG3′	56	8	1	12.5
UBC-822	5′TCTCTCTCTCTCTCTCA3′	45	2	0	0
UBC-823	5′TCTCTCTCTCTCTCTCC3′	45	3	0	0
UBC-835	5′AGAGAGAGAGAGAGAGYC3′	49	9	5	55.56
UBC-845	5′CTCTCTCTCTCTCTCTRG3′	50	2	1	50
UBC-817	5′CACACACACACACACAA3′	49	8	0	0

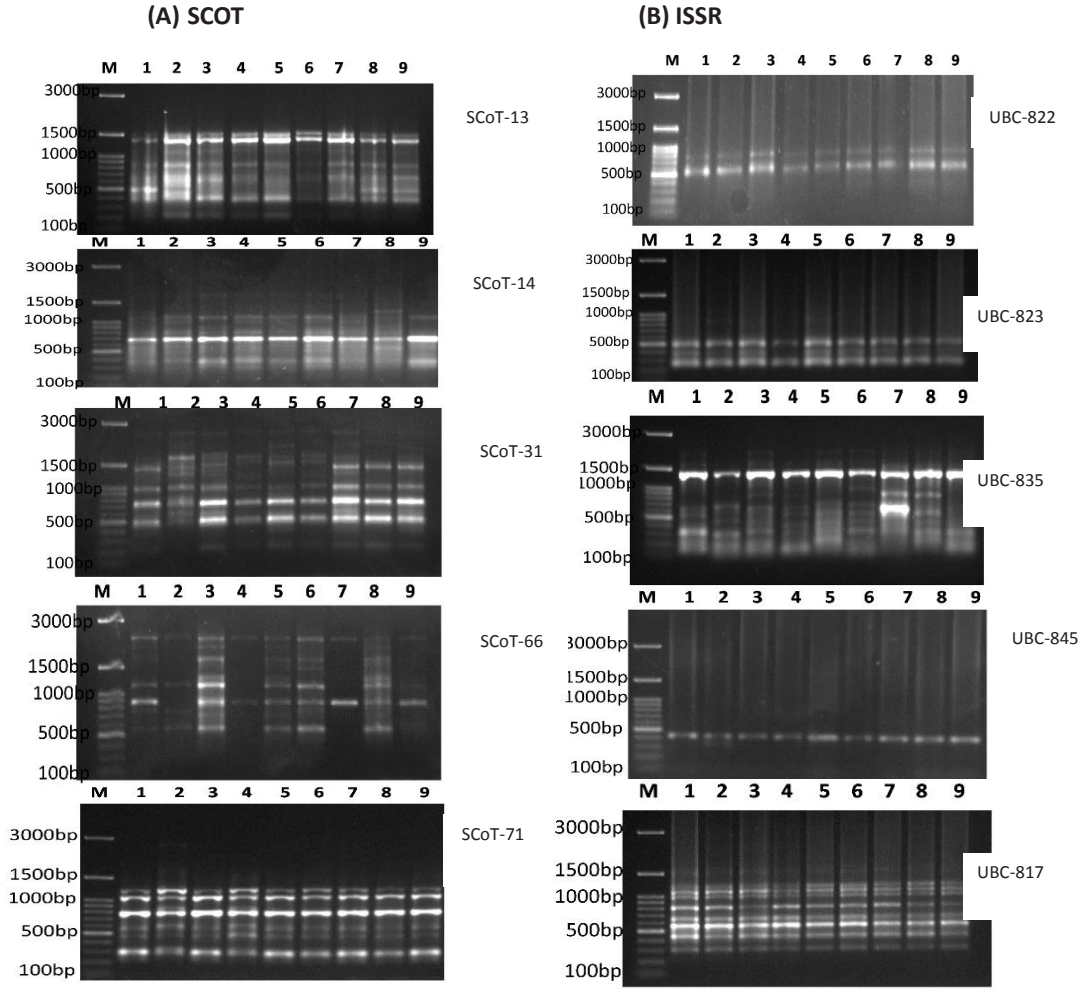

Fig. 2. Banding pattern revealed by SCoT (A) and ISSR (B) primers for the nine study populations of *A. marina* in different habitat type1-9

