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Full Length Article

Genotypic Variations of Mangrove (*Avicennia marina*) in Nabq Protectorate, South Sinai, Egypt

Dalia M. Sabri[1], Sayed A. El-Hussieny[2] and Nagwa Elnwishy[1,3]
[1]*Biotechnology Institute for Postgraduate studies & Research, Suez Canal University, Ismailia, Egypt*
[2]*Nature Conservation Sector, Egyptian Environmental Affairs Agency, Cairo, Egypt*
[3]*Zewail City of Science and Technology, Giza, Egypt*
[*]For correspondence: dotsab@yahoo.com; sayed_bkr@yahoo.com; nelnwishy@zewailcity.edu.eg

Abstract

Mangrove (*Avicennia marina*) in south Sinai is the most prevailing vegetation. In some sites, it is completely terrestrial and totally lost their pneumatophores (aerial roots) and formed high terrestrial dune on the coastal marsh (Sabkha). Thus, the population of the seaside (intertidal) mangroves and the landside (terrestrial) mangrove were investigated to examine the potential genetic variation related to these phenotypic changes. Mangrove was screened in 6 populations collected from 2 sites in Nabq protectorate (Abo Zabad and Rowasisa). Genetic variations were examined by RAPD analysis to explore the genetic diversity among mangrove population of the landside (terrestrial) and seaside (intertidal) areas, and results of the RAPD analysis were supported by DNA sequencing and analysis. DNA sequencing identified all tested samples as *A. marina*, and RAPD banding pattern revealed significant genetic diversity among all tested mangrove populations by 68.33% of polymorphic band. Genetic differences were revealed between the populations of the terrestrial mangrove and intertidal mangroves in each site. These finding suggest that mangroves have moved landward up to 500 meters on land with the effect of wind and erosion, and the genetic variations supported mangrove for a gradual environmental adaptation to the terrestrial land topography, and totally lost their pneumatophores (aerial roots).

Keywords: DNA sequencing; Genetic diversity; Pneumatophores; RAPD; Egypt

Introduction

Mangroves are coastal habitats of trees and shrubs mostly forming forests. They are found in sheltered estuaries and mud flats along lagoons, oceans and sea coasts in the tropics and subtropics. They have developed morphological, physiological and reproductive strategies to adapt to the harsh saline, waterlogged and anaerobic environmental conditions (Spalding *et al.*, 2010). There are 70 mangrove species in the world, eleven of which (16%) are at high threat of extinction (Polidoro *et al.*, 2010).

In Egypt, the most abundant species are *Avicennia marina* (*Avicenniaceae*) and *Rhizophora mucronata* (*Rhizophoraceae*). However, *A. marina* is relatively more tolerant and better adapted to salinity, low rainfall and extreme temperature conditions than *R. mucronata* in Egypt and worldwide. They occupy about 525 hectares distributed in 28 different locations, one large discontinuous stand along the Gulf of Aqaba in Nabq Managed Resources Protected Area, and one small stand at the most southern part of Gulf of Suez in Ras Mohammed National Park (Saenger, 2002).

The values of mangrove are numerous. Firstly, it provides highly economic direct and indirect use pattern values, which can maintain sustainable development in drylands (Elnwishy *et al.*, 2008). Secondly, it embraces about 130 bird species worldwide (Sodhi *et al.*, 1997), at least 13 of which are associated mangrove habitat in Egypt (Goodman *et al.*, 1989). Thirdly, mangrove is an important regulator of sediment movement and costal protection (Sajjaduzzaman *et al.*, 2005). Also, its aerial roots (pneumatophores) help trap terrestrial sand and slow the movement of flood waters full of sediment during flash floods (Thampanya *et al.*, 2006; Kumar *et al.*, 2011). The loss of mangrove vegetation can lead to flushing silt and clay within three to four years post-clearance (Debra and Rachel, 2015). Also, mangroves provide shelter from predation and food availability for small juvenile fish and a wide variety of aquatic animals (Olivier *et al.*, 2015).

For years, the researchers are interested in investigating the genetic diversity among mangrove forests in relation to geographical distribution (Mori *et al.*, 2015), environmental and ecological factors (Maguire *et al.*, 2000), and stress responses. Over time, the molecular techniques have been increasingly developed to evaluate the genetic diversity among mangrove populations; microsatellite and AFLP markers (Dimendra *et al.*, 2013), RAPD markers (Surya *et al.*, 2015) and recently DNA sequencing

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(Huang *et al.*, 2014). Among different genetic markers, randomly amplified polymorphic DNA (RAPD) markers are successfully used to identify and differentiate mangrove populations in phylogenetic studies (Surya *et al.*, 2015).

In Egypt, mangrove is the most prevailing vegetation type in the Nabq Protectorate. In some sites it has been found to be completely terrestrial, form part of the coastal marsh and high terrestrial dune vegetation (Sabkha) and totally lost their aerial roots. This natural difference between mangrove found in the intertidal area and the terrestrial area may bring up a research question on this species. Therefore, this research aims to study the observed phenomena of changes in mangrove structure in different environmental gradients in the study area, and to investigate the genetic variations between the detected terrestrial and the intertidal mangroves.

