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Variability of Natural Populations of *Atriplex Halimus* L. in Morocco as Investigated by RAPD Markers

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Abstract

Atriplex halimus L. (Chenopodiaceae) is a monoecious C_4 perennial shrub native to the Mediterranean Basin, used as fodder shrub for livestock and useful for rehabilitation of degraded rangelands. To assess the levels and patterns of genetic diversity of this species, 99 samples of 11 populations collected throughout the natural range in Morocco plus 9 samples obtained from one population originating from USA, were analyzed using RAPD method and markers. A number of 157 reproducible amplified bands were obtained with 17 primers. Out of 157 amplified bands, 146 (93%) were polymorphic, and only 11 (7%) monomorphic. Global AMOVA analysis showed that the most genetic variation was within populations (66.57%), with the reminder occurring between populations (33.43%). Hierarchical AMOVA analysis revealed that variation among regions (Morocco versus USA) accounted only for 5.87% of the total genetic variation, suggesting that there is not a significant genetic differentiation of populations located at the opposite sides of the Atlantic Ocean. The actual genetic structure could have arisen by a combination of genetic drift effect and limited level of gene flow (0.50). The genetic diversity maintained by this species (0.238) was somewhat higher in comparison to other species with similar life histories and ecological traits. A neighbour- joining dendrogram based on Dice's coefficient resolved five major groups of populations correlated in part with bioclimatic type. Nevertheless, geographic distance did not explain the genetic differentiation among populations (r = 0.103, P = 0.646). The data obtained in this study should have important implications for the conservation and management strategies of genetic variation of Atriplex halimus in Morocco.

Key words: *Atriplex halimus* – Natural populations – RAPD – Genetic structure – Morocco.

Introduction

Among the *Atriplex* species in North Africa, *Atriplex halimus* L. a C_4 perennial shrub, is found in arid and semi-arid environments. It is of interest because of its tolerance to environmental stresses and its use as a fodder shrub for livestock in low rainfall Mediterranean areas [1]. As consequence, *A. halimus* could be considered as a promising species for the reclamation of degraded lands where excessive salinity and low moisture level are the most factors limiting plant growth, but where there is also a need for animal forages, especially in critical drought periods. In Morocco, *A. halimus* L. is widely distributed as a wild species, particularly in habitats that combine relatively high soil salinity with aridity. It's also widely distributed throughout the Mediterranean Basin [1]. The species is using as excellent livestock fodder because of its favourable crude protein content and also as a mean of soil erosion control in depleted rangelands and dust mining owing to its deep root and resistance to harsh environment.

Studies of population genetic structure provide windows to the roles that the fundamental evolutionary forces of selection, gene flow, and drift play in processes such as local adaptation and speciation [2]. Information on the levels and patterns of genetic diversity of natural plant populations over the geographic range of a species, based on molecular markers, has been recognized as fundamental not only to delineate *in situ* or *ex situ* conservation strategies for tree species, but also to establish forms of rational economic exploitation [3]. In spite of their ecological and economic importance and the fact that knowledge about genetic variation provides an important baseline for conservation, management and improvement programmes, only limited information is available on the extent, distribution, and nature of genetic variability in *A. halimus*. This is unfortunate due to the modest economical importance generally attributed to shrubby species, which are not yet seriously considered as cultivated species. Morphological, physiological and isozyme-based studies showed high genetic diversity in Moroccan populations of *A. halimus* [4; 5; 6; 7].

In the study presented here, we therefore applied RAPD analysis and AMOVA technique to determine the pattern and extent of genetic variation within and between natural populations of *A. halimus* from Morocco. Besides, we compared the results obtained with RAPDs to that obtained from previously reported study of our team [4] that used isozymes markers. This study might provide fundamental genetic information for conservation, management and restoration of this natural genetic resource.

Materials and methods

Plant material

Twelve populations of *A. halimus* were studied; 11 natural populations from Morocco and 21-years-old Moroccan plantation originated from Wyoming, USA. Table 1 summarizes their location characteristics. During November 2006, fresh branches were collected haphazardly from nine individuals belonging to 11 populations of A. *halimus* from throughout its geographical area in Morocco, and the branches were stored at -80 °C pending DNA extraction. They correspond to three climatic contexts: semi-arid zones (Settat, Sidi Bouzid and Essouiria populations), arid zones (Kelaâ des Sraghna, Marrakech and Chichaoua populations), and Saharan zones (Ouarzazate, Tafraout, Laâyoune, Es Semara and Wyoming (USA) populations). The seeds of the American population were obtained from the Centre de Production des Semences Pastorales (CPSP) orchard in Kmiss M'touh, El Jadida, Maroc, where the stock plants has been maintained on soil since 1985. The plants of this population utilized in this work were grown in pots, in a mixture of sand, peat and vermiculite as substrate, and maintained in a greenhouse. The experiment was set up as a completely randomized design, with ten replicates, each one of five plants.

