International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Assessment of Genetic Diversity among *Avicennia Marina* Populations on the Arabian Gulf Coast in the Kingdom of Saudi Arabia using PCR - ISSR

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Abstract: ***The cosmopolitan species Avicennia marina (Forsk.) Vierh (Acanthaceae) lives in mangrove forests, which are the most productive and chemically active ecosystems. Mangroves in coastal areas around the world have gotten worse because of human interference, control, and activities in them, such as reclaiming land, growing populations, overgrazing, polluted factory spills, and dumping waste. This has emerged very significantly after the Gulf War, and its damage will remain for a long time until we can grow it again and protect the rest of it. All of the above led to this study, which shows how genetically different A. marina is in different places. The study included testing the genetic accessions of A. marina samples under study collected from different sites along the Arabian Gulf of the Saudi Arabia Kingdom. This was done using the Inter Simple Sequence Repeat (ISSR).11 out of 16 primers were able to bind to cut the DNA of the species and give clear and stable bands, the rest did not give any clear bands so they were excluded. The total number of similar bundles was 1165, and the total number of dissimilar bundles was 1118; hence, the polymorphism rate of the studied species was calculated as 96.0%. This percentage of genetic variability is significant for progress in growth and plant regeneration in the face of unfair practices against it, in addition to adverse environmental conditions in most years. This result concerns knowing the genetic diversity of the plant community is an essential step towards the design of programs for plant breeding as well as preserving it from extinction.***

Keywords: *Avicennia marina*, ISSR, Polymorphism, Arabian Gulf

1. Introduction

Mangroves are primarily found in coastal regions, where tidal regimes have a direct impact. These regions act as transitional zones for the movement of water and goods from the mainland to the sea. Coastal regions capture between 75 and 90 percent of all material movements in water (Klein et al., 2011).

The mangrove ecosystem is heavily influenced by the surrounding environmental factors, such as climate, soil, and water, which act as factors affecting its density and spread. Moreover, Saudi Arabia is characterized by differences in temperature and humidity. Some regions are characterized by a dry climate, in which the temperature rises with the rate of evaporation from the soil surface and therefore the concentration of salts increases in general; it varies between 25 and 45 °C. Where *Avicennia marina* considers the most mangrove species tolerant of high temperatures and salinity in Saudi Arabia (Al Faridan, 2006).

Mangroves are surrounded by soft sediments that attract vibrant societies including bacteria, fungi, large algae, and invertebrates, as well as air roots, trunks, and foliage that host other groups of living organisms where insects, reptiles, amphibians, birds, and mammals thrive in habitats and contribute to their unique character. If its roots are used as farms for many economic fish and crustaceans (Spalding, et al., 2010), and they act as resilience to different pollutants, plastic bags, and waste, then its stems are used as wood for home building, shipbuilding, and boat making due to their strength, durability, and water resistance, and are also used in fuel and heating. As for its leaves and fruits, it is included in the composition of many medicinal drugs (Al - Harith et al., 2017).

The research region is the Arabian Gulf, a shallow peripheral sea of the Indian Ocean, which is one of the most significant sources of crude oil worldwide, not only in the Middle East, with over two - thirds of the world's oil resources concentrated in this Gulf (Khan, 2019). Further, the significance of studying phenotype and genotype as well as their capacity to withstand environmental stress is stressed in numerous earlier studies and research. It is also crucial to use the genetic map of the mangrove to benefit from it industrially, agriculturally, and medicinally for the purposes of developing plants with major crops for humans through scientific methods to produce types of food crops with distinctive characteristics. This is because genes play a role in the development and promotion of the salinity resistance and environmental pollutants.

