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Molecular Phylogeny Using ISSR Analysis in Naturally Growing *Suaeda* Populations in Saudi Arabia

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Abstract: The aim of the present study was to assess the genetic diversity of different *Suaeda* populations collected from Saudi Arabia using Inter Simple Sequence Repeat (ISSR). Seventeen ISSR primers yielded a reproducible banding pattern, the number of polymorphic bands per primer varied between 5 and 14 with a mean of 8 major bands per primer. UPGMA cluster analysis revealed three distinct clusters; The first cluster comprised *Suaeda maritime* populations, the second cluster comprised *Suaeda vermiculata* populations and the third cluster comprised *Suaeda aegyptiaca* populations. The present findings showed clear differences between plant species but no significant differences were observed among geographical distribution.

Key words: Saudi Arabia, ISSR, UPGMA, cluster, species, *Suaeda maritime*

INTRODUCTION

The genus *Suaeda* (Chenopodiaceae family) contains more than 100 species of annual, perennial and some time shrubs; Their leaves are thick, succulent with alternate forms (Abd Elbar and Abd El-Maboud, 2013). In Arabic, Aswad means black and after this Arabic word *Suaeda* was named (Forskal, 1776). The genus *Suaeda* is frequently known as seepweeds or seablites. *Suaeda* is very common in areas of saline/alkaline wetlands or occasionally in upland habitats. Several species of *Suaeda* are used as a vegetable; Native Americans eat *Suaeda* raw seeds. Furthermore, red or black dye is extracted from *Suaeda* seeds.

In Northwestern Europe and the East coast of North America, *Suaeda maritima* is very abundant and the common name is seablite. Saltmarsh areas are the habitat for this species. On the other hand, *Suaeda maritima* is found in land-side edge of mangrove tidal swamps of tropical Asia. In Australia, *Suaeda australis* is very common in tidal zones. *Suaeda vera* (synon. *S. fruticosa*) on the other hand is present in the coasts of the Mediterranean Sea where the common name of this plant is shrubby sea-blite. *S. vera* is a tall plant with a bush growth form. It used to be burned and the ashes used as a source of sodium carbonate in glass industry.

In Saudi Arabia, halophytes are a good source of fodder during adverse climatic conditions. This group of plants has high nutrients content and the water percentage in some of them are very high therefore they are a good source of water for many animals in the region. The most common *halophytic* species in the Arabian Gulf coast of KSA are: *Suaeda monoica*, *Suaeda vermiculata*, *Suaeda maritima*, *Salicornia europaea*, *Halocnemum strobilaceum*, *Arthrocnemum macrostachyum*.

Suaeda monoica is very common South of Jeddah, sometimes it reaches about 4 m height. In areas around Jizan plain, *Suaeda monoica* forms large thickets in association with *Panicum turgidum*, *Dipterygium glaucum*, *Haloxylon salicornicum*, *Tamarix nilotica*. In some areas of the Tihama plain (southwestern region) xerophytic species including *Acacia tortilis* and *Leptadenia pyrotechnica* are seen along with *Suaeda monoica*.

The genus *Suaeda* have widely distributed polymorphic species including *S. maritima, S. calceoliformis* and *S. nigra*. Abiotic factors such as drought conditions and temperature stress in addition to genetic differences resulted in a vast variation in this group of plants. A great variation in growth-form, morphology and structure are seen between and among species. Nevertheless, no available qualitative characters are available to distinguish between related taxa. Therefore, molecular analysis could be used to distinguish distinct between intraspecific taxa, sub-species or even between different populations of the same species (Anderson and Lubberstedt, 2003).

Genetic classification can be achieved by using molecular techniques such as ISSR and AFLP (Donoghue and Sanderson, 1992). It is important to have a quick and reliable technique to confirm the identity of

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species. Proper classification would also allow the efficient exploitation of newly discovered species with valuable biotechnological products.

Molecular markers are considered as an outstanding means for studying genetic diversity and it go's a head of the diversity based on agronomic features or geographic distribution (Shah *et al*., 2009). Several molecular markers like Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs) and Inter Simple Sequence Repeats (ISSR) have been used for studying the genetic diversity and population genetics in many plant species (Lubberstedt *et al*., 2005; Asp *et al*., 2007). Inter Simple Sequence Repeats (ISSR) were developed to detect polymorphisms in microsatellites and inter-microsatellites loci without prior knowledge of the DNA sequence. ISSR involves PCR amplification of template DNA using only one primer having a microsatellite sequence (Archak *et al*., 2003). The markers thus generated can resolve the genome of closely related genotypes. ISSR markers are also suitable for the identification and DNA fingerprinting (Godwin *et al*., 1997).

