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A report on the genome size and ploidy status of wild genotypes of Cymbopogon species

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Abstract
The genome size varies across all species in plants, and their evolutionary dynamics have interested plant breeders. The study of genome in wild crop relative is crucial as it represents a practical gene pool which breeders could use. The genus Cymbopogon is one of the economically significant aromatic plant yielding essential oil and cultivated extensively. The wild Cymbopogon species were collected from different locations in Karnataka state, India. The genome size's characterisation was done using Flow cytometry technique using both chicken red blood cells and internal Standard. This study aims to provide vital information about Genome size as this could be used for breeding and conservation purposes.

Keywords: Cymbopogon, wild crop relative, genome size, ploidy status, flow cytometry

Introduction

The Indian sub-continent is home to many indigenous plant species. Among which aromatic and medicinal plants are investigated for their beneficial values. The genus Cymbopogon is one of the most important essential oil yielding genera of the family Poaceae and includes about 140 species [1]. More than 52 species have reported occurring in Africa, 45 in India, six in Australia and South America, four in Europe, two in North America and the remaining are distributed in South Asia [2]. In India, it grows wild in all regions extending from sea level to an altitude of 4200 m. Several species are endemic to India. East Indian Lemongrass grows wild in India and is cultivated well in Kerala, Assam, Maharashtra and Uttar Pradesh [3].

Most of these species produce characteristic aromatic essential oils that have commercial importance in perfumery, cosmetics and pharmaceutical applications. Cymbopogons are highly stress-tolerant plants that are adapted to diverse edaphic-climatic conditions, occurring widely throughout the tropics and subtropics [4]

Cymbopogon species display wide variation in morphological attributes and essential oil composition at inter and intraspecific level, and over the years, different chemo cultivars varying in their aroma have been selected or crossbreed with other cultivars or closely related species produces different types of essential oil, such as palmarosa oil, lemongrass oil, citronella oil, ginger grass or rusa oil of commercial interest [1].

Phenotypic differences between wild and cultivated plants are generally apparent and studied extensively in many plant species. Genetic mechanisms underlying those phenotypic differences and the domestication processes were studied in several plants [5].

Commercially, the Lemongrass (*Cymbopogon flexuosus*) and Palmarosa (*Cymbopogon martinii*) are the common species that are widely cultivated for their essential oil. These species' oils are used in perfumes, soaps, cosmetics, toiletry, tobacco products, and other related industrial products [6].

Exploration of the genome content in wild plants could help breeders. The nuclear genome is characteristic of a particular organism [7]. Knowledge of its size is essential for identifying species, verifying their taxonomic position, and identifying plant material. Measuring cells' DNA content is a well-established method for monitoring cell proliferation, cell cycle, and DNA ploidy. DNA ploidy indicates the number of chromosomes in a cell; some cell populations can have abnormal DNA content in the cell and due to the anomalies in DNA replication, and therefore, a different ploidy. Variation in DNA content can be linked-to many other factors influencing organism form and function. Under the "Nucleotypic Theory," total DNA content, or the nucleotype, has a different impact on the organism than the phenotype or genotype [8]. DNA content is expected to directly impact nuclear and cell volume, affecting other morphological and ecological features.

Flow cytometry can measure DNA content of cells, and reveals the information on cell position in the cell cycle and the ploidy and DNA content of a given cell population. The DNA content is expressed as a DNA index, which is the DNA's quantity in the test cell population to that in normal diploid cells [9]. Flow Cytometry has several advantages over conventional microscopic techniques to determine the state of the cell cycle of eukaryotic cells. It is a rapid, accurate and convenient technique; observations of mature hybrids' morphological characteristics are possible by performing DNA content measurements at the early stages of seedling development. FCM is also helpful in identifying Intra and interspecific hybrids in hybridisation breeding. A disadvantage of this technique is that it requires a single-cell suspension [10].

In the present study, the wild Cymbopogon species were explored from different ecological locations. Their genome content was determined to provide information related to the genome that could be further explored for their bioactivities and breeding purpose.

Materials and Methods

Collection and Identification of Plant samples

The Cymbopogon species (Sample 1) was collected from Charmadi Ghat, Dakshina Kannada District. The latitude and longitude are 13.05708°N 75.42791°E. The foothills of

Charmadi Ghat are a reservoir for dense flora, particularly medicinal plants. It is a semi-evergreen forest located at an elevation of 240 to 430 m above msl with a steep slope in some areas and represents Western Ghats' rainforests. The area has an annual temperature of 26.9-28 ºC. The soil is lateritic to sandy loam type, and the forest receives an annual rainfall of 3800 mm, mainly during the southwest monsoon season, i.e., June-November with a prolonged dry season of about 6 months.