2. Materials and method

Collect samples

Several field trips were taken to look at the mangrove areas on the Arabian Gulf coast of the Kingdom of Saudi Arabia as shown figure (1). The first location is Dammam, near a new residential area which is a small gathering of mangroves on sewage water. The second location, Sihat Corniche, contains a heavy gathering of mangroves directly opposite the residential buildings, as the area was subjected to the backfill that was observed during the visit, The third location is Tarot Island, which presents a large gathering of mangroves in a residential area in the form of successive groups of trees on the coast, which is polluted with construction waste. The fourth site, Safwa, has large numbers of mangroves and contains a corridor for oil pipelines and a sewage treatment plant, adjacent to agricultural areas. The fifth location is Ras Tanura, The presence of mangroves in Ras Tanura is a series of shrubs

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extending from the entire bridge towards Ras Tanura, with a length of approximately 7 km, and it contains pollution from plastic waste. The sixth location is Jubail, where the presence of mangroves is far from the residential and industrial areas, and they are short shrubs that do not exceed 50 to 70 cm, cultivated in a field with water paths connected to the sea, a clean area without any pollution.

Figure 1: Mangrove distribution in Saudi Arabia (GEOSA & GOOGLE, 2023).

DNA extraction

The young leaves were chosen because they contain a high quantity of cells and a low quantity of polysaccharides.100 mg of plant tissue was ground with liquid nitrogen using a sterilized ceramic pestle and mortar. DNA was isolated from the leaves of plant samples using the kit method. Using the DNeasy Plant Mini Kit manufactured by Qiagen, according to the instructions provided with the kit. The DNA solution is stored at - 20°C until use.

Estimation the DNA purity and concentration

The quantity and purity of genomic DNA were estimated by the NanoDrop spectrophotometer; in beginning, the instrument was set to 0 by taking 1µl of distilled water as a blank. Then, 1 µl of the nucleic acid sample was measured at a wavelength of 260 nm and 280 nm, and OD260/OD280 ratios were recorded to assess the purity of DNA. A ratio of 1.8 to 2.0 for OD260/OD280 indicated good quality DNA.1 O. D. at 260 nm = 50 µg DNA/ml. Therefore, O. D260 × 50 gives the quantity of DNA in µg/ ml.

Testing for DNA intactness

DNA intactness was tested through electrophoresis on 1% agarose gel.4µl of DNA extract was mixed with 2 µl 6x loading dye, then loaded on the 1% agarose gel. Electrophoresis was run at 70V for 60 min in 1X TBE gel buffer, then photographed under UV (Kiti, et al., 2022).

PCR condition

For DNA amplification, 00 decamer ISSR Table (2) primers (Macrogen Korea) were used. PCR reaction was carried out in a volume of 25 µl. PCR was performed as follows: 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, specific annealing temperature (Ta) according to the primer sequence for 30 secs and 72°C for 3 min, and the final extension step at 72 °C for 10 min.

Table 1: ISSR primers Sequences

Primer code	Sequence	
KSU - FBISSR - 1	(AAC) 7G	AACAACAACAACAACAACAACG
KSU - FBISSR - 2	(AAC) 7C	AACAACAACAACAACAACAACC
KSU - FBISSR - 3	(AAC) 7A	AACAACAACAACAACAACAACA
KSU - FBISSR - 4	(AAC) 7T	AACAACAACAACAACAACAACT
KSU - FBISSR - 6	(GTT) 7C	GTTGTTGTTGTTGTTGTTGTTC
KSU - FBISSR - 7	(GTT) 7A	GTTGTTGTTGTTGTTGTTGTTA
KSU - FBISSR - 8	(GTT) 7T	GTTGTTGTTGTTGTTGTTGTTT
KSU - FBISSR - 9	(CACA) 5C	CACACACACACACACACACAC
KSU - FBISSR - 10	(CACA) 5G	CACACACACACACACACACAG
KSU - FBISSR - 11	(CACA) 5A	CACACACACACACACACACAA
KSU - FBISSR - 12	(CACA) 5T	CACACACACACACACACACAT
KSU - FBISSR - 13	(TGTG) 5G	TGTGTGTGTGTGTGTGTGTGG
KSU - FBISSR - 14	(TGTG) 5C	TGTGTGTGTGTGTGTGTGTGC
KSU - FBISSR - 15	(TGTG) 5A	TGTGTGTGTGTGTGTGTGTGA
KSU - FBISSR - 16	(TGTG) 5T	TGTGTGTGTGTGTGTGTGTGT

