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Full Length Research Paper

Nuclear DNA content and genome size of American ginseng

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Flow cytometry analysis of propidium iodide (PI) stained nuclei isolated from leaf tissues was used to estimate the genome size of American ginseng (*Panax quinquefolius* L.) and evaluate inhibitory effects of its secondary compounds on PI intercalation. American ginseng nuclear DNA content was estimated to be 10.05 ± 0.04 pg/2C, and therefore its haploid (1C) genome size is 4914 Mbp. There was no significant inhibition of PI fluorescence of reference standard nuclei co-processed with American ginseng. This indicates that secondary compounds of American ginseng do not interfere with PI intercalation. By comparison, the genome size of American ginseng is 31.30 and 10.03 times bigger than that of *Arabidopsis thaliana* and *Oryza sativa*, respectively, and is 1.58 times bigger than that of its sister species *Panax ginseng* C.A. Meyer (Asian ginseng). The large genome size of American ginseng will have valuable implications on its sequencing, structural and functional genomics research.

Key words: *Panax quinquefolius*, flow cytometry, ginsenosides, propidium iodide.

INTRODUCTION

American ginseng (*Panax quinquefolius* L.) is a perennial herb that is highly valued in the Orient for its medicinal properties. The species belongs to the Araliaceae family and is native to eastern deciduous woodlands of Eastern US and Canada (Catling et al., 1994). *P. quinquefolius* and its sister species *Panax ginseng* C.A.Meyer (Asian ginseng), which is native to Eastern Asia, are the two species in the genus *Panax* that are commonly utilized for medicinal purposes. Their pharmacological properties are mainly attributed to a group of saponins called ginsenosides (Attele et al., 1999).

P. quinquefolius and *P. ginseng* occupy similar habitats despite occurring in different continents; however, they are quite different morphologically and phytochemically (Pritts, 1995). For instance, *P. ginseng* is slightly slender and elongated than *P. quinquefolius*, produces yellow berries as opposed to crimson berries produced by *P quinquefolius*, and its seeds have a slightly rougher texture than those of *P. quinquefolius* (Pritts, 1995).

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Phytochemically, the two species have different number of ginsenosides. Out of the 30 different ginsenosides that have been isolated and characterized in the genus *Panax*, 27 are found in *P. quinquefolius* compared to 20 in *P. ginseng* (Liu and Xiao, 1992; Hon et al., 2003). The two species also have distinctly different ginsenosides profiles. Ginsenoside R_f is specific to *P. ginseng* whereas ginsenoside F_{11} is specific to *P. quinquefolius* (Chan et al., 2000). The species are also genetically different and can be distinctively separated from each other using currently available DNA fingerprinting techniques (Hon et al., 2003).

Thus far, little genomic research advancement has been achieved for *P. quinquefolius* in comparison to *P. ginseng*. For instance, 11,412 expressed sequence tags (EST), 446 nucleotide sequences and 2,789 genomic survey sequences (GSS) have been analyzed and reported for *P. ginseng* in contrast to 5,018 ESTs and 162 nucleotide sequences for *P. quinquefolius* (NCBI, 2011). Moreover, the genome size of *P. ginseng* has been determined but that of *P. quinquefolius* is still unknown. The haploid (1C) genome size of *P. ginseng* is estimated to be 3120 Mbp (Hong et al., 2004), which is

19.87 times that of the model plant *Arabidopsis thaliana* (157 Mbp) (Bennett and Smith, 1991) and 6.37 times that of *Oryza sativa* (490 Mbp) (Bennett et al., 2003).

Genome size is a fundamental parameter in structural and functional genomics studies and having an estimate on a species' genome size is an important first step towards deciding and strategizing for its sequencing (Hong et al., 2004; Gregory, 2005). Over recent years, flow cytometry has become a preferred technique in estimating nuclear DNA content (genome size) of plants because it is fast, easy and accurate compared to other classical methodologies such as Feulgen microspectrophotometry (Doležel and Bartoš, 2005). However, this technique has its limitations in estimating nuclear DNA content in some plants, particularly those rich in secondary compounds. Research reports show that presence of secondary compounds in the cytosol inhibits propidium iodide (PI) intercalation and fluorescence and therefore, could compromise the reliability and accuracy of nuclear DNA content estimation (Price et al., 2000). Since it is unknown what secondary compounds inhibit PI intercalation, it is recommended that the presence of PI inhibitors be determined prior to estimating the genome size of plants with flow cytometry. Presence of inhibitors is confirmed if mean peak position of PI fluorescence of reference standard nuclei is lower in the presence of a target sample (Price et al., 2000).

