

Flow cytometry reveals wide variations in nuclear DNA content among ornamental plant species

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Abstract

Nuclear DNA content (2C value) of six commonly cultivated ornamental plant species *Alocasia wentii*, *Clerodendrum chinense*, *Codiaeum variegatum*, *Spathiphyllum kochii*, *Calathea zebrina* and *Pilea cadierei* was estimated by propidium iodide flow cytometry using chicken red blood cells as internal standards. All histograms showed coefficient of variation below 5%. Genome size varied considerably across species from 0.302 pg in *Alocasia wentii* to 2.461 pg in *Pilea cadierei*. Comparison with congeneric and family level data, *Alocasia wentii* has genome one to two orders of magnitude smaller than related *Alocasia* species and markedly smaller than *S. kochii*. The results show the extent of sparse genome-size record for ornamental plants and show comparative flow cytometry data can generate testable hypothesis about genome evaluation in cultivated taxa.

Summary

Nuclear DNA content (2C value) of six commonly cultivated ornamental plant species was estimated by propidium iodide flow cytometry using chicken red blood cells as internal standards. All histograms showed coefficient of variation below 5%. Genome size varied considerably across species from 0.302 pg in to 2.461 pg in . Comparison with congeneric and family level data, has genome one to two orders of magnitude smaller than related species and markedly smaller than . The results show the extent of sparse genome-size record for ornamental plants and show comparative flow cytometry data can generate testable hypothesis about genome evaluation in cultivated taxa.

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. These variations reflect differences in genome organisation and provide insights into plant diversity and evolution (Leitch et al., 2019). Despite its importance, there are no 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. In addition, it enables efficient determination of ploidy levels and detection of polyploids or mixoploids, which are critical in plant breeding and genetic studies. Compared to conventional chromosome counting, flow cytometer requires only small amount of tissue, does not depend on specific stages of mitosis and allows high through put screening of large sample numbers (Doležal et al., 2007). With appropriate internal reference standards and optimised protocols, flow cytometry enables rapid and reliable comparison of genomes across species (Doležal 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. Global production and trade of ornamental plants has expanded

considerably in recent decades, yet fundamental biological information including genome size remains unavailable or incomplete for many cultivated taxa (Chen et al., 2021). 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 estimates exist for a subset of ornamental taxa, the current studies predominantly focus on methods for improving propagation, production, and postharvest handling, with little-to-no attention to basic genomic characterisation (Chen et al., 2021). Given the diversity and multipurpose utility of ornamental plants alongside their increasing global production; there is a clear need for more fundamental research addressing their genomic attributes. The present study therefore aimed to: (i) estimate the nuclear DNA content (2C value) of six commonly cultivated ornamental plant species using flow cytometry; and (ii) evaluate the extent of interspecific genome size variation among taxonomically diverse ornamental plants maintained under cultivation. The data generated here are intended to serve as baseline genomic reference values for future cytogenetic, breeding, and evolutionary studies.

Materials and Methods

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) Bangalore, India (Fig. 1A). 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. Plants were maintained under outdoor/greenhouse conditions at ambient temperatures of 25–32°C with natural photoperiod (approximately 12 h light/12 h dark) and irrigated twice weekly. Fresh, fully expanded young leaves were selected for all analyses to ensure high nuclear integrity and minimal accumulation of secondary metabolites that could interfere with staining. A minimum of three biological replicates per species were used, where each replicate consisted of leaf tissue sampled from a distinct individual plant (Table 1).

Table 1
Characteristics of the six ornamental plant species and their estimated 2C genome size

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

Sample preparation

Nuclei were isolated using a Nuclear Isolation Buffer (NIB) prepared fresh on the day of use. The NIB consisted of: 1 g/l trisodium citrate dihydrate, 0.05% (v/v) Igepal CA-630 (non-ionic detergent), 2 mg/ml RNase A, 1% (w/v) polyvinylpyrrolidone (PVP-10), and 50 µg/ml propidium iodide (PI). All reagents were of analytical grade. The buffer was kept on ice throughout the extraction procedure to minimise nuclei degradation (Doležel et al., 2007).