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Data recoding

Amplification products of ISSR analyses were subjected to electrophoresis in 1.5% agarose gel. The gels were stained with acridine orange and documented using a gel documentation system (Biorad Gel Doc EZ Imager). Data is stored in binary format, where 1 means presence and 0 means absence.1KB ladder (marker) was used to identify the size of the bands of each genotype. Data generated from ISSR`s markers were analyzed using Jaccard's similarity coefficient. These similarity coefficients were used to construct a dendrogram using the unweighted pair group method with arithmetic average (UPGMA) employing the MVSP Package (version 3.22). To generate the phylogenetic trees based on the unweighted pair - group method with an arithmetic mean algorithm (UPGMA), the group analysis method was used according to Nie and Li's coefficient to determine the possible relationships between the studied species.

3. Results

The study included testing the genetic accessions of *A. marina* samples using the ISSR method to detect the level of polymorphism.11 primers out of 16 primers were able to bind to cut the DNA of the species and give clear and stable bands (multiples with good polymorphism), which are shown in Figure (1) and Table (2). As for the rest of the prefixes, their results were not clear, so they were excluded. The total number of similar bundles was 1165, and the total number of dissimilar bundles was 1118; hence, the polymorphism rate of the studied species was calculated as 96.0%.

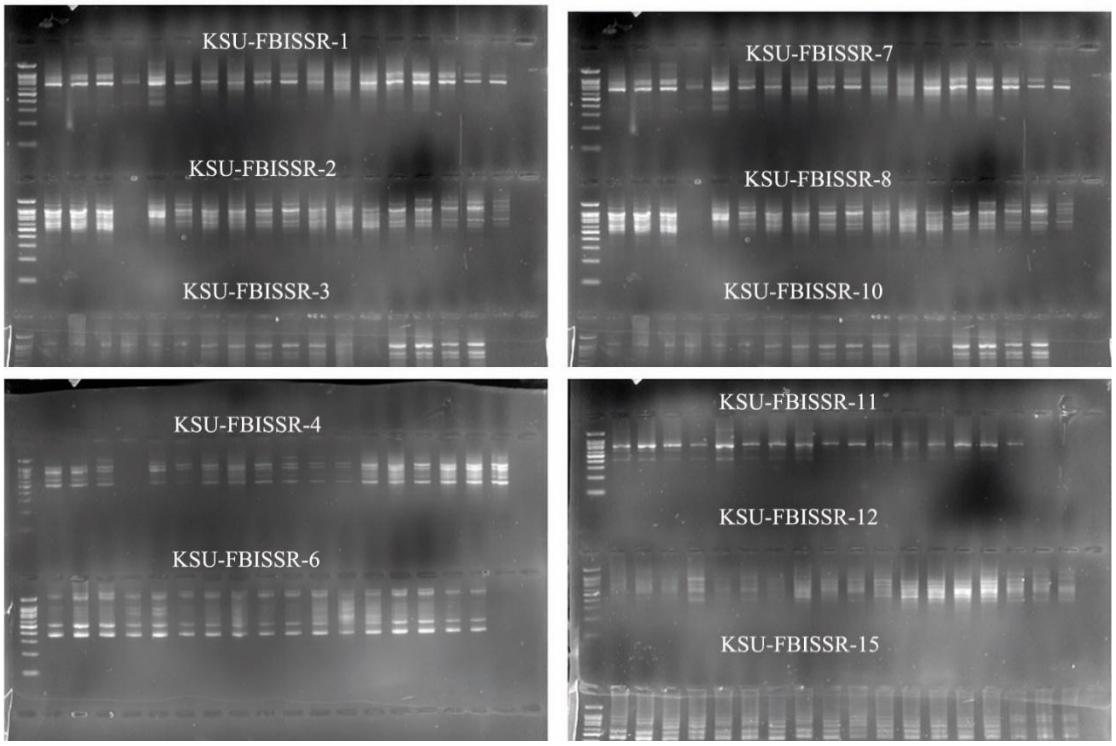

Figure 2: Shows the ISSR patterns resulting from the primers used with the genotypes of *A. marina* samples

