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Note

Evaluation of the nuclear DNA content and GC percent in four varieties of *Fagus sylvatica* L.

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Abstract – The nuclear DNA content of *Fagus sylvatica* has been assessed for the first time by flow cytometry and propidium iodide staining. Three beech varieties were compared to the common beech: the *tortuosa*, the *purpurea* and the *pendula* varieties. Values were 2C = 1.11 ± 0.02, 1.11 ± 0.01, 1.12 ± 0.01 and 1.13 ± 0.01 pg, respectively. These are the first estimates of nuclear DNA content made in the *Fagus* genus. GC percent was estimated in the common beech and in the *tortuosa* variety with mithramycin. It was 40.0 ± 0.2 and 39.8 ± 0.2 %, respectively, values typical of higher plants. © 1999 Éditions scientifiques et médicales Elsevier SAS.

***Fagus sylvatica* / flow cytometry / nuclear DNA content / GC percent**

Résumé – Évaluation de la teneur en ADN nucléaire et pourcentage de GC chez quatre variétés de *Fagus sylvatica* L. La teneur en ADN nucléaire de *Fagus sylvatica* a été estimée pour la première fois par cytométrie en flux et coloration à l'iodure de propidium. Trois variétés de hêtre ont été comparées au hêtre commun: les variétés *tortuosa*, *purpurea* et *pendula*. Les valeurs obtenues étaient respectivement: 2C = 1,11 ± 0,02 pg, 1,11 ± 0,01 pg, 1,12 ± 0,01 pg, et 1,13 ± 0,01 pg. Ce sont les premières estimations de la teneur en ADN nucléaire dans le genre *Fagus*. Les pourcentages de GC ont été estimées pour le hêtre commun et la variété *tortuosa* avec la mithramycine. Elles sont respectivement de 40,0 ± 0,2 % et 39,8 ± 0,2 %, valeurs typiques des plantes supérieures. © 1999 Éditions scientifiques et médicales Elsevier SAS.

***Fagus sylvatica* / cytométrie en flux / contenu en ADN nucléaire / pourcentage de GC**

1. Introduction

The common beech, *Fagus sylvatica* L., is one of the most important broad-leaf trees in Europe, found mainly in mountain areas. Although the common beech is known to possess $2n = 24$ chromosomes [1], no information concerning nuclear DNA content in the whole genus *Fagus* is available. Bennett and collegues [4–8] did not mention it in their extensive survey of Angiosperm genome size. Although they studied the genome of many woody species, Ohri and Ahuja [21] did not measure the DNA content of *F. sylvatica*.

Genome size is, however, an essential parameter in many genetic and molecular biological studies [2]. In Angiosperms, haploid genome size varies from less than one picogram (pg) (*Arabidopsis thaliana*: 0.15 pg) [7] to more than 100 pg (*Fritillaria assyriaca*: 127 pg) [18]. Among techniques used for genome studies, flow

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cytometry is extremely rapid and convenient: it allows accurate determinations of nuclear DNA content [13] and of AT/GC base composition in a genome [15]. Favre and Brown [12] developed a fast and simple flow cytometry protocol for *Quercus* DNA content evaluation, based on high chelating capacity of the nuclear isolation buffer. We used this method to set up experimental conditions for *Fagus*. This study was performed in order to estimate nuclear DNA content in the common beech, compared to three other beech varieties, as well as to evaluate its GC content.

2. Materials and methods

Four varieties were used: the common beech (*F. sylvatica* L.), the purple beech (*F. sylvatica* var. *purpurea* Ait.), the twisted beech (*F. sylvatica* var. *tortuosa* Pépin Willk.) and the weeping beech (*F. sylvatica* var. *pendula* Lodd.). All the samples were collected near Reims, France (49°14'N, 3°59E). The *Petunia hybrida* cv P × Pc6 (2C = 2.85 pg, 41 % GC) [15] was selected as an internal standard. Four plants per variety were randomly chosen and separately analysed. For each plant, two leaves were separately chopped, and two independent measures were performed on each leaf extract.

Healthy leaves were collected from mature trees and rinsed thoroughly with distilled water before slicing. Fresh leaf fragments (ca. 1 cm^2) were chopped at room temperature with a razor blade, together with a leaf fragment of another plant when mentioned, in 500 µL of Galbraith's nuclear isolation buffer [14] with 0.5 % Triton X–100 and sodium metabisulfite (10 mM) as an antioxidant. The crude extract was filtered through 48 µm nylon mesh and kept on ice until further use.