Materials and Methods

Study Area

The study area is located in Nabq Protectorate in Sinai Peninsula at the northern east of Egypt (Fig. 1), where mangrove was distributed discontinuously as mono specific stands for about 15 km along Gulf of Aqaba. It exists in four sites along this distance; Gharquana, Abu Zabad, Rowaisseya and Monquatea northwards. Mangrove starts in the south in the intertidal zone in Gharquana at 28º 06' 13" N and 34º 26' 15" E, and ends in the north in the intertidal zone in Monquatea at 28º 12' 39" N and 34º 25' 26" E. However, a well-developed mangrove is heavily distributed along variable environmental gradients landwards in Abo Zabad (Site one) and Rowaisseya (Site two) as shown in Fig. 2. Thus, they were selected for the phenotypic and genetic studies.

Monitoring Phenotypic Variations

Population demography: A quadrate of 10 x 10 meters (0.01 Hectare) was allocated along about 500 meters long. Monitoring started from the sea side towards land side. Quadrates location was marked using GPS GARMIN etrex Vista Cx.

Individuals heights and total basal area: The total height was measured using telescopic measuring rode, trunk circumference or diameter at 60 cm height were measured using measuring tape or plastic caliper. Individuals which branched beneath 60 cm each of the branches was treated as a separate individual.

Mean number of aerial roots m^{-2} along transects: The number of aerial roots per square meter 1 x 1 m was recorded in both sites using PVC quadrate divided into sub quadrates of 10 x 10 cm to facilitate counting, three, four or five replicates were taken depending on the homogeneity aerial roots density within the main quadrate.

Mean length of aerial roots along transects: The length of aerial roots from the ground surface to the root tip was measured using telescopic measuring rode: 20 random replicates were measured in each population at each site. More than 20 measures were examined at the shoreline (intertidal) due to the mid tide line.

Effect of aspect exposure on productivity outputs: The effect of main aspects (East, West, North and South) on productivity outputs of seeds and flowering buds production was examined on 13 isolated individuals of 3.5 – 5 meters height. Measuring was done by 1 x 1 m PVC quadrate allocated three times at 1.5 to 2.5 meter height for each direction. The calculation was based on the total count of either flowering buds or fruits in the monitored individual.

Investigation on Genetic Variation

Plant sampling: Young and tender mangrove leaves were randomly collected from the two sites. Each site was classified into three populations according to the location; intertidal mangrove populations (P1 and P4), terrestrial mangrove (P2, P3, P5, and P6) as shown in Fig. 2. All samples (six populations) were stored at -20ºC for extraction.

Genomic DNA isolation: Five samples of mangrove leaves from each population were randomly selected for DNA isolation. A modified CTAB protocol (Sunil *et al.*, 2012) was used for DNA isolation to obtain a pure and amplifiable DNA. DNA concentration and purity were determined with a spectrophotometer (Nanodrop, ND-1000) at 260 and 280 nm. DNA quality was assessed for all samples in 0.7% Agarose gel electrophoresis (1xTAE buffer).

Random amplified Polymorphic DNA (RAPD): Ten Oligonucleotide primers, Metabion, Germany, were used in RAPD analysis as described in Table 1. PCR amplification reactions were carried out in 25 µL total volume; 12.5 µL OnePCR master mix (GeneDireX, USA), 40 ng/µL DNA template, and 40 pmol primer. RAPD-PCR was performed in a labcycler (SensoQuest, Germany), starting with initial denaturation for 5 min at 94ºC , followed by 40 cycles (30 sec at 94ºC, 30 sec at 30ºC, 30 sec at 72ºC), then final extension for 7 min at 72ºC. PCR product was separated on 1.5% agarose gel (1X TAE buffer). Amplicon size was determined using 100 bp DNA size ladder (SolisDyne) and photographed under UV light with G:BOX photo documentation system (SYNGENE, England).

RAPD analysis: Genetic similarity/distance between the six populations was estimated by genetic similarity index (Nei, 1978). The banding patterns from RAPD analysis were scored as 1 (present), or 0 (absent) for further calculation of polymorphic bands. Genetic similarity (GS) reflects the proportion of the bands shared between individuals, and Genetic distance (GD) was calculated as GD = 1-GS according to (Moore, 1990). All the previous parameters were calculated using Popgen32 software as described by (Sneath and Sokal, 1973). A phylogenetic relationship based on genetic distance values was performed and a dendogram was constructed after using Unweighted Pair Group Method

Table 1: The RAPD primers sequence

Primer Name	Primer sequence
OPM1	5'-GTTGGTGGCT-'3
OPM6	5'-CTGGGCAACT-3'
OPN4	5'-GACCGACCCA-3'
OPN5	5'-ACTGAACGCC-3'
OPP1	5'-GTAGCACTCC-3'
OPP2	5'-TCGGCACGCA-3'
OPQ1	5'-GGGACGATGG-3'
OPQ20	5'-TCGCCCAGTC-3'
OPT7	5'-GGCAGGCTGT-3'
OPT8	5'-AACGGCGACA-3'

Fig. 1: Geographical location of the study area, Nabq protected area, Egypt

with Arithmetic mean (UPGMA).