ISSR analysis

The five studied primers resulted in repeatable bands in the studied populations (Table 2 & Fig. 2B). The total amplicons yielded by the ISSR primers were 24, the polymorphic amplicons were 6 (25%), while the others (75%) are considered monomorphic and private. The primers [UBC-835 and UBC-817] showed the highest polymorphic amplicons amounting 9 and 8, respectively. Each primer includes distinctive amplicons varying from 2 amplicons in the case of [primers UBC-822 and UBC-845] to 9 amplicons for [primer UBC-835], with an average (4.8/ primer). In line with this, the number of polymorphic bands per primer varies from 0 bands in the case of [primers UBC-822, UBC-823, and UBC-17] to 5 bands for [primer UBC-83], mean (1.2/primer).

Molecular similarities among populations

The molecular similarities using [UPGMA] for SCoT and ISSR are illustrated in Fig. 3A, B. The similarity matrix using ISSR ranged from 0.90 to 1.0, populations 1- 5, 1 – 6, 5 – 6, and 7- 8 showed the highest similarity while the populations 4 - 8 showed the least (Table 3). In case of SCoT marker, the similarity matrix ranges from 0.96 and 0.77; the highest similarity was between populations 5 – 6, and 3 - 8 while the least was between populations 4 - 8 (Table 4). The nine studied populations are then subdivided into categories according to the degree of similarity. For the SCoT marker (Fig. 3A), population 4 appears separate, while 7 & 9 are closely related. The remaining populations occupy another subgroup; populations 3 & 8 as well as 5 & 6 are related while 1 and 2 are relatively diverged populations. Using the ISSR marker, populations 2 and 4 are clustered in a subgroup and the other populations are categorized in the other subgroup (Fig. 3B). Concerning the latter subgroup, populations 5, 1, and 6 are closely related, while population 9 lies nearby, populations 7 and 8 are closely related while population 3 lies nearby. For both markers, we noticed that populations 5, 1, and 6 are closely located, also populations 9 and 7 are neighbors as well as populations 8 and 3.