DNA extraction and PCR reactions

Genomic DNA from leaves of nine plants of each population was extracted with Nucleon Phytopure DNA extraction kits (Amersham Biosciences, UK Ltd), following the instructions given by the supplier. DNA concentration was determined by NanoDrop (NanoDrop Technologies, Wilmington, USA).

RAPD-PCR was performed after the protocol of Williams *et al.* [8], slightly modified for optimization. The reaction mixture, in a final volume of 25 µl, contained: 40 ng of template DNA, 2 mM $MgCl_2$, 10 X reaction buffer, 2 µM of each primer, 0.4 mM dNTPs, and 0.5 U of DNA polymerase (Biotools, Spain). Seventeen primers (OPA-02, OPA-05, OPA-09, OPB-01, OPB-03, OPB-4, OPB-06, OPC-07, OPC-08, OPC-15, OPD-08, OPD-11, OPD-15, OPE-02, OPE-12, OPO-07 and OPP13, OPERON USA), displaying reliable banding patterns were

used. PCRs were run in Primus 96 Plus (MWAG Biotech, Ebersberg, Germany) thermocycler through 45 cycles, each consisting of: 94 °C for denaturation step (1 min), 36 °C annealing

Table 1. List of the populations of *A. halimus* used in the study , with their principal geographic and ecological characteristics and genetic diversity derived from the RAPD data.

Population	Abbreviation	Geographic origin	Latitude North	Longitude West	Altitude (m)	Rainfall (mm)	Genetic diversity ± SD
Kelaâ des Sraghna	K	Kelaâ des Sraghna city	33°50’	7° 24’	465	250	0.236 ± 0.129
Marrakech	M	5 Km N of Marrakech	31° 41’	8° 00’	470	242	0.240 ± 0.131
Chichaoua	C	Chichaoua plateau	31° 32’	8° 46’	340	191	0.227 ± 0.124
Settat	S	20 Km S of Settat	32° 57’	7° 40’	375	391	0.281 ± 0.153
Sidi Bouzid	SB	10 Km N of Safi	32° 24’	9° 14’	15	365	0.267 ± 0.146
Essouiria	E	30 Km S of Safi	32° 03’	9° 19’	15	365	0.167 ± 0.092
Dakhla	D	25 Km NE of Dakhla	23° 43'	15° 55'	7	30	0.239 ± 0.130
Laâyoune	L	Aït Ourir, 15 Km S of Laâyoune	27° 9'	13° 12'	131	50	0.234 ± 0.128
Es Semara	ES	Lafrayrina, 30 Km E of Es Semara	26° 44'	11° 41'	273	12	0.266 ± 0.145
Ouarzazate	O	Tiguida zone, Oued Draâ	30° 56’	6° 54’	1135	78	0.233 ± 0.127
Tafraoute	T	10 Km SE of Tafraoute	29° 43'	9° 01'	1050	168	0.207 ± 0.113
Wyoming (USA)	US	desert plains of southern Wyoming	41° 3'	105° 58'	610	180	0.256 ± 0.140

step (2 min), and a 72 °C extension step (1 min), using the fastest available transitions between each temperature. Amplification products were electrophoresed on 1% agarose gels in TAE buffer, stained with ethidium bromide, and photographed under UV light by using KODAK 1D Analysis Software. Some RAPD-PCR reactions were repeated three times to ascertain the reproducibility of the banding pattern.

Data analyses

RAPD bands were scored as present (1) or absent (0) to compile a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) version 2.02g [9]. Genetic distances among individual plants was calculated according to Dice’s similarity coefficients [10] using the SIMQUAL (Similarity for Qualitative Data) program. The similarity matrix obtained was then used to construct a dendrogram, using the Neighbor-joining method [9] through the NJOIN routine of the NTSYS-pc package. Reliability of fit of the clustering to the original data set was tested with a cophenetic correlation coefficient [11]. A Mantel test used to test whether matrices of genetic distances (FST) between populations were significantly correlated with matrices of geographic distances (1000 permutations; routine MXCOMP of the NTSYS-pc; package; [9]).

