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Full Length Research Paper

Chromosome numbers in Tunisian populations of *Atriplex halimus* L. (Chenopodiaceae)

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***Atriplex halimus* L. (Chenopodiaceae) can be divided in two subspecies: the diploid *halimus* (2n = 2x = 18) and the tetraploid *schwainfurthii* (2n = 4x = 36). This was based on differences in morphology, with respect to habit, size, leaf shape and fruit morphology. Chromosome numbers for 5 populations of *A. halimus* L. from Tunisia (Gabès, Kairouan, Monastir, Sidi Bouzid, Tataouine) 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). Staining was done with the Feulgen reaction for the identification of the chromosome numbers in the nucleus. The results are discussed in relation to adaptation of *A. halimus* L. populations to differing climatic conditions.**

Key words: *Atriplex halimus*, Chenopodiaceae, chromosome numbers, polyploidy.

INTRODUCTION

The genus *Atriplex* (saltbushes) is the dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil salinity with aridity (Osmond et al., 1980). Most saltbush species prosper in areas with annual rainfall ranging from 200 to 400 mm. Saltbushes provides forage for domestic livestock and wildlife, and are effective in erosion control and stabilization of severery disturbed lands (Beadle, 1952; Springfield, 1970; Ansley and Abernethy, 1984; Booth, 1985). The *Index Kewensis* cites 417 species of *Atriplex.*

Among the species of *Atriplex* in North Africa, *Atriplex halimus* L., (Chenopodiaceae) a monoecious, highly outbreeding, C_4 perennial shrub, is found in semi-arid and arid environments. It is of interest because of its tolerance of environmental stresses, its use as a foder shrub for livestock in low rainfall Mediterranean areas (Le Houérou, 1992; Haddioui and Baaziz, 2001), and is considered as a promising forage plant for large-scale plantings. It has been divided into two subspecies: *halimus* (= var. *Typica* Allen or var. *Genuina* Maire et Weill.) and *schweinfurthii* Boiss. (= var. *Glaucoidea* L. Chevall., var. *Ramosissima* L. Chevall or var. *Argutidens* Bornm) (Le Houérou, 1992).

The base chromosome number in the genus *Atriplex* is x = 9 (Nobs, 1975) with variable ploidy levels occurring in several species. For example *A. gardneri* is mostly tetraploid (2n = 36), *A. tridentata* is 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 *A. halimus* L. Diploid populations (2n = 2x = 18) have been reported from Israel (Osmond et al., 1980), and Zhu et al. (2001) reported the existence of a tetroploid (2n = 4x = 36) and a hexaploid (2n = 6x = 54) populations from Tunisia and Morroco, respectively, as well as triploid pla-

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Table 1. Description of the original locations of the populations of *Atriplex halimus*.

Populations	Mean temperature (°C): Max. in hottest month	Mean temperature (°C): Max. in coldest month	Annual precipitation (mm)	Emberger Precipitation-Temperature Quotient[a]
Gabès	32.5	5.8	193	21
Kairouan	37.6	4.4	321	28
Monastir	33.4	9.2	383	44
Sidi Bouzid	37.8	5.7	237	25
Tataouin	39.7	4.7	107	13

nts from Morroco (2n = 3x = 27). Considerable variability has been described within *A. halimus* at both the morphological and isozyme polymorphism 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).

Bearing in mind previous reports of different ploidy levels among *A. halimus* populations, the aim of this work was to determine ploidy levels, using chromosome counting for populations of *A. halimus* collected from different sites in Tunisia, encompassing a wide range of edapho-climatic conditions.

MATERIAL AND METHODS

Plant materials

Five natural populations of *A. halimus* collected from different geographic sites in Tunisia (Gabes, Tataouine, Monastir, Sidi Bouzid and Kairouan) were analysed. The principal characteristics of all sites studied are summarised in Table 1.

Cytogenetic analysis

Seeds of the Tunisian 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 (1 mg/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 30 min, the roots were stained in reagent of shiff. Apical root tips (1 mm long) were squashed in 45% glacial acetic acid under cover slips on microscope slides. At least five slides were observed for each seedling.

