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MOLECULAR CHARACTERIZATION OF JORDANIAN SALTBUSH (*Atriplex* spp.) ACCESSIONS USING INTER SIMPLE SEQUENCE REPEATS MARKERS

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Abstract. The Mediterranean saltbush (*Atriplex* spp.) is a key halophytic shrub adapted to arid and saline environments but is increasingly threatened by overgrazing and climate change. This study assessed genetic diversity and population structure of native Jordanian saltbush genotypes to support conservation and germplasm selection efforts. Genomic DNA was isolated from two species, *Atriplex halimus* L. and *A. leucoclada* (Boiss.), sampled across multiple locations in Jordan. Four reproducible inter-simple sequence repeat (ISSR) primers generated a total of 48 polymorphic bands, revealing high levels of marker polymorphism. Binary ISSR data (2,304 scored entries) were used to estimate genetic diversity indices, pairwise genetic similarities and to construct a cluster dendrogram. Shannon's information index and Nei's gene diversity indicated moderate to high genetic diversity within both species, while cluster analysis revealed clear genetic differentiation among genotypes without strict geographic structuring. Several highly divergent genotypes were identified and have been conserved under greenhouse and field conditions as valuable genetic resources for future breeding, restoration and conservation programs.

Keywords: *Conservation, DNA markers, genetic diversity, ISSR, Saltbush.*

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1. Introduction

Atriplex species (saltbushes) are naturally found in many arid and semi-arid regions of the world, particularly in harsh habitats that are characterized by high soil salinity with aridity (Osmond *et al*., 1980; McArthur & Sanderson, 1984). Many saltbush species grow in areas with low annual rainfall. Saltbushes are used generally as feed for livestock and wildlife and for rehabilitation of degraded lands (Ortíz-Dorda *et*

al., 2005). More than 400 species of *Atriplex* have been identified worldwide. In Mediterranean Basin, 40-50 *Atriplex* species have been found particularly in North Africa and Levant region, where it has been used as feed.

Water deficit is a major stress caused by limited water availability in soil; however, it can be induced by salt stress from irrigation water or saline soils. In order to adapt to salt stress, plants have developed mechanisms to sense and transduce the stress signals that activates pathways controlling ionic/osmotic homeostasis and detoxification (Rodríguez-Milla & Salinas, 2009). During high salt stress condition, compatible solutes may act as cytoplasmic osmolytes facilitating water uptake and its retention; in addition, they serve as protectors and stabilizers of macromolecules and cellular structures (Bohnert & Jensen, 1996).

The Mediterranean saltbush (*Atriplex halimus* L.) is a xero-halophyte that can grow in saline soils and prevailing dry weather utilizing unique adaptation mechanisms and physiological properties (Sadder *et al*., 2013). It belongs to the subfamily Chenopodiaceae and family Amaranthaceae (Stevens 2001 onwards) and it can be found naturally in saline basins in Arabian Peninsula (Al-Turki *et al*., 2000). It is considered as an important forage shrub, which has a medium, with white wooden stems, pale yellowish leathery leaves and spread deep root system (Zoghet & Tag El Din, 1992).

In absence of grazing, *A. halimus* L. can grow up to 4 m in height under saline and dry environmental conditions (Negre, 1961). It is one of the most palatable *Atriplex* species for cattle in the dry lands (Tiedeman & Chouki, 1989). Association of saltbush shrubs with barley showed increased crop yield by 25% (Brandle, 1987). In general, saltbush can produce high biomass yield and high crude protein content (16%) (Hyder, 1981). Moreover, *Atriplex* showed positive effects on the growth rate of wool and body weight in sheep (Abou El Nasr *et al*., 1996).

A number of studies were carried out to investigate genetic diversity between and within *A. halimus* L. populations (Ortíz-Dorda *et al*., 2005; Walker *et al*., 2005). Both morphological characteristics and molecular markers have been applied to study genetic diversity in *Atriplex* spp. Utilized morphological characteristics included fruit dimensions and weight (Hcini *et al*., 2007) and leaf characteristics (Abbad *et al*., 2004). Although morphological characteristics are powerful tools for taxonomy purposes between species of the genera *Atriplex* (Taschereau, 1998; Al-Turki *et al*., 2000), they cannot be used to resolve diversity between individuals within the same species. Furthermore, morphological characteristics were recorded to be highly affected by the environmental conditions, e.g. effect of salinity stress on seed polymorphism in *A. triangularis* L. (Khan & Ungar, 1984) and effect of nutrient levels on fruit polymorphism in *A. sagittata* L. (Mandák & Pyšek, 1999).

