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Estimation of Genome Size Variation in *Hibiscus rosa-sinensis*

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Abstract. **Tropical hibiscus (*Hibiscus rosa-sinensis* L.) is a popular ornamental crop because of its abundant flower color and robust flowering ability. Extensive breeding efforts have produced numerous tropical hibiscus cultivars, thus significantly contributing to the ornamental plant industry. However, limited genetic information is available for this crop, thereby posing challenges to the development of new cultivars. This study aimed to assess the genome size variation of 96 tropical hibiscus accessions using flow cytometry. Root tissue yielded more reliable results than leaf tissue for flow cytometric analyses of this crop. The genome size evaluation unveiled a wide spectrum of genetic diversity within tropical hibiscus that ranged from 3.06 Gbp ('Queen Aussie') to 12.75 Gbp ('Joanne'). Among the accessions, eight exhibited small genome sizes less than 4.2 Gbp, while three accessions possessed significantly large genomes more than 11 Gbp. The remaining 85 accessions had genome sizes that ranged from 6 to 10 Gbp. In addition, F1 hybrids were generated with greater success when parents had comparable genome size. The findings of this study provide valuable insights into the genetic diversity, offer a foundation for future breeding efforts, and directly support the genomic advancement of tropical hibiscus.**

Hibiscus is a polymorphic genus of flowering plants in the Malvaceae family with trees, shrubs, and herbs native to tropical and warm temperate regions (Akpan 2007). Several species in this genus have a long commercial history because of their value as ornamental, medicinal, and edible properties. Tropical hibiscus, *Hibiscus rosa-sinensis* L., a popular ornamental species in the *Hibiscus* genus, is widely cultivated as potted and landscape plants worldwide. This tropical evergreen shrub is highly recognized because of its prolific, large, bright-colored flowers including pure red, pink, yellow, white, and multicolor patterns (Fig. 1). Tropical hibiscus can also be categorized as single, semi-double, and double flower types depending on the variance of the petal number and arrangement (Fig. 1). In addition to being widely used as an ornamental plant in the United States and Europe, tropical hibiscus has substantial cultural importance and is recognized as a national/state flower in Malaysia, the Philippines, and the state of Hawaii (Akpan 2007; Magdalita and San Pascual 2022). Because of widespread interest, the International Hibiscus Society (HIS) has developed a database with records of more than 25,000 tropical hibiscus hybrids that offers a valuable resource for breeders and collectors through genealogy searches (https://www.internationalhibiscussociety.org/).

H. rosa-sinensis is a complex species derived from extensive interspecific hybridization of several *Hibiscus* species, including *H. liliflorus*, *H. fragilis*, *H. boryanus*, *H. arnottianus*, *H. kokio*, *H. storckii*, and *H. denisonii* (Akpan 2007). Although the domestication history of this species is debatable, it is widely recognized that this species is native to China (Kimbrough 1997) or East Africa and the Pacific Islands (van Borssum Waalkes 1966). Locally bred cultivars offer significant advantages for nursery production by enhancing disease and pest resilience, thus meeting market demands and providing better suitability for local growing conditions. As a predominantly outcrossing species, factors such as a complex breeding history (Akpan 2007), self-incompatibility (Pramesti 2021), and ploidy differences (Singh and Khoshoo 1970) have hindered the development of new cultivars that align with market needs. Tropical hibiscus often exhibits polyploidy and has a complex genetic background, complicating breeding efforts and genetic analyses (Braglia et al. 2010; Singh and Khoshoo 1970). Extensive hybridization efforts have been undertaken globally, resulting in the creation of thousands of cultivars. However, there is a significant knowledge gap in the cytological background of tropical hibiscus, including ploidy levels, chromosome numbers, genome sizes, and genetic relationships among different cultivars. This gap complicates the selection of effective parental plants, making it difficult to improve breeding accuracy, shorten breeding cycles, and achieve desired traits. Furthermore, as a woody plant, tropical hibiscus requires several years for new cultivars to be developed and stabilized, leading to a long breeding cycle. Molecular breeding offers a modern approach by integrating genetic information with traditional breeding methods to accelerate and enhance the breeding process. However, the limited availability of genetic resources for tropical hibiscus hinders these advancements, posing a challenge to efficient breeding and genetic improvement.

