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Morphological variability of fruit and chromosome numbers in Tunisian populations of *Atriplex halimus* L. (Chenopodiaceae)

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Abstract — *Atriplex halimus* L. (Chenopodiaceae) can be divided in two subspecies: the diploid *halimus* (2n=2x=18) and the tetraploid *schweinfurthii* (2n=4x=36). This was based on differences in morphology, with respect to habit, size, leaf shape and fruit morphology. *Atriplex halimus* L. is a perennial shrub used as a fodder in the arid and semi-arid parts of North Africa and the Middle East. *A. halimus* is dominant in the semi-arid and sub-humid areas while *A. schweinfurthii* is more common in arid areas. Natural populations of *A. halimus* L. exhibit large levels of plant morphological and physiological variability. However, little is known about the morphological variability of its fruits. We located and sampled ten populations of *Atriplex halimus* L. in Tunisia to assess their fruit diversity. Fruit shape, size, length, width, and weight, measured on samples collected from all ten populations, were highly variable suggested a height phenotypic variability in the natural populations of *Atriplex halimus* L.
In another hand, chromosome numbers for five populations of *Atriplex halimus* L. from Tunisia were determined. Although the Tunisian populations originated from widely-separated sites of contrasting climatic conditions, the chromosome counts showed that all of them were tetraploid (2n = 4x = 36).

Key words: *Atriplex halimus* L., Chenopodiaceae, chromosomes numbers, fruits, morphological variability, Tunisia.

INTRODUCTION

The genus *Atriplex* (Chenopodiaceae family) (Chadefaud 1960) contains various species distinguishable by different morphology, biological cycles and ecological adaptations (Le Houerou 1992). Many species of *Atriplex* are excellent livestock fodder source due to their high protein content, especially when grasses are not available for grazing. *Atriplex*, is an important component of arid land vegetation (Haddioui 2001). *Atriplex halimus* L. biotypes in North Africa are extremely salt-tolerant (Grillot 1956), and are considered as important forage plants in large parts of the pastoral areas of arid and semi-arid zones, especially when other traditional forages are not available (Le Houerou 1992). Moreover, it is considered as a promising forage plant for large-scale plantings (Valderrabano 1996) in arid and semarid parts of the country. *Atriplex halimus* L. is widely distributed throughout the Mediterranean Basin (Ortiz-Dorda 2001). Based on morphological differences, two sub-species are usually recognized: *A. halimus* L. var. *halimus* (2n = 2x = 18) and *A. halimus* L. var. *schweinfurthii* (2n = 4x = 36). The two sub-species show relatively large levels of morphological variability.

The base chromosome number in the genus *Atriplex* is x = 9 (Nobs 1975) with variable ploidy levels occurring in several species, such as *A. gardneri* that is mostly tetraploid (2n = 36), *A. tridentata* mostly hexaploid (2n = 54) (Stutz *et al.* 1979), *A. prostrata* is diploid (2n = 18) and *A. patula* is tetraploid (2n = 36) (Katembe *et al.* 1998). *A. confertifolia* shows variation in ploidy level (Stutz and Sanderson 1983): ploidy levels of 2x, 4x, 6x, 8x and 10x are known in this species, with tetraploids being the most often encountered (Sanderson *et al.* 1990). Highly variable chromosome numbers have been observed in *A. canescens* (Sanderson and Stutz 1994). There is little literature concerning ploidy levels in *Atriplex halimus* L. Diploid populations (2n = 2x = 18) have been reported from Israel (Osmond *et al.* 1980), while Zhu *et al.* (2001) reported the existence of tetraploid (2n = 4x = 36) and hexaploid (2n = 6x = 54) populations from Tunisia and Morocco and triploid plants from Morocco (2n = 3x = 27). Considerable variability has been described within *Atriplex. halimus* L., at both the morpho-

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logical and isozyme levels (LE HOUÉROU 1992; HADDIOUI and BAAZIZ 2001; ABBAD *et al.* 2003). Differences in floral sex ratio (male/female flowers), floral architecture and other vegetative and fruit morphological characteristics, related to population and growth conditions, have been reported recently (ABBAD *et al.* 2003; TALAMALI *et al.* 2003; HCINI *et al.* 2004).