The Wild genotype of Cymbopogon plants (Sample2) was collected from the hilly regions of Devarayan Durga Hills, Tumkur. Which is located at 13.375° N and 77.123° E, the area is 42.27sq.mt; the annual temperature is around 22-25 ºC, about 1290 meters above sea level, having red sandy soil. The Hill receives low rainfall throughout the year with an average temperature of 22.9 °C. The Hill was found to have a plethora of medicinal plants and abundant occurrence of Wild Cymbopogon species. The collected plant samples were submitted to Regional Ayurveda Research Institute for Metabolic Disorder (RARIMD), Bengaluru, India, for Authentication. *Cymbopogon citratus* collected from CIMAP, Bangalore, was used as a reference Standard.

Sample preparation for Flow Cytometry

The young leaf tissue from Sample 1 and Sample 2 was cleaned, dried and weighed to about 50mg. The samples were taken in Petri dishes, and 1ml of buffer solution was added. The leaf samples were chopped using sharp razor blade for approx. 60 seconds at 4 ºC. The reagents used were PI/RNase Staining Buffer, Galbraith's buffer (45mM MgCl2, 30mM Sodium Citrate, 20mM MOPS and 0.015% v/v Triton X-100), 1% PVP-10 and 15mM β-mercaptoethanol and DNA QC Particles. The resulting homogenate is filtered through 40 µm nylon filter to remove large debris. Nuclei were stained using 50 µg/ml propidium iodide (PI), and 50 µg/ml RNase was added to the nuclear suspension to prevent double-stranded RNA staining. The samples were incubated on ice and analysed within 10 minutes [10, 11]. The DNA content was calculated using the formula,

For DNA content,

$$\text{Sample 2C DNA content} = \frac{\text{(sample } G_1 \text{ peak mean)}}{\text{(standard } G_1 \text{ peak mean)}} \times \text{standard 2C DNA content (pg DNA)}$$

For Genome size calculation,

1 pg DNA = 0.978 x 10^9 bp

Result and Discussion

Our country's biodiversity is under threat due to the over-exploitation of natural resources, and natural calamities have contributed to the depletion of bioresources. Therefore, the need to explore and document wild species of plants is most important as many plants could become extinct even before their discovery. The germplasm diversity is vital for plant conservation and improvement. Therefore, there is interest in determining the genetic diversity in Cymbopogon germplasm

The Plant samples (Sample 1 and Sample 2) submitted to RARIMD were identified as *Cymbopogon flexuosus* (Accession No: SMPU/RARIMD/BNG/2019-20/173/RRCBI-mus231) and *Cymbopogon martini* (Accession No: SMPU/RARIMD/BNG/2019-20/352/RRCBI-1052).

The genome size for Sample 1 and Sample 2 were determined using CRB Cells as Standard, and the genome size was found to be 1815 Mbp and 3196 Mbp, respectively. The ploidy was determined by using *Cymbopogon citratus*. The ploidy of sample 1 was tetraploid, and Sample 2 was diploid. The knowledge of genome size is essential to understand the nature of the cell size and phenotypic characters. The plants located in the respective locations are under stress and probably have adapted to the harsh climatic conditions and could contribute to biotic and abiotic stress and continue to evolve as they have not undergone domestication processes.

Analysis of genome variation in wild populations in contrasting environments may deliver insights into how plants adapt to climate variation under natural selection. This may define options for use in plant breeding for climate resilience [12]. The choice of wild germplasm collected in environments similar to the target agricultural production area is a strategy that has been employed. Characterisation of the genome of the adapted genotypes will provide tools for dissection of the genetic basis of performance in these environments and help adapt agriculture to climate change. The variation in genes in wild populations [13] with climate may indicate strategies for developing agricultural crop genotypes adapted to new or variable environments [14]. Adaption to new environments involves adaptation to abiotic or biotic challenges that can be revealed by whole-genome analysis [15].

Table 1: Genome size and ploidy status of Sample 1, Sample 2 and Cymbopogon citratus

Species	Control (CRBC)	*C. martinii* (Sample 1)	*C. flexuosus* (Sample 2)	*Cymbopogon citratus*
M1 mean	122891	97862	172334	183981
2C DNA content(pg)	2.33	1.86	3.28	3.51
Genome size (Mbp)	2279	1815	3196	3440
Ploidy	Diploid	Diploid	Tetraploid	Tetraploid