Apportionment of the observed genetic variation and calculation of the corresponding *F*-values were carried out using three different hierarchical AMOVA [12]. Firstly, global analysis of AMOVA was done to partition the total genetic variation into two hierarchical levels: among populations (FST) and within populations. Secondly, hierarchical AMOVA analysis was used to apportion the variation: (i) between regional groups (Moroccan versus American region) (FCT); (ii) among populations within groups (FSC); (iii) within populations (FST). Thirdly, to investigate possible differences between the three main bioclimatic zones (ecological groups: semi arid, arid and Saharan group) the populations were grouped according to bioclimatic zones and an AMOVA with three hierarchical levels was carried out. The AMOVA was based on genetic distance calculated by the number of pairwise differences between haplotypes. The pairwise genetic differentiations (FST) among the 12 populations was also generated by AMOVA. Population specific FST indices were also computed in order to see which populations are more differentiated from the rest. Genetic diversity of populations was assessed by the average gene diversity over loci among all members of the population. The number of permutations significant testing was set up at 2000 for all analyses. These analyses were done using the package ARLEQUIN version 3.01 [13]. From AMOVA *F* statistics gene flow (number of migrants per generation = N_e m) can be approximated through Wright's island model [14; but see 15] as $N_em = 0.25 (1/FST - 1)$.

Results

The 17 primers used in this study yielded a total of 157 reproducible bands across 108 *Atriplex* individual plants, with 5 to 13 bands per primer, 9.23 as average and their size range from 250 to 2000 bp. Most of these bands (146) were polymorphic, reflecting high level of genetic polymorphism in the 12 *Atriplex* populations analysed. An example of the polymorphism detected with primer OPO-7 is shown in Fig. 1.

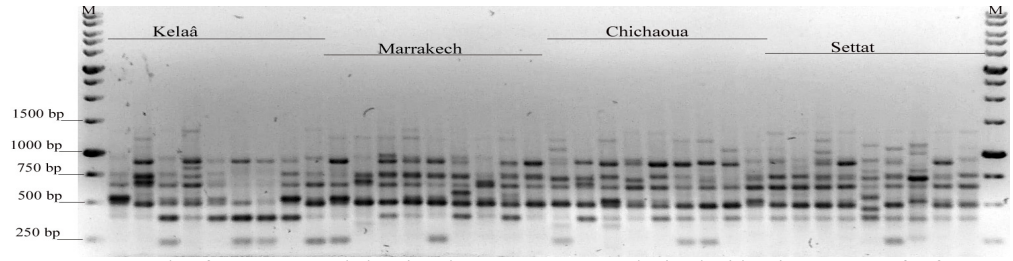

Figure 1. Example of an agarose gel showing the RAPD patterns obtained with primer OPO-7 for four *Atriplex* populations, each represented by 9 individuals. M, 1-kb ladder marker.

According to the global AMOVA analysis, there was significant partitioning of the genetic variation ($P<0.001$), with 33.43% occurring among populations and within-populations variation accounting for the remaining 66.57% (Table 2). This indicates that, together with a high intrapopulational variation, there is also a significant structuring of populations. Although the majority of variation (66.57%) occurred within populations, the level of population differentiation ($FST = 0.334$, $P = 0.000$) was highly significant after 2000 random permutations (Table 2). This structure obtained among the populations corresponds with the low value (0.50) calculated for the average number of individuals exchanged between populations per generation (N_em). A hierarchical AMOVA analysis (Table 2) was performed to see if there is any genetic difference between populations from Morocco and USA. The amount of genetic variation among regions and among populations was 5.87% and 30.81%, respectively, with the reminder (63.32%) occurring within populations, suggesting that there

is not a significant genetic differentiation of populations located on opposite sides of the Atlantic Ocean. When AMOVA was performed, at three hierarchical levels, with three ecological groups designated on the basis of bioclimatic type of populations, very low genetic

Table 2. AMOVA analysis of the RAPD variation in 12 populations of *A. halimus*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F-statistiques
Global					
Among populations	11	1055.056	8.726	33.43	FST= 0.334***
Within populations	96	1668.444	17.379	66.57	
Hierarchical					
Among regional groups	1	120.086	1.611	5.87	FCT=0.059NS
Among populations within groups	10	934.970	8.457	30.81	FSC=0.327***
Within populations	96	1668.444	17.380	63.32	FST=0.367***
Among ecological groups	2	297.963	1.923	7.19	FCT= 0.072*
Among populations within groups	9	757.093	7.416	27.76	FSC=0.299***
Within populations	96	1668.444	17.380	65.05	FST=0.349***
Total	107	2723.500	27.448		

- Significant (P<0.05), *** Highly significant (P<0.001), NS: non significant.