On the other hand, molecular markers are more stable than morphological characters. In addition, molecular markers are important to study inheritance and linkage to important target traits. Several molecular markers have been utilized to assess genetic polymorphism in *Atriplex* spp., e.g. isoenzymes in *A. halimus* L. (Haddioui & Baaziz, 2001; Abbad *et al*., 2004), random amplification of polymorphic DNA in *A. halimus* L. (Ortíz-Dorda *et al*., 2005) and in *Atriplex* spp (Bouda *et al*., 2006), internal transcribed spacer in *A. halimus* L. (Ortíz-Dorda *et al*., 2005; Hcini *et al*., 2010) and inter simple sequence repeats (ISSR) in *A. prostrata* L. (Carter, 2003).

However, there were no available reports accessing genetic diversity of native Jordanian genotypes of *A. halimus* L. Earliest herbarium collections of *A. halimus* L.,

covered samples from several areas from Algeria (1851) to Palestine (1873), did not include any sample from the Arabian Peninsula (Röpert, 2000). Moreover, saltbush is threatened in its natural habitat in Jordan due to unrestricted grazing and successive drought seasons. Therefore, the present study was designed to test the hypotheses that Jordanian populations of *A. halimus* and those of *A. leucoclada* harbor moderate but structured genetic diversity shaped by geographic separation and local environmental pressures. ISSR markers were employed primarily to assess intra-specific genetic diversity and population structure of *A. halimus*, to confirm interspecific differentiation and to identify genetically divergent populations relevant for conservation planning.

2. Materials and methods

2.1. Plant materials

A total of 20 collection sites for *A. halimus* and 18 for were surveyed across Jordan (Table 1).

Table 1. Sample code, collection site. AH represent *A. halimus*, AL represent *A. leucoclada*

Sample code	Collection site
AH1	Wadi Al Butum, west of Amra place, Azraq reserve
AH2	Azraq airpot east boarder, west of Azraq reserve, Azraq
AL2	Azraq airpot east boarder, west of Azraq reserve, Azraq
AH3	West of shomary reserve, Azrak
AH4	AL-Hazem valley, AL-Zarka
AL5	AL-Dahek reserve, north side, AL-Zarka
AL6	Al-Safawi, Al-Mafraq
AL7	Al-Safawi, Al-Mafraq
AL8	Al-Safawi, south of PPC, AL-Mafraq
AH13	Alghawr Deir Alla..alkatarat
AL13	Alghawr Deir Alla..alkatarat
AH14	The castle
AH15	The castle
AH16	Karak
AL16	Karak
AL17	Karak
AH18	Between Tafila and Shobak
AH19	Shobak (AL Mansheya protected area)
AH20	Shobak
AH21	Dead Sea
AL21	Dead Sea
AH22	Dead Sea
AL22	Dead Sea
AH23	Dead Sea
AH24	Dead Sea
AL25	Dead Sea
AH26	Dead Sea
AH28	South almahmeh
AL28	South almahmeh
AL29	Alaghwar alshamalih
AL30	Almafriq om aljamal
AL31	North khalidiya (Dead sea)
AH32	The fist of Al ardaah
AL32	The fist of Al ardaah
AH33	In middle of ardah
AL33	In middle of ardah
AH34	Alghawr
AL34	Alghawr

Note: AH: *A. halimus*, AL: *A. leucoclada*

Initially, ten individuals were sampled per site. Preliminary ISSR analyses revealed highly similar banding patterns among individuals within the same site. Consequently, one representative individual per location was selected for the final analyses to reduce redundancy while maintaining broad geographic coverage.

2.2. DNA Extraction

Total genomic DNA was extracted from ground leaves using wizard genomic DNA purification kit (Promega, USA), following the manufacture protocol. Extracted DNA was stored at -20^{0}C until use. DNA was qualified and quantified using agarose gel electrophoresis and spectrophotometer, respectively.

2.3. ISSR markers

Four ISSR primers were selected based on their reproducibility, clarity of amplification and high polymorphism (Table 2). Despite the limited number of primers, they generated a sufficient number of informative loci to reliably assess genetic diversity and population structure. ISSR analysis was performed according to Brake et al. (2014). Amplification was conducted in a 25 µL reaction mixture containing 40 ng of genomic DNA, 0.3 µM of each primer, 1× Taq DNA polymerase reaction buffer, 1.5 U of Taq DNA polymerase and 0.2 mM of each dNTP. Amplifications were performed in thermos cycler with heated lid (ABI, USA) programmed for an initial 5 min denaturation step at 94^{0}C, followed by 35 cycles of 40 s denaturing at 94^{0}C, 45 s annealing at 50^{0}C and 60 s extension at 72^{0}C. The program was completed with a final extension at 72^{0}C for 10 min. Amplifications were performed at least twice and only reproducible products were taken into account for further data analysis. Amplified products of ISSR were separated on 1.5% agarose gels in 1× TBE buffer. Fragment size was estimated using a 100 base pairs (bp) molecular size ladder (Hyperladder VI, Bioline, USA) and detected by staining with ethidium bromide (10 mg/mL) according to Sambrook et al. (1989). The amplification products were then visualized using a UV-transilluminator and photographed with an AlphaImager gel documentation system.