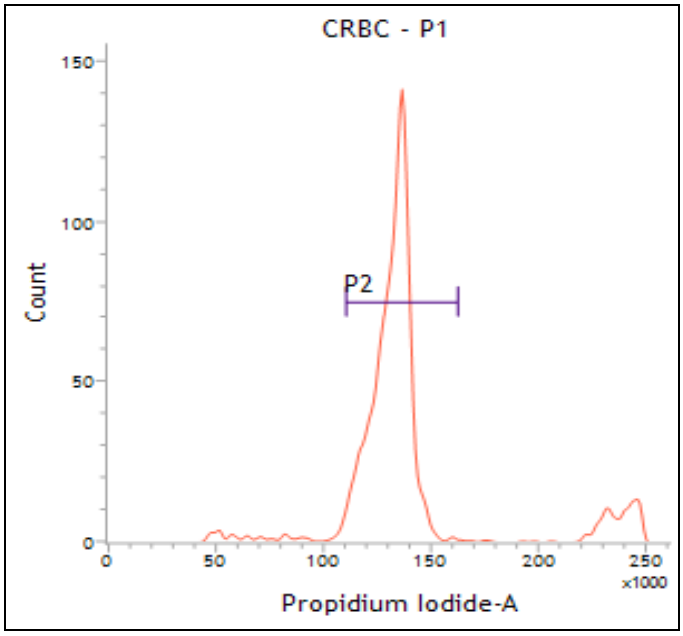


Fig 1: Histogram showing Genome size (CRBC Control)

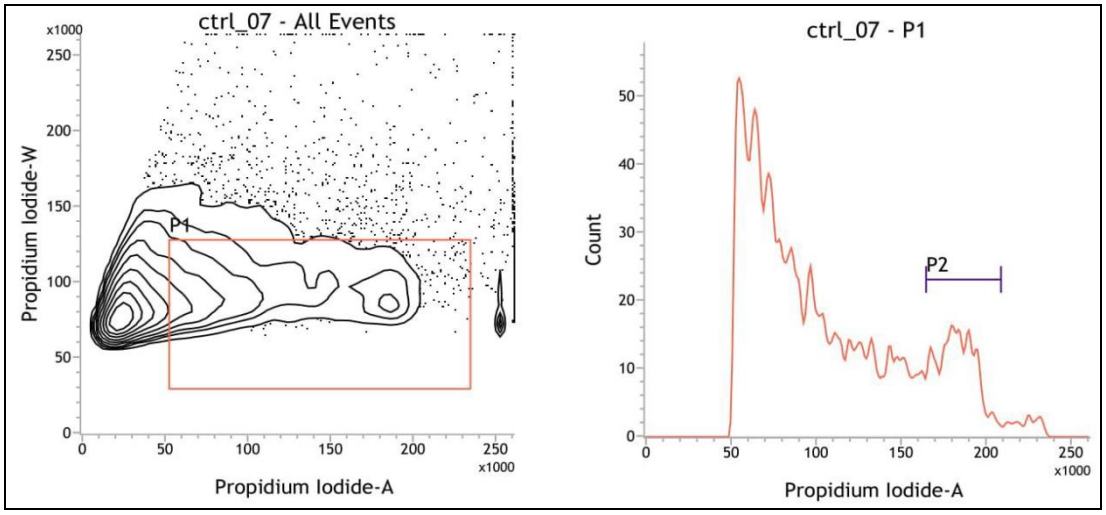
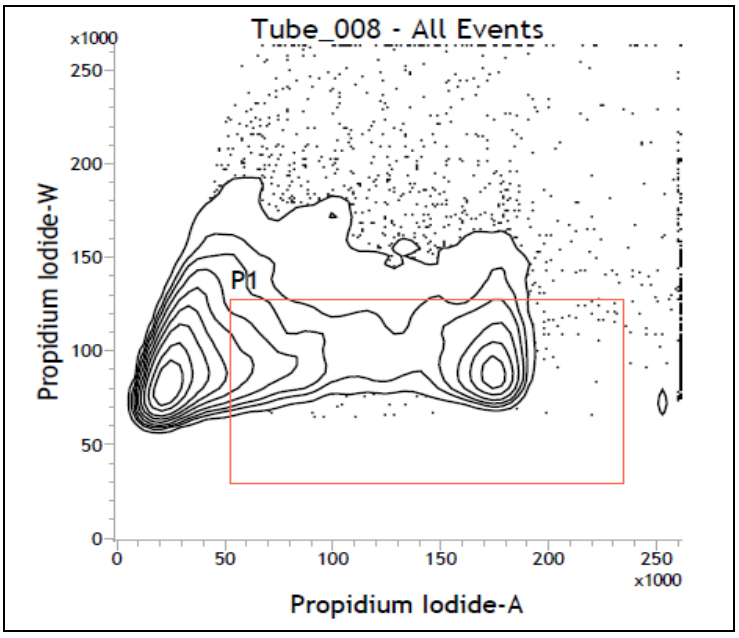


Fig 2: Cytogram of forward scattering and Histogram of DNA content in *C. citratus*