Table 2: Total bands generated from all primers, and divergent bands, and their percentage for study samples based on the results of the ISSR

Primers	Total No. of Bands	Polymorphicbands	Monomorphicbands	Polymorphism%
KSU - FBISSR - 1	116	113	3	97.4
KSU - FBISSR - 2	125	123	2	98.4
KSU - FBISSR - 3	72	67	5	93.1
KSU - FBISSR - 4	107	103	4	96.3
KSU - FBISSR - 6	121	118	3	97.5
KSU - FBISSR - 7	56	49	7	87.5
KSU - FBISSR - 8	113	109	4	96.5
KSU - FBISSR - 10	133	128	5	96.2
KSU - FBISSR - 11	98	94	4	95.9
KSU - FBISSR - 12	119	114	5	95.8
KSU - FBISSR - 15	105	100	6	95.2
Total	1165	1118	48	96.0

The results were put through cluster analysis so that a dendrogram could be made of the genetic entries. Figure (3) shows the number of series, clusters, and groups that carry the studied genetic entries, and their arrangement in the kinship tree, as well as the percentage of similarity between them. The dendrogram obtained during this study using the ISSR technique showed the genetic relationship tree. The cluster analysis was divided into two main series. The first

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series (SI) included only one sample, S1, of the plant collected from Sihat, and the second series (SII) included the remaining samples in the study .

The second series (SII) was divided into two clusters. The first sub - cluster included the two samples, S2 and D1 (Sihat and Dammam, respectively), and the second sub - cluster included the rest of the samples from other sites in the branched series.

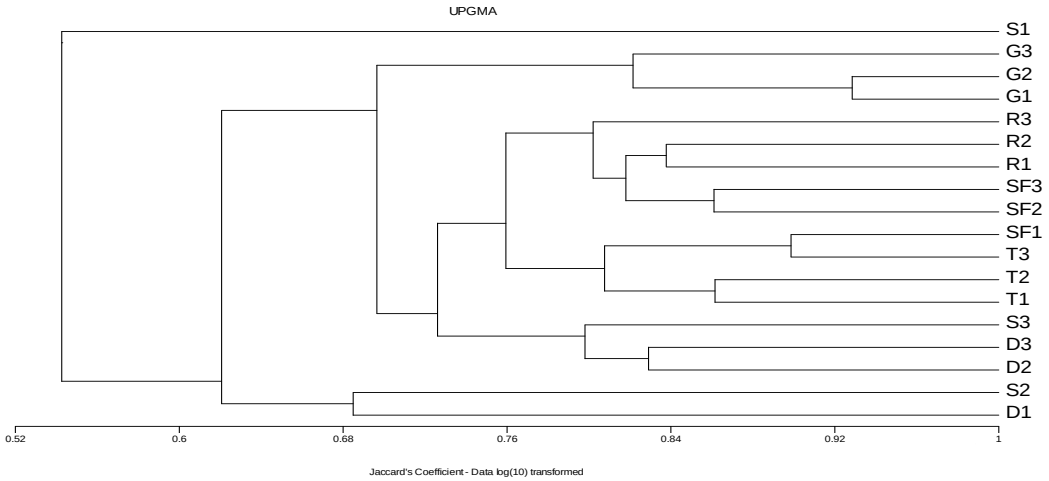

Figure 3: Shows the Dendrogram of *A. marina* samples based on the ISSR results

It is clear from the matrix of the similarity percentage of the ISSR results, table (3), that the genetic distance between the genetic accessions ranged between (0.38 - 0.9), as the two genotypes T3 and SF1 had the highest genetic affinity ratio with a similarity rate of (0.9), which gave a great similarity in the genotype, while the genetic accessions of D1 and S1 (Sihat, Dammam) recorded the lowest similarity ratio of (0.38) and between these two values was the affinity ranges between the remaining genetic accessions.

