Determining the genome content of ornamental plants using flow cytometry

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Short Report

Keywords: Flow cytometry, Ornamental plants, Genome size, Nuclear DNA content, Plant breeding

Posted Date: April 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9111048/v1

Additional Declarations: No competing interests reported.

Abstract

Genome size is a key genomic trait influencing plant growth, development, and breeding potential. Here, nuclear DNA content of six commonly cultivated ornamental plant species was estimated using flow cytometry using propidium iodide staining. Nuclei were isolated from young leaf tissues and stained with propidium iodide (PI). Chicken red blood cells served as an internal reference standard for genome size calculation. All samples produced high-quality DNA histograms with coefficients of variation below 5%. Genome size considerably varied among the studies species, ranging from 0.302 pg in 0.302 pg in *Alocasia wentii* to 2.461 pg in *Pilea cadierei*. Intermediate genome sizes were observed in *Clerodendrum chinense*, *Codiaeum variegatum*, *Spathiphyllum kochii*, and *Calathea zebrina*. The observed variation suggests genomic diversity in ornamental plants and demonstrates the flow cytometry as a tool, for rapid estimation of genome size.

1. Introduction

Genome size defined as the amount of nuclear DNA within a cell is a highly variable feature of plant genomes. Among angiosperms, genome size differs by several orders of magnitude and has been linked to biological traits such as cell size, growth rate and ecological adaptability among others. The variation reflects differences in genome organisation and provides insights into plant diversity and evolution (Leitch et al., 2019). Despite its importance, we still lack information regarding many cultivated ornamental plants.

Flow cytometry has gained importance because it allows rapid, precise and reproducible measurement of nuclear DNA content from limited sample volume. With appropriate internal reference standards and optimised protocols, flow cytometry enables rapid and reliable comparison of genomes across species (Doležel et al., 2005; Basak et al., 2018; Nix et al., 2024).

Ornamental plants constitute taxonomically and morphologically diverse group that is of significant horticulture and economic value. Genome size variations within the ornamental species can provide useful information for evolutionary comparisons and hybridisation strategies (van de Peer et al., 2021). Despite its relevance, genome size information remains incomplete for many cultivated ornamental plants.

Although genome size has been reported for some ornamental taxa, current research on ornamental plants is mainly focused on methods or techniques for improving propagation, production, and postharvest or postproduction handling. Given the diversity and multipurpose use of ornamental plants, along with their increasing production worldwide, require more fundamental research on their growth and development, flower and leaf colour, fragrance, and growth form (Chen et al., 2021). Therefore, the present study is aimed to estimate the nuclear DNA content of six ornamental plant species using flow cytometry and to assess interspecific variation in genome size.

2. Materials and Methods

2.1 Plant Materials

Young and healthy leaf tissues were collected from six commonly cultivated ornamental plants from two locations: the herbal garden at the National Centre for Biological Sciences (NCBS) and the nursery at Institute of Stem Cell Science and Regenerative Medicine (inStem), Bangalore, India (Fig. 1). A total of six plant species were collected (in minimum three replicates). *Alocasia wentii, Clerodendrum chinense, Pilea cadierei, Codiaeum variegatum, Spathiphyllum kochii, Calathea zebrina* plants were collected. These plants were maintained under normal growth conditions, and fresh fully expanded young leaves were selected to ensure high nuclear integrity and minimal accumulation of secondary metabolites (Table 1).

Table 1
Genome size of various plants species studied in the current study

Common Name	Sample	Origin	Mean Channel Number	Genome Content (pg)	Mbps*
New Guinea Shield	*Alocasia wentii*	New Guinea	6.539	0.302	295.356
Madras Mallige	*Clerodendrum chinense*	Nepal, the Himalayas, Assam, the Andaman and Nicobar Islands	17.447	0.687–0.920 (0.804)	786.312
Aluminium Plant	*Pilea cadierei*	Vietnam	53.791	2.461	2406.858
Garden Croton	*Codiaeum variegatum*	Malaysia and the Pacific	14.603	0.676	661.128
Peace Lily	*Spathiphyllum kochii*	Tropical regions of the Americas and south-eastern Asia.	35.562	1.744–2.183 (1.964)	1920.792
Zebra Plant	*Calathea zebrina*	South-eastern Brazil.	20.467	0.584–0.998 (0.791)	773.598
1pg = 978 Mbps					

2.2 Sample preparation

Nuclei from the plant cells were isolated using Nuclear Isolation Buffer (NIB) containing 50 µg/ml propidium iodide (PI) prepared in in 1 g/l trisodium citrate dihydrate supplemented with 0.05% (v/v) of non-ionic detergent (Igepal CA-630), 2 mg/ml RNase A, 1% (w/v) PVP - Polyvinylpyrrolidone.