Genome size, often known as the DNA content or C-value, is correlated with seed mass, growth rate, and ploidy level in most plants (Knight and Beaulieu 2008; Knight et al. 2005; Ohri 1998). It is widely used in genome evaluations (Knight et al. 2005), genetic complexity studies (Barré et al. 1998), hybrid identification (Šiško et al. 2003), and infrageneric classification (Ohri 1998; Zonneveld 2001), and it is often used as an informative tool to increase the interspecific hybridization efficiency of ornamental breeding (Denaeghel et al. 2017). Flow cytometry is an efficient tool used to analyze cellular and nuclear characteristics; for example, it is used for cell cycle analyses (Pozarowski and Darzynkiewicz 2004), hybrid verification (Keller et al. 1996), ploidy level analyses (Costich et al. 1993), and genome size estimations (Doležel and Bartoš 2005). These applications provide valuable information that can improve the understanding of the fundamental mechanisms during plant growth, development, and functionality. Additionally, flow cytometry frequently contributes to plant breeding programs (Eeckhaut et al. 2005; Ochatt 2008), particularly those involving ornamental crops (Li et al. 2022; Marasek et al. 2006). Genome sizes of several tropical hibiscus relatives have been studied, including *H. syriacus*, *H. sinosyriacus*, *H. moschetous*, *H. paramutabilis*, and *H. sabdariffa* var. sabdariffa, and they range from 2 Gbp to 4 Gbp (Deepo et al.

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Fig. 1. The various flower morphology of tropical hibiscus cultivars.

2022; Mohammad et al. 2020). The genome size of hibiscus species enhances the understanding of basic biology of the crop and provides prebreeding information for new cultivar development.

In this study, genome sizes were evaluated by conducting flow cytometry of a breeding collection of 96 tropical hibiscus accessions across commercially available and economically valuable cultivars as well as experimental accessions. This cytogenetic knowledge can help identify hybridization barriers and speculate crossing compatibility as well as provide basic information regarding tropical hibiscus genome sequencing to hasten the breeding process with improved ornamental traits.

Material and Methods

Plant material. Ninety-six accessions of *H. rosa-sinensis* were assessed to determine variations in genome size. These plants were maintained at the Tropical Research and Education Center, University of Florida, Homestead, FL, USA. The hybrid lines were generated by controlled pollination using accessions with contrasting flower colors and petal phenotypes. The pollen was removed from the paternal flower by a cotton swab, followed by rubbing the swab and releasing pollen over a clean stigma of fully opened maternal plants. Fruits were harvested within 1 to 3 months after pollination. Seeds were germinated in a petri dish with cotton and 10 mL of water-soluble fertilizer solution prepared according to the dilution instructions (JR Peters, Allentown, PA, USA) and then placed in a growth chamber at 24 °C for 2 to 3 weeks. The germinated seedlings were transplanted to half-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) in 250-mL glass jars and cultivated for at least 3 months. The seedcoat of ungerminated seeds was removed to accelerate germination. Four F1 progenies germinated successfully and were used in this study: ADRHO (Anderson Double Red × Hollywood Orange); HDDR (Hawaiian Dot × Double Red); NHY (Nairobi × Hollywood Yellow); and SYYP (Sanibel Yellow × Yoder Pink).

Sample preparation and flow cytometry. The solution buffer was prepared as a general-purpose buffer with some modifications (Loureiro et al. 2007). Then, 50 mL of general-purpose buffer solution was configured with 1 mL of propidium iodide (1 mg/mL aqueous; Thermo Fisher Scientific, Waltham, MA, USA) to stain the nuclei and 250 µL RNase A (DNase and protease-free; 10 mg/mL; Thermo Fisher Scientific) to prevent staining of double-stranded RNA and 48.75 mL of GBP. *Oryza sativa* L. ssp. *japonica* cv. Nipponbare was used as the reference standard.

Root sample preparation. For each cultivar, tissues of three young roots (length, ≈1–3 cm) were collected randomly to represent three replications. In each case, young root tissues of tropical hibiscus and coleoptile of rice seedling were added at the same time to the same petri dishes with 1 mL of solution buffer and then chopped together using a sharp razor blade for approximately 60 s. Then, 0.5 mL of nuclear suspension was transferred to a 96-well flat plate through a 20-µm filter for analysis. All processes were maintained on ice until the samples were analyzed.

Leaf sample preparation. Healthy leaf sections (1 cm × 1 cm) were randomly collected from 'Anderson Double Red', 'Tahitian Maid', 'Brilliant Red', 'Yellow Wings', 'TX-19871', and NHY. To avoid the masking of rice nuclei by mucilage from tropical hibiscus leaves, leaf tissues from the tropical hibiscus accessions and *O. sativa* were chopped separately in two different petri dishes with 0.5 mL of solution buffer. For flow cytometry, 0.25 mL of each nuclear suspension was subsequently added to the same immunoassay microplate well via a 20-µm filter.