Briefly, 2 ml of NIB was dispensed into a sterile plastic Petri dish. Two to three young leaf tips (approximately 1 cm in length) were placed in the buffer and chopped for approximately 60 seconds using a sharp, sterile scalpel blade, producing a fine homogenate. The resulting suspension was then incubated at room temperature for 5 minutes to allow complete nuclear staining by PI, followed by

RNase A digestion at 37°C for 15 minutes to eliminate RNA-associated PI fluorescence. The homogenate was subsequently filtered through a 30 µm nylon mesh filter into a clean 5 ml FACS tube to remove cell wall debris and undigested tissue fragments. Filtered samples were kept on ice and analysed within 30 minutes of preparation to minimise nuclei deterioration.

Microscopy

Prior to flow cytometric analysis, a 10 µl aliquot of each nuclear suspension was examined under a bright-field microscope (Olympus FluoView FV1000) to confirm the presence of intact, morphologically normal nuclei and to assess the degree of cellular debris. Samples with excessive debris or apparent nuclear clumping were discarded and re-extracted from fresh tissue. Only preparations exhibiting clearly defined, intact nuclei with minimal background debris were proceeded to flow cytometric analysis.

Flow cytometric analysis

PI-stained nuclear suspensions were analysed using a benchtop BD FACS Fortessa X-20 SORP flow cytometer (BD Biosciences, USA). PI fluorescence was excited using a 561 nm solid-state laser, and emission was collected through a 585/20 nm band-pass filter. Forward scatter (FSC) and side scatter (SSC) parameters were used to set the acquisition gate and exclude aggregates and debris from analysis. Prior to sample acquisition, instrument performance and fluorescence linearity were verified using a DNA QC particle kit (BD Biosciences) following the manufacturer's instructions.

For each sample, a minimum of 5000 nuclei within the gated G0/G1 population were acquired to ensure statistical robustness (Doležel et al., 2007). Frequency distribution histograms of PI fluorescence intensity were generated for data analysis using BD FACS Diva software (BD Biosciences). Only histograms with a CV below 5% for the G0/G1 peak were considered acceptable for genome size estimation.

CRBC with a known 2C DNA content of 2.33 pg (Doležel & Bartoš, 2005) were used as the internal reference standard. The 2C nuclear DNA content of each sample was estimated in picograms using the following formula (Doležel et al., 2007):

$$\text{DNA content (pg)} = \frac{\text{CRBC DNA content} \times \text{sample mean fluorescence channel no.}}{\text{CRBC mean fluorescence channel no.}}$$

Results and discussion

Nuclear isolation and histogram quality

Genome size estimation of six ornamental plant species was carried out using flow cytometry with CRBC as the internal reference standard (2C = 2.33 pg). Initial bright-field microscopic examination

confirmed the presence of intact nuclei with minimal debris in all samples (Fig. 1B), indicating that the isolation protocol was well-suited to the tissue types examined. Flow cytometric analysis yielded clear, well-resolved DNA content histograms for all species, with coefficients of variation below 5% for the G0/G1 peak meeting the accepted threshold for reliable genome size estimation (Doležal et al., 2007). The consistent histogram quality across species suggests that the PI-based NIB protocol was effective even for species with high secondary metabolite content, such as *Codiaeum variegatum* (Bairagi et al., 2019).

Genome size variation

A wide range of genome sizes was observed among the six species analysed (Table 1; Fig. 1C-E). The smallest genome was recorded in *Alocasia wentii* (New Guinea Shield Plant), with a 2C DNA content of 0.302 pg (approximately 295 Mbp). The largest genome was found in *Pilea cadierei* (Aluminium Plant), with a 2C DNA content of 2.461 pg (approximately 2,407 Mbp). This represents an approximate 8-fold range across the species examined. Intermediate genome sizes were observed in *Clerodendrum chinense*, *Codiaeum variegatum*, *Spathiphyllum kochii*, and *Calathea zebrina*, with 2C DNA contents ranging from approximately 0.676 to 1.964 pg (Fig. 1F).

The genome size recorded for *Spathiphyllum kochii* in the present study is broadly consistent with values reported for other members of the Araceae family, which tend to exhibit relatively small to medium-sized genomes (Sliwinska et al., 2018). The large genome size observed in *Pilea cadierei* (Urticaceae) is notable and to our knowledge represents one of the first reported estimates for this species. Future chromosome counts and ploidy analysis would help interpret this value in an evolutionary context.