The objective of this study was to assess the phylogenetic relationships and genetic diversity/similarity of different *Suaeda* species collected from several locations in Saudi Arabia using ISSR marker.

MATERIALS AND METHODS

Plant material: Fresh young fleshy shoots of different *Suaeda* species were collected from different locations in Saudi Arabia (Table 1) during the period of Spring 2016/2017.

Total genomic DNA extraction: Total genomic DNA was extracted from each individual plant separately. Shoots were first ground into a fine powder using liquid nitrogen in a pestle and mortar and then extracted using Dellaporta *et al*. (1983) protocol with some modifications. Quantity and quality of the DNA were checked using a fluorometer (Hoefer DyNA Quant 200; Pharmacia Biotech,Piscatawaym, N J). The stock DNA samples were diluted with sterile TE buffer to make a working solution of 10 ĺ g/mL to be used in PCR analysis.

ISSR analysis: A total of 24 ISSR primers (Biotechnology Laboratory of British Columbia University. Vancouver, British Columbia, Canada) (Table 2) were used for PCR amplification. The primers were dissolved in sterilized distilled water at a concentration of 10 pmol/µL. Amplification reactions were performed in volumes of 25 µL using "Ready-To-Go PCR Beads" kit (GE Healthcare Life Sciences). Each reaction contains thermostable polymerases (2.5 Units of recombinant puReTaq DNA polymerase), dNTPs (200 µM each dNTP in 25 µL reaction volume) and buffer [1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris, (pH 9)], 20 pmol/µL ISSR primer and 25 ĺ g of template DNA. PCR amplification was performed in Eppendorf Master Cycler Gradient PCR machine. The PCR program used contain, one cycle of denaturation for 5 min at 95°C; 35 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min and one final cycle for 10 min at 72°C.

Table 1: *Suaeda* spp. collected from different locations in Saudi Arabia

Code #	Species	Location
C_1	*Suaeda maritime*	Ras-Tanwarh
C_2	*Suaeda maritime*	Jazeerah Abu-Ali
C_3	*Suaeda maritime*	Jazeerah Abu-Ali
C_4	*Suaeda maritime*	Al-Zor
C_5	*Suaeda maritime*	Darain
C_6	*Suaeda maritime*	Darain
C_7	*Suaeda maritime*	Al-Jabeel
C_8	*Suaeda vermiculata*	Jazeerah Abu-Ali
C_9	*Suaeda vermiculata*	Jazeerah Abu-Ali
C_{10}	*Suaeda vermiculata*	Ras-Tanwarh
C_{11}	*Suaeda vermiculata*	Al-Zor
C_{12}	*Suaeda vermiculata*	Al-Zor
C_{13}	*Suaeda vermiculata*	Darain
C_{14}	*Suaeda aegyptiaca*	Al-Jabeel
C_{15}	*Suaeda aegyptiaca*	Ras-Tanwarh
C_{16}	*Suaeda aegyptiaca*	Al-Zor
C_{17}	*Suaeda aegyptiaca*	Al-Halfmoon

Table 2: List of ISSR primers used in this study

Primer name	Primer sequence (5'→3')
UBC 801	ATATATATATATATATT
UBC 802	ATATATATATATATATG
UBC 803	ATATATATATATATATC
UBC 804	TATATATATATATATAA
UBC 805	TATATATATATATATAC
UBC 806	TATATATATATATATAG
UBC 807	AGAGAGAGAGAGAGAGT
UBC 808	AGAGAGAGAGAGAGAGC
UBC 809	AGAGAGAGAGAGAGAGG
UBC 810	GAGAGAGAGAGAGAGAT
UBC 811	GAGAGAGAGAGAGAGAC
UBC 812	GAGAGAGAGAGAGAGAA
UBC 813	CTCTCTCTCTCTCTCTT
UBC 814	CTCTCTCTCTCTCTCTA
UBC 815	CTCTCTCTCTCTCTCTG
UBC 816	CACACACACACACACAT
UBC 817	CACACACACACACACAA
UBC 818	CACACACACACACACAG
UBC 819	GTGTGTGTGTGTGTGTA
UBC 820	GTGTGTGTGTGTGTGTC
UBC 821	GTGTGTGTGTGTGTGTT
UBC 822	TCTCTCTCTCTCTCTCA
UBC 823	TCTCTCTCTCTCTCTCC
UBC 824	TCTCTCTCTCTCTCTCG