Genome size estimation. The DNA content and genome sizes were measured using a flow cytometer (Attune Autosampler; Attune Acoustic Focusing Cytometer, Invitrogen, Singapore). The data were collected with clear and well-defined peaks for both the internal reference and hibiscus samples. Each run was analyzed using a minimum of 2000 nuclei but an average of approximately 5000 nuclei. The 2C genome size was calculated as the standard 2C value (pg) = (sample peak mean/standard peak mean) × nuclear DNA content of the reference standard (pg). The 2C standard of rice was 0.795 pg (Temsch et al. 2022). The mean genome sizes and standard deviations (*SD*s) were calculated using Microsoft Excel (Redmond, WA, USA). The Wilcoxon signed-rank test was conducted in R using the *wilcox.test* function to test the significance of differences in genome sizes resulting from leaves and roots.

Results

Tropical hibiscus flow cytometry optimization with leaves and roots. Tropical hibiscus is a crop containing high mucilage, which increases the sample viscosity and prevents nuclei isolation for flow cytometric analysis. Although the abundance of mucilage varied among tropical hibiscus accessions, it still posed a challenge to the leaf sample preparation for flow cytometry. Based on this characteristic, leaves and roots from tropical hibiscus accessions 'Anderson Double Red', 'Tahitian Maid', 'Brilliant Red', 'Yellow Wing', 'TX-19871', and F1 progeny ADRHO were selected to perform flow cytometry to compare the feasibility of genome size measurements. Not all accessions were able to generate sample peaks to estimate the genome size when using leaves as materials (Fig. 2A–2C). This may be attributed to the presence of a high mucilage content in those accessions, which inhibited nuclei isolation during filtering process. The peaks from root samples were clearer and without significant background noise (Fig. 2D–2F), which was a positive signal of reliable estimation. The genome sizes of 'TX-19871', 'Yellow Wing', and ADRHO were estimated using root and leaf samples, respectively. The Wilcoxon signed-rank tests confirmed that genome size differences between root and leaf tissues were consistent across all three cultivars (Table 1). The data indicated that, in the case of tropical hibiscus, root samples outperformed leaf samples when flow cytometry was conducted, and that the genome size estimation based on leaves was likely to be less accurate.

Genome size variation of tropical hibiscus. To study the pattern of DNA content variation in a tropical hibiscus germplasm, we