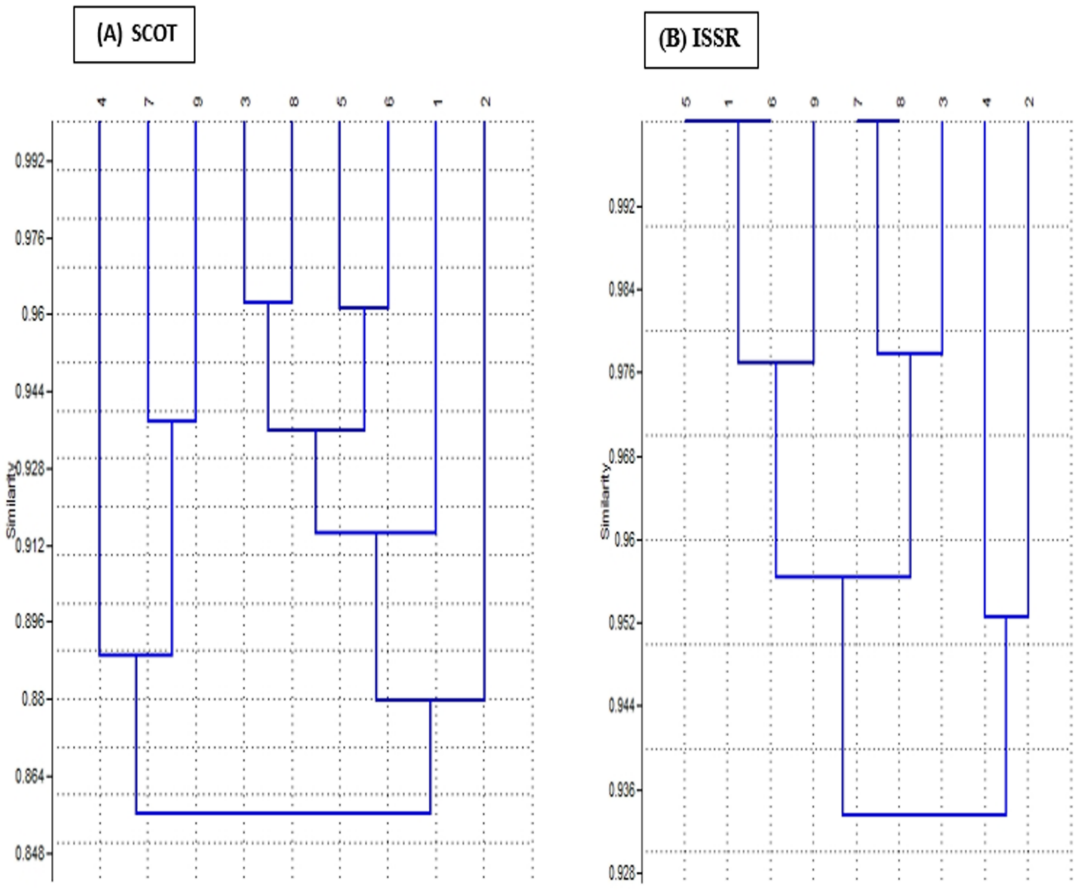

Fig. 3. UPGMA cluster analysis based on Jaccard's similarity coefficient for the nine study populations of *A. marina*. Using (A) SCoT and (B) ISSR markers. For population habitat type1-9

TABLE 3. Similarity matrix among nine populations of Avicennia marina according to Dice coefficient as revealed by ISSR markers

ISSR	1	2	3	4	5	6	7	8	9
1	1.00								
2	0.95	1.00							
3	0.98	0.93	1.00						
4	0.95	0.95	0.93	1.00					
5	1.00	0.95	0.98	0.95	1.00				
6	1.00	0.95	0.98	0.95	1.00	1.00			
7	0.95	0.91	0.98	0.90	0.95	0.95	1.00		
8	0.95	0.91	0.98	0.90	0.95	0.95	1.00	1.00	
9	0.98	0.93	0.95	0.93	0.98	0.98	0.93	0.93	1.00

TABLE 4. Similarity matrix among nine populations of *Avicennia marina* according to Dice coefficient as revealed by SCoT markers

SCoT	1	2	3	4	5	6	7	8	9
1	1.00								
2	0.90	1.00							
3	0.91	0.89	1.00						
4	0.82	0.87	0.79	1.00					
5	0.91	0.87	0.95	0.85	1.00				
6	0.89	0.88	0.94	0.85	0.96	1.00			
7	0.90	0.88	0.86	0.92	0.86	0.84	1.00		
8	0.95	0.85	0.96	0.77	0.94	0.92	0.85	1.00	
9	0.93	0.85	0.89	0.86	0.86	0.84	0.94	0.90	1.00

The heat map (Fig. 4) provides an accurate view of the molecular relationship among the population in this study. The red color indicates much representation, while the blue indicates a low degree of representation, and the degree of color is correlated with the genetic similarity between the populations. Heat map analysis as well as UPGMA using both markers showed identical relationships among the populations. The heat map using SCoT (Fig. 4A) showed the populations' genetic similarity arrangement 2, 4, (7, 9), 1, (8, 3), and (5, 6). The ISSR (Fig. 4B) showed the relationships 3, (7, 8), (2, 4), 9, and (6, 1, 5). The principal component analysis [PCA] using the SCoT marker (Fig. 5A), populations (7 & 9), (3 & 8), and (5 & 6) appear to be related to each other, while using ISSR marker Figure (5B) showed closer genetic similarity among populations (2 & 4), (7 & 8) as well as (5,1 & 6).

Photosynthetic pigments

Concerning all photosynthetic pigments (chlorophyll a & b and carotenoid), the populations inhabiting littoral and intertidal localities (except littoral population # 6) showed significantly increased pigment values than salt plain populations. For chlorophyll a, populations 9 (littoral) and 3 (intertidal) reached the highest values 0.79 and 0.78mg/g respectively. Alternatively, salt plain populations 4 and 7 showed the lowest content values 0.15 and 0.16mg/g respectively (Fig. 6A). Populations 2 and 8 (both are intertidal), amounted to the highest chlorophyll b contents 0.21mg/g (for both populations), while the least value 0.08mg/g fresh weight was recorded in population 7 which inhabits