Characterization and conservation of *A. halimus* genetic resources are particularly important for the rehabilitation of disturbed areas affected by salt and low rainfall (HADDIOUI 2001). However, few genetic investigations have been carried out on this species. It is necessary to find more discriminating criteria, which could provide information about the genotypes, by more direct measurements of morphological diversity among populations belonging to the same species. Our objective was to describe the morphological variability of fruit of ten natural populations of *Atriplex halimus* L. originating from different localities in Tunisia area and to determine chromosome numbers for five populations of *Atriplex halimus* L. encompassing a wide range of edapho-climatic conditions.

MATERIAL AND METHODS

Source of fruits - Fruits of ten natural populations of *Atriplex halimus* L. growing in different eco-geographical sites in Tunisia were collected. The main characteristics of these sites are summarised in Table 1.

Table 1 — Populations of *Atriplex halimus* L. used in this study and their main characteristics.

Population	Code	Floor/Under-floor	severity
Tunis	A	Semi-arid superior	mild
Siliana	B	Semi-arid means	moderated
Monastir*	C	Semi-arid lower	soft
Sousse	D	Semi-arid lower	soft
Kairouan*	E	Arid superior	moderated
Kasserine	F	superior Arid	harsh
Sidi Bouzid*	G	Arid superior	moderated
Gabès*	H	Arid lower	soft
Médenine	I	Arid lower	soft
Tataouine*	J	Arid lower	soft

* Population for chromosome counts

Measured variables - Two qualitative characters have been recorded on individual fruits, these were the shape and the dry fruit size, and four quantitative variables were recorded on 200 individual fruit of each population (Table 2).

Table 2 — Variables recorded on single fruits of *A. halimus* collected from 10 populations in Tunisia.

Variables	Abbreviation	Units
Dry fruit weight	DFW	(g)
Dry fruit length	DFL	(mm)
Dry fruit width	DFWi	(mm)
DFL-DFWi ratio	DFL/DFWi	

Statistical analysis - Analysis of variance (DAGNELIE 1975) was carried out on all four quantitative fruit variables, followed by mean separation using Duncan Multiple Range Test (5%). Then, to study the structuring of variability in *A. halimus*, a hierarchical classification and a canonical discriminant analysis, based on FISHER (1936), were carried out on the adjusted means of 200 individuals, representing ten populations (Tunis, Siliana, Monastir, Sousse, Kairouan, Kasserine, Sidi Bouzid, Gabès, Médenine, and Tataouine respectively designated as A, B, C, D, E, F, G, H, I and J). Statistical analysis was performed on quantitative variables using SAS (1990). Finally, the data was clustered using hierarchical average-linkage cluster analysis.

Chromosome counts - Seeds of five populations Gabes, Tataouine, Monastir, Sidi bouzid and Kairouan were sown in Petri dishes, on paper towels wetted with tap water. Root tips were pre-treated with colchicine (1mg/1ml) for 12 h at 4 °C to arrest mitosis and fixed in 3:1 ethanol:acetic acid. After hydrolysis in 5 N HCl for 30mn, then the roots were stained in reagent of shiff. Apical root tips (1mm long) were squashed in 45% glacial acetic acid under cover slips on microscope slides. At least five slides were observed for each seedling.

RESULTS

Comparison of fruit morphology between populations of Ariplex halimus L.- The geographic distribution of *Atriplex halimus* L. encompasses the arid and semiarid parts of Tunisia (GOUNOT and LE HOUEROU 1955; GOUNOT and LE HOUEROU 1967; CARRIÈRE 1986); this distribution was verified by the information collected during the germplasm collection (Fig. 1).

Fruits of ten populations of *Atriplex halimus* collected from Tunisia (Fig. 2) display clear morphological differences in size and shape. With regard to the size, large fruits were found in the regions of Tunis, Monastir, Sousse and Siliana, fairly big ones in the regions of Kairouan, Kasserine,