differentiation was observed between groups (FCT=0.072, P=0.01) even though that it is slightly significant (Table 2). This indicates that climatic conditions have had little effect on populations structuration which imply that there is no local adaptation of studied populations.
As calculated by the average gene diversity over loci among all members of the population, intrapopulational diversity of *A. halimus* populations exhibited high levels for populations of Settat (S), Sidi Bouzid (SB) and Es Semara (ES) (Table 1). The pairwise FST values and geographic distances between 12 populations are shown in Table 3. All the pairwise genetic distances (FST) between populations were highly significant (P<0.001), indicating that all populations may be considered different from each other. It ranged from 0.067 (C / M; 66 Km) to 0.607 (T / E; 315 Km), indicating that populations C / M were the most similar and populations T / E were the most different. Population specific FST's indices were also obtained (data not shown) after Arlequin program execution. The results showed that populations E and T had the highest values, respectively FST = 0.356 and FST = 0.344, meaning that these two populations are the most differentiated from the rest of the studied populations. On the other hand, the population S showed the lowest value of the FST (0.321) implying that it is the least differentiated from the remaining populations. Geographic distance did not explain the genetic distance among populations: the matrix of 66 pairwise genetic distances (FST) among 12 populations was not significantly correlated with the corresponding matrix of geographic distances (r = 0.103, Mantel t-test = 0.375, P = 0.646).
Genetic relationships among populations were further reconstructed by neighbour-joining (NJ) clustering of Dice's similarity coefficients (Fig. 2). The correlation coefficient between cophenetic values of the dendrogram and the Dice's coefficients was r = 0.86 (P<0.001), indicating that the clustering was congruent with the original data set [9]. The 108 plants of *A. halimus* analysed belonged to 103 different haplotypes, reflecting high intrapopulational diversity. Only in three populations five pairs of plants (two in population Kelaâ, two in

Chichaoua and one in Dakhla) showed identical RAPD phenotype, suggesting that the sampled pairs belonged to the same genotype. The NJ dendrogram resulted in five main clusters, the population Essouiria was separated from the others and form with three

Table 3. Matrix of pairwise FST values and corresponding geographic distances (in Km, above diagonal) for 12 populations of *A. halimus* analysed by RAPD. Abbreviations as in Table 1.

	K	M	C	S	SB	E	D	L	ES	O	T	US
K		84	120	153	205	225	1245	1006	908	288	273	8216
M	0.086[a]		66	166	167	187	1176	922	824	204	219	8315
C	0.092	0.067		201	124	144	1125	648	576	171	186	8279
S	0.177	0.156	0.156		211	231	1314	1088	990	370	378	8254
SB	0.146	0.161	0.142	0.199		40	1165	953	855	371	295	8184
E	0.579	0.582	0.595	0.488	0.579		1185	983	885	401	315	8202
D	0.185	0.171	0.245	0.247	0.153	0.590		477	585	1140	981	8277
L	0.258	0.190	0.197	0.186	0.234	0.572	0.235		222	1024	522	8251
ES	0.248	0.248	0.275	0.248	0.210	0.574	0.266	0.278		926	411	8397
O	0.337	0.317	0.317	0.258	0.303	0.601	0.334	0.307	0.172		213	8458
T	0.353	0.359	0.391	0.318	0.327	0.607	0.385	0.330	0.233	0.272		8389
US	0.348	0.373	0.388	0.330	0.306	0.594	0.340	0.378	0.167	0.289	0.273	

[a] All FST values were significantly larger than a random FST value ($P<0.001$)

individuals from Settat population one cluster (I). The second cluster (II) consisted of most of the populations from Saharan bioclimate zones, which concerned the populations of Tafraout, Ouarzazate, Es Semara and USA. The third cluster (III) included the Laâyoune population and most of the Settat's population individuals. The forth cluster (IV) grouped the Dakhla and Sidi Bouzid populations plus two individuals from Chichaoua population. The last cluster (V) comprised the individuals of arid zones populations, Kelâa, Marrakech and Chichaoua, which were intermingled. In this dendrogram, all populations of *A. halimus* could be discriminated from each other, except for the populations Kelâa, Marrakech, and Chichaoua of arid zones, which were mixed irrespective of their origin from different habitats. This clear discrimination of populations on dendrogram was strengthened with the high proportion of genetic differentiation (FST = 0.334) obtained among them. Besides, the Essouiria and Sidi Bouzid populations (collected from the Atlantic littoral) were not grouped together, although are the most geographically closely located populations (40 Km apart). Overall, genetic relationships among the 12 populations were not related to geographic distances among them, which are confirmed by the lack of significant association between genetic and revealed geographic distances (Fig. 2). On the other hand, the population Wyoming originated from USA (US) was not isolated in the cluster analysis, which agrees with the findings of the hierarchical AMOVA analysis indicating that the among-regions genetic variation was very weak. The dendrogram shows, also, that most individuals from a given population tend to cluster together and are, therefore, more genetically similar than individuals from different populations.

Discussion

An understanding of the extent and distribution of genetic variation within and between wild *A. halimus* populations is essential for devising strategies, which will efficiently maintain genetic diversity in *ex situ* and *in situ* programs of germplasm conservation. Furthermore, it

contributes to the understanding of the available genetic variability and its potential use in breeding programs.