Table 2. ISSR primers name and sequence, numbers of polymorphic markers, % primer efficiency and unique bands

Primer name	Sequence 5'–3'	Number of polymorphic markers	% Primer efficiency	Unique bands
UBC 840	(GA)8YT	9	19	0
UBC 842	(GA)8YG	11	23	1
UBC 855	(AC)8YT	16	33	3
UBC 834	(AG)8YT	12	25	1
Total		48		

Note: Y = CT

2.4. Data analysis

The DNA bands were scored visually from the gel photographs. Only clear and reproducible band are scored. Amplification products were scored as either present (1) or absent (0). A data matrix was prepared to calculate genetic similarities between each pair of genotypes. A similarity matrix was calculated by the un-weighted pair group method with arithmetic mean (UPGMA) using Jaccard's coefficients supported in the software package SPSS version 11. Primer efficiency was calculated (Brake *et al*.,

2014). Population structure was inferred using STRUCTURE v2.3.4 (Pritchard *et al*., 2010). As ISSR markers are dominant, the recessive allele model was applied following Falush et al. (2007). The most likely number of clusters (K) was determined using the ΔK method of Evanno et al. (2005). K = 2 to K = 7 were chosen in order to estimate the number of populations (K). Assuming no prior population information, each run included 30,000 MCMC (Markov Chain Monte Carlo) repetitions after a 30,000-step burn phase associated allele frequencies and an admixture model (Cicatelli *et al*., 2013).

For ISSR markers, observed heterozygosity (Ho) was estimated as band frequency, while expected heterozygosity (He) equivalent to Nei's gene diversity for biallelic loci (Nei, 1973), was calculated assuming Hardy-Weinberg equilibrium using He = 2p(1-p). In addition, power of discrimination (D) (Tessier *et al*., 1999) were also calculated. Polymorphic information content (PIC) was calculated following the original definition of Botstein et al. (1980). The applicability and interpretation of PIC for different marker systems have been further discussed by Chesnokov and Artemyeva (2015). Genetic diversity was estimated using Shannon's information index (H′), calculated for dominant ISSR markers as $H' = -\sum(p\ln(p)+q\ln(q))$, where p is the frequency of band presence and q=1-pq is the frequency of band absence across loci (Lewontin, 2014).

3. Results

Four hundred and fifty-two bands were produced by the ISSR primers used in this study. The four primers were able to generate 48 polymorphic markers. The molecular size of the scored bands ranged from 150 to 1800 bp for all primers. The highest primer efficiency was 33% for the primer UBC 855 while the lowest was 19% for the primer UBC 840. Three unique bands were produced by the primer UBC 855 and 1 band was produced by each of the primers UBC 842 and UBC 834.

The scoring data were used to generate genetic similarity matrix. two genetic similarity matrix were generated; the first was for the samples belongs to the *A. halimus* (Table 3) and the second was for the *A. leucoclada* (Table 4) samples. The highest genetic similarity value for the samples belonging to *A. halimus* was 0.91 between samples collected form The castle and Dead sea, while the lowest was 0.00 between different samples from different part of Jordan. In the second matrix, the genetic similarity values ranged from 0.0 between more than one sample to 0.67 between sample collected from South almahmeh and Alaghwar alshamalih.

The dendrogram constructed based on the genetic similarity values between 20 plants belonging to *A. halimus* shows that the samples are divided into two groups, the first include one sample collected from AL-Zarka and the second group includes 21 samples collected from different parts of Jordan. The second group is subdivided into further groups (Figure 1). Large group of this dendrogram which includes 11 samples out of 20 shows clustering of samples collected from the same geographical zone.

Like the dendrogram constructed for *A. halimus*, the dendrogram constructed for *A. leucoclada* is divided into two main groups, the first group contain one sample come from Dead Sea while the second group include the rest of samples. The second group then subdivided into two subgroups, the first contain 7 samples and the second include 10 samples (Figure 2).

