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Short note

A flow cytometric evaluation of the nuclear DNA content and GC percent in genomes of European oak species

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Summary – The nuclear DNA content and GC% have been assessed for the first time by flow cytometry in *Quercus petraea* (Matt) Liebl, *Q pubescens* Willd and *Q robur* L. Values were, respectively, 2C = 1.87, 1.86 and 1.84 pg with 41.7, 42.1 and 42.0 GC%. These estimates of DNA content were greater than those previously obtained by Feulgen microdensitometry. This difference is discussed in reference to effects of tanning in sample preparation.

nuclear DNA content / GC percent / European oaks / flow cytometry

Résumé – Évaluation par cytométrie en flux du contenu en ADN nucléaire et du pourcentage de GC chez des chênes européens. *Le contenu en ADN nucléaire et le pourcentage de GC ont été évalués pour la première fois par cytométrie en flux chez* Quercus petraea *(Matt) Liebl,* Q pubescens *Willd and* Q robur *L. Les valeurs enregistrées, respectivement 1,87, 1,86 et 1,84 pg avec 41,7, 42,1 et 42,0 GC%, sont comparables pour les trois espèces mais les contenus en ADN se sont révélés supérieurs à ceux obtenus antérieurement par microdensitométrie. Cette différence est discutée en relation avec les effets de tannage lors de la préparation des échantillons.*

contenu en ADN nucléaire / pourcentage de GC / chênes européens / cytométrie en flux

INTRODUCTION

Karyotypic studies in genus *Quercus* (Fagaceae) have shown that the species are diploid, 2n = 2x = 24 (Darlington and Wylie, 1955; Moore, 1982) although occasional polyploids have been found (Butorina, 1993). Recently, a detailed karyomorphological analysis in *Q petraea* (Matt) Liebl, *Q robur* L and *Q rubra* L showed that the different chromosomes can be identified and paired on the basis of C-band patterns (Ohri and Ahuja, 1990). These authors also assessed nuclear DNA content by Feulgen microdensitometry, obtaining data coherent with previous reports (Bennett and Smith, 1991). In this note, we explore an alternative approach to determining nuclear DNA content in *Quercus* species by using a flow cytometer. In conjunction with

this procedure, both intercalating and base specific dyes are used to enable calculation of the AT/GC base composition (= GC%) in a genome. The simple technique developed herein could be extended to estimate the ploidy level of *Quercus* plants on the basis of genome size relative to a standard plant of known ploidy.

MATERIALS AND METHODS

The plant material was leaves of in vitro cloned plantlets of *Q petraea* (Matt) Liebl, *Q pubescens* Willd and *Q robur* L. *Petunia hybrida* cv Px Pc 6 (2C = 2.85 pg; 41% GC) was selected as an internal standard. The *Q petraea* and *Q robur* clones originated from donor trees in north-eastern France (Lorraine). The *Q pubescens* donor tree was from the French southern Alps (Provence).

Three to four leaves of a single in vitro grown 2-month-old plantlet were chopped with a razor blade together with a leaf fragment of *Petunia* in 1 mL of Marie's nuclear isolation buffer (Marie and Brown, 1993) with 2.2 μL β-mercaptoethanol added fresh so that the buffer was used within 3 h. The crude suspension of nuclei was filtered through 30 μm nylon.

The total nuclear DNA was assessed after incubation with RNase (Boehringer), five units per mL, and ethidium bromide (Sigma), 30 μg per mL, as intercalating dye. The proportion of AT was measured separately using bisbenzimide Hoechst 33342 (Aldrich), 3 μg per mL, as base-specific dye and applying the fifth root relationship of Godelle et al (1993):

$$\text{AT\% of } Quercus = \text{AT\% of } Petunia \times (R_{Ho}/R_{Eb})^{1/5}$$

where R_{Eb} = Intensity $_{Quercus}$ / Intensity $_{Petunia}$ for ethidium bromide and R_{Ho} = Intensity $_{Quercus}$ / Intensity $_{Petunia}$ for Hoechst 33342.