Recently, genetic variability of *Atriplex* was analysed by Bouda *et al*. [16]. The analysis was

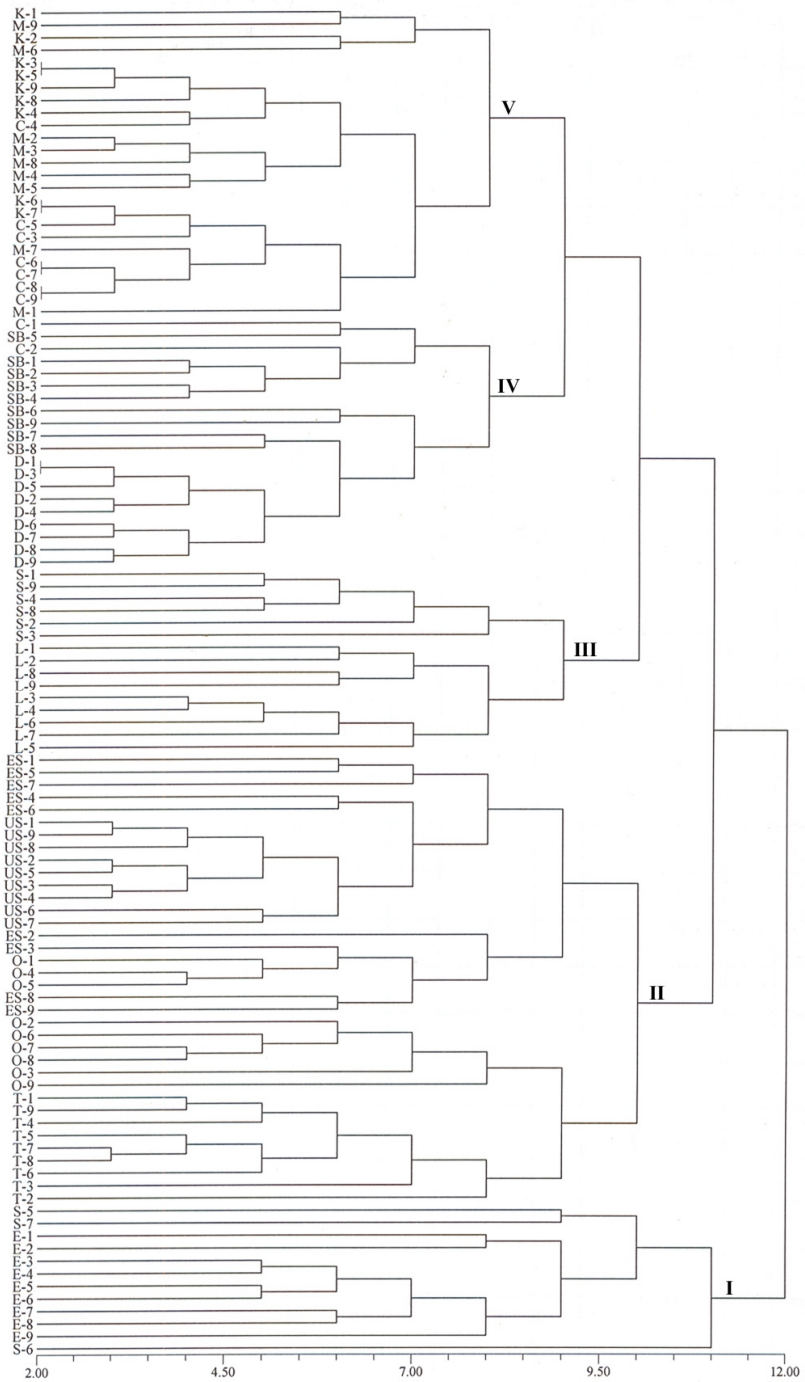

Figure 2. Neighbor-joining dendrogram of genetic relatedness among 108 individuals from 12 *A. halimus* populations, based on Dice's similarity coefficients. Populations' names refer to Table 1.

done for seven *Atriplex* species introduced to semi-arid zones of Morocco by using RAPD and ITS markers. This study is expanded in the present work on the native species in Morocco, *A. halimus*, with RAPD markers.

Within-population genetic diversity values were high suggesting that individuals within populations display a large proportion of genetic loci of high allelic variation. The characteristics of *A. halimus* as a geographically widespread, outbreeding, long-lived perennial plant species may contribute to the observed high levels of genetic diversity [17; 18; 19]. Besides, *A. halimus* is a polyploid species; and according to Soltis and Soltis [20], polyploids, both individuals and populations, maintain higher levels of heterozygosity than do their diploid progenitors. Moreover, most polyploids are polyphyletic incorporating genetic diversity from multiple progenitor populations. This enables *A. halimus* to have much better adaptability to diverse ecosystems, which may contribute to their success in nature by its widespread distribution.