Table 3: Showing the matrix of similarity percentages based on the ISSR results

Similarity matrix	D1	D2	D3	S1	S2	S3	T1	T2	T3	SF1	SF2	SF3	R1	R2	R3	G1	G2	G3
D1	1.00																	
D2	0.75	1.00																
D3	0.72	0.83	1.00															
S1	0.38	0.52	0.53	1.00														
S2	0.69	0.71	0.65	0.49	1.00													
S3	0.66	0.77	0.83	0.58	0.75	1.00												
T1	0.62	0.69	0.77	0.57	0.62	0.78	1.00											
T2	0.59	0.68	0.73	0.61	0.64	0.82	0.86	1.00										
T3	0.58	0.65	0.68	0.63	0.65	0.73	0.85	0.81	1.00									
SF1	0.63	0.74	0.77	0.57	0.68	0.78	0.82	0.76	0.90	1.00								
SF2	0.58	0.74	0.71	0.66	0.70	0.81	0.77	0.81	0.83	0.80	1.00							
SF3	0.54	0.73	0.72	0.58	0.69	0.79	0.76	0.77	0.76	0.78	0.86	1.00						
R1	0.50	0.68	0.69	0.58	0.64	0.77	0.73	0.74	0.76	0.76	0.81	0.84	1.00					
R2	0.54	0.64	0.65	0.55	0.60	0.70	0.71	0.70	0.78	0.76	0.81	0.82	0.84	1.00				
R3	0.55	0.70	0.73	0.56	0.64	0.74	0.73	0.74	0.75	0.77	0.80	0.78	0.83	0.80	1.00			
G1	0.52	0.59	0.66	0.48	0.61	0.69	0.69	0.68	0.70	0.72	0.68	0.67	0.69	0.69	0.77	1.00		
G2	0.56	0.62	0.69	0.47	0.60	0.70	0.73	0.72	0.71	0.73	0.69	0.68	0.70	0.68	0.80	0.93	1.00	
G3	0.53	0.63	0.68	0.47	0.58	0.75	0.71	0.75	0.69	0.74	0.72	0.69	0.71	0.62	0.68	0.81	0.83	1.00
	D1	D2	D3	S1	S2	S3	T1	T2	T3	SF1	SF2	SF3	R1	R2	R3	G1	G2	G3

4. Discussion

ISSR technology was used to look at the genetic differences between *A. marina* samples from different places and environments on the Arabian Gulf coast. This showed that ISSR is a powerful tool for studying genetic diversity among the study samples and has a lot of potential for other uses in *A. marina* breeding and cultivation, such as figuring out how to make the fish grow faster. This includes characterizing the collection of plant genetic resources and its communities, as well as studies of genetic mapping and selection using genetic markers.

The results of the DNA fingerprint obtained using the ISSR technique were able to distinguish between the genotypes of the samples well, as the percentage of variation between the genetic inputs was 96%. These results agree with the study (Hnia et al., 2013) that found that genetic differentiation among *K. obovata* populations was relatively high. The results of the genetic diversity and cluster analysis suggest that geographical isolation of populations of plant species mainly results in low gene flow and random genetic drift, leading to genetic differentiation (Shao - Bo et al., 2010).

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The high percentage of genetic variation may be due to the difference in genotypes, whose difference may reach the level of species (Triest, et al., 2021) With the findings of (Kumar et al.2011), these indications are important for the plant to be able to adapt to these changes, and as it is known, DNA is the stable genetic material that is not affected by the environment, so it is used as a genetic indicator because it is characterized by stability. Because DNA, unlike other genetic indicators, is stable genetic material that is unaffected by the environment, it is utilized as a genetic indicator. Environmental factors have a significant impact on appearance characteristics These indicators are unique in that they can follow genetic changes across generations and can detect vast numbers of polymorphic variations, which enabled them to detect any difference, no matter how minute, between the closest individuals. (Naqvi, 2007).

5. Conclusion

The results of this study has successfully demonstrated a large set of polymorphic ISSR markers that can be used to analyze the genetic diversity of *A. marina* along the Arabian Gulf coast of Saudi Arabia. The analysis of genetic diversity showed a high percentage of genetic diversity (96.0%), so we can rely on the ISSR technique as a valuable tool for future population genetics studies and conservation efforts, not only for this species. In general, this study highlights the importance of genetic diversity analysis in understanding the adaptation potential and conservation of species Halo plant in changing environments.

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