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Electrophoresis: The PCR products were separated by gel electrophoresis according to their molecular weight on 1.4% (w/w) agarose gels and stained with ethidium bromide (10 mg/mL). Bands were visualized using UV transilluminator and documented by using the Gel Documentation System of Alpha Innotech. The length of the amplified ISSR fragments was estimated by running 100 pb DNA Ladder (Bio Rad) in the gel as standard size marker.

Data analysis: Because we used dominant markers (ISSR), amplified bands were scored for presence (1) or absence (0) of bands. For statistical analysis, data was analyzed using NTSYS-pc Software (Rohlf, 2000). Cluster analysis demonstrating genetic relationships of accessions were generated using Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) and simple matching coefficient.

RESULTS AND DISCUSSION

The aim of the present study was to assess the genetic diversity of different *Suaeda* species collected from several locations in Saudi Arabia using ISSR marker. DNA extraction protocol used Dellaporta *et al*., 1983 resulted a good quality and high quantity DNA samples. The average yields from 300-5,000 mg of the fleshy stems were 10-30 mg/mL DNA.

Clear amplified polymorphic DNA products were obtained from the screening using 24 ISSR primers on 19 different populations. Results showed that 17 primers gave reproducible products. Primers used here revealed the presence of high polymorphisms in DNA fragments ranged from 100-2000 bp. Representative banding pattern using primers UBC 807, 810 and 811 are shown in Fig. 1.

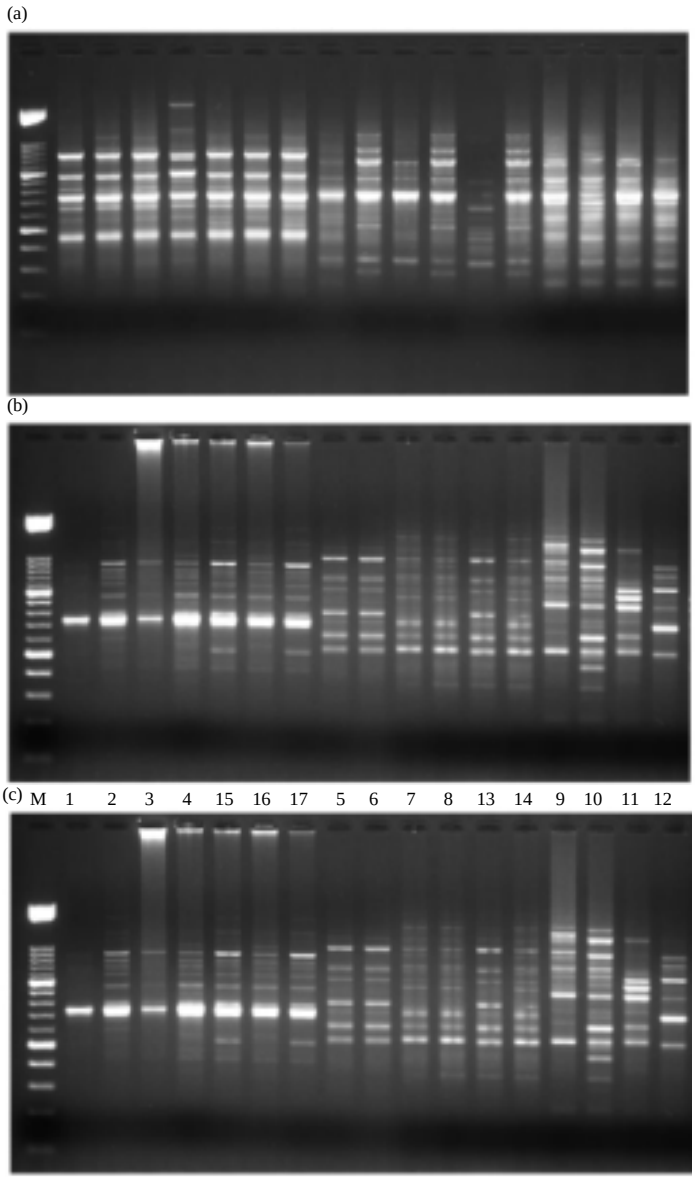

Fig. 1: a-c) ISSR profiles of 17 *Suaeda* plants using UBC 807, UBC 810, UBC 811 primers. Lane: M: Molecular marker (100 bp Ladder) Lanes 1 to 17 are *Suaeda* spp.