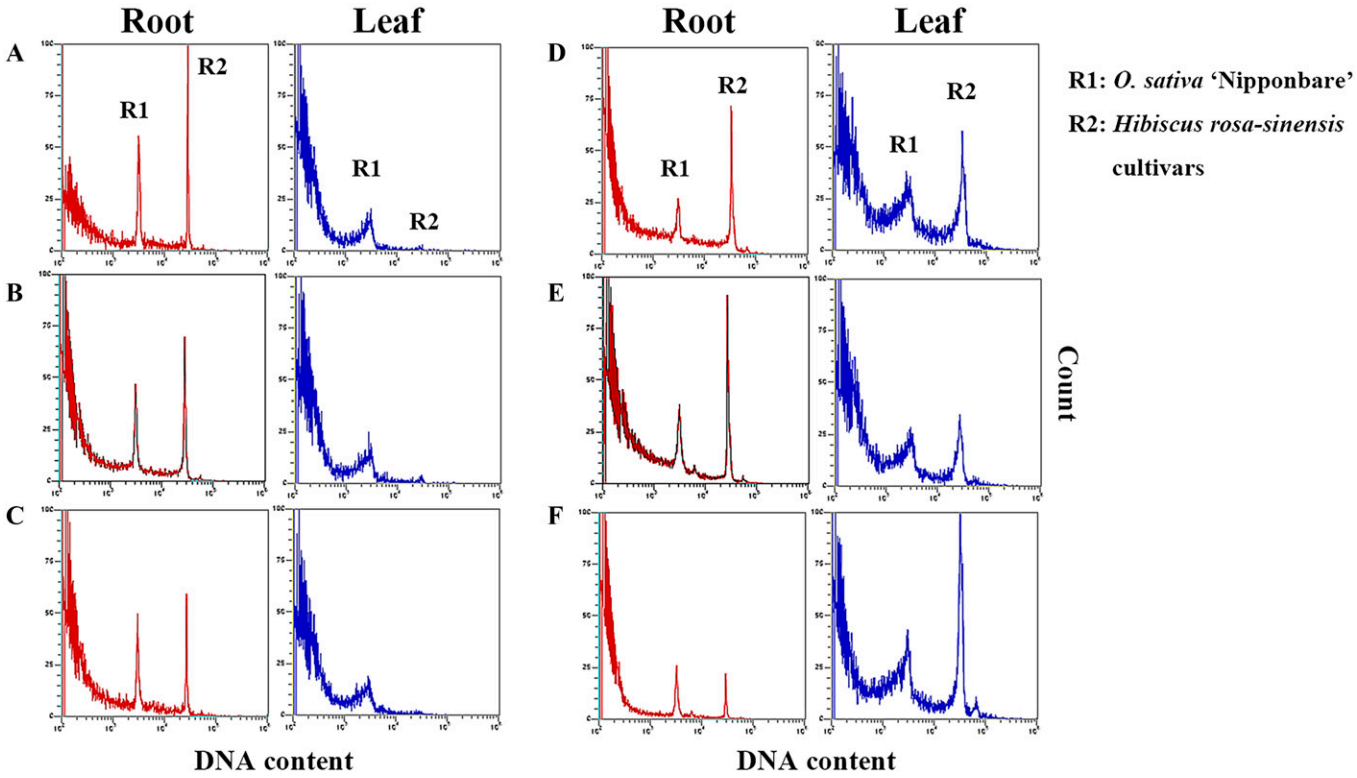

Fig. 2. Histogram of nuclear DNA contents in roots and leaves of tropical hibiscus accessions: (**A**) 'Anderson Double Red'; (**B**) 'Tahitian Maid'; (**C**) 'Brilliant Red'; (**D**) 'TX-19871'; (**E**) 'Yellow Wing'; and (**F**) F1 ADRHO.

sampled 96 commercially important tropical hibiscus accessions to conduct a high-resolution flow cytometric analysis. We observed a significant variation in DNA content among these accessions, with a 4.1-fold difference ranging from 3.13 ± 0.03 pg to 13.04 ± 0.10 pg (Table 2). Most accessions had a relatively low *SD* in DNA content, indicating that a consistent DNA content was estimated for each cultivar (Table 2). Genome sizes were calculated based on DNA content, which varied widely (Table 2), with the smallest genome size being 3.06 Gbp ('Queen Aussie') (Fig. 3A, Table 2) and the largest being 12.75 Gbp ('Joanne') (Fig. 3C, Table 2). Among the accessions studied, 85 accessions had genome sizes ranging from 6 to 10 Gbp, including 'Versicolor Pink', which had a genome size of 7.10 Gbp (Fig. 3B, 3D, Table 2). In contrast, only eight accessions had genome sizes between 3.06 and 4.11 Gbp, while three accessions were within the 11.05 to 12.75 Gbp range (Fig. 3D, Table 2).

Genome size estimation in tropical hibiscus F1 hybrids. To investigate the potential impact of genome size on the success of hybridization in tropical hibiscus, we crossed different accessions and obtained four successful hybridizations. Root tissues were collected from F1 hybrids for flow cytometric analysis. All the hybrids displayed good performance according to flow cytometry, with distinct sample peaks and minimal *SD*s (Table 3). These hybrids, which originated from diverse parental groups, exhibited similar genome sizes from 6.85 to 7.26 GB (Table 3). The parental genome sizes were relatively consistent within HDDR, NHY, and SYYP, with differences of less than 0.5 GB (Table 3). The only hybrid whose parents differed in size by more than 1 GB was ADRHO (Table 3). These results revealed that the F1 hybrids that resulted from these crosses tended to possess genome sizes within a relatively narrow and consistent range, with only minor variations compared with their parent plants.

Discussion

Tropical hibiscus production faces several challenges, including a limited germplasm pool, shortage of locally adapted cultivars, self-incompatibility, and insufficient genomic information. Traditional breeding methods alone cannot fully address these issues. Genome size is regarded as a constant characteristic of a species and has garnered increasing attention because of its role in understanding genetic diversity, supporting genomic studies, and guiding prebreeding evaluation. In *H. syriacus*, for example, variations in genome size and ploidy have been used to guide artificial polyploidy manipulation and genome doubling efforts (Lattier et al. 2019). These approaches highlight the potential of cytogenetic tools to provide more precise insight into the genetic basis of tropical hibiscus, further helping to accelerate the development of improved cultivars.

