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# Estimation of nuclear DNA content in Algerian populations of *Atriplex halimus* and *Atriplex canescens* by flow cytometry

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## Resumen

*Estimación del contenido de ADN nuclear en poblaciones argelinas de* Atriplex halimus *y* Atriplex canescens *por citometría de flujo .*

*Atriplex halimus* es un arbusto perenne nativo de la cuenca mediterránea con una excelente tolerancia a la sequía y la salinidad. *Atriplex canescens*, de origen americano, se adapta muy bien a los climas desérticos. El objetivo de este trabajo fue estimar el contenido de ADN nuclear en poblaciones argelinas de *A. halimus* y *A. canescens* por citometría de flujo, un método eficaz para estimar los niveles de ploidía y para determinar el contenido de ADN nuclear en las plantas. La citometría de flujo es una técnica que permite la estimación rápida de contenido de ADN nuclear. Con respecto al ADN nuclear, el ADN contenido 2C de la población de Cala Tarida (España) se estimó en 2.46pg. No hubo diferencias significativas entre las poblaciones de *A. halimus*, cuyo contenido 2C de ADN oscilaron entre 4,91 y 5.04pg. Sin embargo, el contenido 2C de ADN de *A. canescens* oscilaron entre 3,14 y 3,27.

**Palabras clave:** *Atriplex halimus*, *Atriplex canescens*, Citometría de flujo, ADN nuclear.

## Abstract

*Atriplex halimus* is a perennial native shrub of the Mediterranean Basin with an excellent tolerance to drought and salinity, also, *Atriplex canescens*, four-wing saltbush, is adapted to desert climates. The aim of this work was to estimate nuclear DNA content in Algerian populations of *A. halimus* and *A. canescens* by flow cytometry, an efficient method to estimate ploidy levels and to determine nuclear DNA content in plants. Flow cytomertry is a technique which permits rapid estimation of nuclear DNA content. With respect to nuclear DNA, the 2C DNA content of population Cala Tarida (Spain) was estimated to be 2.46pg. There was no significant difference among the populations of *A. halimus*, whose 2C DNA content ranged from 4.91 to 5.04pg. However, the 2C DNA content of *A. canescens* ranged from 3.14 to 3.27.

**Key words:** *Atriplex halimus*, *Atriplex canescens*, Flow cytometry, Nuclear DNA content.

## Introduction

Polyploidy, which is known to occur in numerous dryland shrubs, is present in one third of the known *Atriplex* species (Stutz 1989). Polyploidy is one of the most important mechanisms in plant evolution. As in the majority of the Chenopodiaceae, the base chromosome number in *Atriplex* is considered to be x = 9 (Nobs 1975). *A. halimus*, perennial shrub, is found in semi-arid and arid environments. It is interest because of its tolerance of environmental stresses, its use as a foder shrub for livestock in low rainfall Mediterranean areas (Le Houerou 1992, Cibilset et al. 1998, Zervoudakis et al. 1998, Haddioui & Baaziz 2001), and is considered as a promising forage plant for large-scale plantings (Valderrábano et al. 1996). Also, *Atriplex canescens* (Pursh) Nutt., fourwing saltbush, is adapted to desert climates and is a valuable forage shrub because it is abundant, palatable, provides large quantities of forage, is nutritious, and grows rapidly (Cibilset et al. 1998, McArthur et al. 1983, Peterson et al. 1987).

In nature, considerable variation in nuclear DNA content occurs both within and among plant species. Manipulation of ploidy level is an important tool for plant breeding in a number of crops. Estimation of nuclear DNA content is one of the important applications of flow cytometry. Flow cytometry is increasingly employed as the method of choice for determination of nuclear DNA content and ploidy level in plants (Galbraith et al. 1997). Compared to conventional chromosome counting flow cytometry turned out to be highly competitive in terms of simplicity, accuracy and costs.

The aim of this work was to estimate nuclear DNA content in *A. halimus* and *A. canescens* based on flow cytometry. This would allow elucidation of the relationships between ploidy, species, morphology and edapho-climatic conditions for these important shrubs.

## Materials and methods

### Plant material

Plants of nine populations collected from Algeria: Five populations of *A. halimus* and four populations of *A. canescens* and two populations which preliminary analyses had shown to be diploid (2n= 2x = 18) (Cala Tarida, Spain) and tetraploid (2n= 4x = 36) (Buteria, Italy), respectively, were analysed. Details of the original locations of these populations are given in table 1. Plants were grown from seeds in a peat-soil mixture, in a greenhouse for about four weeks. Leaves of these plants were used for flow cytometry. For most populations, three plants were analysed. For each plant, one measurement was conducted in each analysis; an analysis being performed on three different days to give 12 measurements per population.