Table 3. Similarity matrix for *Atriplex halimus* accession

Proximity Matrix

Case	Matrix File Input																			
	AH1	AH2	AH3	AH4	AH13	AH14	AH15	AH16	AH18	AH19	AH20	AH21	AH22	AH23	AH24	AH26	AH28	AH32	AH33	AH34
AH1	1.000																			
AH2	.471	1.000																		
AH3	.450	.412	1.000																	
AH4	.063	.091	.143	1.000																
AH13	.350	.467	.368	.167	1.000															
AH14	.263	.357	.278	.100	.167	1.000														
AH15	.389	.429	.412	.200	.294	.583	1.000													
AH16	.471	.429	.412	.200	.294	.583	.538	1.000												
AH18	.250	.364	.188	.000	.133	.273	.250	.250	1.000											
AH19	.261	.263	.400	.067	.182	.353	.412	.263	.118	1.000										
AH20	.263	.357	.438	.222	.400	.500	.583	.583	.167	.353	1.000									
AH21	.167	.231	.111	.000	.059	.154	.143	.143	.375	.111	.071	1.000								
AH22	.444	.400	.471	.182	.353	.538	.909	.615	.231	.389	.667	.133	1.000							
AH23	.550	.444	.579	.059	.273	.389	.444	.444	.235	.429	.316	.222	.500	1.000						
AH24	.500	.467	.444	.000	.263	.500	.375	.467	.308	.238	.313	.286	.438	.647	1.000					
AH26	.381	.412	.400	.143	.300	.278	.500	.333	.188	.217	.278	.176	.471	.500	.444	1.000				
AH28	.211	.385	.222	.111	.176	.308	.200	.286	.300	.100	.133	.273	.188	.333	.429	.467	1.000			
AH32	.045	.125	.048	.111	.053	.214	.125	.125	.083	.100	.063	.273	.118	.143	.176	.222	.455	1.000		
AH33	.500	.333	.455	.111	.304	.227	.333	.400	.095	.280	.350	.091	.381	.417	.304	.280	.182	.083	1.000	
AH34	.364	.316	.381	.133	.286	.143	.316	.250	.053	.318	.200	.167	.300	.409	.227	.381	.150	.150	.435	1.000

Table 4. Similarity matrix for *Atriplex leucoclada* accession

Case	Matrix File Input																	
	AL2	AL5	AL6	AL7	AL8	AL13	AL16	AL17	AL21	AL22	AL25	AL28	AL29	AL30	AL31	AL32	AL33	AL34
AL2	1.000																	
AL5	.400	1.000																
AL6	.125	.063	1.000															
AL7	.063	.067	.300	1.000														
AL8	.313	.538	.063	.143	1.000													
AL13	.267	.286	.154	.167	.385	1.000												
AL16	.235	.176	.063	.143	.250	.286	1.000											
AL17	.071	.000	.100	.250	.000	.000	.167	1.000										
AL21	.167	.429	.000	.143	.333	.200	.250	.000	1.000									
AL22	.091	.100	.000	.000	.000	.125	.100	.000	.100	1.000								
AL25	.438	.222	.118	.125	.222	.250	.294	.143	.222	.083	1.000							
AL28	.250	.267	.067	.071	.188	.133	.267	.182	.267	.111	.235	1.000						
AL29	.294	.235	.200	.063	.167	.118	.235	.154	.235	.091	.278	.667	1.000					
AL30	.313	.250	.063	.231	.250	.200	.429	.167	.333	.100	.571	.267	.235	1.000				
AL31	.176	.056	.333	.154	.118	.000	.118	.182	.118	.000	.235	.385	.429	.188	1.000			
AL32	.231	.071	.333	.100	.071	.000	.154	.286	.071	.000	.214	.273	.333	.154	.556	1.000		
AL33	.400	.421	.143	.150	.350	.316	.227	.050	.286	.059	.526	.182	.217	.286	.182	.158	1.000	
AL34	.211	.375	.118	.125	.294	.333	.222	.000	.294	.083	.333	.105	.150	.222	.105	.000	.526	1.000

Cluster analysis based on ISSR data grouped accessions into several genetically distinct clusters, reflecting varying degrees of genetic similarity among populations. The observed clustering did not strictly correspond to geographic proximity, suggesting that genetic relationships among accessions are not solely structured by spatial distance at the scale sampled.