Leaves are commonly used for genome size estimation of plants because of their accessibility and reliable results (Pellicer and Leitch 2014). Traditionally, leaves have been used for flow cytometry of many *Hibiscus* species (Deepo et al. 2022, 2023). However, recent studies emphasized the importance of revisiting assumptions and methodological variations in flow cytometry results, including tissue type (Nix et al. 2024). For species with a high mucilage content, such as some crops in the Malvaceae family, flow cytometry involves significant challenges. The mucilage can cause cell aggregation, leading to inaccurate fluorescence signals and unreliable genome size estimates. This study compared the suitability of leaves and roots for a flow cytometric analysis of tropical hibiscus. Root tissues from tropical hibiscus were more appropriate for flow cytometric sample preparation because the high mucilage content in leaves interfered with nuclei isolation, resulting

Table 1. Comparison of genome size between roots and leaves of 'TX-19871', 'Yellow Wing', and 'F1 ADRHO'.

	DNA content (Gbp/2C) mean ± *SD*		*P* value
Accessions	Root	Leaf	(Wilcoxon signed-rank test)
TX-19871	8.66 ± 0.0025	9.07 ± 0.7928	0.5
Yellow Wing	6.84 ± 0.0529	7.29 ± 0.1037	0.25
F1 ADRHO	7.16 ± 0.0887	7.91 ± 0.0615	0.25

SD = standard deviation.

Table 2. Genome size estimation of tropical hibiscus cultivars by flow cytometry.