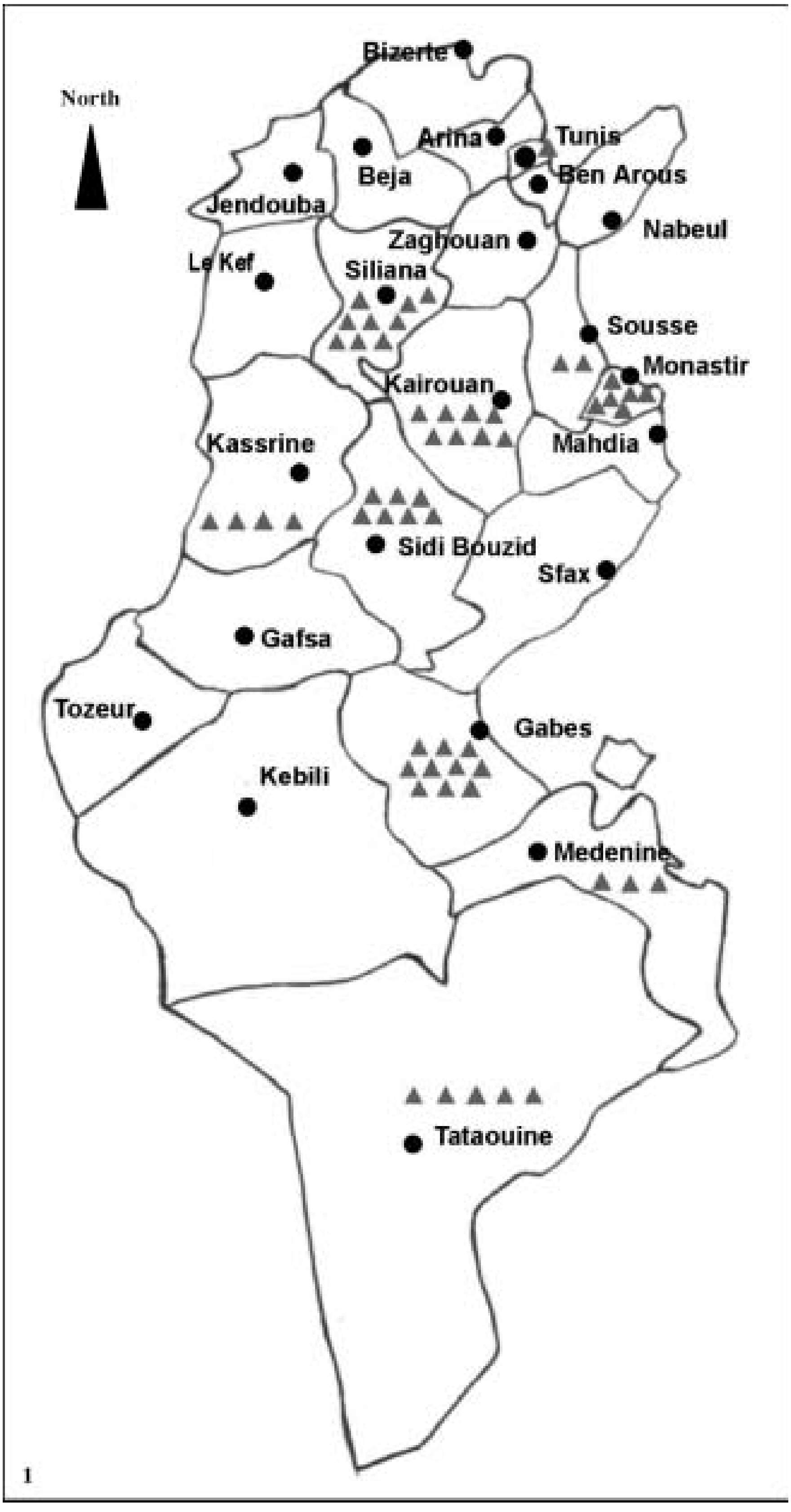

Fig. 1 — Geographical distribution of *Atriplex halimus* L. in Tunisia.