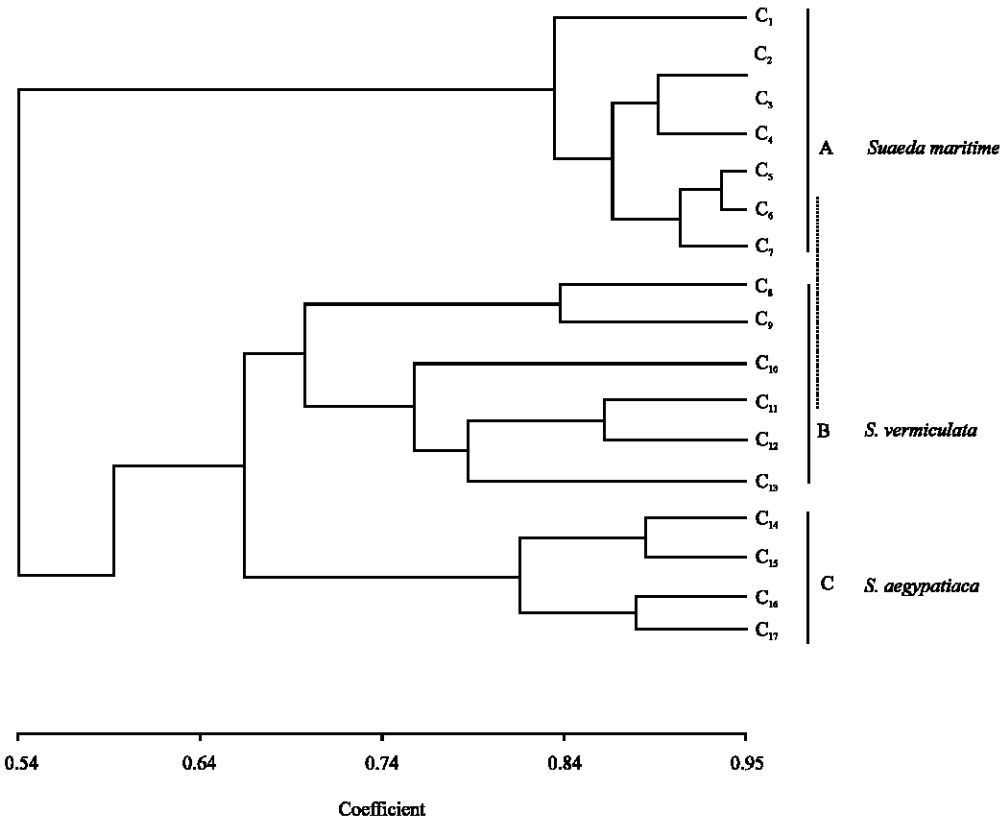

Fig. 2: A dendrogram of phylogenetic relationships among 17 genotypes of *Suaeda* ssp., based on Nei and Li's similarity coefficient

Table 3: Similarity matrix for Nei and Li's coefficients of 17 genotypes of *Suaeda* spp. obtained from ISSR Primers