	2C value (pg)		Genome size (Gbp)		
Tropical hibiscus cultivar	Mean	*SD*	Mean	*SD*	Source
Queen Aussie	3.13	0.03	3.06	0.03	Emerald Goddess Gardens
Brilliant	3.15	0.05	3.08	0.05	Collaborator
Queen Rose	3.15	0.02	3.08	0.02	Emerald Goddess Gardens
Queen snow	3.15	0.05	3.08	0.05	Emerald Goddess Gardens
Red Hot	3.22	0.07	3.15	0.07	Local Nursery
Lion Tail Yellow	4.13	0.08	4.04	0.08	Emerald Goddess Gardens
Lion Tail Peach	4.15	0.09	4.06	0.09	Emerald Goddess Gardens
Lion Tail Red	4.20	0.06	4.11	0.06	Emerald Goddess Gardens
Carnation	6.20	0.05	6.06	0.05	Local Nursery
pride of Hankins	6.37	0.13	6.23	0.13	Emerald Goddess Gardens
Yellow Wing	7.00	0.05	6.85	0.05	Emerald Goddess Gardens
Tahitian	7.09	0.05	6.94	0.05	Collaborator
Moorea Mangy Blue	7.12	0.00	6.97	0.00	Collaborator
Kona	7.13	0.00	6.97	0.00	Collaborator
Tahitian Maid	7.19	0.06	7.03	0.06	Emerald Goddess Gardens
Yoder Pink	7.19	0.05	7.03	0.05	Emerald Goddess Gardens
Brilliant Red	7.20	0.06	7.04	0.05	Emerald Goddess Gardens
Fiji Island	7.22	0.10	7.06	0.10	Collaborator
Ruth Wilcox	7.22	0.00	7.06	0.00	Collaborator
Marilyn	7.23	0.09	7.07	0.09	Emerald Goddess Gardens
Versicolor Pink	7.26	0.05	7.10	0.05	Emerald Goddess Gardens
Double Peach	7.26	0.05	7.10	0.05	Local Nursery
Double Red	7.29	0.05	7.13	0.05	Local Nursery
Anderson Double Red	7.29	0.12	7.13	0.11	Emerald Goddess Gardens
Cajun Line	7.29	0.05	7.13	0.05	Collaborator
Taiwan Magical River	7.32	0.09	7.16	0.09	Collaborator
Hawaii Dot	7.32	0.10	7.16	0.10	Emerald Goddess Gardens
Jane Cowl	7.35	0.06	7.19	0.06	Emerald Goddess Gardens
Hot Shot	7.42	0.10	7.26	0.10	Local Nursery
Yellow Wing	7.46	0.11	7.29	0.10	Emerald Goddess Gardens
Nairobi	7.46	0.12	7.29	0.11	Emerald Goddess Gardens
Hollywood Red	7.56	0.06	7.39	0.06	J. Berry Nursery
Senibel Yellow	7.66	0.07	7.49	0.06	Emerald Goddess Gardens
California	7.80	0.16	7.63	0.15	Emerald Goddess Gardens
Margurite	7.80	0.06	7.63	0.06	Emerald Goddess Gardens
Arizone	7.84	0.00	7.66	0.00	Emerald Goddess Gardens
Hollywood Yellow	7.87	0.06	7.70	0.06	J. Berry Nursery
Boreas White	7.98	0.06	7.80	0.06	Local Nursery
Seminole Pink	8.12	0.06	7.94	0.06	Emerald Goddess Gardens
Sunkist	8.12	0.13	7.94	0.13	Emerald Goddess Gardens
Adonis Pearl	8.16	0.11	7.98	0.11	Local Nursery
Peach Blow	8.20	0.07	8.02	0.06	Emerald Goddess Gardens
Painted Lady	8.27	0.11	8.09	0.11	Emerald Goddess Gardens
TX-19530	8.27	0.00	8.09	0.00	Collaborator
Hula Girl	8.31	0.06	8.12	0.06	Emerald Goddess Gardens
Wally's Yellow	8.34	0.07	8.16	0.07	Emerald Goddess Gardens
Hollywood Orange	8.34	0.06	8.16	0.06	J. Berry Nursery
El Capitolio Red	8.42	0.06	8.24	0.05	Collaborator
Bon Tempe	8.42	0.07	8.24	0.07	Emerald Goddess Gardens
Night Fire	8.46	0.12	8.27	0.12	Collaborator
Aehig Pelawn	8.46	0.07	8.28	0.06	Collaborator
Fairy Music	8.50	0.00	8.32	0.00	Emerald Goddess Gardens
Symphony	8.54	0.00	8.35	0.00	Plant Hawaii Nursery
Sunscape	8.54	0.07	8.35	0.07	Local Nursery
TX-18084	8.57	0.07	8.38	0.07	Collaborator
TX-19482	8.65	0.07	8.46	0.07	Collaborator
Yellow Tequila	8.65	0.07	8.46	0.07	Local Nursery
TX-19871	8.73	0.00	8.66	0.24	Collaborator
Simones Gold	8.77	0.07	8.58	0.06	Plant Hawaii Nursery
TX-19261	8.77	0.07	8.58	0.07	Collaborator
Fiesta	8.89	0.36	8.70	0.35	Local Nursery
Kalei's Valentine	8.90	0.19	8.70	0.19	Plant Hawaii Nursery
Sharlenes Beauty	8.91	0.55	8.71	0.54	Plant Hawaii Nursery
Vermillian Queen	8.97	0.12	8.77	0.12	Emerald Goddess Gardens
Kaliponi Ekahi	8.97	0.12	8.77	0.11	Plant Hawaii Nursery
Evangeline	8.97	0.11	8.78	0.11	Emerald Goddess Gardens
Swoon	9.05	0.08	8.85	0.08	Plant Hawaii Nursery
Mary's Miracle	9.05	0.25	8.86	0.24	Plant Hawaii Nursery
Ivy's Beauty	9.13	0.14	8.93	0.14	Plant Hawaii Nursery
David Orr	9.13	0.14	8.93	0.14	Plant Hawaii Nursery
Fair Damsel	9.17	0.08	8.97	0.07	Plant Hawaii Nursery
Halo	9.21	0.22	9.01	0.21	Plant Hawaii Nursery

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Table 2. (*Continued*)

Tropical hibiscus cultivar	2C value (pg)		Genome size (Gbp)		Source
	Mean	*SD*	Mean	*SD*	
Tangerine Dream	9.22	0.00	9.02	0.00	Emerald Goddess Gardens
September Mouring	9.22	0.25	9.02	0.24	Plant Hawaii Nursery
Minoakaakala	9.26	0.15	9.05	0.14	Plant Hawaii Nursery
TX-20463	9.26	0.19	9.06	0.18	Collaborator
TX-19871	9.28	0.81	9.07	0.79	Collaborator
Sunset Pink	9.30	0.19	9.10	0.19	Emerald Goddess Gardens
Claude Monet	9.34	0.12	9.13	0.11	Plant Hawaii Nursery
TX-20463	9.35	0.12	9.14	0.12	Collaborator
Prima Donna	9.42	0.15	9.22	0.14	Plant Hawaii Nursery
Seduction	9.51	0.07	9.30	0.06	Plant Hawaii Nursery
Pink Nectar	9.60	0.00	9.39	0.00	Emerald Goddess Gardens
Blessing Indeed	9.60	0.13	9.39	0.13	Plant Hawaii Nursery
Cele Tinney	9.64	0.07	9.43	0.07	Emerald Goddess Gardens
Marmalade Skies	9.65	0.28	9.43	0.27	Plant Hawaii Nursery
Stardust	9.68	0.07	9.47	0.07	Plant Hawaii Nursery
Sunny Yellow	9.69	0.20	9.47	0.20	Local Nursery
Fort Myers	9.73	0.01	9.52	0.01	Emerald Goddess Gardens
Double Delite	9.77	0.08	9.56	0.08	Emerald Goddess Gardens
Karens Beauty	10.00	0.24	9.78	0.23	Plant Hawaii Nursery
Kawaihapai Beauty	10.04	0.08	9.82	0.08	Plant Hawaii Nursery
Hulili	10.19	0.75	9.97	0.73	Plant Hawaii Nursery
Hollywood Pink	11.29	0.01	11.05	0.01	J. Berry Nursery
Hawaiian Sunset	11.92	0.16	11.66	0.16	Emerald Goddess Gardens
Joann	13.04	0.10	12.75	0.10	Emerald Goddess Gardens