Initially, experimental conditions were established using DAPI, 3 µg per mL, in nuclear isolation buffer. Subsequently, total nuclear DNA was assessed after a 30 min incubation with RNase, 100 µg (5U) per mL, and propidium iodide staining, 50 µg mL^{-1}. The proportion of GC was measured separately, using mithramycin, 30 µg mL^{-1}, as specific dye [15].

Stained nuclei were passed through an EPICS V cytometer (Coulter, Fl, USA) equipped with an Argon ion laser (Spectra-Physics 2025-05) exciting at 514 nm for propidium iodide, 458 nm for mithramycin, or 351 + 364 nm for DAPI (for further information on the method, see [10, 18]). At least 2 500 nuclei were examined each time to assess the intensity of 2C *Fagus* nuclei relative to 2C *Petunia* nuclei.

Conversion of mass values into base-pair number was carried out according to Bennett and Smith [6]:

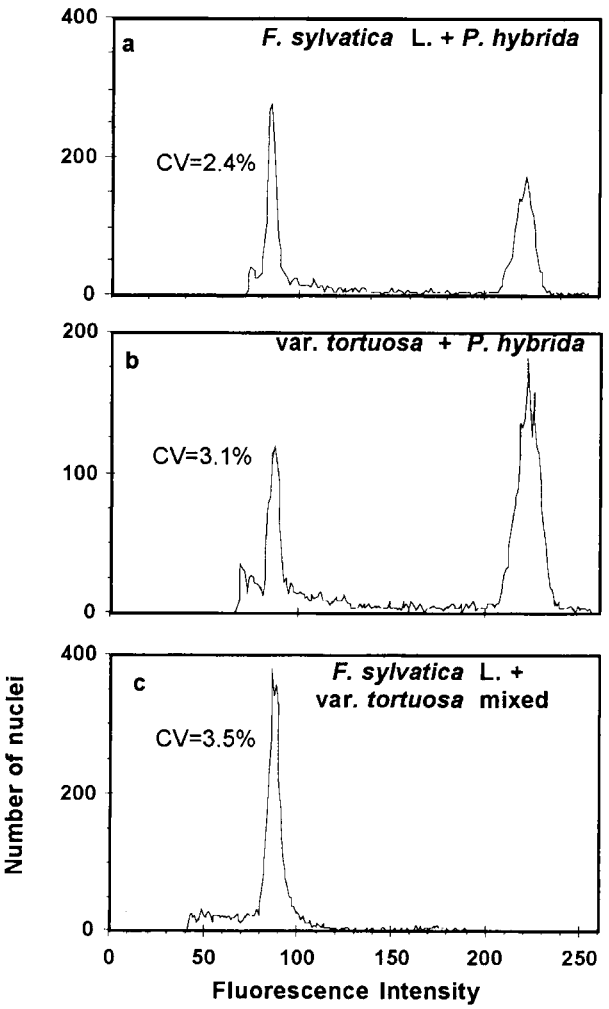

Figure 1. Histograms of relative propidium iodide fluorescence in nuclei from leaves of: a) *F. sylvatica* (common beech) and b) *F. sylvatica* var. *tortuosa*, both chopped in Galbraith buffer (0.5 % Triton X-100) in presence of *P. hybrida* leaf as an internal standard. c) Leaves of both beech varieties were mixed and chopped, without *P. hybrida*. The single peak of these 2C nuclei has the same dispersion (CV = 3.5 %) as analyses of individual specimens made on the same occasion.

1 pg = 965 Mbp. The proportion of GC was determined using the relationship of Godelle et al. [15]:

$$\%GC_{Fagus} = \%GC_{Petunia} \cdot (R_{Mi}/R_{Pi})^{1/3}$$

where $R_{Mi} = intensity_{Fagus}/intensity_{Petunia}$ for mithramycin

$R_{Pi} = intensity_{Fagus}/intensity_{Petunia}$ for propidium iodide

Statistical *t*-test was performed for DNA content comparison.

Table I. Flow cytometric comparison and 2C nuclear DNA content of four *F. sylvatica* varieties (propidium iodide staining). Four independent trees per variety, each test involving two different leaves of the test sample, and two different measures per leaf extract. The mean channel number of each *Fagus* tree and *Petunia* sample, the mean ratio and their corresponding standard deviation (S.D.), and the DNA content are given.

