

Full Length Research Paper

Preliminary molecular analysis of the genetic diversity of some *Atriplex* species present in the Northeast of Algerian

MÂALEM Souhaïl^{1, 3*}, KHOUFI Sahari², RAHMOUNE Chaâbane³ and BENNACEUR M'Barek²

¹Department of Life Sciences and Nature, Exact Sciences and Life Sciences and Nature Faculty, Tebessa University, 12000 Tébessa, Algeria.

²Laboratory of Plant Physiology and Biotechnology, National Institute of Agronomic Research of Tunisia (INRAT) Street Hedi Karray, 2080 Ariana, Tunisia.

³Laboratory of Ecotoxicology and Abiotic Stresses, Sciences life and nature Department, Sciences Faculty, Constantine Mentouri University, Road Ain el Bey, 25000 Constantine, Algeria.

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In the arid and semi arid areas, salt bush (*Atriplex*) represents an important forage resource. The characterization of the genetic diversity of these species is useful for their classification, their conservation and their improvement. In this context, we used the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique to test the genotypes of three different species of *Atriplex*: *Atriplex halimus*, *Atriplex canescens* and *Atriplex nummularia*. The Obtained results showed 319 amplified bands which were mostly polymorphic. The analysis of the results generated four different groups where the genotypes of two introduced species (*A. canescens* and *A. nummularia*) were clustered into two independent groups. However, genotypes of the autochthonous species (*A. halimus*) was subdivided into two different independent groups where we noted that individuals of one of these groups are genetically the nearest between them, by report, to the existing others within the same group. In general, results reveal inter and intra high level of genetic diversity in the *Atriplex* studied species.

Key words: *Atriplex*, *Chenopodiaceae*, genetic diversity, molecular analysis, steppe, Algeria.

INTRODUCTION

The *Atriplex* genre is a dicotyledonous that belongs to the family of *Chenopodiaceae*. It contains several distinguishable species by their morphology, their cycle of development and by their ecological adaptation (Barrow and Osuna, 2002). They are distributed in most regions of the globe, and their total number is appraised to 400 species (Kaocheki, 1996), of which 48 are own to regions of the Mediterranean basin. In the arid and semi-arid regions, the *Atriplex* species are endowed of an aerial and root-like biomass which is very important. They constitute an efficient tool and relatively little expensive in

the struggle against the erosion and the desertification (Essafi et al., 2007). In countries of the Maghreb, the present state of the steppe is not satisfactory because in addition to the impact of the human factor of which the most prominent is the overgrazing, the natural constraints, such as the drought and the salinization of soils led to a state of alarming deterioration that touched the almost-totality of the land of pastures (Dutuit et al., 1991).

In Algeria, the caused problems are the direct or indirect reasons of the forage production fall (Houmani, 1997). This genre endowed of the halophilic and xerophytic character would in association with other plant species, rehabilitate the severely degraded land grazing (Le Houérou, 1989; Dutuit et al., 1991; Valderrabano et al., 1996). However, the genetic structure of the *Atriplex*

*Corresponding author. E-mail: soubenyou@yahoo.fr. Tel: 00213 790 72 30 47. Fax: +213 37 49 02 48.