RESULTS AND DISCUSSION

For the Tunisian populations, the chromosome number in a somatic metaphase nucleus was observed to be 36 (Figure 1). Thus, since the base chromosome number in the genus *Atriplex* is x = 9 (Nobs, 1975), these populations are tetraploid (2n = 4x = 36).

Polyploidy is one of the most important mechanisms in plant evolution. About 30-35% of phanerogamous species are polyploid. The ploidy levels frequently identified are tetraploid and hexaploid. Within the genus *Atriplex*, the diploid state has been found in 26 species recorded in California (Nobs, 1975) and 27 endemic species in Australia (Nobs, 1979). More recently, meiotic chromosome counts of n = 9 (2n = 18) have been determined from wild plants of *Atriplex* (Subgenus Theleophyton) *billardierei* gathered in New Zealand and on Chatham Island (De Lang et al., 1997). The existence of polyploidy has been found in *Atriplex canescens* (Stutz et al., 1975; McArthur, 1977; Sandeson and Stutz, 1994), in *A. tridentata* (Stutz et al., 1979), in *A. confertifolia* (Stutz and Sanderson, 1983; Sanderson et al., 1990). *A* diploid population of *A. halimus* has been reported previously from Israel (Osmond et al., 1980). Although the Tunisian populations came from differing edapho-climatic conditions, all were adapted to arid/semi-arid environments and all were tetraploid. Population Cala Tarida, adapted to more favourable conditions of water availability, is diploid. These results could be considered as an example of a better adaptation of polyploid populations of woody shrubs to more extreme environments (Sanderson et al., 1989; Stutz, 1989). With respect to their morphology, the tetraploid populations from Tunisia studied here correspond to subspecies *schweinfurthii*. 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 *A. halimus* are soil type, since subspecies *schweinfurthii* is often found on gypsiferous or saline soils (Le Houérou, 1992), and the seasonal distribution of precipitation.

The general observation that subspecies *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; Stutz, 1989). However, the reasoning for this was based on their lower growth rates and smaller size

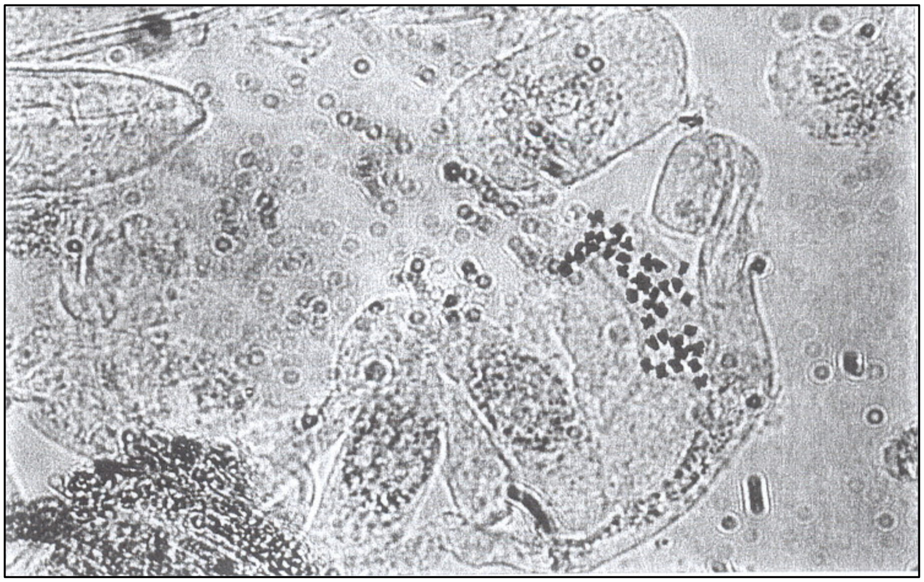

Figure 1. Chromosome numbers in Kairouan population of *Atriplex halimus* L. from Tunisia, chromosome number observed = 2n = 36.

(Sanderson et al., 1989), so the greather growth rates and stature of subspecies *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 *A. halimus* are extremely heterogenous in terms of their morphology, ecology, productivity and palatability 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 better adaptability to diverse ecosystems, which may contribute to their success in nature.

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