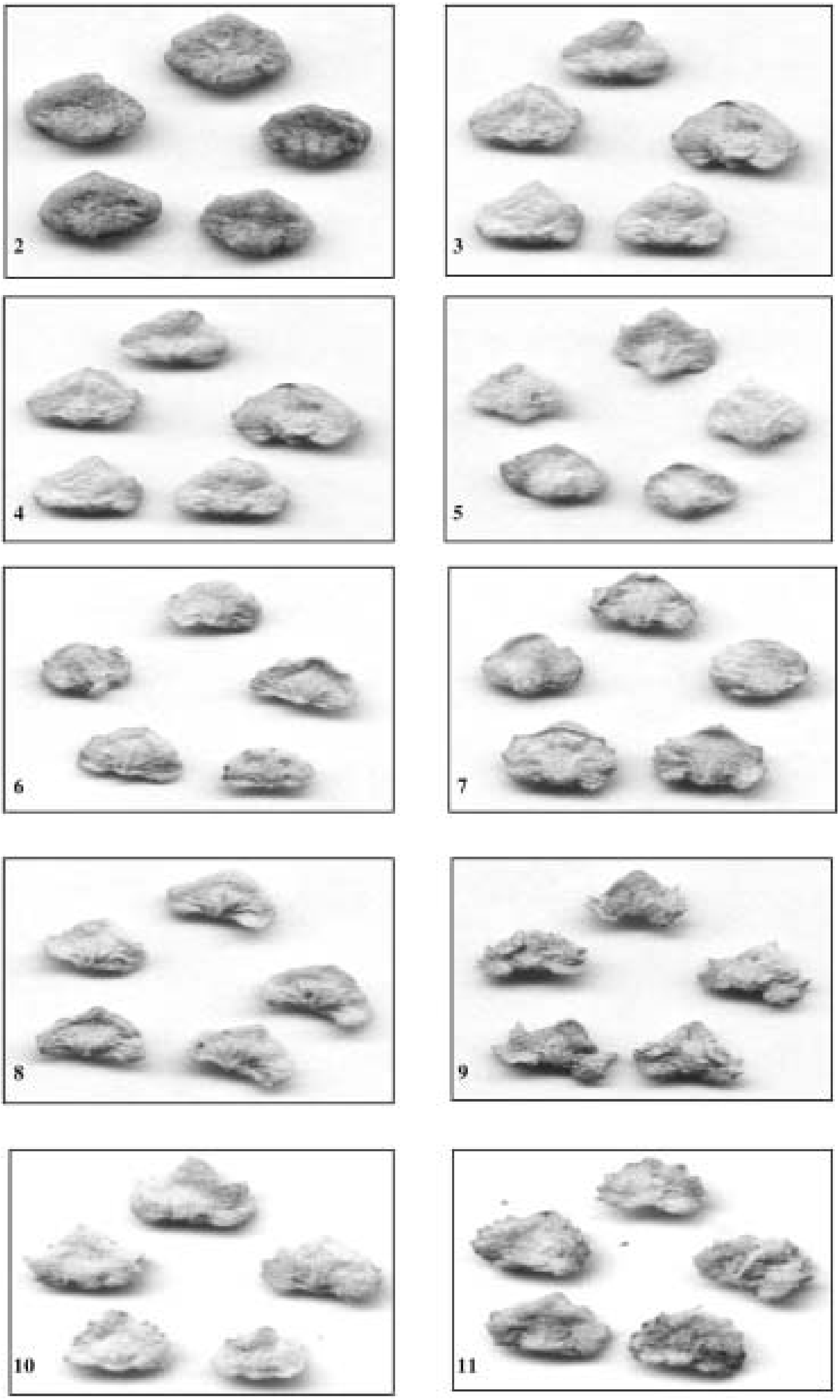

Fig. 2 — Comparison of fruit morphology between populations of *Atriplex halimus* L. 2. Tunis, 3. Monastir, 4. Sousse, 5. Siliana, 6. Kairouan, 7. Kasserine, 8. Sidi Bouzid, 9. Gabes, 10. Medenine, 11. Tataouine.

and Sidi Bouzid, and small-size fruits in Gabes, Médenine, and Tataouine. The shape of fruits differed from smooth to whole margin, for those collected from Tunis, Monastir, Sousse and Siliana, and with toothed margins for populations collected from Gabès, Médenine and Tataouine.

A. halimus is a sub-species having bracteoles with whole wing margins, whereas *A. schweinfurtii* is characterized by bracteoles having distinctly toothed wings on the margins (FRANCLET and LE HOUEROU 1971).

Analysis of variance and mean comparisons - An assessment of intrapopulation versus interpopulation variability of quantitative fruit descriptors in *Atriplex halimus* L. was carried out on all three fruit quantitative traits (DFW, DFL and DFWi). A wide range of variation was found among different populations (Table 3). The comparison of means (Table 4) reveals that for the DFWS, DFLS and DFWi characters, populations of the North of Tunisia, (A, B,C and D) are similar and have the largest mean values for fruit traits, whereas populations collected from southern Tunisia (H, I and J) have the smallest.