SD = standard deviation.

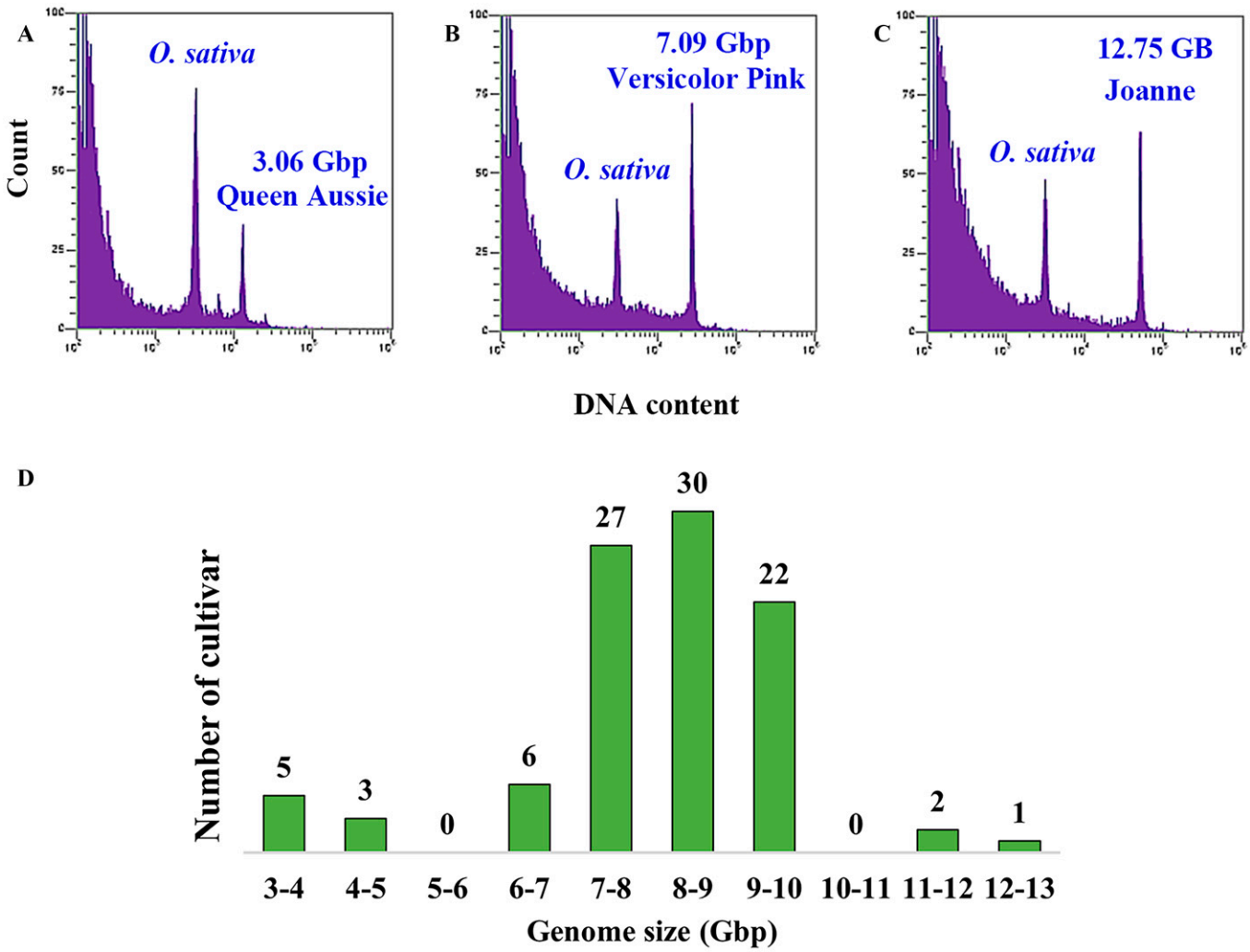

Fig. 3. A histogram of 'Brilliant', 'Versicolor Pink', and 'Joanne' according to flow cytometry (**A–C**) and the distribution of genome sizes of tropical hibiscus germplasm (**D**).