DNA sequencing analysis: DNA sequencing analysis was performed on three DNA samples from each population. PCR reactions were was amplified with 18S ribosomal RNA primer (F:5'-TTAAGCCATGCATGTCTAAG-3', R:5'-GACTACGACGGTATCTAATC-3'). The thermal protocol consists of one cycle of initial denaturation for 2 min at 94ºC, followed by 35 cycles (30 sec at 94ºC, 30 sec at 52ºC, 3 min at 72ºC), then final extension step for 10 min at 72ºC. Purification of the PCR product was performed using QIAquick PCR Purification Kit (Qiagen). Then PCR product was subjected to cycle sequence PCR using BigDye Terminator v3.1 Cycle Sequencing Kit. 20 µL of each reaction contained 8 µL Terminator ready reaction mix was added to 3.2 pmol Primer, DNA template (quantity was calculated

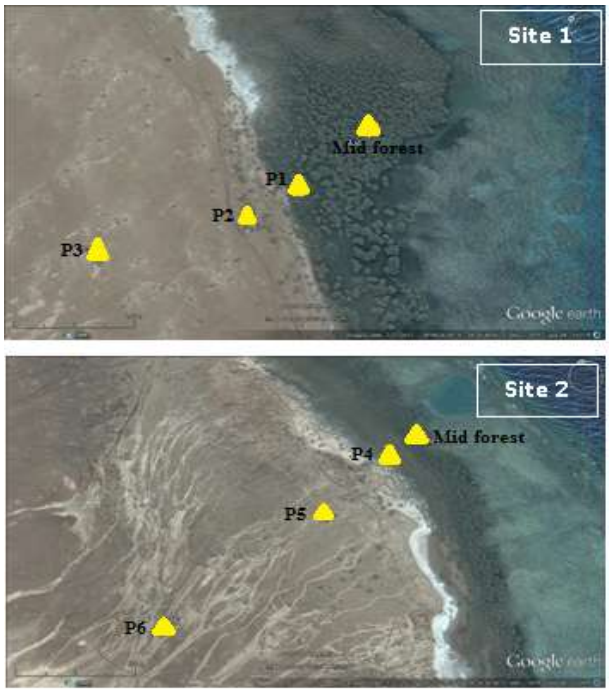

Fig. 2: Location of collected populations

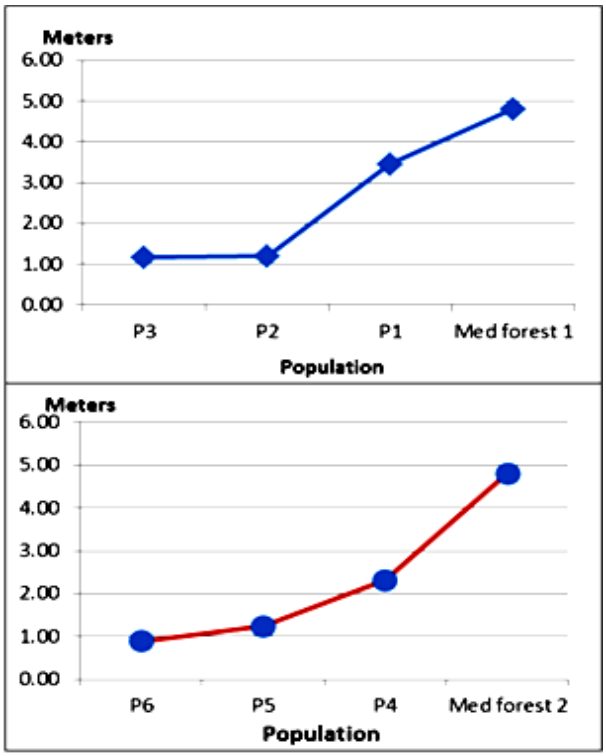

Fig. 3: Total height (m)

according to the PCR product size), and MilliQ water. Thermal profile for Cycle Sequencing PCR: 1 min at 96ºC, 25 Cycles (10 sec at 96ºC, 5 sec at 50ºC, 4 min at 60ºC), followed by another purification by Centri-Sep spin

column (PRINCETON SEPARATIONS), DNA sequencing was applied by 3500 Genetic Analyzer (Applied Biosystems). Finally, DNA sequencing data analysis was conducted using DnaSp 5.10 and MEGA6 software for multiple sequence alignment, similarity index, and genetic tree.

Statistical Analysis

Statistical analysis was carried out using the SPSS BASE 10.0 (SPSS Inc., Chicago, IL, USA) packages. Data were tested by ANOVA. F-protected LSD separated means ($p < 0.05$).

Results

Generally, Abu Zabad and Rowaisseya sites showed well presence of *A. marina* development along variable environmental gradients with two different forest widths. Site one (Abu Zabad) was 100m wide landward from shoreline, while site two (Rowaisseya) was more dense 500 m.

Morphological Variations

Individuals heights and total basal area: Change in individual mean and maximum heights followed the same pattern in both sites, where tree heights increase gradually starting from terrestrial mangrove (2 m) until the intertidal area (5 m). However, moving further into the sea, mangrove increased till it reached its maximum values in the mid forest (9.12 m) (Fig. 3). Study of changes in the mean height (m) and basal area (m^2 ha^{-1}) of mangrove from landward (terrestrial) – seaward (intertidal), revealed certain patterns in changes. Basal area increased gradually, moving seaward till it reached its maximum values at intertidal zone (Fig. 4).

Mean number of aerial roots along transects: Aerial roots density (number m^{-2}) started inland at zero aerial roots in all terrestrial populations (P2, P3, P5, P6). Then a gradual increase was observed at shoreline (P1 and P4) passing from shoreline seaward direction. However, the highest average density was 197.33 m^{-2} and 439 aerial roots m^{-2} were observed in mid forest in the seaside in site one and site two, respectively (Fig. 5).

