CYTOMETRIC INVESTIGANTIONS OF GENOME SIZE AND LEVEL OF PLOIDY IN SOME PLANTS WITH ORNAMENTAL POTENTIAL

INVESTIGAȚII CITOMETRICE ALE DIMENSIUNII GENOMULUI ŞI NIVELULUI DE PLOIDIE LA UNELE PLANTE CU POTENȚIAL ORNAMENTAL

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Abstract. *Cytometric determination of nuclear DNA content has proved to be useful in studying the variation in interspecific and intraspecific DNA content in plants. The ability to estimate DNA content stimulated a vast array of applications, which ranged from basic research to breeding and seed production. The main objective of this study is to establish the genome size specific to species of plants with ornamental potential and to examine the ploidy level of the species using the technique of flow cytometry. Our results showed that all the investigated species are diploid. We also revealed the quantity of DNA specific to Allium ursinum (65,21 pg 2C DNA) and Lilium martagon (91,56 pg 2C DNA).*

Key words: genome size, ploidy level, ornamental potential plants.

Rezumat. *Determinările citometrice ale conținutului de ADN nuclear s-au dovedit a fi un parametru util în studierea variațiilor intra- şi interspecifice ale cantității de ADN la plante. Posibilitatea estimării cantității de ADN a stimulat un areal vast de aplicații, începand cu cercetarea fundamentală şi până la încrucişări şi producția de semințe. Obiectivul principal al studiului de față este de a determina mărimea genomului specifică unor specii de plante cu potențial ornamental şi de a examina gradul de ploidie al acestor specii folosind tehnica citometriei în flux. Rezultatele noastre au demonstrat că toate speciile investigate sunt diploide. De asemenea, a fost determinată cantitatea de ADN specifică speciilor Allium ursinum (65,21 pg 2C DNA) şi Lilium martagon (91,56 pg 2C DNA).*

Cuvinte cheie: marimea genomului, grad de ploidie, plante cu potential ornamental.

INTRODUCTION

The nuclear DNA content and the ploidy level of a cell can be measured at high speed by flow cytometry. Cytometric determination of nuclear DNA content has proved to be useful in studying the variation in interspecific and intraspecific DNA content in plants.

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The first flow cytometers quantified DNA in cells by measuring absorbance of UV light (Kamentsky et al., 1965). This approach was soon abandoned for fluorescence (Dittrich and Gohde, 1969; Van Dilla et al., 1969). DNA analysis by flow cytometry has been used extensively in biomedical research to detect aneuploidy (Kawara et al., 1999), apoptosis (Vermes et al., 2000) and to monitor cell cycle kinetics and its perturbations (Rabinovitch, 1994). Attempts to apply the method in plants were hampered by difficulties in preparation of suspensions of intact cells and nuclei suitable for flow cytometry. In the first successful experiment, Heller (1973) stained nuclear DNA with ethidium bromide.

The ability to estimate DNA content stimulated a vast array of applications, which ranged from basic research to breeding and seed production, and included estimation of nuclear genome size (Hulgenhof et al., 1988), ploidy screening (De Laat et al., 1987), detection mixoploidy (Roux et al., 2001) and aneuploidy (Roux et al., 2003), assessment of the degree of polysomaty, reproductive pathways (Matzk et al., 2000), and cell cycle kinetics (Sandoval et al., 2003).

For cell DNA quantity measurements, the results are expressed in picograms (pg) and the status of the nuclei is expressed in C values. A haploid nucleus has a C quantity of DNA, and a diploid nucleus has a 2C quantity of DNA (Dolezel et al. 2007; Roberts A.V. 2009). The cytometric method implies also the simultaneous measurement of DNA content in the nuclei of an internal control which has the role of calibrating the 2C DNA content for the investigated species (Greilhuber et al., 2005). As for the investigations on ploidy level, the calibrator is an external control belonging to the same species as the analyzed one (Dolezel et al. 2007).

The main objective of the study is to establish the genome size specific to species of plants with ornamental potential and to examine the ploidy level of the species using the technique of flow cytometry.

MATERIAL AND METHOD

The biological samples were received from the University of Agricultural Sciences and Veterinary Medicine „Ion Ionescu de la Brad", and consist of dried seeds belonging to the following species with ornalemntal potential: *Parnassia palustrus* (Botanical Garden, Iasi); *Allium ursinum* (Barnova); *Lilium martagon; Aconitum degenii* (Botanical Garden, Iasi); *Centaurea phrygia (*Botanical Garden, Iasi).