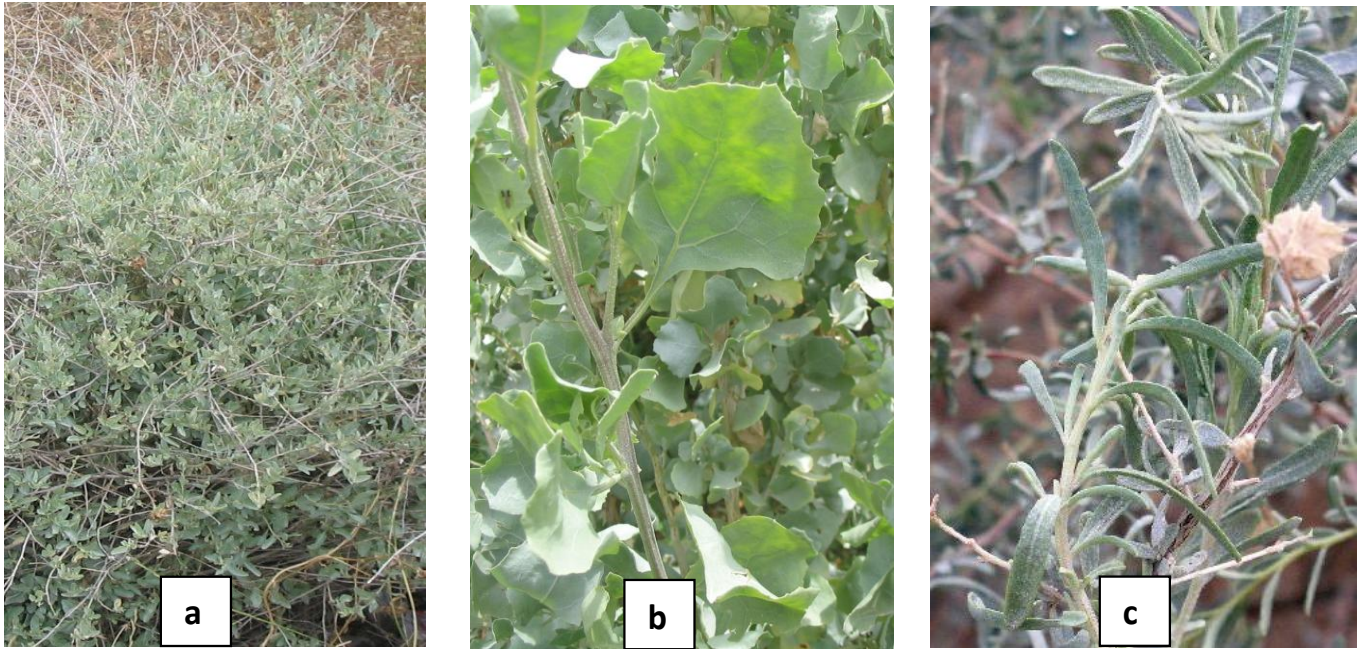


Figure 1. Photos of studied species: *Atriplex halimus* (a), *Atriplex nummularia* (b), *Atriplex canescens* (c).

genre remains little known, notably as for the Mediterranean species and again less for the Algerian. Little study is carried on the assessment of their genetic diversity where its master is necessary for the good management and the valorization of this plant genetic resource (Abbad et al., 2004). For example; the works of Bouda et al. (2008) and Haddioui and Baazizes (2001) carried on the molecular analysis of the genetic diversity of some *Atriplex* species existing in Morocco and in USA. And the works of Ortiz-Dorda et al. (2005) that had the same aspect, but these researches are realized on existing populations in all countries of the Mediterranean basin.

These works are among the rarest studies that have treated the previous subject and which had used RAPD-PCR technique. The works of Byrne et al. (2008) and Anderson et al. (2008) are behind the availability of specific primers simple sequence repeat (SSR)-PCR for the *Atriplex nummularia* but we ignore their efficiency for the rest species of *Atriplex* genre notably *Atriplex halimus* that is the local species. The objective of this work was to contribute to the study of the genetic variability of the three species of *Atriplex* genre of which one is autochthonous (*A. halimus*), whereas the other two (*Atriplex canescens* and *A. nummularia*) are originally from North America and Australia, respectively. For that we opted for the RAPD-PCR technique. Although it is not a specific discrimination tool, it remains, nevertheless, a very efficient means in the case of absence of more specific methods or in preliminary phase in the molecular studies of genetic diversity (Abdelhamid and Küpfer,

2004; Ortiz-Dorda et al., 2005; Kadri et al., 2006).

MATERIALS AND METHODS

The samples of studied *Atriplex* species were collected on different sites, of the region of Tebessa (Figure 1). First, young leaves from adult plants growing in the pastoral nursery of Thlidjen (situated in the Southeast of the city of Tebessa) were taken at random and 3 samples were taken from each species. The samples: H8, H9 and H10 represent the *A. halimus* species; C1, C2 and C3 represent the *A. canescens* species and N1, N2 and N3 represent the *A. nummularia* species. Other taking of out station was realized on other sites, and this is only for the case of the autochthonous species (*A. halimus* samples: H1, H2, H3, H4 and H6) mentioning that the two other *Atriplex* species (*A. nummularia* and *A. canescens*) do not exist in the natural state in the African continent. The sampling sites, the name of species as well as the numbers of the studied individual are presented in Figure 2.