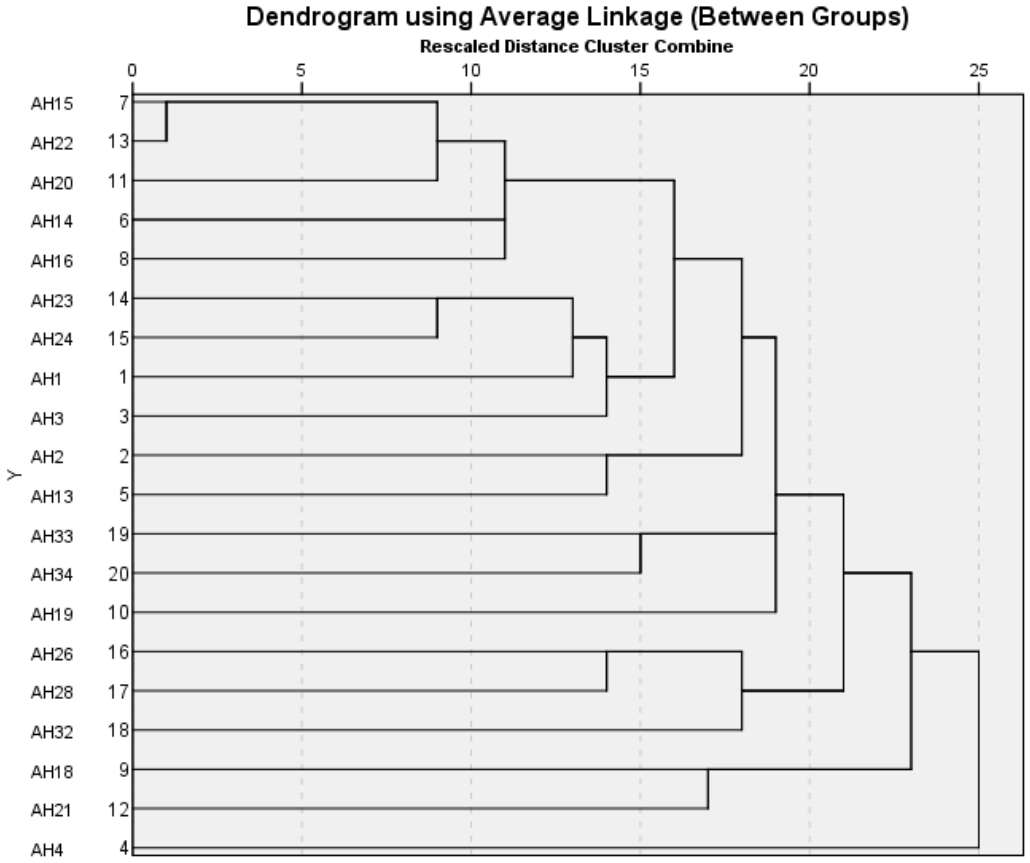


Figure 1. Dendrogram for *A. halimus* collected from different part of Jordan

In Figure 3, the genetic structure analysis (with k=2 up to K=7) revealed unique genetic content for the majority of accessions for both *Atriplex halimus* and *Atriplex leucoclada*. By applying the Evanno method (Δk), it is possible to determine the uppermost hierarchical level of structure by calculating the rate of change in log-likelihoods and their variance between successive k values. For *A. halimus*, the ΔK peaks strongly at K=5, confirming that K=5 is the optimal number of clusters. On the other hand, for *A. leucoclada*, the ΔK peaks strongly at K=3, confirming that K=3 is the optimal number of clusters. This means that the Jordanian accession for saltbush can be assigned into five major genetically distinct groups, while orache accessions can be grouped into three genetically distinct families.

All four ISSR primers were highly informative in both *A. halimus* and *A. leucoclada* and populations (Tables 5 and 6). In *A. halimus*, PIC values ranged from 0.865 to 0.888, with D values between 0.911 and 0.978, confirming the strong discriminatory capacity of all primers. Estimates of Ho (0.185-0.323) and He (0.218-0.296) were comparable between the two species, with ISSR_840 generally showing the highest He values, whereas ISSR_834 displayed the lowest Ho in both populations. Similarly, in *A. leucoclada*, polymorphic information content (PIC) values ranged from 0.865 to 0.920, while the power of discrimination (D) was consistently high (0.948-0.978). Observed heterozygosity (Ho) varied between 0.197 and 0.286 and expected heterozygosity (He) ranged from 0.272 to 0.335.