salt plain locality (Fig. 6B). The highest total chlorophyll content was recorded in populations 9 (littoral) and 3 (intertidal) amounting to 0.91 and 0.90mg/g respectively, while population 7 (salt plain) showed the least total chlorophyll content (0.25mg/g) (Fig. 6C). Chlorophyll a: b ratio showed a wide range scale where, the maximum ratios amounted to 6.9 and 6.4 in populations 9 and 3, respectively, while the least ratios amounted to 1.0 and 1.1 in populations 2 and 4, respectively (Fig. 6D). Population 1 (intertidal) showed the maximum carotenoid contents (0.47mg/g), while the least value (0.23mg/g) was recorded in salt plain populations 4 and 7 (Fig. 6E).

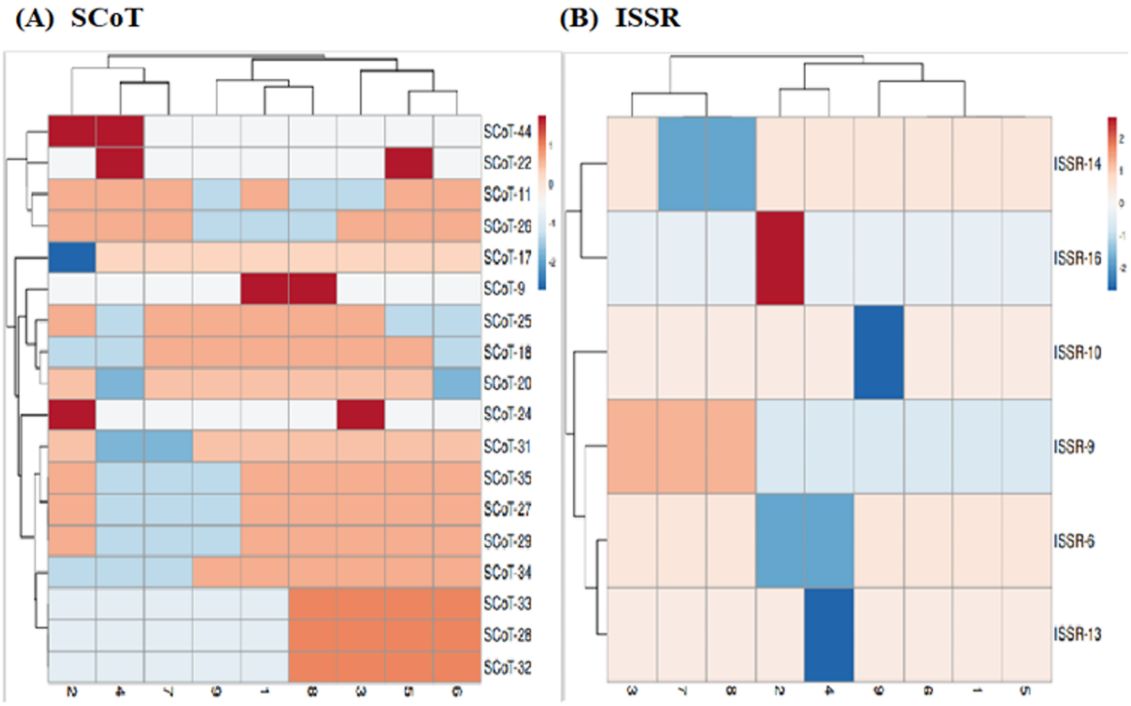

Fig. 4. Heat map analysis for the nine study populations of *A. marina* [SCoT analysis (A), ISSR analysis (B). The different populations groups are in columns and the obtained bands are in rows. For population habitat type1-9]