Briefly, 2 ml od NIB was dispensed into sterile petri plate and two to three young leaf tips (approximately 0.5 inch in length) were placed in the buffer. The leaf tissue was finely and vigorously chopped using surgical blade. The resulting suspension, including the residual leaf fragments was transferred to a 5 ml tube and gently agitated by repeated pipetting using a dropper to ensure thorough mixing. The homogenate was subsequently filtered through 30 µm nylon mesh to remove debris. The resulting clean suspension was used for further analysis.

2.3 Microscopy

After extraction, sample suspension was observed in bright-field microscope to assess the presence of intact nuclei and cellular debris.

2.4 Flow cytometric analysis

PI-stained nuclear samples were analysed using benchtop FACS Fortessa X-20 (BD Biosciences). The PI fluorescence was excited using 561 nm solid state LASER and the emission was collected using 585/20 band pass filter. Prior to sample acquisition, instrument performance and fluorescent linearity was verified using DNA QC kit (BD Biosciences) following manufacturer instructions. For each sample, frequency distribution of histograms of PI-stained nuclei were generated and used for data analysis. Only histograms exhibiting coefficient of variation (CV) less than 5% for the G_0/G_1 signal were considered acceptable for genome size estimation. Chicken blood cells (CRBC) with known DNA content of 2.3 pg per diploid nucleus (Yang et al., 2019) were used as internal reference standard for genome size calculation. The nuclear DNA content of the samples was estimated in picogram using the following formula relative to CRBC standard (Doležel et al., 2007).

$$\mathrm{DNAcontent\ (pg)} = \frac{\mathrm{CRBCDNAcontent} \times \mathrm{samplemeanfluorescencechannelno.}}{\mathrm{CRBCmeanfluorescencechannelno.}}$$

3. Results and discussion

Genome size estimation of six ornamental plant species was carried out using flow cytometry with CRBC as an internal reference standard (2C = 2.30 pg). Initial bright-field microscopic examination confirmed the presence of intact nuclei with minimal debris (Fig. 2). Flow cytometric analysis of extract showed a clear and well resolved DNA histograms with coefficients of variation below 5%. The estimated nuclear DNA content of the studied ornamental plants is summarized in Table 1.

A wide range of genome sizes was observed among the species analyzed. The smallest genome size was in *Alocasia wentii* (New Guinea Shield), with a DNA content of 0.302 pg (approximately 295 Mbp), whereas *Pilea cadierei* (Aluminium Plant) exhibited the largest genome size of 2.461 pg (approximately 2407 Mbp). Intermediate genome sizes were observed in *Clerodendrum chinense*, *Codiaeum variegatum*, *Spathiphyllum kochii*, and *Calathea zebrina*, with DNA contents ranging from approximately

0.676 to 1.964 pg (Fig. 3). The results demonstrate substantial interspecific variation in genome size among commonly cultivated ornamental plants. Such variation in nuclear DNA content is a well-recognized feature of angiosperms and reflects differences in genome organization, repetitive DNA content, and evolutionary history (Sliwinska et al., 2018). Genome size has been linked to influence several biological traits, including cell size, growth rate, and developmental timing, which may indirectly affect ornamental characteristics such as leaf size, plant architecture, and flowering behaviour. In this study, PI staining was employed for genome size estimation due to its stoichiometric binding to double-stranded DNA. In contrast, DAPI may introduce bias associated to base composition particularly differences in GC/AT content (Basak et al., 2018; Nix et al., 2024).

From a breeding perspective, nuclear DNA content can support the selection of compatible parental lines, assist in planning hybridization strategies, and support approaches involving polyploidy for trait improvement. The observed genome size diversity among the studied species suggests that the ornamental plants are genetically diverse group with considerable scope for breeding and improvement. Additionally, genome size estimation using flow cytometry is rapid and easy (Nix et al., 2024). Future studies can integrate flow cytometry with complementary cytogenetic and molecular approaches such as chromosome analysis, ploidy determination, and genome sequencing to gain deeper insights into understand genome structure, organisation and evolutionary relationship among ornamental plants (Zheng et al., 2021; Kishi-Kaboshi et al., 2018).

Declarations

Conflict of interest

None

Author Contribution

HSK: Analysed, data curation, Manuscript- writing original RK: Analysis, data curationSM: Manuscript-writing original; review editingHK : Supervision, Manuscript- review editing

Acknowledgement

The authors acknowledge the Central Imaging & Flow Cytometry of National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru.

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Figures

Figure 1

Representative images of ornamental plants used in this study. *Clerodendrum chinense* (Madras mallige) (A), *Pilea cadierei*(Aluminium plant) (B), *Spathiphyllum kochii* (Peace lily) (C) and *Codiaeum variegatum* (Garden croton) (D).