Ginsenosides are secondary metabolites and are present in all parts of *P. quinquefolius* plant. Given the structural complexity of ginsenosides, it was speculated that they will have an inhibitory effect on PI intercalation of nuclei thus, hindering accurate genome size estimation of *P. quinquefolius*. Inhibitory effects of secondary compounds on PI intercalation have been reported in sunflower (Price et al., 2000). In this study, we sought to determine if ginsenosides interfere with PI intercalation and fluorescence of reference standard nuclei and proceeded to estimate the genome size of *P. quinquefolius* using flow cytometry. Knowing the genome size of *P. quinquefolius* will not only expand the C-value database of Araliaceae family, but will also provide information that would progress functional and structural genomic research of this important medicinal plant, and together with other genomic data advance studies on biochemical and evolutionary divergence of *Panax* species.

MATERIALS AND METHODS

Plant materials

Young leaves from 4 to 6 weeks old seedlings of *P. quinquefo* and reference plant standards were used in this study. Two reference plant standards including barley (*Secale cereale* L. cv. Daňkovské, 2C = 16.19 pg) and pea (*Pisum sativum* cv. Ctirad, 2C = 9.09 pg) (Doležel et al., 1998) were assessed for use in this study. Plants were grown in the greenhouse from seeds planted in 150 × 150 mm round pots (Dillen Products, Middlefield, OH) using Sunshine Mix 1 (SUNGRO Horticulture, Seba Beach, Canada), under natural light conditions, temperature of 21°C ± 3, and were hand watered daily.

Nuclei preparation

Nuclei from samples and reference plant standards were isolated following the protocol outlined in Obae and West (2010). Briefly, that included thinly slicing leaves with a sharp scalpel blade in Arumuganathan and Earle's (AE) nuclei isolation buffer (Arumuganathan and Earle, 1991) and staining isolated nuclei with PI.

Flow cytometry

A FACS Calibur flow cytometer equipped with a 15 mW 488 nm air-cooled argon-ion laser and Cell Quest Pro software (BD Bioscience, San Jose, CA) was used. Prior to running samples, linearity of the instrument was checked using the BD DNA QC Particles kit (BD Bioscience, San Jose, CA). Doublets, disintegrated nuclei, and other cell debris were eliminated from analysis by gating forward and side scatter profiles of samples. The gate was uniformly maintained across all samples in each run. For each sample 7,000 to 10,000 events (nuclei) were collected and the resulting histograms were analyzed using FCS Express version 3 De NOVO software (FCS Research, Los Angeles, CA).

Test for PI inhibitors

To test for inhibitory effects of ginseng's secondary compounds on PI intercalation, mean fluorescence peak positions of reference standard nuclei that were processed separately and those that were co-processed with *P. quinquefolius* were compared.

Data collection and analysis

A total of 10 plants of *P. quinquefolius* were used, and each plant sample was analyzed in triplicates with replicate measurements carried out on different days. Nuclear DNA content of *P. quinquefolius* was estimated using the linear relationship according to the following equation:

$$\text{Sample 2C nuclear DNA content (pg)} = 16.19 \times \frac{\text{Sample 2C peak mean}}{\text{Reference standard 2C peak mean}}$$

Conversion from picograms (pg) to base pairs (bp) was done as follows: 1 pg DNA= 978 Mbp (Doležel et al., 2003). The results were analyzed using analysis of variance (SAS Institute, Inc. 2003).

RESULTS AND DISCUSSION

Fluorescence peaks of *P. quinquefolius* and *P. sativum* cv. Ctirad (pea) overlapped (Figure 1); so, pea was ruled out as an appropriate internal reference standard for use in estimating the genome size of *P. quinquefolius*. The fluorescence peak of *S. cereale* L. cv. Daňkovské was clearly separated from that of *P. quinquefolius* and was therefore, used as an internal reference standard in estimating *P. quinquefolius* genome size (Figure 2).

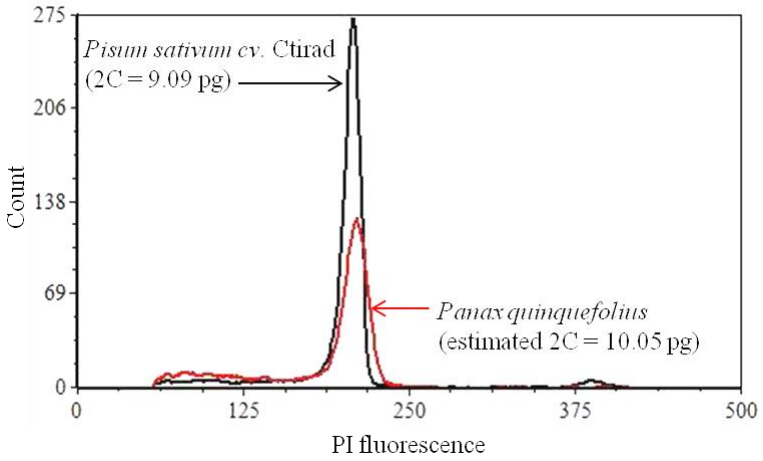

Figure 1. Histograms of *P. sativum* cv. Ctirad and *P. quinquefolius* nuclei showing overlap of their PI fluorescence.