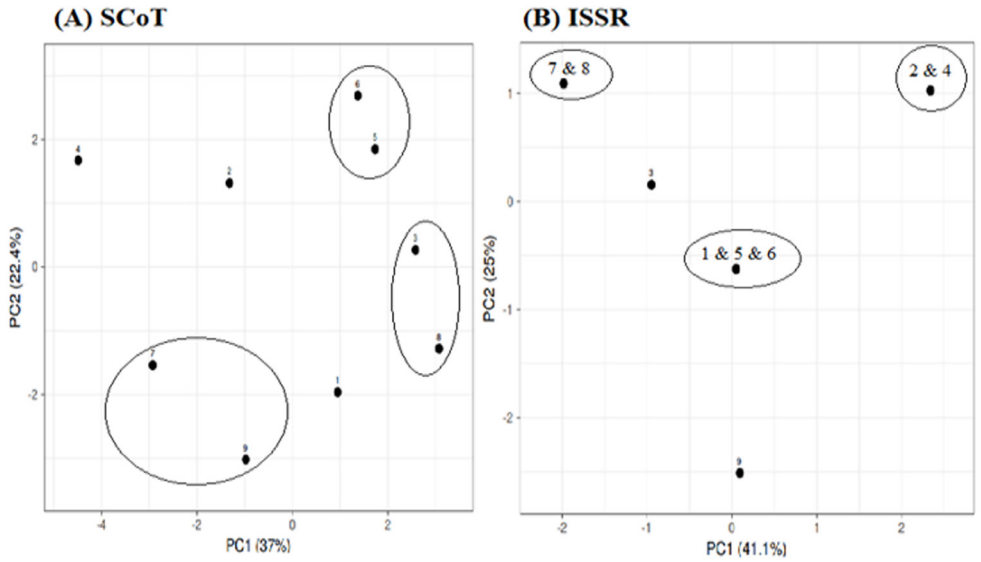

Fig. 5. Principal Component Analysis (PCA) of the PCR data showing the two-dimensional (PC1 and PC2) plot of the nine study populations of *A. marina*. SCoT analysis (A), ISSR analysis (B). For population habitat type1-9