The AMOVA revealed that most of the variation (66.57%) is found within populations, which agrees with the data collected by allozyme analysis [4]. The prevalence of genetic diversity within population for *A. halimus* in Morocco is consistent with the general trend in other outcrossing species revealed through RAPD variation [21; 22; 23].

Altough FST has a theoretical range of zero (indicating no genetic divergence) to one (indicating fixation for alternative alleles in subpopulations); the observed maximum is usually much less than one [24]. Wright [25] suggested that the range 0 - 0.05 indicates little differentiation, 0.05 - 0.15 moderate, 0.15 - 0.25 large differentiation and above 0.25 indicates very large differentiation. Considering the genetic structure of *A. halimus* populations, RAPD markers revealed significant genetic subdivision (FST= 0.334, P=0.000). According to Wright's interpretation of FST values [25], one can say that the *A. halimus* populations evaluated here are very largely differentiated. The FST value obtained was comparable to that obtained for plants in general (Φ_{ST}= 0.35±0.25 from 78 entries) [23] using RAPD and AMOVA-derived Φ_{ST} approach utilized here. This high genetic structuring, an indication of the extent and distribution of genetic diversity, can arise within populations as a result of joint action of natural selection, genetic drift, migration, and mutation [25]. According to Slatkin [26], genetic drift causes population differentiation, if flow value is less than one migrant per generation. As pointed out by Telles *et al*. [27], when gene flow is restricted, the population tends to have smaller effective size and greater inbreeding and, as a result, a greater probability of intrapopulational differentiation. A high rate of gene flow homogenizes the genetic differences among populations, even in the presence of intensive selection.

Plant species differ markedly in the way genetic diversity is partitioned between populations and among individuals within population. The pattern of partitioning is correlated with the mating system and life history traits [28]. Species that are primarily outcrossing and long-lived retain most of their genetic variability within populations. Our results are consistent with this pattern. Therefore, most genetic variation apportioned within population is not surprising and is possibly due to high level of gene flow at population level. Nevertheless, the populations of *A. halimus* are slightly more differentiated than expected for an outcrossing–long lived perennial–wind pollinated shrub. In their compilation of studies using RAPD markers for evaluating population differentiation, comprising >106 plant species, Nybom and Bartish [23] used Φ_{ST} values to indicate population differentiation. They report an average Φ_{ST} of 0.28 for outcrossing, 0.25 for long-lived perennial and, also, 0.25 for wind seed dispersal species. The level of population differentiation (33.43%) obtained in this investigation was comparable to that found between 51 *A. halimus* populations (29.18%) from ten countries in the Mediterranean basin, analysed by RAPDs [29]. However, it was substantially higher than that (21.40%) reported for the annual widespread *Atriplex tatarica* [30].

Referring to the isoenzyme-based study, Haddioui and Baaziz [4] found that genetic diversity was essentially explained by the within population component. The proportion of the total diversity found within populations was 92%, leaving only 8% of the diversity between populations. Our results are in concordance with this trend. However, in the present study genetic differentiation (FST=0.334) between populations based on RAPD variation was observed to be more than four times higher than that (FST=0.08) based on isoenzyme analysis. The estimated gene diversity values for *A. halimus* (0.339 – 0.385), even considering the small number of allozyme loci examined [4], were higher than our estimates (0.167–0.281) RAPD-based. Furthermore, Hwang *et al.* [31] reported that genetic differentiation between populations of *Chamaecyparis formosensis* based on RAPD variation was observed to be about three times higher than that based on allozymes analysis. This comparison should be made with caution, as data obtained with isoenzyme markers, or with any other type of markers, are not directly comparable. This is due to the nature of the marker itself, since enzymes are markers that are not neutral, and generally are related to adaptive traits. The RAPD (about 95% of the bands) are non–coding regions of the genome and part of the repetitive DNA, and, therefore, are evolutionary neutral. Another important fact is related to the genome sampling. RAPD is a comparatively simple technique, which allows sampling a much greater number of loci than isozyme markers [32]. It is important to emphasize that dominance is a characteristic of the RAPD technique that can cause bias estimate of homologous parameters (Φ_{ST} versus FST). This bias does not occur with codominant markers (as is the case for isozymes) [33]. The greater sensitivity of RAPDs to population divergence may be derived from rapid evolution of non–coding repetitive DNA sequences detected by RAPDs [34].

Genetic differentiation among populations was not related to geographic distance in *A. halimus*. Similarly, no correspondence between geographic and genetic distances has been found in the widespread, long-lived, perennial species *Haloxylon ammodendron* [35], in the short-lived monocarpic forb *Gentianella germanica* [36], in the perennial forb *Lychnis viscaria* [37] and in the annual widespread heterocarpic *Atriplex tatarica* [30]. In contrast, significant correlations between genetic and geographic interpopulation distances has been found in the long-lived woody perennial species *Quercus petrae* [38], in the outcrossing woody species *Prunus mahaleb* [39] and in the rare perennial *Tradescantia hirsuticaulis* [40]. All these case studies are consistent with the view that a close relationship between geographic and genetic distances may only be expected if gene flow preventing isolation by distance is a simple function of geographical distance and if such gene flow is not overlaid by strong effects of genetic drift. The absence of such a correlation therefore suggests an important role of genetic drift in *A. halimus*, in line with the observed pronounced differentiation among populations.