Extraction and purification of the DNA

Genomic DNA was extracted from young leaves using the cetyltrimethyl ammonium bromide (CTAB), according to the method described by Porebski et al. (1997): briefly, 200 mg of plant tissue was ground to a fine paste in approximately 500 μ l of CTAB buffer then CTAB/plant extract mixture was transfer to a microfuge tube; also the CTAB/plant extract mixture was incubated for about 15 min at 55°C in a recirculating water bath. After incubation, the CTAB/plant extract mixture was allowed to spin at 12000 g for 5 min down cell debris. Subsequently, the supernatant was transferred to clean microfuge tubes. To each tube was added 250 μ l of Chloroform:Iso Amyl Alcohol (24:1) and the solution was mixed by inversion. After mixing, the tubes were spun at 13000 rpm for 1 min. The upper aqueous phase was transferred only (contains the DNA)

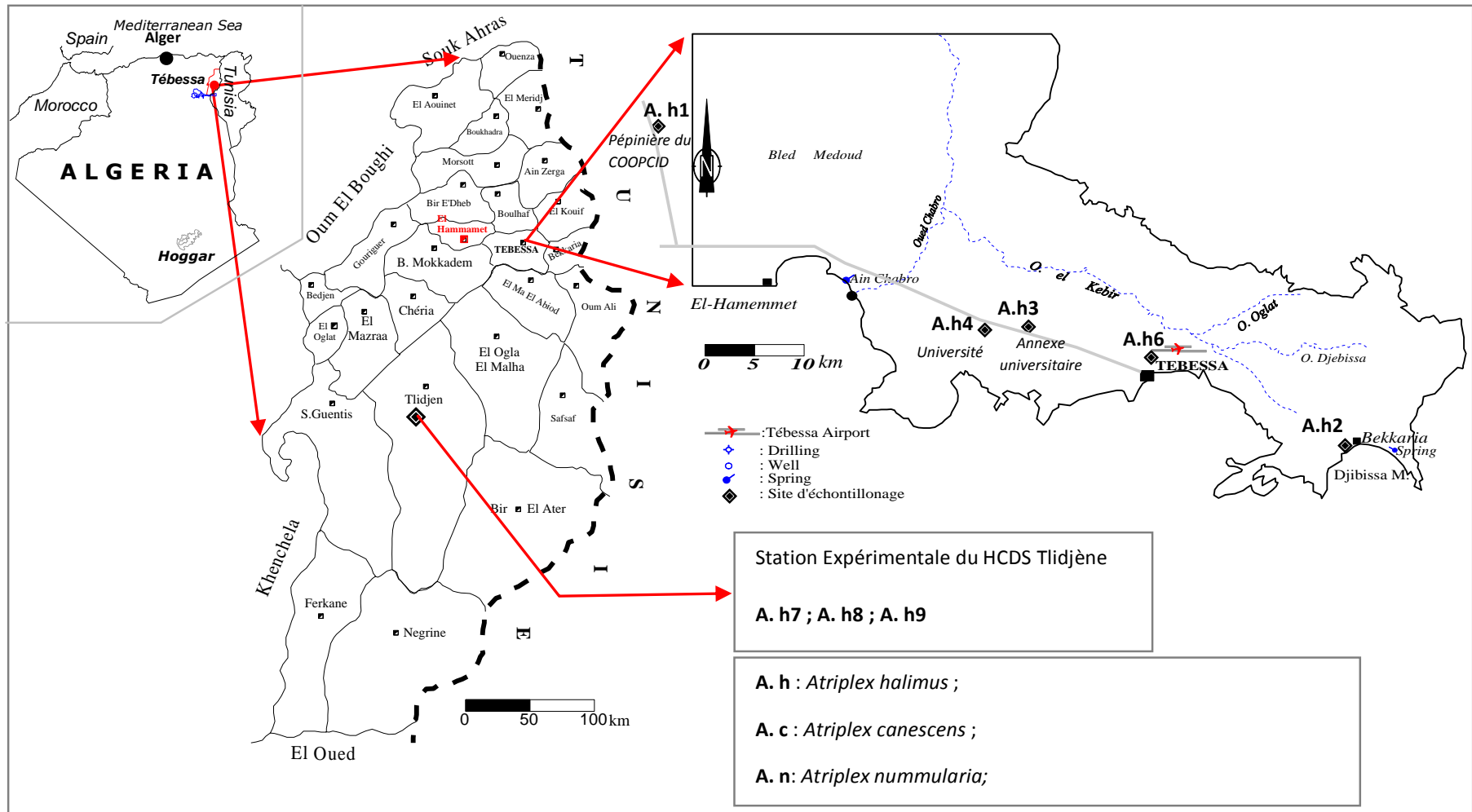


Figure 2. The representation of codification and the repartition of the studied genotypes on the plantation sites on the map of the city of Tebessa.