Mean length of aerial roots along transects: Measured length of aerial roots passing landward-seaward showed a start of zero value, where no aerial roots exist for individuals and forming sand dunes in site one (P3 and P2), and in site two (P5 and P6), then gradual increase in the average lengths of aerial roots starting from P1 and P4 towards sea was observed (Fig. 6).

Effect of aspect on productivity: The mean number of flowering buds and fruits m^{-2} in a mangrove stand was greater at east west sides than north and south, flowering buds showed high sensitivity to this aspect so that 50.68 and 47.03% of total recorded flowering buds were at the west

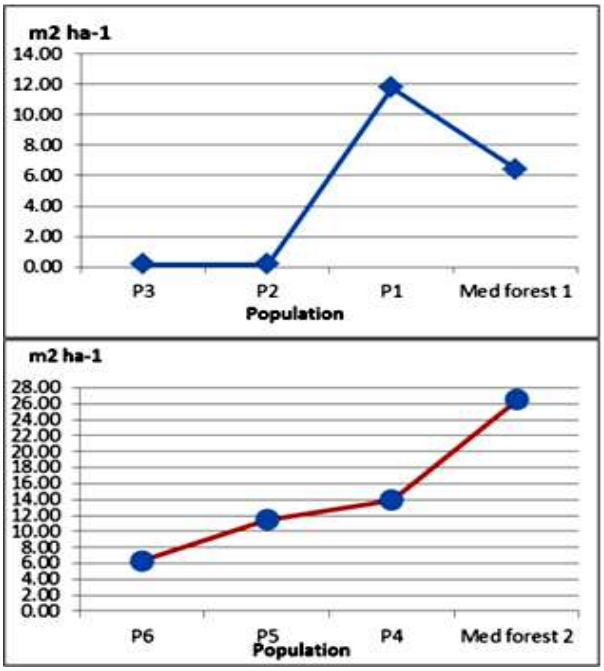

Fig. 4: Total basal area (m^2ha^{-1})

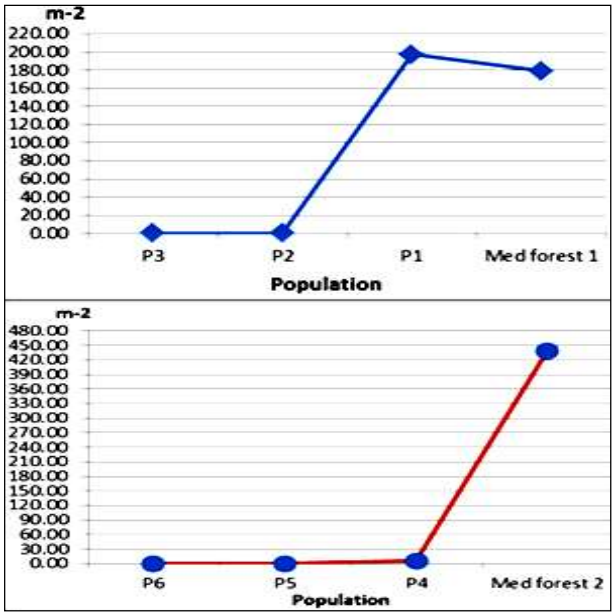

Fig. 5: Density of aerial roots (m^{-2})

and east orientations respectively while only 1.37 and 0.91% of them were at the north and south orientations respectively (Fig. 7). Fruits' spatial distribution showed less sensitivity toward the studied aspect, so 33.7 and 29.67% of the total seeds count were at the west and east orientation respectively while 49.47 and 17.07% of the total seeds count were at the northern and southern orientations respectively.

Measuring both parameters at the same time and

Table 2: Mid spring mean number of flowering buds and fruits m^{-2}

Direction	No. of Flowering Buds	No. of Fruits	Conversion %
East	103	136	56.90
West	111	154	58.11
North	3	89	96.74
South	2	78	97.50

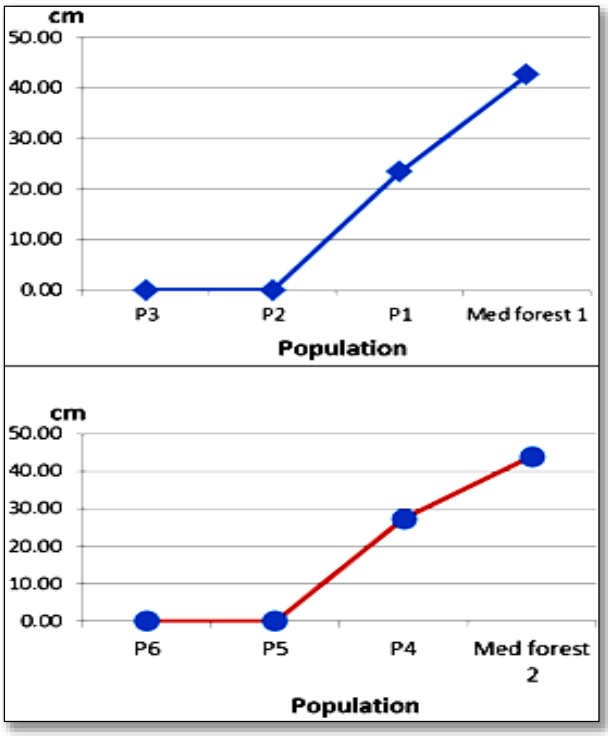

Fig. 6: Length of aerial root (cm)

further prospected conversion of the flowering buds into seeds is of interest since reviewing the total count of flowering buds and fruits for each direction at that definite time and the fruits' size for each direction prove different responses of a single individual to the studied aspect in term of flowering and seed formation and further conversion of flowering buds into fruits (Table 2).