Genotypes	ISSR Primers																
1	1.00																
2	0.85	1.00															
3	0.88	0.93	1.00														
4	0.83	0.87	0.89	1.00													
5	0.79	0.86	0.85	0.85	1.00												
6	0.81	0.85	0.88	0.84	0.92	1.00											
7	0.79	0.86	0.87	0.85	0.88	0.91	1.00										
8	0.60	0.60	0.58	0.60	0.62	0.63	0.63	1.00									
9	0.55	0.54	0.50	0.52	0.56	0.56	0.54	0.83	1.00								
10	0.54	0.47	0.47	0.46	0.47	0.52	0.51	0.69	0.76	1.00							
11	0.57	0.53	0.53	0.51	0.53	0.55	0.55	0.63	0.75	0.81	1.00						
12	0.55	0.50	0.48	0.49	0.52	0.53	0.53	0.65	0.71	0.73	0.85	1.00					
13	0.55	0.57	0.56	0.56	0.55	0.56	0.59	0.64	0.68	0.70	0.79	0.76	1.00				
14	0.50	0.52	0.50	0.52	0.52	0.50	0.52	0.60	0.64	0.60	0.66	0.68	0.79	1.00			
15	0.50	0.52	0.49	0.54	0.55	0.52	0.52	0.63	0.63	0.57	0.66	0.67	0.76	0.88	1.00		
16	0.54	0.52	0.52	0.55	0.54	0.53	0.52	0.63	0.65	0.62	0.68	0.65	0.73	0.79	0.83	1.00	
17	0.59	0.58	0.57	0.60	0.60	0.57	0.56	0.65	0.64	0.56	0.62	0.66	0.72	0.79	0.83	0.87	1.00

The pair-wise genetic distance estimates of the 17 populations in this study were analyzed and are given in Table 3. The similarity matrix is based on Nei and Li's similarity coefficient (Nei, 1978; Nei and Li, 1979). The genetic distance (Nei and Li's similarity) ranged from 0.930-0.450. Maximum similarity was observed between 2 populations of Suaeda maritime Code # C_2 and C_3 (0.93). Both the populations (C_2 and C_3) were collected from Jazeera Abu-Ali.

Cluster analysis by the Unweighted Paired Group Method of Arithmetic mean (UPGMA) showed 4 clusters (Fig. 2). Cluster A consisted of 7 populations (Code # C_1-C_7) of *Suaeda maritime* with a 0.93-0.79 Nei

and Li's similarity range. Cluster B consisted of 6 populations (Code # C_8-C_{13}) belonging to *S. vermiculata* with a range of 0.85-0.63 Nei and Li's coefficients in the similarity matrix. Cluster C consisted of four populations (Code # C_{14}-C_{17}) of *S. aegyptiaca* with 0.88-0.79 Nei and Li's similarity range.

Suaeda maritime plants collected from Jazeerah Abu-Ali (C_2 and C_3) were the 2 most closely related populations among the 17 populations studied with the highest value in the similarity matrix (0.93). Similarly, *Suaeda maritime* plants collected from Darain (C_5 and C_6) also belonging to the same cluster (A) and showed the second highest similarity among the populations with a value of 0.92 in the similarity matrix. Among the 6 *S. vermiculata* populations, plants collected from Al-Zor (C_{11} and C_{12}) showed highest similarity (0.85) whereas among *S. aegyptiaca* populations, plants collected from Al-Jabeel and Ras-Tanwarh (C_{14} and C_{15}) showed the highest value (0.88). In the present study the average similarity among the 19 individuals was more than 60%. Previous studies reported genetic diversity of *Suaeda* using RAPD analysis. The genetic diversity in *Suaeda nudiflora* was studied using RAPD of 5 populations in India. Results showed that RAPD analysis revealed high polymorphic number of bands per population per primer. Further more, dendogram analysis grouped the studied populations into 2 major groups based on ecotypic adaptability (Jena and Das, 2006). In a different study, the morphological and molecular characteristics of *Suaeda australis* grown in Korea were studied. Molecular analysis using ITS and psbB-psbH spacer were used and indicated that the *S. australis* plants collected from Korea should instead be reclassified to *S. maritima* (L.) Dumont (Lee *et al*., 2007).

CONCLUSION

ISSR analysis could be used an efficient identification and phylogenetic assessment of the *Suaeda* populations growing in Saudi Arabia. The 3 species of *Suaeda* used in this project clustered separately and there was no mixing among the populations of different species. The findings of this study clearly showed that ISSR analysis is a reliable tool that can be used in a genetic diversity studies and in identification of plant species as previously been noted in other plant species (Godwin *et al*., 1997; Casasoli *et al*., 2001; Archak *et al*., 2003; Geleta *et al*., 2012).

RECOMMENDATIONS

The present results provide evidence of a genetic similarity and/or divergence between the studied populations. This indicates that there is no mixed population and the genomic purity is maintained in the population. This will greatly help in the collection and cataloguing of the germplasm in the form of a germplasm bank.

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