to a clean microfuge tube. To each tube was added 50 µl of 7.5 M ammonium acetate followed by 500 µl of ice cold absolute ethanol. Next, invert the tubes slowly several times to precipitate the DNA. Generally, the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 h at 20°C after the addition of ethanol

to precipitate the DNA.

Following precipitation, the DNA can be pipette off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice

cold, 70% ethanol and slowly invert the tube. Repeat (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold, 70% ethanol). After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm for 1 min.

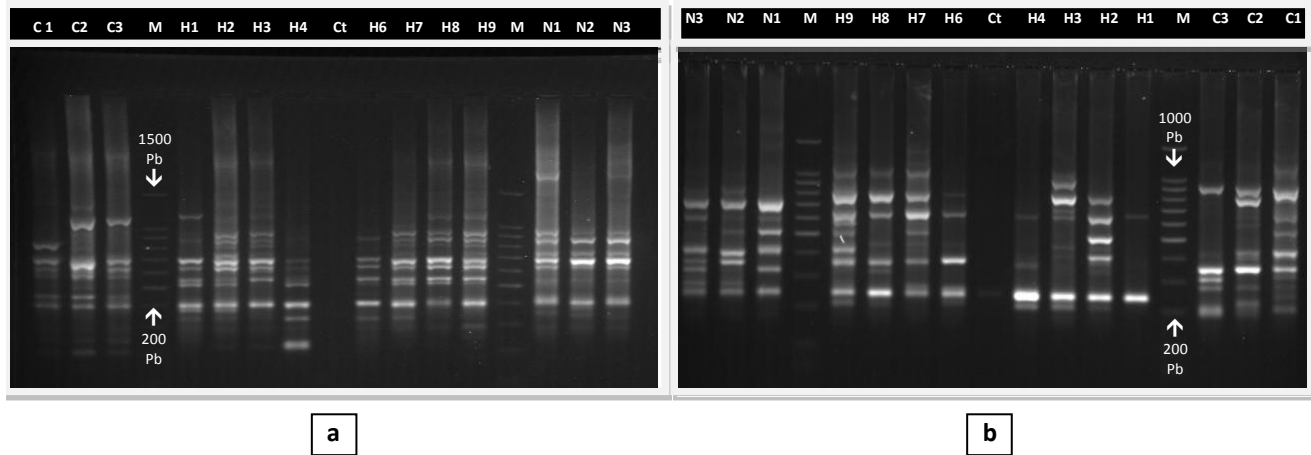


Figure 3. Example of agarose gel showing an amplified DNA profile of 14 genotypes belonging to 3 *Atriplex* species (RAPD-PCR reaction, using Primer OPA02 (Picture a) and OPC08 (Picture b)). C, H and N correspond respectively to the species: *A. canescens*, *A. halimus* et *A. nummularia*; M: Molecular marker (100Pb Ladder); Ct: negative control (without ADN).

Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). The DNA was not allowed to over dry because it will be hard to re-dissolve. Also, resuspend the DNA in sterile DNase free water (approximately 50 to 400 μ l H₂O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 μ g/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 μ l RNaseA in 10ml H₂O). After resuspension, the DNA is incubated at 65°C for 20 min to destroy any DNases that may be present and store at 4°C. Finally, agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.

Used primers

We tested 40 different primers by reason of the polymorphous bands quality produced. Only 20 was kept which include the following: OPA2, 5, 7, OPB1, 3, 6, OPC7, 8, 15, OPD8, 11, 15, OPE12, 18, OPG3, OPI16, OPK9, OPL2, OPN7, OPP8.