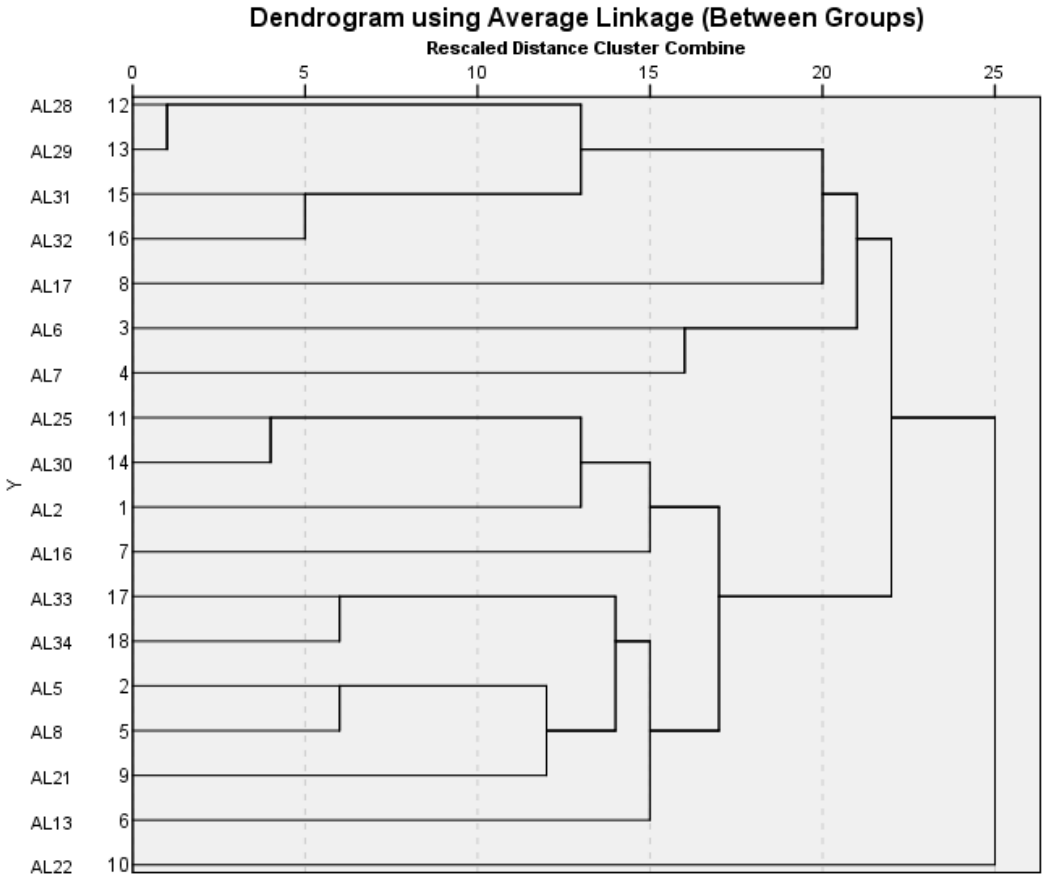


Figure 2. Dendrogram for *A. leucoclada* collected from different part of Jordan

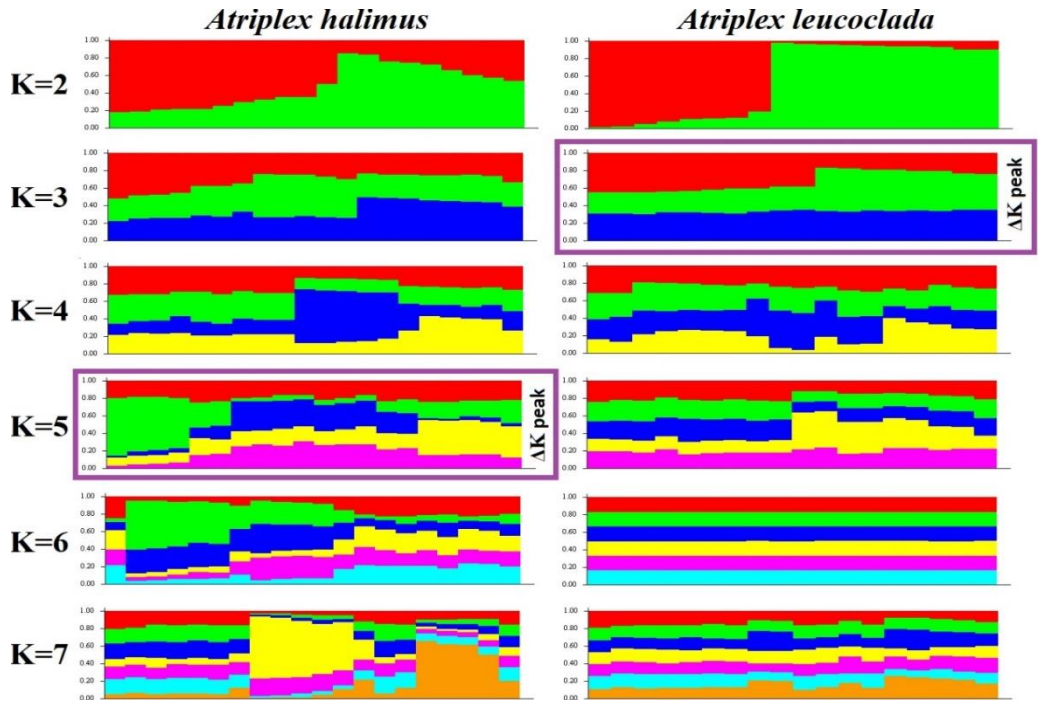


Figure 3. Structure analysis for both *Atriplex halimus* (left panels) and *Atriplex leucoclada* (right panels) accessions using K range (2-7). The data showed a peak ΔK values at K=5 for *A. halimus* and K=3 for *A. leucoclada* accessions. Revealing the optimal number of clusters for each species