Flow cytometry analysis of ploidy level and DNA quantity. This method requires a cytometer equiped with a excitation source (laser) and a detector suitable for capturing fluorescence emission of propidium iodide (PI). Because PI also stains double-stranded RNA, a previous RNase treatment is reqiured.

Isolating and staining the nuclei. For the estimation of the specific DNA quantity, the tissue corresponding to 3-20 seeds is placed in a plastic Petri dish together with the internal standard tissue (*Allium cepa* seeds - 32,97pg/2C), they are chopped into fine pieces using a sharp razor blade together in 500 µl nuclei isolation/staining buffer containing 50 µg/ml PI and 50 µg/ml RNase. The nuclear suspension is filtered through a 30- or 50-µm nylon mesh to remove large debris. The sample is incubated for 20 to 60 min at room temperature. For ploidy level analysis

the internal standard is prepared separately from the target sample, following the same steps and is analyzed before the other samples.

Flow cytometric analysis was performed on a FC 500 Cytometer (Beckman Coulter). Approximately 10000 events (20-50 events/sec) were acquired on flow cytometer and analyzed using CXP 2.2 software. The cytometer was calibrated with Flow Check fluorescent beads (Beckman Coulter) before the determinations.

RESULTS AND DISCUSSIONS

Cuantification of DNA quantity specific to the investigated species

Estimation of DNA quantity for the target sample has been obtained following the formula:

$$\text{sample 2C DNA content (pg DNA)} = \frac{\text{sample G1 peak mean}}{\text{standard G1 peak mean}} \text{ X standard 2C DNA content}$$

Considering a 1:1 ratio of AT:GC base pairs and ignoring the presence of modified nucleotides, Dolezel et al. (2005) stated that 1 pg DNA =0.978 x 10^9 bp. This formula was used to convert the amount of DNA expressed in picograms, in number of base pairs. We worked with three replicates per species in three different days to avoid cytometer fluctuations.

Two species with ornamental potential were investigated for the detection of specific DNA quantity: *Allium ursinum* and *Lilium martagon* (figure 1).

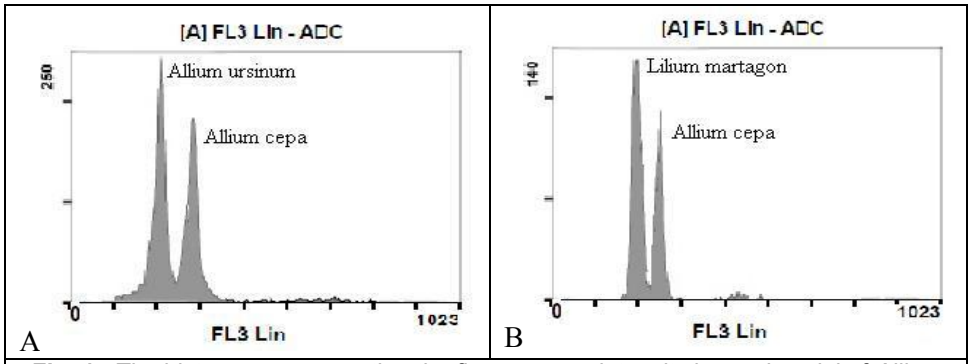

Fig. 1 - The histogram representing the fluorescence intensity in seed nuclei of *Allium ursinum* (A) and *Lilium martagon* (B) stained simultaneously with the internal standard *Allium cepa* (32,97pg 2C ADN).

The amount of DNA 2C specific to each species was calculated based on the above mentioned formula. The determined genome size in *Allium ursinum* is 65,21pg 2C DNA, and for *Lilium martagon* – 91,56pg 2C DNA (table 1).

Table 1

Ploidy level and DNA quantity specific to the analyzed species

Species	Ploidy level	ADN 1C (pg) amount	ADN 1C (bp) amount	ADN 2C (pg) amount
Allium ursinum	2n = 14	32,6	31,88 x 10^9 bp	65,21
Lilium martagon	2n = 24	45,78	44,77 x 10^9 bp	91,56

Our data confirm the previous results obtained during other scientific studies.

Analyzing the specific content of DNA for nearly 300 species of angiosperms, Zonneveld and his team (2005), reported for *Lilium martagon* a DNA amount value of 93.2 pg (DNA - 2C). According to their studies, Baranyi and Greilhuber (1999), found a value of 60.34pg 2C DNA specific to *Allium ursinum*; Labani and Elkington (1987) – 71,39pg 2C ADN for the same species and Ohri et al. (1998) – 63, 57pg.