Amplification of DNA and electrophoresis

The amplification was done in a total volume of 25 μ l containing 2 μ l of extract of DNA (10 ng/ μ l), 5 μ l of tampon of the polymerization enzyme (5 x), 2 μ l of MgCl₂ (2.5 mM), 2.5 μ l of dNTPs (2 mM), 3.8 μ l of oligonucleotide primers (2 μ M), 0.2 μ l of Taq polymerase DNA (5 U/ μ l). The process of the polymerase chain reaction (PCR) consists of initial first denaturation (3 min to 95°C) follow-up of 45 cycles of amplification (30 s of denaturation to 95°C, 1 min of hybridization to 37°C, 2 min of extension to 72°C) and in a final extension (10 min to 72°C). The PCR products are separated by electrophoresis on agarose gel to 2% (TBE p/v) containing the bromide of ethidium, during 90 min. DNA bands were observed and photographed under the ultraviolet light.

Bioinformatics analysis of data

For each of the individuals and for every primer, the visual reading

of the gels allowed us to note the polymorphous bands. Thus, we assigned the value 1 for the present bands and 0 for the absent bands. After the introduction of the corresponding data to the gotten binary matrix, the SIMQUAL program (similarity quantitative for dated program) of the software NTSYS-pc (Tea Taxonomy numerical and Multivariate Analysis System for personnel to compute), Version 2.0 permitted to elaborate a dendrogram and a matrix based on the SM coefficient (Simple matching) of genetic similarity between the different studied *Atriplex* genotypes (*A. halimus* (H1, H2, H3, H4, H6, H7, H8 and H9), *A. Nummularia*(N1, N2 and N3) and *A. canescens* (C1, C2 and C3)).

RESULTS AND DISCUSSION

Genetic prints

This survey was permitted to detect some specific genetic prints for the 14 studied genotypes. The 20 retained primers in this test generated a very big number of polymorphous bands characterized by a molecular weight between 750 to 10000 bp. Figure 3 presents an example of profile generated by the primers OPA02 and OPA08. The 20 restraints primers produced a whole of 319 polymorphous bands. For all genotypes, the most elevated number of polymorphous bands is gotten by the primer OPI 16 with 26 bands, whereas the weak is obtained by the primers OPE 18 with only 7 bands. On the average, 17 bands by primers were produced (Figure 3).

Genetic similarity

The matrix of similarity (Figure 4), display a variation of the genetic similarity coefficient which comprises between (0.533 and 0.831), with an average of 0.682. The most elevated similarities were observed between the

	C1	C2	C3	H1	H2	H3	H4	H6	H7	H8	H9	N1	N2	N3
C1	1													
C2	0.762	1												
C3	0.759	0.796	1											
H1	0.655	0.712	0.727	1										
H2	0.564	0.589	0.599	0.69	1									
H3	0.58	0.63	0.652	0.693	0.771	1								
H4	0.665	0.69	0.73	0.796	0.668	0.727	1							
H6	0.639	0.696	0.737	0.828	0.699	0.708	0.831	1						
H7	0.549	0.592	0.621	0.674	0.734	0.774	0.677	0.69	1					
H8	0.577	0.614	0.655	0.74	0.705	0.74	0.73	0.743	0.777	1				
H9	0.552	0.577	0.599	0.646	0.762	0.759	0.655	0.655	0.796	0.749	1			
N1	0.552	0.533	0.567	0.602	0.586	0.583	0.574	0.586	0.564	0.599	0.592	1		
N2	0.618	0.586	0.627	0.699	0.627	0.618	0.702	0.696	0.611	0.671	0.621	0.69	1	
N3	0.567	0.574	0.596	0.605	0.539	0.542	0.596	0.602	0.561	0.589	0.533	0.727	0.712	1

Figure 4. Correlation matrix generated by RAPD Markers and representing the similarity coefficient between the genotypes studied.

following genotype combinations: (H4-H6:0.831), (H7-H9: 0.796) and (C2-C3: 0.796). The lowest similarities were obtained with combinations (H9-N3: 0.533), (N1-C2: 0.533) and (N3-H2: 0.539).