Table 3. Genome size information of F1 hybrids and corresponding parents.

	Genome size (Gbp)		Genome size (Gbp) of parents	
F1 progeny	Mean	*SD*	♀ (female)	♂ (male)
ADRHO	7.16	0.09	7.13	8.16
HDDR	6.85	0.05	7.16	7.12
NHY	7.26	0.10	7.29	7.70
SYYP	7.13	0.06	7.49	7.03

SD = standard deviation.

in poorly defined peaks (Fig. 2). Similar improvements have been observed in other mucilage-rich members of the Malvaceae family. For instance, in okra (*Abelmoschus esculentus* L.), roots are preferred over leaves for flow cytometric analyses (Salameh 2014). Similarly, in *Drimia indica*, a perennial herbaceous flowering plant in Asparagaceae family, young root tissue is favored because of its lower mucilage content (Nath et al. 2014). These findings suggest that roots may be a better alternative to leaves for flow cytometric studies of species with high mucilage content, ensuring more accurate and reliable results.

We estimated genome sizes of various tropical hibiscus accessions with diverse phenotypes. The results revealed a significant variation in genome size that ranged from 3.06 to 12.75 Gbp (Table 2). Most accessions exhibited genome sizes between 7 to 10 Gbp, which were substantially larger than those reported for relatives species (Deepo et al. 2022; Mohammad et al. 2020) such as *H. sabdariffa* (3337 Mbp), *H. sinosyriacus* 'Seobong' (4077 Mbp; 2n = 4×), and *H. moscheutos* 'Luna Red' (2014 Mbp; 2n = 2×). Polyploidy is one of the major evolutionary forces that drives large increases in the nuclear DNA content. Polyploids typically exhibit larger 2C value than those of their diploid progenitors (Leitch and Bennett 2004). 'Brilliant' is the only cultivar identified as tetraploid, with 84 chromosomes, and it also has the lowest ploidy of this species (Deepo et al. 2022). Based on the genome size of 'Brilliant' (3.08 Gbp) (Table 2), we can infer that *Hibiscus* sp. seems to have a high level of ploidy and most likely possesses a significant variation in ploidy levels among accessions. Tropical hibiscus progenitors, such as 'Queen Rose', 'Queen Aussie', 'Queen Snow', 'Red Hot', and 'Lion Tail' series, displayed genome sizes similar to that of 'Brilliant'. This similarity indicates that these cultivars likely originated during the early evolutionary stages of the species. The long history of breeding tropical hibiscus has resulted in a highly complex hybrid group. Modern ornamental cultivars, which are widely available on the market, tend to have larger genomes, thus highlighting the extensive genetic diversity within tropical hibiscus.

Difficulties achieving successful cross-hybridization of tropical hibiscus have posed challenges for the development of new cultivars. Under typical tropical conditions, tropical hibiscus has shown a low seed-setting rate (Sharma and Sharma 1962); this phenomenon was also observed during our controlled pollination experiments. In this study, genome estimation of F1 progeny revealed that offsprings and parents generally maintained similar genome sizes (Table 3). This suggests that similar genome sizes may indicate the comparable genetic background, potentially playing a critical role in successful hybridization. Genome size information of progenies and their parents provides valuable insights into the inheritance patterns of genome size in this species. It is notable that current commercial cultivars exhibit diverse genetic backgrounds, indicating the potential for significant differences in ploidy levels and genetic diversity. Great distinctions in genome size and ploidy may result in challenges in hybridization caused by difficulties in chromosome pairing and structural incompatibilities. Conducting a karyotyping analysis is essential to determining the ploidy of various cultivars. Leveraging cytometric knowledge as a reference for cross-pollination will help accelerate the development of new desirable cultivars of this crop.

In conclusion, our study highlighted the considerable genomic variations present within tropical hibiscus. These findings provide a foundation for advancing the cytometric understanding of this species and offer opportunities for further exploration of genomic research and molecular breeding.

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