Table 5. PIC, D, Ho, He and H' for ISSR markers used in *A. halimus* populations

marker	PIC	D	Ho	He	H'
ISSR_840	0.898	0.943	0.289	0.296	0.458
ISSR_842	0.865	0.911	0.245	0.249	0.511
ISSR_855	0.888	0.978	0.323	0.286	0.444
ISSR_834	0.888	0.978	0.185	0.218	0.362

Table 6. PIC, D, Ho, He and H' for ISSR markers used in *A. leucoclada* populations

marker	PIC	D	Ho	He	H'
ISSR_840	0.920	0.974	0.229	0.305	0.468
ISSR_842	0.895	0.948	0.286	0.335	0.509
ISSR_855	0.888	0.978	0.250	0.272	0.424
ISSR_834	0.888	0.978	0.197	0.295	0.460

Shannon's information index (H′) revealed comparable levels of genetic diversity between *A. leucoclada* and *A. halimus* across the four ISSR primers. In *A. halimus*, H′ values ranged from 0.362 to 0.511, whereas in *A. leucoclada* they ranged from 0.460 to 0.509 as well, with very similar primer-specific patterns observed in both species. The highest diversity was detected for ISSR_842 in both species (H′ ≈ 0.51), while the lowest values were associated with ISSR_834 (H′ ≈ 0.41). Overall, these results indicate moderate to high levels of genetic diversity detected by ISSR markers in both *Atriplex* species.

4. Discussion

Plant conservation is very demanding and requires space and labor. Genetic diversity can aid in reducing the number of individuals to be considered for conservation. Genotypes with high genetic similarity (homology) can be represented by one individual. Therefore, we investigated the genetic diversity between individual genotypes from two Jordanian *Atriplex* spp.

Despite the importance of *Atriplex* spp. as slat and water tolerant plants, little is known about the Jordanian species of *Atriplex*. The *Atriplex* species has resistance mechanism to salt and water stresses, which make this plant as potential crop for biosaline agriculture (Alam *et al*., 2022). Therefore, this study investigates the genetic diversity between individual genotypes from two Jordanian *Atriplex* spp.

Natural populations of *Atriplex* spp. represent a huge genetic diversity, as they spread from sub-humid to arid regions including North Africa and the Arabian Peninsula (Zoghet & Tag El Din, 1992; Le Houerou, 1992). They are found along the Mediterranean basin from Iraq in the east to Morocco in west (Sankari, 1978; Röpert, 2000) and even in east of Africa in Ethiopia and Tanzania (Miller *et al*., 1996). The DNA polymorphic analysis in this study revealed high genetic diversity within species. The degree of diversity reach 1 for both species. Similarity values of zero observed between some accessions pairs reflect the absence of shared ISSR bands under the Jaccard similarity metric rather than a complete lack of genetic relatedness. Such values are expected when using dominant markers and presence-absence coefficients and have been reported in previous ISSR-based studies of genetically diverse plant populations.

The results of the study were in agreement with several researchers (Sampson & Byrnei, 2012, Elframawy *et al*., 2016; Bouda *et al*., 2016). Polyploidy is common in *Atriplex* spp. (Flores-Olvera & Mercado-Rua, 1997). Polyploidy species are expected to

maintain high levels of heterozygosity and diversity because of genome doubling and/or hybridization during formation. The high genetic diversity of genotypes are potential genetic resources for future studies, e.g. to study salt responsive genes in *Atriplex* spp. The study demonstrates high genetic diversity among natural collection of *A. halimus* L. The conserved genotypes are potential genetic resources for future studies, e.g. to study salt responsive genes in *A. halimus* L. Similar studies found high genetic diversity in *A. halimus* L. using other marker technologies as isozymes among Moroccan populations (Haddioui & Baaziz, 2001) and RAPD DNA markers and Internal transcribed spacer (Ortíz-Dorda *et al*., 2005). In another study, natural populations of *A. prostarta* from USA were analyzed using ISSR markers (Carter *et al*., 2003), where detectable genetic diversity was illustrated between studied genotypes.

Unique individuals with high diversity are very important candidates for breeding purposes (Muehlbauer *et al*., 2006). As distinctive material, such individuals are expected to have new desired traits for that species and to widen the genetic background of available lines (Erskine *et al*., 1998). In the case of *A. halimus* L., the diversity is important to detect interesting candidate genes, e.g. salt tolerant genes. These genes could be transformed into related crop species in the *Amaranthaceae* family that are less tolerant, e.g., sugar beet (*Beta vulgaris*), chard (*B. vulgaris* subsp. cicla) and spinach (*Spinacia oleracea*). Otherwise, such candidate genes from *A. halimus*, once identified, can be used to determine their homologous counterparts in these crops. These genes can be then, either over expressed or combined together through classical breeding in one line. On the other hand, diversity is important in *Atriplex* spp for other important utilizations. *Atriplex* spp are being used for bioremediation by growing on saline water from coal bed methane extraction and industrial brines (Browning *et al*., 2006; Jordan *et al*., 2009). In addition, *Atriplex* spp are used to produce huge biomass (Glenn *et al*., 1999) and as livestock fodder with good protein content (Khan *et al*., 2000).