Table 3 — Analysis of variance.

Variables	MS(a)	MS(r)	F
DFW: Dry fruit weight	0.023	0.003	7.76**
DFL: Dry fruit length	21.260	0.851	24.97**
DFWi: Dry fruit width	10.826	0.085	126.61**
DFL/DFWi: DFL-DFWi ratio	0.095	0.077	1.24*

$F_{theoric}$ =1.83 (5%); 2.32 (1%)
MS(a): Factoriel mean square
MS(r): Residuel mean square
**Highly significant
*Non significant

Canonical discriminant analysis - The first three components scores account for 98.02; 1.04 and 0.62% of the total variation, respectively. Axis 1 was highly correlated with DFWi and DFL, axis 2 with DFW and axis 3 with DFL/DFWi (Table 5). The first canonical discriminant root was strong enough to separate populations from southern Tunisia from those collected from central and northern parts of the country. However, very little separation was achieved among populations on the second canonical discriminant root. Populations in the first group have smaller DFL and DFWi, as compared with the second group of populations (Fig. 3).

Table 4 — Comparison of means by the Duncan test.

Population	DFW	DFL	DFWi	DFL/ DFWi
A	0.200a*	4.800b	4.040b	1.193a.b
B	0.194a.b	4.850b	3.910b.c	1.124a.b
C	0.215a	5.727a	4.272a	1.355a
D	0,180a.b.c	4.917b	3.925b.c	1.254a.b
E	0.160b.c.d	4.660b	3.855b.c	1.208a.b
F	0.159b.c.d	4.767b	3.765c	1.275a.b
G	0.150c.d.e	4.475b	3.347d	1.345a
H	0.122d.e	3.000c.d	2.682e	1.125b
I	0.117e	3.185c	2.670e	1.222a.b
J	0.125d.e	2.487d	2.057f	1.216a.b

* Populations having the same letter are not significantly different.

Cluster analysis - The results of cluster analysis suggest that geographic origin partially accounts for the genetic distances among the populations (Fig. 4). It is clear that populations from the north and center of Tunisia (A, B, C, D, E, F, G) diverge from those located in the south of the country.

The dendrogram produced from the average linkage cluster analysis reveals that the diploid sub-species *halimus* from north Tunisia (A, B, D) diverges from populations located in the center of Tunisia (E, F), which are proposed to contain the dipoid sub-species *halimus* and the tetraploid *schweinfurthii*.

It should be noted that D and C populations, which are close geographically, do not cluster together in the dendrogram ordination. This situation was observed for E, F and G, from the center of the country. Populations from southern Tunisia, H, I and J, cluster together. The grouping of the southern populations supports the proposed origin of *A. schweinfurthii* as a tetraploid sub-species.

Table 5 — Canonical discriminant analysis.

Canonical axis	Axis 1	Axis 2	Axis 3
Eigen values	7.51	0.08	0.04
Proportion (%)	98.02	1.04	0.62
Cumulative (%)	98.02	99.06	99.68
Variables defining the axis and their correlations	DFL (+0.771) DFWi (+0.984)	DFW (+0.731)	DFL/DFWi (+0.960)