Variety	Tree sample number	Mean channel number *Fagus/Petunia*	Relative intensity ratio (SD)	2C nuclear DNA pg (SD)	2C nuclear DNA Mbp
F. sylvatica L.	1	84/218	0.384 (0.004)		
	2	89/229	0.388 (0.002)		
	3	89/221	0.403 (0.002)		
	4	82/210	0.390 (0.001)		
	mean		0.391 (0.008)	1.11 (0.02)	1 070
tortuosa	1	85/217	0.392 (0.002)		
	2	81/209	0.388 (0.002)		
	3	82/212	0.388 (0.005)		
	4	85/216	0.391 (0.002)		
	mean		0.390 (0.002)	1.11 (0.01)	1 070
pendula	1	91/228	0.399 (0.001)		
	2	88/225	0.391 (0.001)		
	3	88/222	0.396 (0.000)		
	4	83/208	0.398 (0.003)		
	mean		0.396 (0.004)	1.13 (0.01)	1 090
purpurea	1	88/225	0.391 (0.000)		
	2	84/216	0.388 (0.000)		
	3	92/232	0.398 (0.002)		
	4	83/213	0.390 (0.000)		
	mean		0.392 (0.004)	1.12 (0.01)	1 080

Table II. Base composition in GC % in *Fagus sylvatica* and the *tortuosa* variety.
Two independent *F. sylvatica* trees and one *tortuosa* tree, each test involving two different leaves of the test sample, and two different measures per leaf extract. The mean relative fluorescent intensity of each *Fagus* and *Petunia* sample for both stainings and the mean GC % per variety are given.

Variety	Tree sample number	Relative fluorescent intensity ratio *Fagus/Petunia* (propidium iodide)	Relative fluorescent intensity ratio *Fagus/Petunia* (mithramycin)	Base composition GC % (SD)
F. sylvatica L.	1	0.383	0.352	
	2	0.388	0.364	
	mean	0.386	0.359	40.0 (0.2)
tortuosa	1	0.392	0.357	39.8 (0.2)

3. Results and discussion

In a first set of experiments, nuclei of common beech stained with propidium iodide were run concurrently with nuclei of *Petunia hybrida* (*figure 1a*). Two distinct major peaks were visible, one for *Petunia* (relative fluorescence: channel 222), the second for *F. sylvatica* (relative fluorescence: channel 86), with a low coefficient of variation (2.4 %). Similar fluorescence distribution was obtained for *Petunia* and *tortuosa* nuclei run simultaneously (*figure 1b*). In order to verify whether the fluorescence channels were identical for the common beech and the *tortuosa* variety, both nuclei populations were run concurrently (*figure 1c*). One single peak was observed (relative fluorescence: channel 87; CV = 3.5 %), indicating that DNA content in the *tortuosa* variety is the same as in the common beech. Furthermore, in replicated analyses of common beech with or without *tortuosa*, the coefficients of variation were tight and independent of whether or not two varieties were present.

We then measured DNA content for all four varieties. *Table I* shows mean relative fluorescence after propidi-

um iodide staining. In 16 histograms, the average coefficient of variation for the peak of 2C nuclei for *Petunia* was 2.2 % and that of *Fagus* 3.1 %, altogether acceptable. 2C DNA values converted to pg amounts and to Mbp are listed on *table I*. They range from 1.11 ± 0.02 pg for the common beech to 1.13 ± 0.01 pg for the *pendula* variety. These results show a relatively uniform nuclear DNA content among the varieties of *F. sylvatica*, except that the *pendula* differs significantly from the *tortuosa* variety at $P = 0.001$. No clear intraspecific variation was evident, although it has been observed in several diploid species [3, 9, 17, 19].

Compared to *Quercus*, the only genus of the *Fagaceae* family whose genome size is known, *F. sylvatica* genomes are smaller: according to a flow cytometry estimation [12], the genome size of *Q. robur* is: 2C = 1.84 ± 0.01 pg and of *Q. petraea*: 2C = 1.87 ± 0.02 pg. Using microdensitometry methods, Greilhuber evaluated the genome of *Q. petraea* to 2C = 1.8 pg [16], and Ohri and Ahuja [20] to 1.58 pg. Although their DNA contents are different, these two genera have the same number of chromosomes ($2n = 24$) and the chromosome morphology is similar, as shown by C-banding [20, 21].

This analysis revealed that *F. sylvatica* is situated at the low end of the range of known 2C genome sizes, as for instance *Musa acuminata* (1.2 pg), *Vitis vinifera* (1.0 pg) or *Phaseolus augustii* (1.1 pg) [4, 11].