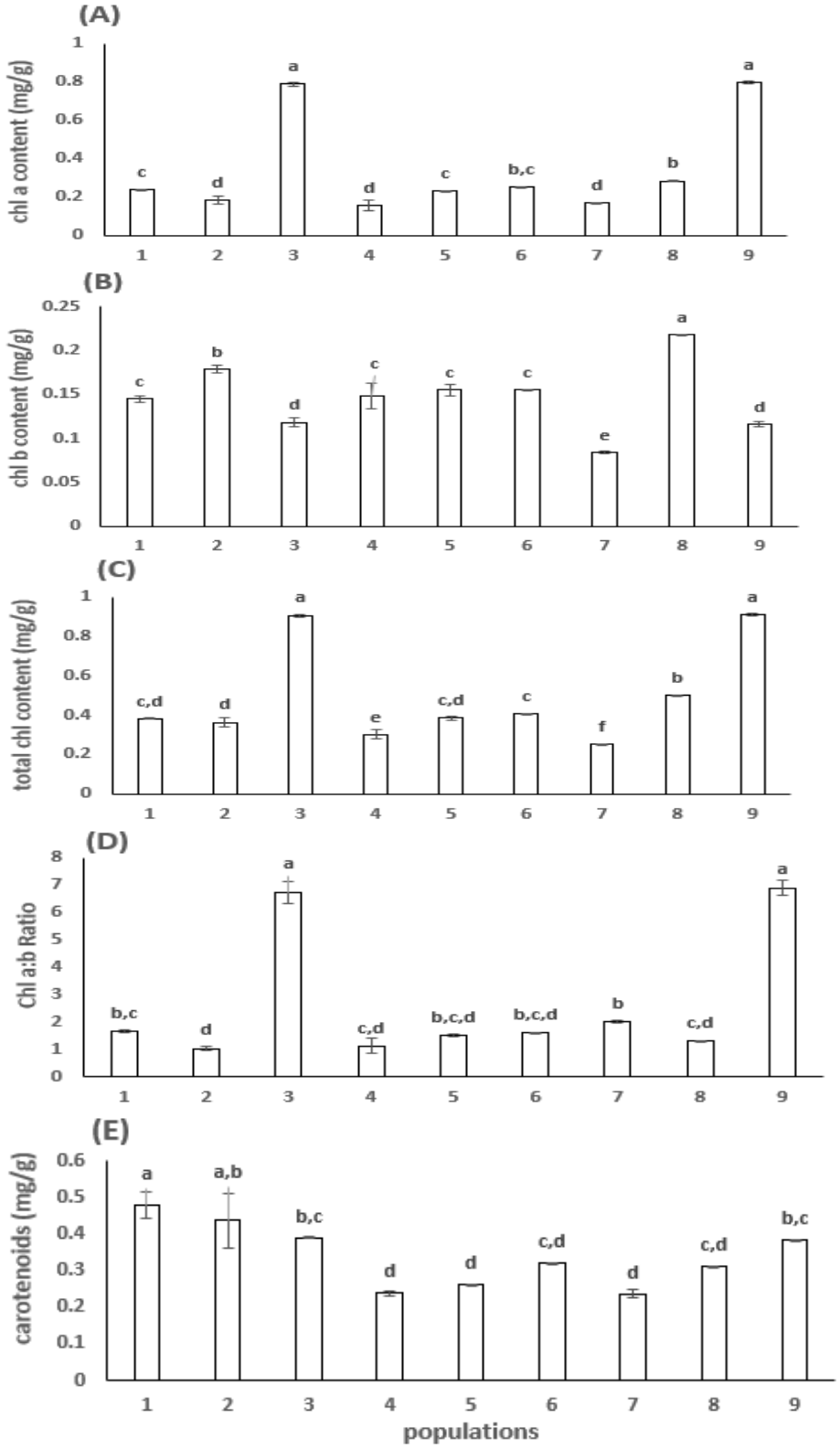

Fig. 6. Pigment content (mg/g fresh weight) of the nine study populations of *A. marina* [(A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll, (D) chlorophyll a:b ratio, (E) carotenoids of population habitat types 1-9, see table 1. Different small letters indicate significant differences among populations at P≤ 0.05. Values are expressed as the mean ± SE, n= 5]

Discussion

The SCoT results monitor polymorphism in the coding sequence, which can be linked to particular functional genes, whereas the ISSR results determine the microsatellite polymorphism, which is frequently not associated with particular functional genes (Xiong et al., 2011). The number of bands generated per primer is a significant measurement showing the marker's effectiveness in the investigation of DNA variation (Luo et al., 2011). In the present investigation, the SCoT markers generate more bands per primer (8.8) than ISSR markers (4.8). Several genetic diversity studies demonstrated that SCoT markers produce more bands than ISSR, Agarwal et al. (2015) recorded 5.3 bands for SCoT and 3.1 for ISSR in their study on *Alhagi maurorum*, Amom et al. (2020) recorded (13.8) and (11.5) for SCoT and ISSR, respectively in their study on five bamboo species in India. *Cicer arietinum* in Iran showed 9.5 and 4.75 (Pakseresht et al., 2013), and Amirmoradi et al. (2012) recorded 12.4 and 11.5 for SCoT and ISSR, respectively. Abdein (2018) recorded 173 bands for SCOT and 54.54 for ISSR in his study on Pumpkin in Saudi Arabia.

The molecular variation using SCoT markers showed 40.91% polymorphism, while ISSR markers showed 25% polymorphism. A similarly wide range of molecular relationships is common among different studies of mangrove populations around the world. A study of 14 different populations of *A. marina* in Australia and Japan, using microsatellite markers found that some populations showed high levels of molecular variations, while the other populations exhibit little or no molecular variations, the latter populations include Port Albert, Victoria, Australia; Bunbury southern Western Australia; Iriomote Island, Japan (Maguire et al., 2000a); these populations are similar to the nine populations in this study because they are located at the far ends of the ecological distribution range margins. The low molecular variation among populations inhabiting different habitats was recorded by many authors; similar studies conducted by Maguire et al. (2000b) and Giang et al. (2003), demonstrated the genetic relationships and molecular variation using five molecular markers among six *A. marina* communities throughout Vietnam's whole coast; these studies showed modest molecular diversity among local populations, however, high molecular variations among *A. marina* communities of the northern, central, and southern sectors. Another studies conducted by Arnaud-Haond et al. (2006), Do et al. (2019) revealed a shortage of genetic variation and a significant degree of inter-breeding in *A. marina* communities of the north sector of Vietnam, similar low genetic diversity was reported in Pemba-Metuge, Mozambique (Amade et al., 2021).