The high level of variation found within populations suggests that sampling from a few localities for either breeding or conservation could capture a large proportion of the variation within the species. Since the level of genetic differentiation observed might be related to adaptive variation, structured progeny trails may be required to assess the performance of different populations in the different regions for traits of interest such as resistance to salinity and biomass production. Genetic divergence was particularly large among populations of *A. halimus*, which may be explained by a combination of genetic drift and restricted gene flow. However, isolation by distance has not played an important role in establishing the genetic structure of this species. Thus, the present study suggests that the evolutionary potential of *A. halimus* is high, because of the substantial level of genetic diversity within species and genetic differentiation among the populations.

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References

1. H.N. Le Houérou, The role of saltbushes (*Atriplex spp*) in arid land rehabilitation in the Mediterranean Basin: a review. Agroforestry Systems, **18**, 107 –148 (1992).
2. S. A. Foster, R. J. Scott, W. A. Cresko, Nested biological variation and speciation. Philos. Trans. R. Soc. Lond. Biol. Sci., **353**, 207–218 (1998).
3. G.F. Moran, Patterns of genetic diversity in Australian trees species. New Forests, **6**, 49–66 (1992).
4. Haddioui, M. Baaziz, Genetic diversity of natural populations of *Atriplex halimus* L. in Morocco: an isoenzyme-based overview. Euphytica, **121**, 99–106(2001).
5. A Haddioui, M. Baaziz, Effect of salinity on seed germination and early growth of five natural populations of *Atriplex halimus* in Morocco. Physiol. Mol. Biol. Plants, **12**, 247 –51(2006).
6. Haddioui, S. Bouda, M.M.Ould Mohamed Lemine, S. Hammada and M. El Hansali, Effect of salinity on growth of five natural populations of *Atriplex halimus* L. in Morocco. Journal of Agronomy, **7**: 97 –201 (2008a).
7. Haddioui, M.M.Ould Mohamed Lemine, L.H. Zinelabidine, M. El Hansali, S. Bouda, Variabilité phénotypique de la phénologie végétative et de la biomasse d'une espèce d'intérêt écologique et économique au Maroc: *Atriplex halimus* L.ecologia mediterranea, **34**, 65 –272 (2008b).
8. Williams, G.K., A.R. Kubelik, A.J. Livak, J.A. Rafalski and S.V. Tingey SV (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic ac Res **18**: 6531 –6535.
9. Rholf, F.J. (1998): NTSYS-pc: Numerical taxonomy and multivariate analysis system Version 2.02. Applied Biostatistics, New York.
10. Dice, L.R. (1945): Measures of the amount of ecological association between species. Ecol **26**: 297 –302.
11. Sokal, R.R., F.J Rohlf, the Comparison of dendrograms by objective methods. In: Official News Bulletin of the International Association for Plant Taxonomy, Edition International Bureau for Plant Taxonomy and Nomenclature, pp. 33-40 (1962).
12. Excoffier, L., P.E. mouse and J.M Quatro JM (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data Genet **131**: 479 –491.
13. Excoffier, L., G. Laval and S. Schneider (2005): Arlequin ver 30: An integrated software for population genetics data anlysis. Evolutionary Bioinformatics Online 1:47 –50.
14. Wright, S. (1951): The genetical structure of populations. Ann of Eugenetics **15**: 323 –354.
15. Slatkin, M. and N.H. Barton (1989): A comparison of three indirect methods for estimating average levels of gene flow. Evolution **43**: 1349 –1368.
16. Bouda, S., F.F. Del Campo, A. Haddioui, M. Baaziz and L.E. Hernandez (2008): RAPD and ITS-based variability revealed in *Atriplex* species introduced to semi-arid zones of Morocco. Scientia Horticulturae **118**: 172–179.
17. Loveless, M.D. and J.L. Hamrick JL (1984): Ecological determinants of genetic structure in populations. Ann.
18. Rev. Ecol. Syst. **15**: 65 –95.
19. Hamrick, J.L. and M.J.W. Godt (1990): Allozyme diversity in plant species. In: *Plant Population Genetics,*
20. *Breeding, and Genetic Resources* (eds) Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 43–63. Sinauer, Sunderland, MA.
21. Richter, T.S., P.S. Soltis and D.E. Soltis (1994): Genetic variation within and among populations of the narrow endemic, *Delphinium viridescens (Ranunculaceae).* American Journal of Botany **81**:1070–1076.
22. Soltis, P.S. and D.E. Soltis (2000): The role of genetic and genomic attributes in the success of polyploids.
23. Proceedings of the National Academy of Science of the USA **97**: 7051–7057.
24. Huff, D. R., R. PEAKALL and P. E. SMOUSE (1993): RAPD variation within and among natural population of outcrossing buffalograss [*Buchloedoctyloides* (Nutt.) *engelman*].Theor. Appl.Genet. **86**: 927–932.
25. Nesbitt, K.A., B.M. Potts, R.E. Vaillancourt, A.K. West, and J.B. Reid (1995): Partitioning and distribution
26. of RAPD variation in a forest tree species, *Eucalyptus globulus* (*Myrtaceae*). Heredity **74**: 628 –637.