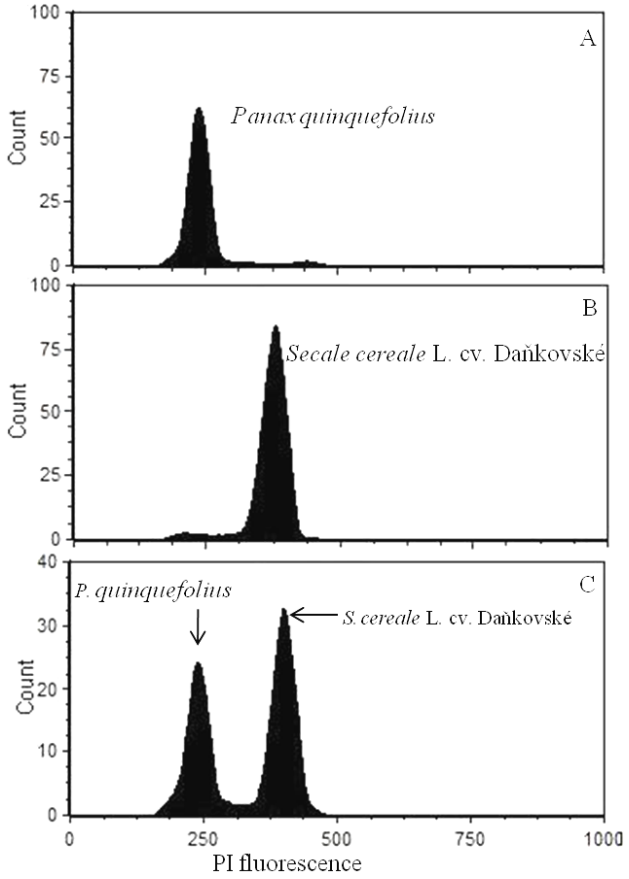

Figure 2. Histograms of separately processed nuclei of: (A), *P. quinquefolius*; (B), *S. cereale* L. cv. Daňkovské; (C), co-processed nuclei of both specimens.

Comparison of PI fluorescence peaks of separately processed *S. cereale* L. cv. Daňkovské nuclei and those co-processed with *P. quinquefolius* did not show any significant shift on their mean PI fluorescence peak positions (Figure 2), suggesting that saponins in *P. quinquefolius* do not inhibit PI intercalation or

Table 1. Nuclear DNA content (mean ± SD) and genome size of *P. quinquefolius* estimated by flow cytometry using *S. cereale* L. cv. Daňkovské (2C = 16.19 pg) as an internal reference standard.

Plant	2C Nuclear DNA	1C Genome size	
Sample	Content ± SD (pg)[y]	Mbp[z]	CV (%)
1	10.01 ± 0.03	4895	3.62
2	10.01 ± 0.12	4895	4.01
3	10.10 ± 0.02	4939	3.99
4	10.06 ± 0.03	4919	4.49
5	10.04 ±0.06	4910	3.47
6	10.06 ± 0.02	4919	4.28
7	10.01 ± 0.04	4895	3.52
8	10.06 ± 0.06	4919	3.14
9	10.08 ± 0.03	4929	4.34
10	10.03 ± 0.05	4905	3.98
All plants	10.05 ± 0.04	4914	3.88

[y], Values are means from three replicate measurements per plant sample, values are not significantly different from each other at $P \leq 0.05$ (Tukey's test); [z], 1 pg DNA = 978 Mbp (Doležel et al., 2003).

fluorescence.

Estimates of nuclear DNA content of *P. quinquefolius* ranged from 10.01 to 10.10 pg/2C among the plants tested (Table 1). Slight variations in nuclear DNA content estimates were noted among *P. quinquefolius* plants used; however, those variations were not statistically significant ($P > 0.05$) and therefore, were attributed to instrument drift.

The haploid genome size of *P. quinquefolius* is estimated to be 4,914 Mbp (Table 1). Haploid genome size of *A. thaliana* (model plant) and *O. sativa* are 157 and 490 Mbp, respectively (Bennett and Smith, 1991; Bennett et al., 2003), whereas that of *P. ginseng* is 3,120 Mbp (Hong et al., 2004). Therefore, the estimated genome size of *P. quinquefolius* (4,914 Mbp) is 31.30 times that of *A. thaliana*, 10.03 times that of *O. sativa*, and 1.58 times that of its sister species *P. ginseng*. Based on records from plant DNA C-values database (Bennett and Leitch, 2010), the genome size of *P. quinquefolius* falls in the upper end of C-value distribution of its family – Araliaceae (6 records, range 1,369 to 5,330 Mbp/1C).

In conclusion, the noted large genome size of *P. quinquefolius* will have important implications on structural and functional genomics research and provides information for consideration in genome sequencing undertakings of this economically important medicinal plant. The now known genome size of *P. quinquefolius* together with other genomic data would be utilized in advancing biochemical and evolutionary studies of the genus *Panax*.

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