Analysis of the ploidy level specific to the investigated species

For ploidy estimation, the channel number of the sample G1 peak is determined and the ploidy of the sample is calculated according to the following equation:

$$\text{sample ploidy level} = \frac{\text{external standard G1 peak mean}}{\text{sample G1 peak mean}} \text{ X external standard ploidy level}$$

If the linear scale is used and the G1 peak of the diploid external standard is set at channel 50, the G1 peak of a triploid should appear at channel 75, a tetraploid at 100, and so on.

For analysis of seed ploidy, two seeds were included in one sample. Detection of a single G0/G1 peak in the resulting histogram indicates that both seeds are of the same ploidy. If two G0/G1 peaks appear, the seeds differ in ploidy.

The ploidy level was determined for the following species: *Aconitum degenii, Allium ursinum, Centaurea phrygia* and *Lilium martagon.*

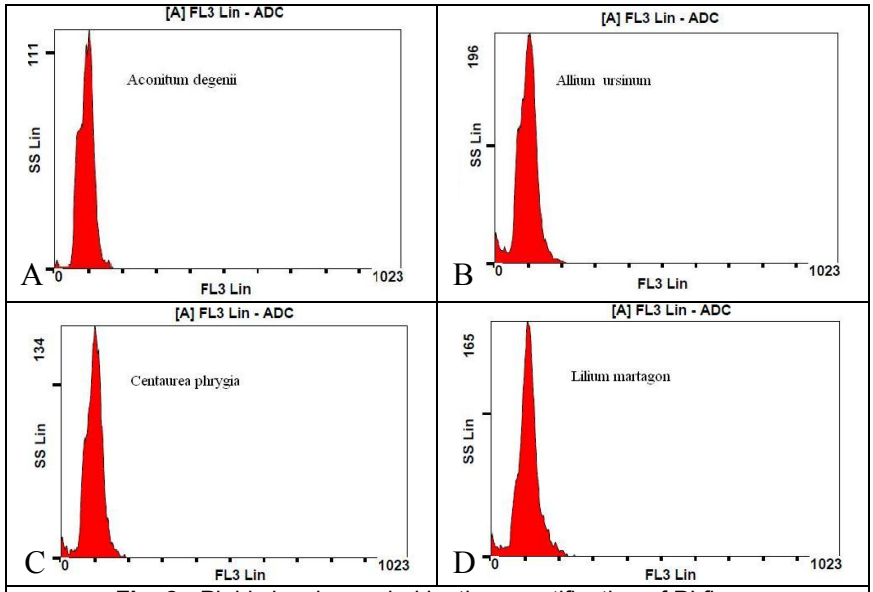

Fig. 2 - Ploidy level revealed by the quantification of PI fluorescence intensity detected with FL3 for A. *Aconitum degenii,* B. *Allium ursinum,* C. *Centaurea phrygia* and D. *Lilium martagon.*

The histogram reveals that the analyzed samples belonging to *Aconitum degenii* species are diploid, the G1 peak appears at channel 50 (figure 2A).

This result is similar to the previous studies of Joachimiak and his collaborators 1999, who revealed the diploid status (2n=16) of this species. For the other investigated species, the presence of the diploid cytopypes was also certified by histograms with the same pattern as the one for *Aconitum degenii* (figure 2-B,C,D).

The diploid status of *Allium ursinum* was also mentioned by Ricroch et al., (2005), Ohri et al., (1998) and Baranyi and Greilhuber, (1999) in their previous studies.

As for *Centaurea phrygia,* its diploid status was showed in the research studies of Dydak (2009) and Koutecky (2007). Koutecky mentions the existence of two cytotypes of this species in the area of the Carpathian mountains. The tetraploid cytotype belongs to the Western Carpathians (Slovakia, Poland) and the diploid cytotype - found also in our study - belongs to the Estern Carpathians (Romania).

The samples belonging to *Lilium martagon* anaysed by us, are diploid. Our results are confirmend by Zonneveld et al., (2005) and Ambrozova et al. (2011) in their previous studies.

CONCLUSIONS

1. Using the technique of flow cytometry, the genomic size specific to *Allium ursinum* (65,21pg 2C DNA) and *Lilium martagon* (91,56 pg 2C DNA) species were determined. Our data certify the values obtained in previous studies.

2. The ploidy level specific to the species *Aconitum degenii, Allium ursinum, Centaurea phrygia* and *Lilium martagon* was determined. All the analyzed samples are diploid. Our results are confirmed by the studies of other different researchers from abroad.

Acknowledgments: *This work was financially supported by PNII 52-174/2008.*

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