### Flow cytometry

Flow cytometric estimation of nuclear DNA content was performed with a Partec PA II flow cytometer, using propidium iodide (PI) as the fluorescent stain. Samples of growing leaf tissue of *A. halimus* and tomato (*Lycopersicon esculentum* Mill., cv. Stupicke polni) (20 mg fresh weight each) were prepared together. Leaf material was chopped with a razor blade for 30-60 s, in a plastic Petri dish containing 0.4 mL of extraction buffer (Partec CyStain PI Absolute P Nuclei Extraction Buffer; Partec GMBH, Münster, Germany). The resulting extract was passed through a 30-µm filter into a 3.5-mL plastic tube, to which was then added 1.6mL of Partec CyStain PI Absolute P Staining Buffer, to give final PI and RNase concentrations of 6.3 µg mL-1 and 5.0 µg mL-1 respectively. Samples were kept in darkness for 30 min. before analysis by flow cytometry (preliminary assays had shown no effect of storage time on the measurements).

All stages of the extraction and staining were performed at 4°C. For cytometry, 20-mW argon ion laser light source (488 nm wavelength) (Model PS9600, LG-Laser Technologies GmbH, Kleinosthein, Germany) and RG 590 longpass filter were employed. *A. halimus* and *A. canescens* nuclear DNA was estimated by the internal standard method, using the ratio of the *A. halimus*/tomato G0/G1 peak positions (Galbraith et al. 1997, Dolezel 1997). The precision and linearity of the flow cytometer were checked on a daily basis using 3-µm calibration beads (Partec). The gain of the instrument was adjusted so that the peak representing the G0/G1 nuclei of the internal standard was positioned on channel 100. At least 5000 nuclei were analysed in each sample and the 2C

| Species | Populations | Latitude | Longitude | Altitude m.a.s.l. | Mean T of coldest month °C | Soil parameters | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | pH saturated paste | EC dS $m^{-1}$ | Soluble Na meq $kg^{-1}$ | Soluble K meq $kg^{-1}$ |
| *A. halimus* | El-Biodh | 33º54'27"N | 00º20'43"W | 989 | -8 | 7.61 | 4.69 | 3.40 | 0.63 |
| | El-Kasdir | 33º42'52"N | 01º23'31"W | 981 | -6 | 7.66 | 3.18 | 1.34 | 0.39 |
| | El-Kheiter | 34º08'29"N | 00º04´´15"E | 989 | -8 | 7.80 | 32.80 | 92.7 | 9.27 |
| | Maamoura | 34º37'29"N | 00º33'00"E | 1106 | -6 | 7.89 | 1.30 | 2.08 | 0.11 |
| | Oran | 35º38'23"N | 00º36'46"W | 92 | -5 | 7.29 | 25.10 | 86.7 | 2.08 |
| *A. canescens* | Ain El Hadjar I | 34º45'31"N | 00º07'08"E | 1008 | -8 | 8.09 | 1.84 | 2.69 | 1.53 |
| | Ain El Hadjar II | 34º45'42"N | 00º08'50"E | 1036 | -10 | 8.08 | 0.93 | 1.96 | 0.05 |
| | Maamoura | 34º36'34"N | 00º33'03"E | 1106 | -6 | 7.93 | 1.61 | 2.37 | 0.06 |
| | Mzila | 35º65'45"N | 03º83"50"E | 1280 | -9 | 7.38 | 26.12 | 78.30 | 1.90 |

Tabla 1. Descripción de las localizaciones originales de las poblaciones de *Atriplex halimus* y *Atriplex canescens*.
Table 1. Description of the original locations of the populations of *Atriplex halimus* and *Atriplex canescens*.

nuclear DNA content of the unknown sample was calculated according to a formula: Sample 2C DNA content = (sample peak mean/standard peak mean) x 2C DNA content of standard.

## Results and discussion

Representative histogram of the flow cytometric analyses of El Kasdir and Maamoura populations is shown in figure 1. The coefficient of variation (= (100 x standard deviation) / mean) values of *A. halimus* L. G0/G1 peaks ranged from 1.5 to 4.4 %. The results of nuclear DNA content analysis are shown in Table 2. With respect to nuclear DNA, the 2C DNA content of population Cala Tarida (Spain) was estimated to be 2.46pg. There was no significant difference among the populations of *A. halimus*, whose 2C DNA content ranged from 4.919 to 5.049pg. However, the 2C DNA content of *A. canescens* ranged from 3.14 to 3.27.