Genetic Variation

RAPD analysis: The genetic relationship among six different populations of mangrove has been carried out using RAPD markers. Eight primers out of ten were reproducible and resulted in reliable amplified bands. The amplified amplicons were varied from 6 to 11 for each primer with a total number of 60 bands for all primers and an average of 7.5 bands per primer. 41 bands were polymorphic with a percentage of 68.33%, and the amplified amplicons were sized between 3695 and 129 bp (Table 3 and Fig. 8). The analysis of the banding

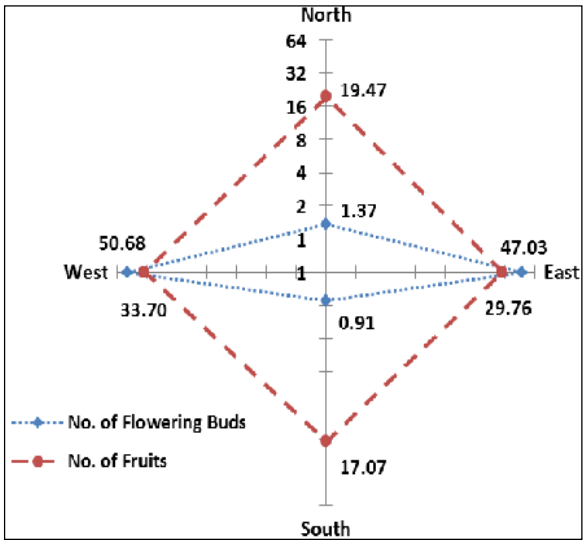

Fig. 7: Overall average of percentage of spatial distribution of flowering buds and fruits m^{-2} (scale log =2)

pattern among the six populations revealed high genetic diversity among mangrove populations. The percentage of genetic diversity was 68.33% of polymorphic bands. In addition, results of banding patterns were used to calculate the Nei (1978) genetic similarity index among mangrove populations Table (4). The genetic identity among the six populations ranged from 0.7915 to 0.9616, and the maximum similarity was recorded among P5 and P6 populations, while the genetic distance ranged from 0.2339 to 0.0391.

Phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic mean (UPGMA), the dendogram construction clustered the three groups of each site together (Fig. 9). Also P3 from site one was diverged from P1 and P2, whereas P4 was diverged from P5 and P6 in site two.

DNA sequencing: In order to sequence 18S ribosomal RNA region, PCR products of 1000 bp were amplified using 18S primer for all samples, then samples were tested and analyzed on agarose gel electrophoresis (Fig. 10). DNA sequencing results were initially used to identify mangrove samples. A search was made using Basic Local Alignment Search Tool (BLAST) on NCBI website to determine the similarity with any published sequences on gene bank. The contig from the overlapped sequences from all samples were found to have 99% matching score with *A. marina* 18S ribosomal RNA sequence (Accession no.: AY289641). The genetic tree was constructed with contig sequence (Fig. 11). Sequences alignment was done using ClustalW, and the maximum composite likelihood model was used to analyze the number of base substitutions per site from averaging over all sequence pairs between groups. Results revealed maximum divergence between P3 and all other populations (Table 5).

Table 3: RAPD banding pattern for Mangrove populations

Total					Mangrove Populations											
					P1		P2		P3		P4		P5		P6	
Primer name	Amplicon size	No. bands	Poly. bands	%	Poly. bands	%	Poly. bands	%	Poly. bands	%	Poly. bands	%	Poly. Bands	%	Poly. bands	%
OPT7	1324-293	7	2	28.57	0	0	1	14.29	0	0	1	14.29	0	0	0	0
OPT8	1551-270	7	7	100	5	71.43	3	42.86	6	85.71	4	57.14	2	28.57	0	0
OPQ1	1685-145	8	6	75	0	0	2	25	2	25	4	50	3	37.5	3	37.5
OPQ20	3695-260	7	4	57.14	1	14.29	2	28.57	4	57.14	2	28.57	2	28.57	2	28.57
OPP1	1837-129	11	11	100	11	100	1	9.09	4	36.36	8	72.73	4	36.36	2	18.18
OPP2	1100-300	6	6	100	0	100	2	33.33	0	100	4	66.67	2	33.33	1	16.67
OPM6	780-303	6	1	16.67	1	16.67	0	0	1	16.67	0	0	0	0	0	0
OPN4	1249-243	8	4	50	2	25	2	25	2	25	3	37	1	12.5	1	12.5
Total	3695-129	60	41	68.33	20	33.33	13	21.67	19	31.67	25	41.67	14	23.33	9	15