Comparison with congeneric and family-level genome size data provides a broader evolutionary context. The 2C DNA content of 0.302 pg (~ 295 Mbp) observed here for *Alocasia wentii* is substantially smaller than the values reported for other Araceae species such as *A. longiloba* (27.5 pg 2C) and *A. clypeolata* (11.02 pg 2C) against is also below 15.3 pg 2C reported for terrestrial Araceae (Kocjan et al. 2022). Such pronounced variation within a single genus may indicate that *A. wentii* belongs to a distinct small-genome lineage. The genome size estimated for *Spathiphyllum kochii* is similarly smaller than the 2C values reported for wild Costa Rican *Spathiphyllum* species (~ 18–22 pg for *S. cf. silvicola*, *S. wendlandii* and related taxa) (Kocjan et al. 2022). As chromosome numbers are generally conserved across many Araceae genera, the observed difference may reflect variation in breeding history, ploidy level, or other genome evolutionary processes associated with cultivated *Spathiphyllum* lineages.

In contrast, the genome size determined for *Pilea cadierei* (Urticaceae) appears to represent the first reported 2C DNA content estimate for this species. The absence of comparable genome size data from closely related species currently limits evolutionary interpretation. Nevertheless, its relatively large genome compared with the other species examined in this study suggests the possibility of genome expansion or polyploidy. Further cytogenetic analyses or chromosome level analyses would be necessary to confirm either mechanism.

Variations in nuclear DNA content is a well-recognised feature of angiosperms and reflects differences in genome organisation, repetitive DNA content, transposable element accumulation, and evolutionary history, including ancient and recent polyploidy events (Sliwinska et al., 2018; Wendel et al., 2016). Genome size has been shown to influence several biological traits, including cell size, stomatal size, growth rate, and developmental timing, which may indirectly affect ornamental characteristics such as leaf morphology, plant architecture, and flowering behaviour (Beaulieu et al., 2008).

Methodological considerations

In this study, PI staining was selected for genome size estimation because of its stoichiometric, intercalating binding to double-stranded DNA, which is independent of base composition. In contrast, DAPI fluorescence can be biased by differences in AT/GC content, potentially introducing systematic errors when comparing species with divergent base compositions (Basak et al., 2018; Nix et al., 2024).

Implications

From a breeding perspective, nuclear DNA content data can support the selection of compatible parental lines, assist in hybridisation planning and inform strategies involving induced polyploidy for trait improvement (van de Peer et al., 2021). The substantial genome size diversity observed among the studied species suggests the broad taxonomic range and genomic heterogeneity of cultivated ornamental plants. These baseline genome size estimates also provide a prerequisite for genome sequencing projects where 2C DNA content informs sequencing depth requirements and assembly strategy (Michael & VanBuren, 2015).

Future studies should integrate flow cytometry with complementary cytogenetic and molecular approaches for chromosome counting, ploidy determination, and whole-genome sequencing to gain deeper insight into genome structure, organisation, and evolutionary relationships among ornamental plants (Zheng et al., 2021; Kishi-Kaboshi et al., 2018).

Conclusion

Flow cytometric estimation of nuclear DNA content revealed substantial genome size variation among six studied ornamental plant species cultivated in southern India. Genome size ranged from 0.302 pg in *Alocasia wentii* to 2.461 pg in *Pilea cadierei*, representing an approximately 8-fold difference across the species examined. These are the first reported 2C genome size estimates for several of the species studied, and the data provide valuable baseline genomic reference information. The results demonstrate the utility of flow cytometry using a PI-based NIB protocol with CRBC as a standard for rapid, reliable genome size determination. These data may serve as a foundation for future studies on cytogenetics, genome evolution, ploidy assessment, and breeding of ornamental species.

Declarations

Conflict of interest

None

Ethics and consent to participate

All plant material used in this study consisted of leaf tissue collected from cultivated specimens maintained in the herbal garden of the National Centre for Biological Sciences, Bengaluru, India. No wild plants were collected and the six ornamental species examined are common cultivated taxa that are not endangered, protected or subject to specific national or international collection restrictions. No specific permissions or licences were required.

Consent to participate, and consent to publish declarations

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Author Contribution

H. S. K.: Investigation, Methodology, Writing – original draft. R. K.: Validation, Methodology, Writing – review & editing. M. S.: Validation, Writing – original draft. H. K.: Supervision, Validation, Writing – review & editing.

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Data availability statement

All data generated or analysed during this study are included in this article.