Inter and intra-specific polymorphism

In this study, we choose on the dendrogram (Figure 5) a value on the ladder of the coefficient similarity distinguishing the number of the produced groups. According to the dendrogram generated by the RAPD approach, we separated the studied genotypes into 4 distinct groups: The first (G1) contains all individuals only belonging to the American origin species: *A. canescens* (C1, C2 and C3), the second group (G2) contains also and exclusively individuals of the Australian species *A. nummularia* (N1, N2 and N3). However, the individuals of the local species (*A. halimus*) was shared between the groups (G3) and (G4) containing respectively the genotypes (H1, H4 and H6) and (H2, H3, H7, H8 and H9). These results also show that individuals of G3 are genetically the nearest between them, by report, to the existing others within the same group. In this survey, the couple H4-H6 is unusually the most genetically converging, with the most elevated similarity coefficient (0.831). The individuals of G4 also belonging to the local species aggregated with those of G1, to the second place. However, the individuals of the Australian species appear as the most distant between them. This reveals that the level of intra-specific genetic variability, of this

last species, is so raised. The nature of the reproduction of the species (*A. nummularia*) would be able to explain this character, viewing that the plants of this species have exclusively allogamy mode of reproduction, contrary to the other two (Francllet and Houérou, 1971).

The dendrogram also permits us to notice that the group 4 (G4) is itself subdivided into 2 other groups; the first is composed of the genotypes: H2 and H3 and the second is composed of the genotypes H7, H8 and H9. The genetic convergence recorded between the last genotypes (G4) is justified by the fact that all their plants are reproduced within the same pastoral nursery of Thlidjene (Figure 2) and by consequence their seeds could belong to plantations having some common parents, or even, they could be collected on the same plant or have been multiplied again by cuttings, in this same nursery. Paradoxically, the genotypes H4 and H6 of the *Atriplex halimus*, existing in the spontaneous state (Figure 2) record the biggest similarity in this survey are geographically, relatively distant from one another. But, Tunisians (Chalbi et al., 1997) and Moroccans studies (Abbad et al., 2004) led on this species, reveal that the polymorphism existing within these populations is more important when they are situated in a different climate, that is on a north-south orientation axis that is parallel to climatic aridity, which is not the case in our study. More difficulty conceived is the fact that genotypes of the G3 are nearer to those of the G1 than those of the G4, whereas groups 3 and 4 correspond to the same species (*A. halimus*) and the G1 characterizes another species (*A. canescens*).