Poly. Bands: Polymorphic bands

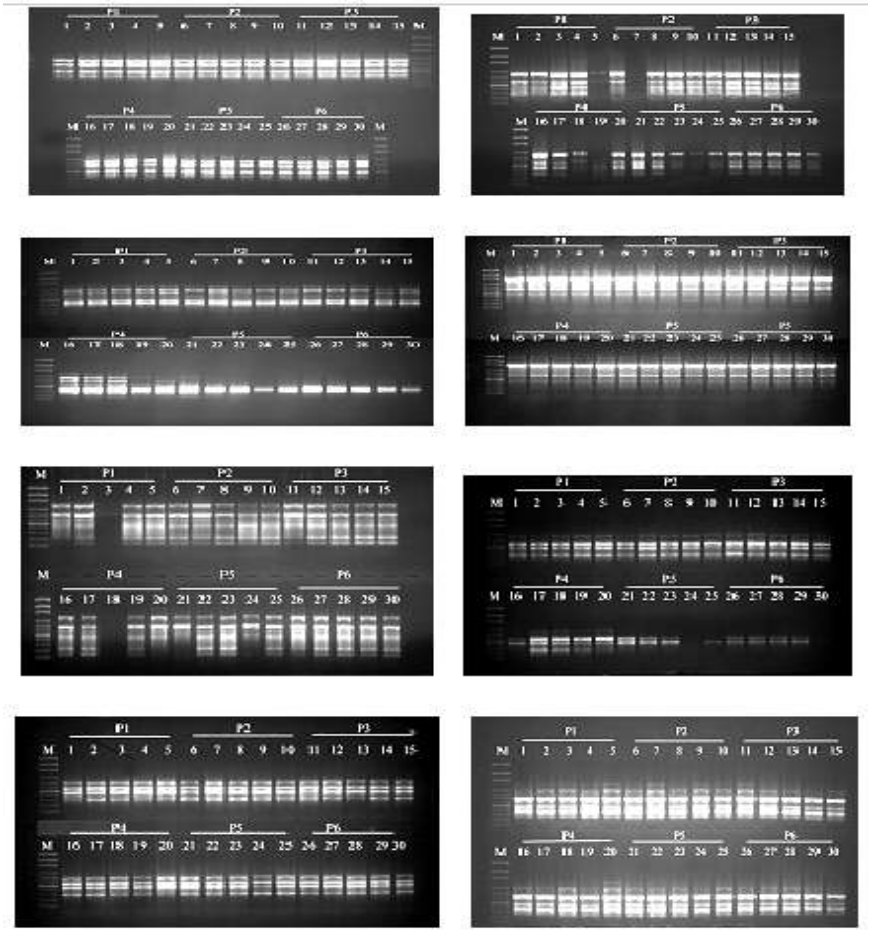

Fig. 8: RAPD-PCR profile for mangrove populations using OPT7, OPT8, OPQ1, OPQ20, OPP1, OPP2, OPM6 and OPN4 primers

Discussion

Generally, the two sites showed a similar pattern in all phenotypic parameters. The observed variation of areal roots heights from zero in the terrestrial area to maximum height in the middle forest in the intertidal zone was mostly due to the need of performing better gas exchange with the surrounding atmosphere under water logging conditions to avoid hypoxia in the oxygen poor mud (Alongi and Mukhopadhyay, 2015).

The absence of the areal roots in the terrestrial individuals can be attributed to the accumulation and

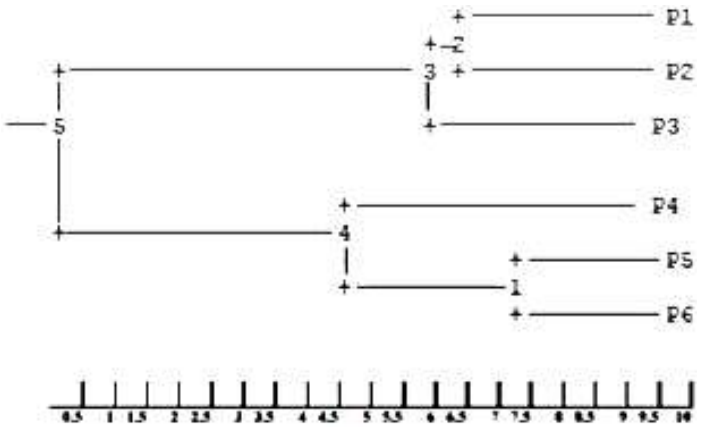

Fig. 9: Dendrogram based Nei's (1978) Genetic distance: Method = UPGMA, Modified from NEIGHBOR procedure of PHYLIP Version 3.5

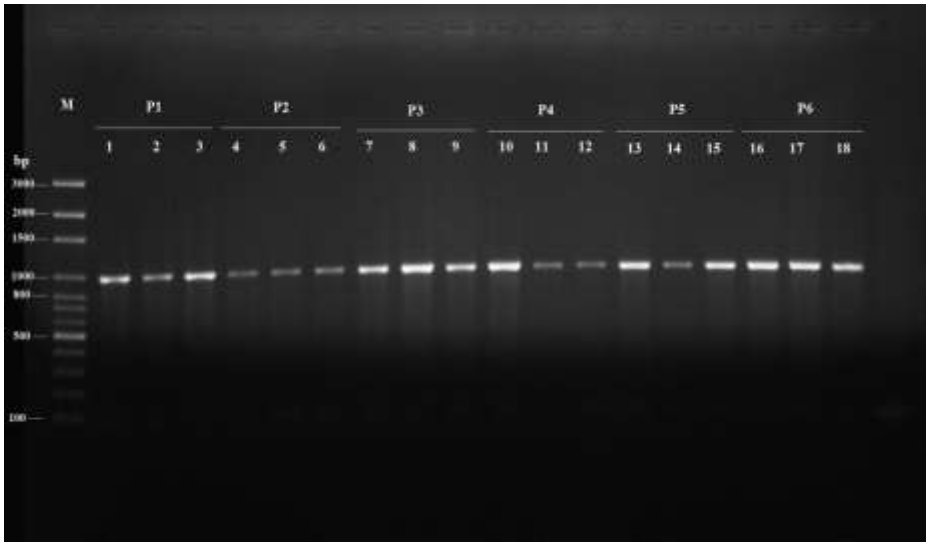

Fig. 10: Agarose gel electrophoresis analysis for the amplified PCR product with 18S primer

translocation of metals from sediment to roots (Souza *et al.*, 2015). Also the use of ground freshwater from the sediment may have resulted in adaptation to the aerial roots in the terrestrial area (Saenger, 2002).