Franclet & Le Houérou (1971) and Le Houérou (1992) divided *A. halimus* L. into two subspecies, *A. halimus* S.E. and *A. schweinfurthii*. This was based on differences in morphology, with respect to habit, size, leaf shape and fruit morphology. With respect to their morphology, populations of *A. halimus* studied here were tetraploid and correspond to ssp. *schweifurthii*. Haddioui & Baaziz (2001), who studied isozyme polymorphism in *A. halimus* L., found a high degree of within-population genetic diversity. In the current study, there were no significant differences among plants within populations with respect to nuclear DNA content. This kind of approach, together with studies of morphology and isoenzyme polymorphism, as well as molecular techniques could be employed on a wider (and more detailed) geographical scale to ascertain the phylogenetic relationships within the species (David et al. 2005, Hcini et al. 2006).

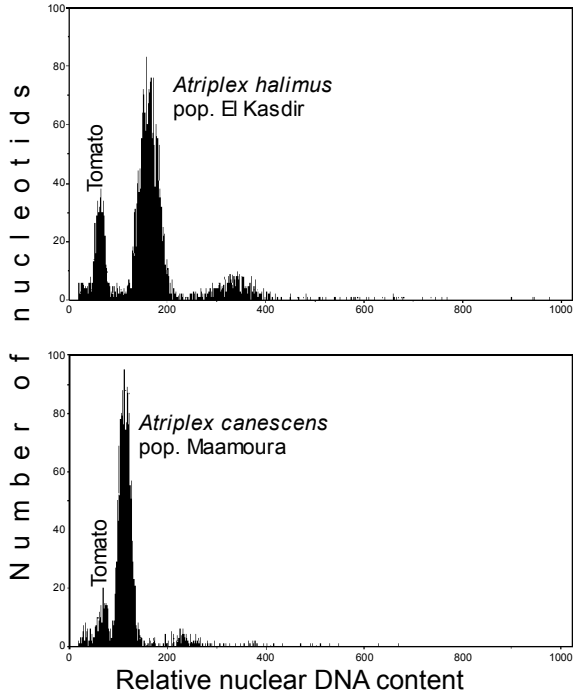


Figure 1. Análises de citometría de flujo. **a:** *Atriplex halimus*, población de El Kasdir. **b:** *Atriplex canescenes* población de Maamoura. Las Hojas se cortaron y entonces se marcaron con yoduro de propidio empelando tomate como estandar interno (contenido 2C de ADN nuclear: 1.96 pg).
Figure 1. Flow cytometric analyses. **a:** *Atriplex halimus* population of El Kasdir. **b:** *Atriplex canescenes* population of Maamoura. Leaf samples were chopped and then stained with propidium ioide using tomato (2C nuclear DNA content: 1.96 pg). as internal standar.

| Populations | 2C nuclear DNA content (pg) | |
|---|---|---|
| | Mean | s.d. |
| ***Atriplex halimus*** | | |
| El-Biodh (Naama) | 4.993 | 0.086 |
| El-Kasdir (Naama) | 5.005 | 0.099 |
| El-Kheiter (El-Bayad) | 4.994 | 0.058 |
| Maamoura (Saida) | 4.919 | 0.028 |
| Oran (I.A.P) | 5.049 | 0.016 |
| Cala Tarida | 2.460 | 0.079 |
| Butera | 4.826 | 0.083 |
| ***Atriplex canescens*** | | |
| Ain-El-Hadjar I (Saida) | 3.147 | 0.026 |
| Ain-El-Hadjar II (Saida) | 3.274 | 0.054 |
| Maamoura (Saida) | 3.247 | 0.058 |
| Mzila | 3.143 | 0.052 |

Figura 1. Cantidades medias estimadas de ADN nuclear (2C) (pg) ± s.d. para las poblaciones estudiadas *Atriplex halimus* y *Atriplex canescens.*

Figure 1. Estimated mean nuclear DNA (2C) amounts (pg) ± s.d. for the studied populations of *Atriplex halimus* and *Atriplex canescens.*

Flow cytometry can be used to determine (DNA) ploidy (Lysac &Dolezel 1998, Emshiller 2002), although cytological studies are required for confirmation (Bennett et al. 2002). This protocol showed to be convenient (sample preparation is easy), rapid (several hundreds of samples can be analysed in one working day), it doses not require dividing cells, it is non-destructive (one sample can be prepared, e.g., from a few milligrams of leaf tissue), and can detected mixoploidy. Therefore the method is used in different areas ranging from basic research to plant breeding and production.

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