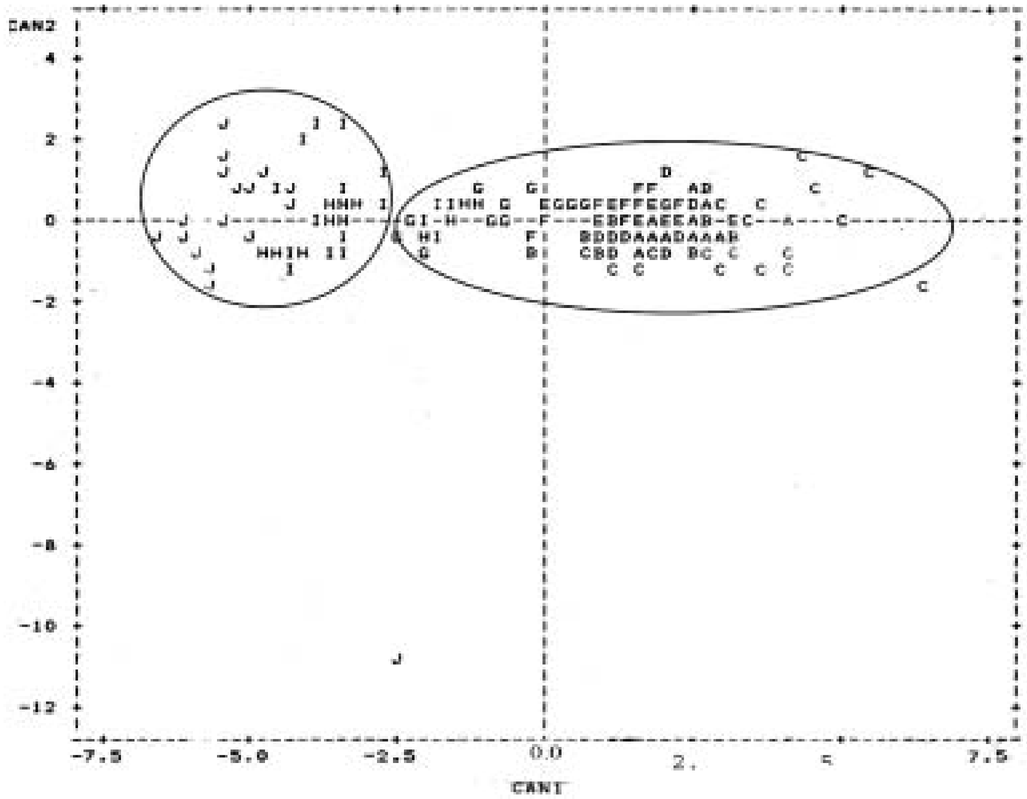

Fig. 3 — Plot of 10 A. halimus populations along the first two canonical discriminant roots.

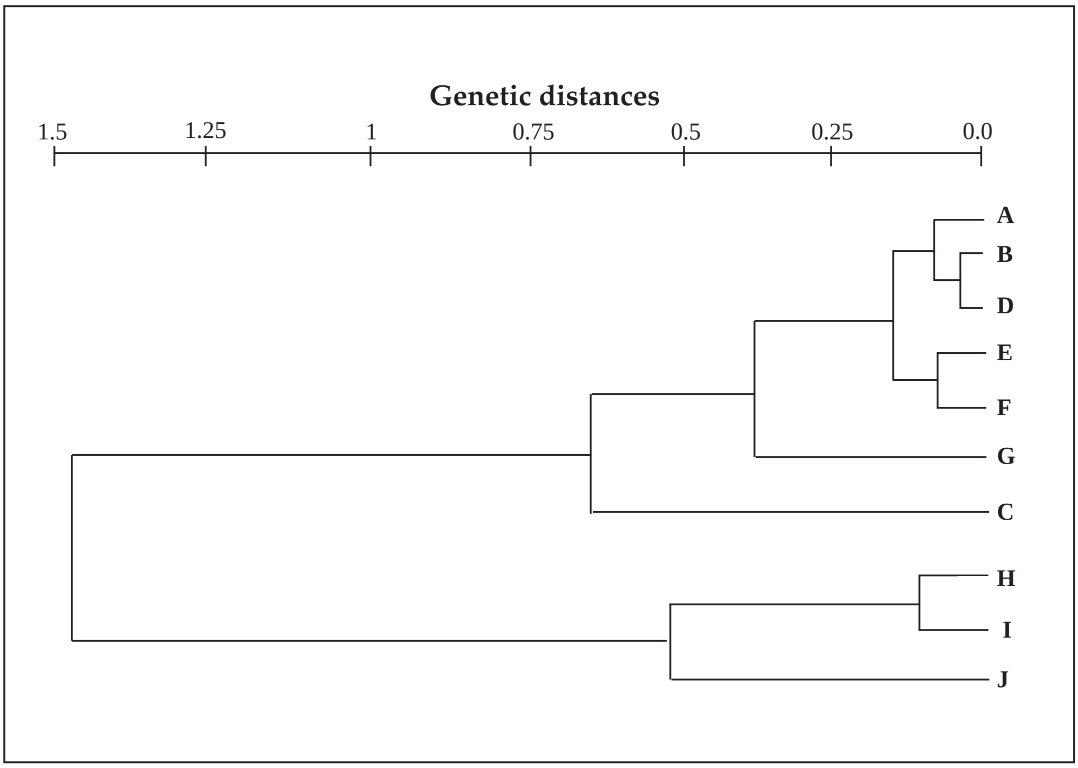

Fig. 4 — Representation of ten natural populations of *Atriplex halimus* L. in average linkage cluster analysis.