An EPICS V cytometer (Coulter, FL, USA) was used with an argon laser (Spectra-Physics 2025-05) at 488 or 351 + 364 nm, for ethidium bromide or Hoechst, respectively, taking emissions of > 590 or 408–530 nm. Each event was analysed for pulse versus integral to avoid doublets, as explained in a general review of the method by Marie and Brown (1993). Nuclear DNA content values and GC% were calculated from five to six different samples of 5 000–6 000 isolated nuclei for each dye. Conversion of mass values into base-pair number was done according to the factor 1 pg = 965 Mbp (Arumuganathan and Earle, 1991).

RESULTS AND DISCUSSION

Results are given in table I. They show a relatively uniform nuclear DNA content and base composition among the three species.

The DNA values are generally 12% greater than values previously published for the genus *Quercus* that are all about 1.6 pg per 2C interphasic nucleus: *Q petraea* 2C = 1.6 pg (Band, 1984 in Bennett and Smith, 1991), 1.8 pg (Greilhuber, 1988) or 1.58 pg (Ohri and Ahuja, 1990), *Q robur* 2C = 1.59 pg (Ohri and Ahuja, 1990), *Q rubra* 2C = 1.61 pg (Ohri and Ahuja, 1990). Olszewska and Osiecka (1984) gave a lower value for *Q sessilis:* 2C = 1.0 pg.

This difference may be due to different microdensitometry methods used by these authors. Plant extracts from *Quercus* species are prone to browning and tanning that can substantially interfere with Feulgen microdensitometry, as demonstrated

Table I. 2C nuclear DNA content in pg and Mbp, and base composition in GC% in *Q robur, Q petraea* and *Q pubescens.*

	2C nuclear DNA content		*Base composition*
	pg (SD)	*Mbp*	*GC%*
Q robur	1.84 (0.01)	1776	42.0
Q petraea	1.87 (0.02)	1805	41.7
Q pubescens	1.86 (0.04)	1795	42.1

SD: standard deviation.

by Greilhuber (1988) for *Pinus*. He stated that "a significant part of the reports on fluctuating genomic DNA contents can be attributed to unrecognized stoichiometric errors induced by plant tannins". Correspondingly, microdensitometry tends to underevaluate the DNA content of nuclei. To evaluate this fluctuation, we tested five isolation buffers for cytometric assessment of *Quercus* spp and found that buffers with a high chelating capacity (Marie's buffer and that of Galbraith et al, 1983) ensured greater stability and uniformity. It is noteworthy that our cytometric data for *Q petraea* concorded with the results obtained by Greilhuber (1988), who has paid particular attention to overcoming tanning during Feulgen microdensitometry.

The basic genome size is not expected to vary between various tissues of a plant. Notably, flow cytometric data from leaf tissue and root apices has always been concordant in our laboratory, eg, with *Medicago* spp (Blondon et al, 1994) and with *Actinidia* spp (Blanchet et al, 1992). Of course, meristematic activity and endoreplication may increase nuclear DNA, but this cytometric calculation is based on only the first subpopulation of nuclei, the 2C peak, avoiding the higher levels. Yet another source of variation can be the in vitro procedure. However, the propagation procedure from axillary buds used here has never been shown as responsible for karyotypic variation and it is recognized as highly reliable in terms of genetic fidelity. Starting with clonal material, we found that the in vitro procedure had not introduced variability, as evidenced by our tight standard deviations.

Unfortunately, there are no available microdensitometry or flow cytometry data on the DNA content in other Fagaceae genera *(Castanea, Fagus, Nothofagus,* etc). Therefore, a comparison of genome size within Fagaceae is presently not possible. The GC% values presented here are the first for the genus *Quercus* and the Fagaceae family. The values are typical for higher plants. This type of data can be useful in planning molecular procedures for DNA polymorphism analysis or the study of genetic architecture of genomes by RFLP and PFGE (choice of rarely cleaving restriction enzymes) or RAPD (choice of primers), and in speciation studies (Godelle et al, 1993).

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