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Figures

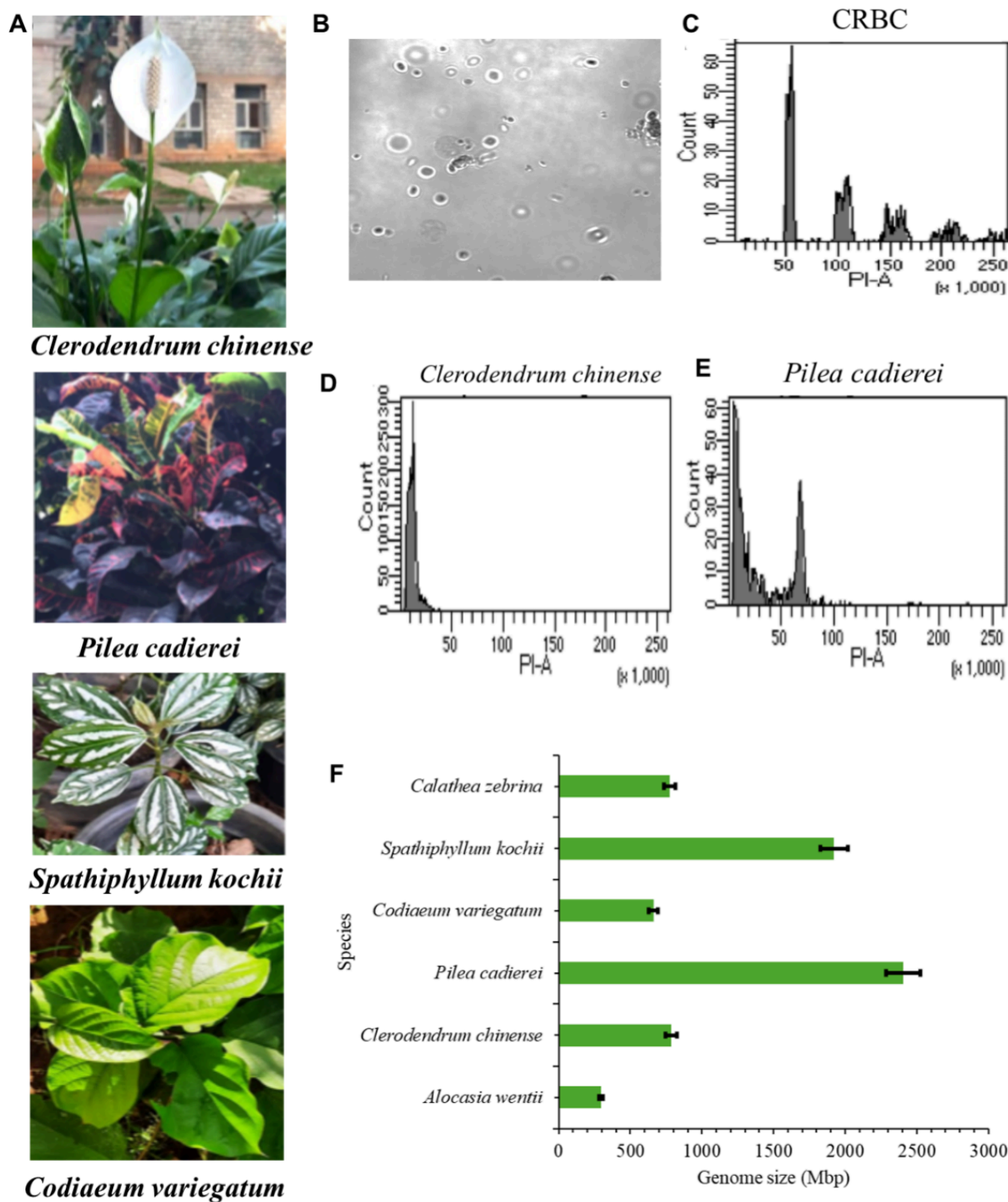


Figure 1

Ornamental plants used in this study (A), Bright field view of nuclei extracted from *Clerodendrum chinense* (B), Histogram of fluorescence intensity of CRBC nuclei (C), *Clerodendrum chinense* (D), *Pilea cadierei* (E) stained with hypotonic PI and Genome size estimates (F). Bars represent mean genome size values calculated from nuclear DNA content measurements using chicken red blood cells (CRBC; 2C = 2.33 pg) as the reference standard. Error bars indicate variation among biological replicates.