The differences in aerial root length in the intertidal area are mostly due to the variation in the sedimentation levels at some locations according to the site specific hydrodynamics, and sedimentation increase rates, average 0.32 and 0.37 cm/year (Xia *et al.*, 2015). This may have resulted in relative rise in substrate level. Which gave relative shortening in aerial roots lengths. Changes of mean length of areal roots in the intertidal individuals might be caused by the excessive erosion, tide, related hydrodynamics and associated sediment accretion which can result in exposing cable roots (Kathiresan and Bingham, 2001). In fact, similar patterns in change of mangrove height showed gradual increase moving toward sub-tidal part of forests and increase in mean height around lagoons and creeks (Dahdouh-Guebas *et al*., 2004; Simard *et al.*, 2006). However, *A. marina* indicated the ability to adapt its pneumatophore (areal roots) to micro-topographical irregularities (Dahdouh-Guebas *et al.*, 2007). This indicated that mangrove's aerial roots are highly adapted to localized topographic differences and are important in explaining the changes in intertidal hydrology, which is highly linked to changes in topography, which respectively conserve soil carbon content as the roots biomass of mangrove are important source of organic carbon accumulation in mangrove soils (Ranju *et al*., 2017; Thi *et al*., 2018) and have an impact on modifying the chemistry and the distribution carbon, nitrogen and sulfur in the sediment (Efrén *et al*., 2017) due to the presence of *Bruguiera praviflora, B. sexangula, B. gymnorrhiza, R. apiculata* and *R. mucronata,* which are the best carbon sequestring microorganisms (Endang *et al*., 2017).

The observed variation of the total height of the

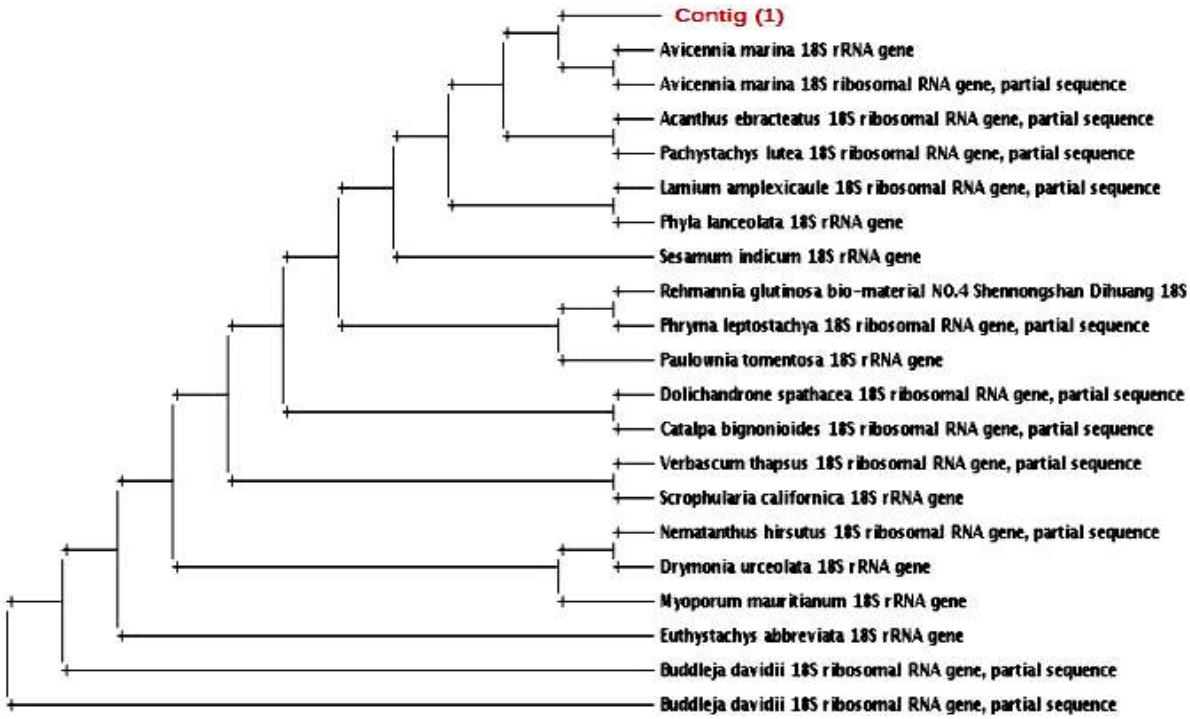

Fig. 11: Genetic tree for Contig sequence for all the tested groups

Table 4: Nei's unbiased genetic similarity (above diagonal) and genetic distance (below diagonal) among 6 mangrove populations