Chromosome counts - For the Tunisian populations, the chromosome number in a somatic metaphase nucleus was observed to be 36 (Figure 5). Thus, since the base chromosome number in the genus *Atriplex* is x = 9 (NOBS 1975), these populations are tetraploid (2n = 4x = 36). With respect to their morphology, the tetraploid populations from Tunisia studied here correspond to subspecies *schweinfurthii*.

DISCUSSION

The qualitative and quantitative character analyses of ten populations of *Atriplex* demonstrated the existence of some degree of polymorphism in their fruits. The ten populations show clear morphological differences, and considerable variability has been described within *A. halimus*, at both the morphological and isozyme polymorphism levels (FRANCLET & LE HOUEROU 1971; LE HOUEROU 1992; HADDIOUI and BAAZIZ 2001; ABBAD *et al.* 2003). Differences in floral sex ratio (male/female flowers), floral architecture and other vegetative and fruit morphological characteristics, related to population and growth conditions, have been reported recently (ABBAD et *al.* 2003; HCINI et *al.* 2004; TALAMALI *et al.* 2003). Based on differences in morphology, with respect to habit, size, leaf shape and fruit morphology, *A. halimus* has been divided into two subspecies: *halimus* (= var. *typica* Aellen or var. *genuina* Maire & Weill.) and *schweinfurthii* Boiss. (= var. *glaucoidea* L. Chevall., var. *ramosissima* L. Chevall or var. *argutidens* Bornm) (FRANCLET & LE HOUEROU 1971; LE HOUEROU 1992). In Tunisia, FRANCLET and LE HOUEROU (1971) reported that subsp. *halimus* occurred in sub-humid and semi-arid areas whilst subsp. *schweinfurthii* was found in arid areas (annual rainfall < 400 mm).

Although the Tunisian populations came from differing edapho-climatic conditions, all were adapted to arid/semi-arid environments and all were tetraploid. Regarding distribution, *halimus* generally grows in higher-rainfall (> 400 mm/year) zones, in western Mediterranean areas such as France and Spain and on Atlantic coasts, whilst *schweinfurthii* is adapted to arid zones (100-400 mm rainfall/ year) in North African and Eastern Mediterranean countries (LE HOUÉROU 1992). Much of the Mediterranean basin became more arid in the period 1000-500 BC (LE HOUÉROU 1981); and *schweinfurthii* may have subsequently populated such areas. Other factors which may influence the distrbution of diploid and tetraploid poipulations of *Atriplex halimus* L. are soil type, since ssp. *schweinfurthii* is often found on gypsiferous or saline soils (LE HOUÉROU 1992), and the seasonal distribution of precipitation.

The general observation that ssp. *schweinfurthii* populates more arid zones could be considered as an example of a better adaptation of polyploid populations of woody shrubs to more extreme environments (SANDERSON *et al.* 1989;

Fig. 5 — Chromosome numbers in Kairouan population of *Atriplex halimus* L. from Tunisia, chromosome number observed = 2n = 36.

STUTZ 1989). However, the reasoning for this was based on their lower growth rates and smaller size (SANDERSON *et al*. 1989), so the greather growth rates and stature of ssp. *schweinfurthii* populations argue against this. Altenatively, the greater genetic heterozygosity of polyploids, at both individual and populations levels, may give them a selective advantage in unstable environments (SANDERSON *et al.* 1989; SOLTIS and SOLTIS 2000). Both subspecies of *Atriplex halimus* L; are extremely heterogenous in terms of their morphology, ecology, productivity and oalatability to herbivores. (LE HOUÉROU 2000).

According to SOLTIS and SOLTIS (2000), 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. In this way, polyploid may have a much betteradaptability to diverse ecosystems, which may contribute to their success in nature.

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