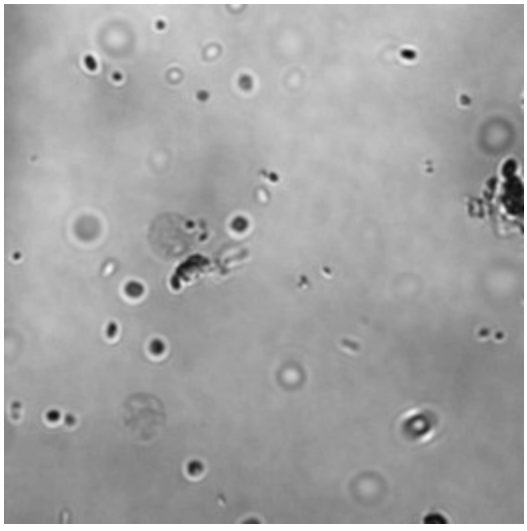

Figure 2

Bright filed view of nuclei extracted from *Clerodendrum chinense*, commonly known as Madras Mallige.

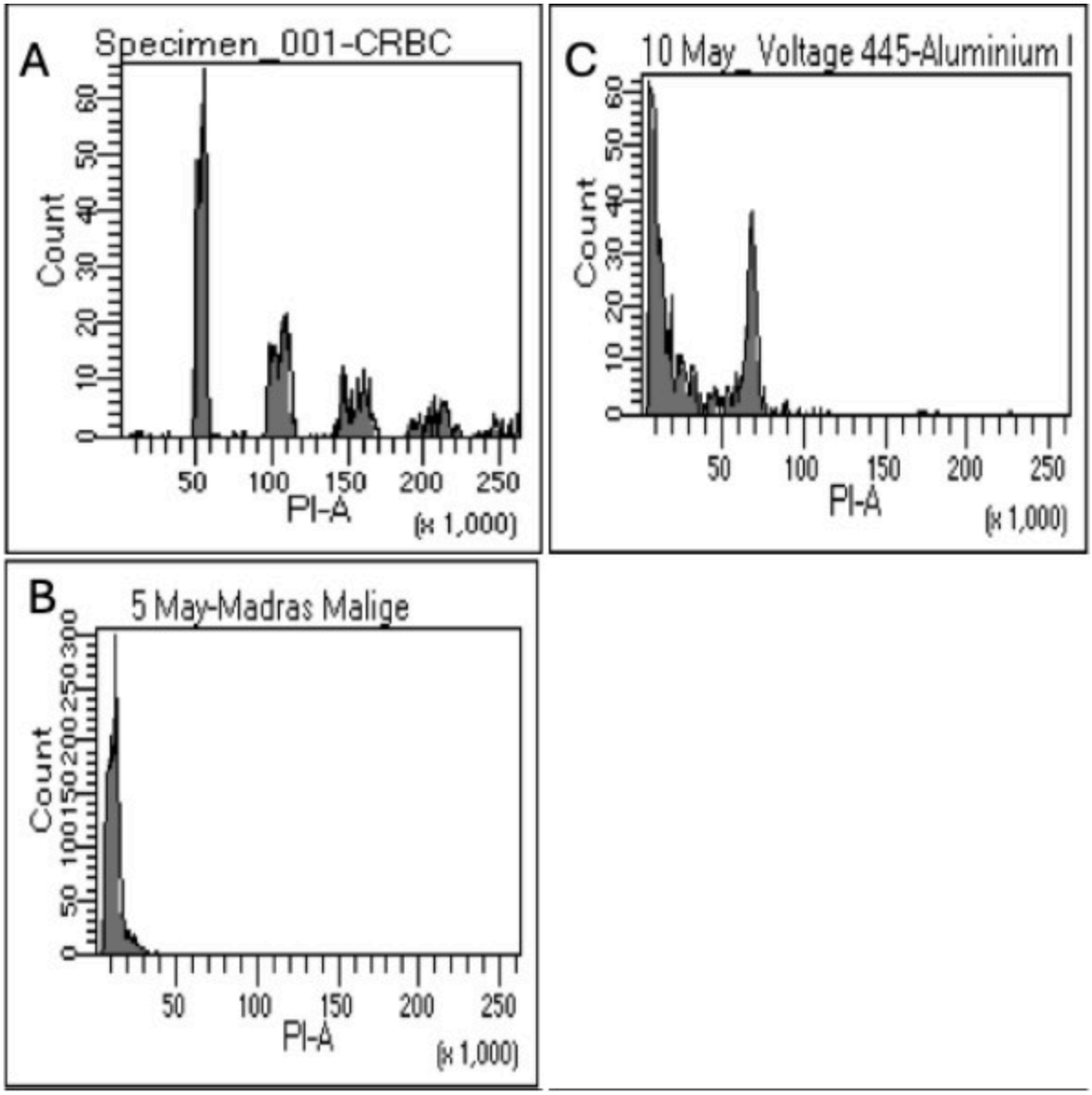

Figure 3

Histogram of fluorescence intensity of CRBC nuclei (A), *Pilea cadierei* (Aluminium plant) (B), *Clerodendrum chinense* (Madras mallige) (C) stained with hypotonic PI (A).