Genome Size of Pecan as Determined by Flow Cytometry

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Knowledge of the amount of nuclear DNA in a plant's cells contributes to an understanding of the genome of that organism, and allows comparison with other organisms of known genome size. The size of the unreplicated haploid genome, or 1C value is used to characterize species. The 1C value, expressed in picograms (pg), may be converted to an estimate of the number of megabase pairs (Mbp). One picogram equals ≈ 960 Mbp. Species vary widely in genome size, from about 0.2 pg for *Arabidopsis thaliana* L. (which makes an excellent model system due to small genome size) to ≈ 90 pg in *Fritillaria davisii* Turrill (Bennett and Smith, 1976). Furthermore, closely related species may vary greatly in genome size (Price, 1976). To our knowledge, there has been no previous report of genome size in the walnut family *Juglandaceae*. The purpose of this work was to determine the genome size of pecan, *Carya illinoensis* ($2n = 32$), as a component in the development of a molecular genetics research strategy.

Samples of nuclei for flow cytometry were obtained from newly expanded pecan leaves ('Bryozski-Vlasek', CSV 14-12), the distal 5 mm of root tips ('Little Jewel' seedling rootstock) and embryos from mature seeds (selection 74-20-16, CSC 7-1). Pecan tissue was chopped together with a leaf of *Pisum sativum* var. Minerva Maple using a new razor blade in a modified Galbraith et al. (1983) buffer as described by Johnston et al. (1999). The buffer consisted of (per l), 4.26 g $MgCl_2$, 8.84 g sodium citrate, 4.2 g 3-[morpholino]propane sulfonic acid, 1 mL Triton X-100, 1 mg boiled ribonuclease A, pH 7.2. The resulting slurry was filtered through a 53- μm nylon filter, and propidium iodide (PI) was added to a final concentration of 50 $mg \cdot kg^{-1}$. The stained samples were stored in the dark on ice and analyzed by flow cytometry after 1 h. The flow

cytometer was a Coulter Epics Elite (Coulter Electronics, Hialeah, Fla.) equipped with a water-cooled laser tuned at 514 nm and 500 mW. Fluorescence emission at >615 nm was detected with a photomultiplier screened by a long pass filter. Offset and linearity of the flow cytometer were checked with fluorescent check beads (Coulter Electronics) as recommended by the manufacturer. The mean 2C DNA content of pecan nuclei was calculated by the formula: DNA content = (mean fluorescence pecan/mean fluorescence pea) (mean DNA content of the pea standard). The 2C DNA content of pea is 9.56 pg (Johnston et al., 1999).

Samples from pecan root and embryo produced distinct and consistent peaks, while leaf tissue did not. Genome sizes (2C) estimated from 2C root (1.64 pg) and embryo nuclei (1.66, 1.68 and 1.69 pg; mean = 1.68 pg) were similar. The mean 2C genome size of pecan, therefore, is ≈ 1.7 pg (1C = 0.85 pg or 816 Mbp). This is comparable to the genome size of certain species of oak (*Quercus petraea* (Matt.) Liebl., 1C value = 0.80 pg) (Bennett and Smith, 1991), but much larger than the diploid peach, *Prunus persica* (L.) Batsch ($2n = 16$, 1C value = 0.28 pg) (Bennett and Leitch, 1995). The genome of the hexaploid plum, *Prunus domestica* L. ($6n = 48$, 1C value of 0.92 pg) (Bennett and Leitch, 1995) is only slightly larger than that of pecan.

Genome size has taxonomic value and may have importance for the characterization of diversity in the National Clonal Germplasm Repository collections. The technique reported here may be applied to accessions of related species to assess patterns of genome size differences related to patterns of polyploidy. Repository Provenance collections (populations of pecan collected across the range of the species) could be evaluated for genome size to determine the extent of intraspecific variation in the trait, which in other species has been linked to drought tolerance (Wakamiya et al., 1993). One of the most practical applications of the genome size information is in the development and evaluation of DNA clone libraries. Knowledge of genome size is necessary to determine the number of known-size fragment clones required, at a given probability, in order to have coverage of all DNA sequences represented in a genome library (Clarke and Carbon, 1976).

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Fig. 1. Relative red fluorescence of PI-stained nuclei from pecan embryo (K), and pea leaves (F) as visualized against background noise (E).

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