The populations of *A. marina* in this study lie at the ecological range edges of this species, the molecular variation of these isolated populations may depend on the habitat types (Arnaud-Haond et al., 2006; De Ryck et al., 2016). The molecular variations may change according to the location of the population, the populations of *A. marina* found at the range edges have much lower molecular variation and much more DNA similarity when compared to the innermost populations in Vietnam. This was attributed to the increased environmental stress, the small population size, and the shortage of pollinators (Arnaud-Haond et al., 2006). Similarly, *A. marina* populations in this study inhabiting isolated localities in the Gulf of Aqaba in the most northward distribution and partially closed southward by Tiran and Sanafir Islands in the Gulf of Aqaba Isthmus, this resulted in almost completely isolated habitats of low water currents. Sea currents and local geomorphology were also identified to be crucial factors for molecular variations of *A. marina* populations in South and East Africa (De Ryck et al., 2016).

Similarly, studies emphasized that obstacles, like the Central American Isthmus, can hamper the flow of genes and form molecular discontinuities among *A. marina* populations (Villesen et al., 1999). In the Pacific and Caribbean estuaries, some obstacles may create genetic breaks for comparable mangrove species such as *Rhizophora mangle* L. and *Avicennia germinans* (L.) Stearn (Cerón-Souza et al., 2012). Eckert et al. (2008) emphasized the reduced genetic diversity in formerly marginal populations with distribution constraints in northern Vietnam (Giang et al., 2003; Cerón-Souza et al., 2012). The Philippines (Arnaud-Haond et al., 2006), and Japan (Maguire et al., 2000a, b). Although, the horizontal floating propagules of mangrove species such as *A. marina* and *R. mucronate* demonstrated comparable spread patterns under natural circumstances in Gazi Bay, Kenya (Xiao et al., 2011).

The vast expanse propagation of *A. marina* is

less effective than that of *Rhizophora* due to the limited viability of *A. marina* seedlings (Binks et al., 2019). As appears, the most genetically similar populations are geographically close to each other (Sreekanth & Anupama, 2021) and the long-distance dispersal is not the usual propagule scenario for *A. marina* because of the seedling characteristics (Van der Stocken et al., 2018) and the extensive roots (pneumatophores) which may limit seedling establishment away from dropped fruits (Villesen et al., 1999; Van der Stocken et al., 2015). This may explain the high molecular similarities between *A. marina* populations inhabiting different habitats in this study such as the clustering of the littoral population at Gharquana (no. 9) in the same subgroup with salt plain populations at Rowaisseya and Abu-Zabad (no. 4 and 7) in the case of UPGMA cluster analysis (Fig. 3A) and the close relationship between these populations in heat map analysis (Fig. 4A) for SCoT. In addition, the rising sea level may suppress the sedimentation rate and submerging of littoral and intertidal mangroves. As a result, this may force these populations to migrate landwards (Krauss et al., 2014) which may result in similar molecular structure within populations inhabiting different habitats in the same local area.

Considering populations inhabiting the same coastline as demonstrated by Triest (2008), the largest molecular variations occurred within the populations rather than between populations. Alternatively, the clustering of the population that inhabits similar habitats in different clusters seems to be attributed to the variations in the local abiotic conditions where molecular variation within different populations inhabiting similar habitats in a small area may be associated with urban environments and sediment pollution (Melville & Burchett, 2002) or may be attributed to the reproductive ecology and pollination in *A. marina* populations by wind or insects (Tomlinson, 2016).

A. marina flowers are protandrous with extended periods of flowering and about 16000 pollen grains and four ovules per flower these help to avoid self-pollination (Clarke & Myerscough, 1991), this may interpret the high molecular variations among some local intertidal population at Monquatea'a (no. 1) with salt plain population at Rowaisseya (no. 4).

Alternatively, the high level of polymorphism using SCoT markers (40.91%) may be also attributed to the reproductive ecology of this species which resulted in high heterozygosity and polymorphism levels (Tomlinson, 2016). Similar polymorphisms were recorded within an individual population (45.61%) and (85.35%) among three *A. marina* populations inhabiting the Kerala coast, Southern India (Sreekanth & Anupama, 2021).