27. H. Nybom, I.V. Bartish, Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in Plant Ecology, Evolution and Systematics, **3**, 93–164 (2000).
28. Hartl, D.L. (1987): A primer of population genetics, 2nd edn. Sinauer Associates Inc. Publishers. Sunderland,
29. Massachuetts.
30. Wright, S. (1978): Evolution and the Genetics of Populations, vol. 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago. USA.
31. Slatkin, M. (1985): Gene flow in natural population. Annual Review of Ecology and Systematics. **16**: 393 –
32. 430.
33. Telles, M.P.C., A.S.G. Coelho, L.J. Chaves, J.A.F. Diniz-Filho, F. D'ayala Valva (2003): Genetic diversity and population structure of *Eugenia dysenterica* DC. (*cagaiteira – Myrtaceae*) in Central Brazil: spatial analysis and implications for conservation and management. Conservation Genet **4**: 685 –695.
34. HAMRICK, J. L. and M. J. W. GODT (1989): Allozyme diversity in plant species, pp. 43–63 in Plant
35. Population Genetics, Breeding and Genetic Resources, edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER and B. S. WEIR, Sinauer Associates, Inc., Sunderland, Mass. USA.
36. J. Ortíz-Dorda, C. Martínez-Mora, E. Correal, B. Simón,J.L. Cenis, Genetic Structure of *Atriplex halimus*
37. populations in the Mediterranean Bassin. Ann. Bot., **95**, 827 –834 (2005).
38. Mandak, B., K. Bimova, I. Plackova, V. Mahelka and J. Chrtek (2005): Loss of Genetic variation in
39. geographically marginal populations of Atriplex tatarica (Chenopodiaceae). Annals of Botany **96**: 901–912.
40. Hwang, S.Y, H.W. Lin, Y.S. Kuo and T.P. Lin (2001): RAPD variation in relation to population
41. differentiation of *Chamaecyparis formosensis* and *Chamaecyparis taiwanensis.* Bot. Bull. Acad. Sin. **42:** 173 –179.
42. Zucchi, M.I., R.P.V. Brondani, J.P. Pinheiro, L.J. Chaves, A.S.G. Coelho and R. Vencovsky R (2003): Genetic structure and gene flow in *Eugenia dysenterica* DC in the Brazilian Cerrado utilizingSSR makers. Genetics and Molecular Biology **26**: 449 –457.
43. Lynch, M. and B. G. Milligan (1994): Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**: 91–99.
44. Plomion, C., N. Bahrman, C.E. Durel and D.M. O'Malley (1995): Genomic mapping in *Pinus pinaste*
45. (Maritime pine) using RAPD and protein markers. Heredity **74**: 661 –668.
46. Sheng, Y., W. Zheng, K. Pei and K. Ma (2005): Genetic Variation Within and Among Populations of a Dominant Desert Tree *Haloxylon ammodendron* (*Amaranthaceae*) in China Annals of Botany **96**: 245–252.
47. M. Fischer, D. Matthies, RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (*gentianaceae*). American Journal of Botany, 85(6), 811–819 (1998).
48. G. Berge, I. Nordal, G. Hestmark, The effect of breeding systems and pollination vectors on the genetic variation of small plant populations within an agricultural landscape. Oikos, **81**, 17 –29 (1998).
49. P. Bruschi, G.G. Vedramin, F. Bussotti, P. Grossoni, Morphological and molecular diversity among Italian populations of *Quercus petraea* (*Fagaceae*). Ann Bot, **91**, 707 –716 (2003).
50. P. Jordano, J. A. Godoy, RAPD variation and population genetic structure in *Prunus mahaleb* (*Rosaceae*), an animal-dispersed tree. Molecular Ecology, **9**, 1293–1305 (2000).
51. M.J.W.Godt, J.L. Hamrick, Genetic diversity and population structure in *Tradescantia hirsuticaulis* (*Commelinaceae*). American Journal of Botany, **80,** 959 –966 (1993).