The GC content was then determined for the common beech and the *tortuosa* variety, after propidium iodide and mithramycin stainings. Results are listed in *table II*. In *F. sylvatica*, the GC content was 40.0 ± 0.2 %; in the *tortuosa* variety, 39.8 ± 0.2 %. These values are not significantly different and they are typical for higher plants. Compared to the GC content found in the *Quercus* genus, they are slightly lower. The GC content was evaluated at 41.7 % for *Q. petraea*, 42.0 % for *Q. robur*, and 42.1 % for *Q. pubescens* [12]. Other values in the *Fagaceae* family have not yet been determined.

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References

[1] Becker M., Taxonomie et caractères botaniques, in: INRA (Ed.), Le Hêtre, Tec Doc, Paris, 1981, pp. 35–46.

[2] Bennett M.D., The genome, the natural karyotype and biosystematics, in: Grant W.F. (Ed.), Plant Biosystematics, Academic Press, San Diego, CA, 1984, pp. 41–66.

[3] Bennett M.D., Variation in genomic form in plants and its ecological implication, New Phytol. 106 (1987) 177–200.

[4] Bennett M.D., Leitch I., Nuclear DNA amounts in angiosperms, Ann. Bot. 76 (1995) 113–176.

[5] Bennett M.D., Leitch I., Nuclear DNA amounts in angiosperms - 583 new estimates, Ann. Bot. 80 (1997) 169–196.

[6] Bennett M.D., Smith J.B., Nuclear DNA amounts in angiosperms, Phil. Trans. R. Soc. London B 274 (1976) 227–274.

[7] Bennett M.D., Smith J.B., Nuclear DNA amounts in angiosperms, Phil. Trans. R. Soc. London B 334 (1991) 309–345.

[8] Bennett M.D., Smith J.B., Heslop-Harrison J.S., Nuclear DNA amounts in angiosperms, Proc. R. Soc. London Ser. B 216 (1982) 179–199.

[9] Blondon F., Marie D., Brown S., Kondorosi A., Genome size and base composition in *Medicago sativa* and *M. truncatula* species, Genome 37 (1994) 264–270.

[10] Brown S.C., Bergounioux C., Tallet S., Marie D., Flow cytometry of nuclei for ploidy and cell cycle analysis, in: Negrutiu I., Gharti-Chhetri G. (Eds.), A Laboratory Guide for Cellular and Molecular Plant Biology, Birkhäuser, Basel, Switzerland, 1991, pp. 326–345.

[11] Dolezel J., Dolezelova M., Novak F.J., Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*), Biol. Plant. 36 (1994) 351–357.

[12] Favre J.M., Brown S., A flow cytometric evaluation of the nuclear DNA content and GC percent in genomes of European oak species, Ann. Sci. For. 53 (1996) 915–917.

[13] Galbraith D.W., Flow cytometric analysis of plant genomes, in: Darzynkiewicz Z., Crissman H.A. (Eds.), Methods in Cell Biology, vol. 33., Academic, San Diego, CA, 1990, pp. 549–563

[14] Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabady E., Rapid flow cytophotometric analysis of the cell cycle in intact plant tissues, Science 220 (1983) 1049–1051.

[15] Godelle B., Cartier D., Marie D., Brown S.C., Siljak-Yakovlev S., Heterochromatin study demonstrating the non-linearity of fluorometry useful for calculating genomic base composition, Cytometry 14 (1993) 618–626.

[16] Greilhuber J., "Self-tanning" a new and important source of stoichiometric error in cytophotometric determination of nuclear DNA content in plants, Plant Syst. Evol. 158 (1988) 87–96.

[17] Laurie D.A., Bennett M.D., Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation, Heredity 55 (1985) 307–313.

[18] Marie D., Brown S.C., A cytometric exercise in plant DNA histograms, with 2C values for seventy species, Biol. Cell 78 (1993) 41–551.

[19] Michaelson M.J., Price H.J., Johnston J.S., Ellison J.R., Variation of nuclear DNA content in *Helianthus annuus* (*Asteraceae*), Am. J. Bot. 78 (1991) 1238–1243.

[20] Ohri D., Ahuja M.R., Giemsa C-banded karyotype in *Quercus* L. (oak), Silvae Genet. 39 (1990) 216–219.

[21] Ohri D., Ahuja M.R., Giemsa C-banding in *Fagus sylvatica* L., *Betula pendula* Roth and *Populus tremula* L., Silvae Genet. 40 (1991) 72–75.