Population ID*	P1	P2	P3	P4	P5	P6
P1	****	0.9456	0.9426	0.9252	0.7915	0.8084
P2	0.0559	****	0.0611	0.9087	0.8204	0.8595
P3	0.0591	0.9408	****	0.9083	0.8054	0.8034
P4	0.0777	0.0958	0.0962	****	0.9325	0.9032
P5	0.2339	0.1980	0.2164	0.0699	****	0.9616

*P1: intertidal mangrove in site1; P2: landward mangrove in site1; P3: end of landward mangrove in site1; P4: intertidal mangrove in site 2; P5: landward mangrove in site 2; P6: end of landward mangrove in site 2

Table 5: The evolutionary divergence over sequence pairs between groups

Population ID*	P1	P2	P3	P4	P5
P2	0.001				
P3	0.021	0.021			
P4	0.001	0.001	0.020		
P5	0.001	0.001	0.021	0.001	
P6	0.001	0.002	0.020	0.001	0.001

*P1: intertidal mangrove in site1; P2: landward mangrove in site1; P3: end of landward mangrove in site1; P4: intertidal mangrove in site 2; P5: landward mangrove in site 2; P6: end of landward mangrove in site 2

populations in the intertidal area than the terrestrial area in both sites was properly because the surrounding individuals caused shades. These shades forced other individuals to exceptionally elongate strait vertically for some meters without common branching to overcome shading.

The observed total productivity of mangrove in both sites showed greater production of buds and flowers in west and east aspects sides. This is mostly due to the exposure to more light and less shading (Wangondu *et al.*, 2010). Also, a higher production can be due to the assimilation process is favorable for a crop of new leaves and organs before herbivory and senescence, or that the plant is most efficient to transfer assimilates directly towards growing organs rather than store them in different tissues (Wright and Schaik, 1994). The increase of the productivity has great protection for coastal areas during cyclone and wind speed (Rahdarian *et al.*, 2017).

As regards genetic differences, first investigation on the genetic composition of mangrove forest in Nabq area required confirmation that the tested mangrove species is *A. marina* according to the phenotypic characterization. DNA sequencing succeeded to identify all samples on the gene bank as *A. marina*, it did not succeed in evaluating the genetic variation among the different populations due to the significant similarity among the DNA sequences in the amplified regions. Therefore, RAPD technique was used to identify the tested samples. RAPD protocol is distinguished as a rapid and less expensive method, and commonly used to identify many plant species (Khan *et al.*, 2010). The most important advantage for RAPD technique in the genetic variation analysis that can differentiate between populations below species level, it can distinguish coding and non-coding regions (Vanijajiva *et al.*, 2005).

In this study, RAPD analysis showed a high level of polymorphism (the variation at the level of individual

genes) among the six populations, and they were differentiated by dendogram into two apparent clusters; one for each site regardless of their type either terrestrial or intertidal. The high level of polymorphism is a very good indicator of the genetic variability which is the main force for mangrove plants preservation (Mahesh and Satish, 2008). That variation could be a result of the variation in environmental factors, pollen flow, and natural selection to help mangrove plants to adapt to different sites conditions (Faisal and Anis, 2002). Some other factors were described in earlier studies as the reason for the low genetic diversity such as low population size and inbreeding (Tyge *et al.*, 2015; Xia *et al.*, 2015). Dendogram also revealed that at site one (Abu Zabad), the terrestrial population (P2) was more similar to the intertidal population P1 than P3. However, P1 and P2 were different in respect to phenotypic characters. This result could be due to the limited distance that mangrove forest occupied landward, the total distance was around 100 m from the shoreline. The P2 samples were collected at 35 m from the shoreline, therefore enormous change in the genetic composition might not have happened as they were still close to the shoreline and the intertidal zone.

As regards second site two (Rowissya), *A. marina* trees extended on land to almost 500 m from the shoreline, and P5 samples were collected about 100m from the shoreline far from the effect of the intertidal zone conditions. This explained the maximum similarity among the two terrestrial populations (P5, P6) and their descent from the intertidal population, P4. Such a genetic variation between intertidal and terrestrial mangroves maybe attributed to an ability of mangrove to survive in underground fresh water and saline waters. This gives them a competitive advantage in saline environment (Ball, 1988). However as halophytes, they complete their life cycles in saline waters up to 4.7% (Por *et al.*, 1977). In addition, the maximum similarity recorded among the terrestrial P5 and P6 populations is probably due to intrinsic factors like mixed inbreeding mating system (Goodwillie *et al.*, 2005), or limited pollen and propagule dispersal (Mori *et al.*, 2015).

In spite of the similarity in the phenotypic characters between the intertidal (P1and P4) and the terrestrial (P2, P3, P5 and P6) populations, the genetic similarity/distance was expressed differently. RAPD pattern was a very successful tool to detect the genetic differences among all six populations. However, further investigation should be done to give more explanation about the reasons for the genetic distance between the two sites.

Conclusion

The first genetic investigation of mangrove in Nabq protectorate revealed significant genetic diversity among the six populations. Intertidal populations genetically descended from the terrestrial population at each site. However, in spite of phenotypic similarity between intertidal stands in both sites, genetic differences were observed. A similar pattern was observed in the terrestrial stands. The phenotypic variation between inland and intertidal individuals in term of aerial roots is a result of adaptation of climatological conditions, shore line extension in responding to dominant wind direction and absence of respiration by areal roots due to the absence of wetland. However, further investigation is required to give more explanation about the reasons for the genetic distance between the two sites.

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