The measurements of photosynthetic pigments showed that the littoral and intertidal populations in all localities (1, 2, 3, 8, and 9) have higher pigment content than sand mound and salt plain populations 4, 5, and 7, while littoral population at Abu-Zabad (no. 6) showed intermediate pigment contents. These results show that photosynthetic pigments increased seaward whereas the level of salinity decreased. Similar results were obtained in a greenhouse experiment on *A. marina* seedlings in Saudi Arabia where, increasing salinity from 150 to 1200mM NaCl caused a 45, 20, and 53% decline in chlorophyll a, chlorophyll b, and carotenoid pigment contents, respectively (Barhoumi et al., 2021). Similarly, Bhar et al. (2013) discovered that the total chlorophyll content in low-saline habitats was higher than chlorophyll content in moderate and highly saline habitats. Studies on another mangrove species *Aegiceras corniculatum* showed a 27% reduction in total chlorophyll when treated by 250m M NaCl (Kumar & Bandhu, 2004).

Concerning carotenoid contents in the studied populations, the seashore populations amounted to higher values than inland populations, this may be attributed to the salinity effect, similar results were obtained in a study on *A. corniculatum* where the plant showed 1.6 fold reduction in carotenoid contents when treated with 250mM NaCl (Kumar & Bandhu, 2004).

Conclusions

The genetic relationships across populations of A. *marina* in this study demonstrated a wide range of polymorphism when tested by SCoT & ISSR molecular markers, which may be attributed to the reproductive ecology of *A. marina* populations. Photosynthetic pigments of *A. marina* populations are affected by variations of salinity levels stress. The difference in photosynthetic pigment contents in the studied populations is correlated

to combined actions of the environmental conditions and the genetic of populations. There should be more studies to investigate population health & genetic relationships among *A. marina* populations inhabiting the Red Sea coast.

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الاختلافات الجزيئية ومحتوى صبغ البناء الضوئي في نبات المنغروف الأسود *Avicennia marina* النامي في الموائل شبه الاستوائية

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تعتبر موائل أشجار المانغروف أنظمة بيئية مهمة لقيمتها البيئية والسلع والخدمات التي تقدمها. تتعرض هذه الموائل الساحلية حاليا للخطر بسبب الأنشطة البشرية المفرطة. وتمثل مجموعات المنغروف الأسود *Avicennia marina* (Forssk.) Vierh. (*Avicenniaceae*) التي تنمو في منطقة محمية نبق الحد الأقصى لخط العرض الشمالي لغابات المانغروف التي تنمو بشكل طبيعي في منطقة المحيطين الهندي والهادئ وشرق إفريقيا. في هذه الدراسة، تمت دراسة تسعة مجموعات من نوع *A. marina* التي توجد في خليج العقبة لتقييم العلاقة الوراثية بين هذه المجموعات بإستخدام طرق Start Codon Targeted (SCoT) وInter Simple Sequence Repeat (ISSR) كواسمات الحمض النووي DNA. تم تقييم الاختلافات في محتوى أصباغ البناء الضوئي في المجموعات النامية في الموائل المختلفة. كان عدد النطاقات متعددة الأشكال 18 و6 لـ SCoT وISSR، على التوالي. أظهرت نسبة تعدد الأشكال مدى واسع بلغ 40.9 لـ SCoT و 25 لـISSR ، بينما تراوح عدد نطاقات الأمبليكون من 10-8 في SCoT و9-2 في.ISSR. أظهرت العلاقات الوراثية بين المجموعات باستخدام تحليل SCoTو ISSR وجود علاقة وثيقة بين المجموعات في الموائل القريبة المختلفة مثل التلال الرملية و الساحلية، أو بين مجموعات من نفس أنواع الموائل في منطقة الدراسة. كان محتوى أصباغ البناء الضوئي للكلوروفيل أ و ب والكاروتينات في المجموعات الساحلية ومجموعات المد والجزر أعلى من ذلك الموجود في مجموعات التلال الرملية والسهول المالحة. وتتميز مجموعات المنجروف الموجودة في محمية نبق بنطاق واسع من تعدد الأشكال بين الموائل المختلفة. هناك حاجة إلى مزيد من الدراسات لدراسة العلاقات الوراثية بين مجموعات *A. marina* التي تنمو علي الجانبين الشرقي والغربي للبحر الأحمر.