In total, four ISSR markers were used to investigate polymorphism among saltbush genotypes. Some ISSR primers produce unique bands for same samples; three bands for primer UBC 842 and one band for primers UBC 842 and UBC 834. These bands will be useful for gene identification (Brake *et al*., 2014). The primer UBC 855 was shown to be informative enough to assess molecular polymorphism in *A. halimus* L. and *A. leucoclada* genotypes (Table 2).

The dendrogram revealed genetically distinct clusters among *Atriplex* accessions; however, the present study did not include quantitative environmental or land-use variables. Consequently, direct associations between genetic clustering and ecological gradients such as soil salinity, climate or grazing history cannot be inferred. The lack of a strict geographic pattern may reflect a combination of historical gene flow, seed dispersal and local demographic processes rather than current environmental conditions. Future studies integrating molecular data with detailed environmental and land-use information will be required to disentangle the relative contributions of ecological and anthropogenic factors to population genetic structure in *Atriplex*.

The collection areas for the investigated genotypes from different part of Jordan were having different environmental conditions. Our data revealed high genetic diversity among *A. halimus* L. genotypes that would contribute in distribution and adaptation. However, wild *A. halimus* L. plants in several areas were found in sparse populations and very specific habitats namely the salt marshes. Moreover, the collection site has witnessed unprecedented destruction of *A. halimus* L. nature populations, they are threatened by sever grazing and sequential drought conditions, which prevent shrubs

from reaching full maturity to reproduce. Therefore, it is important to preserve the saltbush (*A. halimus* L.) as native plant species and important genetic pool for current global challenges.

The genetic structure data revealed three major genetic groups for orache (*A. lecuclada*), which agrees with the dendrogram revealing group1 (Accessions AL28, AL29, AL31, AL32, AL17, AL6 and AL7), group 2 (accessions AL25, AL30, AL2 and AL16) and group 3 (accessions Al33, AL34, AL5, Al8, Al21 and AL13). This major finding would help in conservation selection and focus the future studies to these three genetic groups. On the other hand, the genetic structure data revealed three five genetic groups for saltbush (*A. halimus*), while the dendrogram showed six major clades. This limited discrepancy is explained by the admixture nature of the genetic material reveled between the *A. halimus* accessions, where each of the five assigned groups by genetic structure analysis showed multiple colors indicating multiple genetic admixtures present in all genetic groups. Nonetheless, considering (five or six) groups would likewise help in future research assignment and studies.

The consistently high PIC and power of discrimination values observed for all ISSR primers in both *A. lecuclada* and *A. halimus* confirm their high efficiency in detecting genetic variation and resolving genotypic differences, as reported for dominant molecular markers in plant population studies (Nei, 1973; Roldán-Ruiz *et al*., 2000). The moderate levels of expected heterozygosity detected in both species indicate the maintenance of substantial genetic diversity despite exposure to arid and saline environments, where strong selective pressures often reduce within-population variability. The systematic tendency for observed heterozygosity to be lower than expected heterozygosity is characteristic of ISSR data and reflects the dominant nature of these markers, which prevents direct discrimination between heterozygous and homozygous band-presence genotypes, as well as potential departures from Hardy-Weinberg equilibrium (Lynch & Milligan, 1994). The overall similarity in diversity indices between A. leucoclada and A. halimus suggests that both species retain comparable adaptive potential, which may contribute to their ecological success and physiological resilience under drought and salinity stress conditions typical of arid and semi-arid habitats.

The comparable Shannon's diversity indices observed in *A. leucoclada* and *A. halimus* suggest that both species maintain similar levels of genetic variation at the loci surveyed, despite differences in their ecological distribution and conservation status. The moderate to high H′ values are consistent with expectations for outcrossing, perennial halophytic shrubs exposed to heterogeneous and stressful environments, where genetic diversity may contribute to local adaptation. The consistency of diversity patterns across ISSR primers further supports the reliability of the marker system for detecting genome-wide variation. Importantly, the presence of appreciable genetic diversity in *A. halimus* populations from Jordan highlights the conservation value of these native populations and underscores the need to preserve genetically divergent genotypes under increasing grazing pressure and recurrent drought conditions.

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