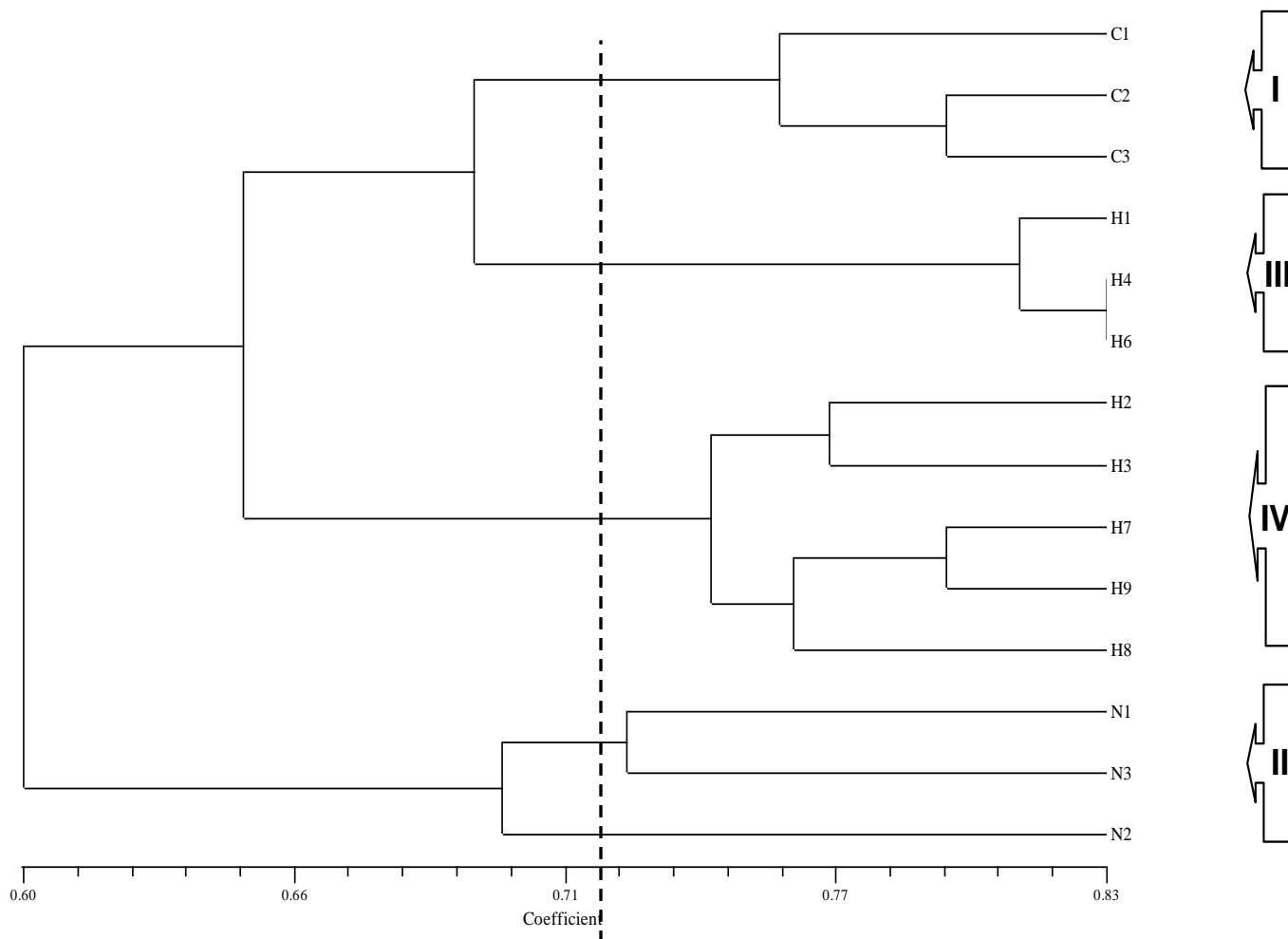


Figure 5. Dendrogram representing the genetic distances existing between genotypes generated by RAPD markers. (Based on the similarity coefficient (simple matching (SM))). The formed groups:(I, II, III and IV) are mentioned on the right of figure. The vertical discontinue line indicates the point of separation of groups.

This big divergence recorded between the genotypes of the G3 and G4 could find its origin in the big genetic diversity characterizing the populations of *A. halimus*. According to an isoenzymatic study achieved in Morocco, an ample genetic convergence has been recorded between the populations of *A. halimus* and that was due to differences of allele's frequencies rather than to their polymorphism (Haddioui and Baaziz, 2001). Ortiz-Dorda et al. (2005), find also that the large genetic diversity characterizing the populations of *A. halimus* can be due, in addition to their ecological adaptation, to their allogamy mode of reproduction, leading to an elevated level of gene flux. According to the study of Soltis and Soltis (2000), the polyploidy characters of the plants present a big genetic diversity from multiple ancestors and generate a more heterozygosis elevated level. Concerning the interspecific genetic relation, the dendrogram (Figure 5) situate the local species *A. halimus* (originated from North Africa) in inter-medium position and this

function in the genetic distance that separates the three studied species. This corresponds with their geographical origins, noting that the two exotic species (*A. nummularia* and *A. canescens*) are introduced respectively from Australia and North America. Therefore, a Morocco study similar to ours situated the *A. canescens* in an inter-medium position between *A. halimus* and *A. nummularia*.

Conclusion

The results of this study show that the molecular RAPD primers, in spite of their non-specific characters showed a strong faculty to the genetic characterization between the *Atriplex* species and their high polymorphism level. The RAPD-PCR technique permits, in cases of a preliminary study and/or no more specific markers availability, the realization of some exploitable discrimination. In our case, the different studied species were isolated in

separate groups. However, the interpretation of some cases of separations and/or the genetic bringing together of some species is still strongly discussed. Generally, we noted within the same group that the studied genotypes of the local species (*A. halimus*) appeared to look more genetically like the genotypes of the American species (*A. caescens*).

Individuals of the Australian species were characterized by an important dissimilarity between them, which could be due to their exclusively allogamy reproduction mode. The surprising bringing together observed between the G3 of the *A. halimus* species and the G1 of the *A. canescens* species could be explained by the theory of gene flux link to the mode of allogamy reproduction of these species in question. We also thought that the large existing divergence between the genotypes of *A. halimus* species, belonging to the two groups, G3 and G4, would find its origin in hétérozygote and polyplôide nature characterizing this last species. It is important to retain the fact that the result of the interspecific genetic variability analysis shows that the local species is situated in an inter-medium position that go in pair with its geographic situation.

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