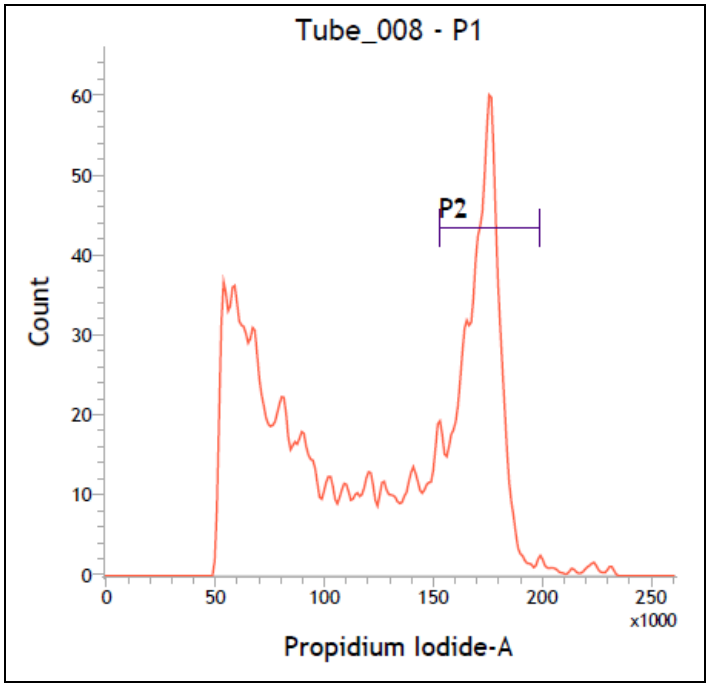


Fig 3: Cytogram of forward scattering and Histogram of DNA content in Sample 1 *C. flexuosus*

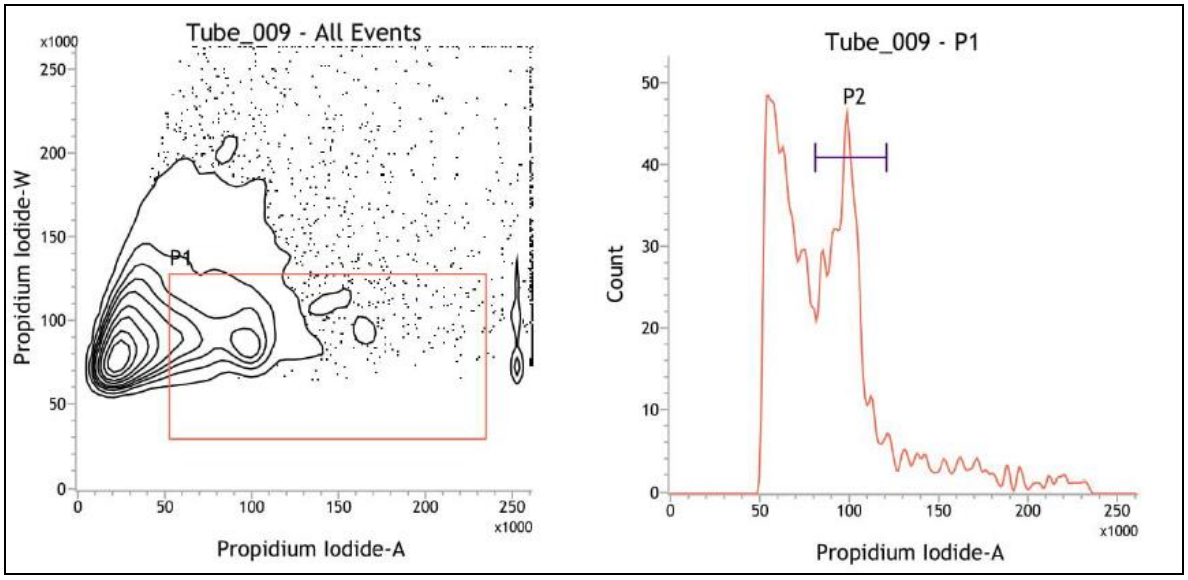


Fig 4: Cytogram of forward scattering and Histogram of DNA content in Sample 2 *C. martinii*

Table 2: Propidium Iodide Mean for CRBC, Sample 1, Sample 2 and *C. citratus*

Name	Events	%Parent	%Grandparent	%Total	Propidium Iodide-A Mean
CRBC	2,153	86.12	26.90	26.90	132,944
Cymbopogon citratus	467	15.84	4.81	4.81	183,981
C. flexuosus (Sample 1)	1,088	38.60	12.10	12.10	172,334
C. martini (Sample 2)	977	41.95	10.35	10.35	97,862

Conclusion

Knowing the genome size in wild Cymbopogon species could help provide conservation strategies and understand the relation between wild plants and cultivated plants. These crop wild relatives represent a primary source of diversity for use in plant breeding. As there is very little information available on the wild species from their cultivars were developed, and in some cases, the wild plant populations may even be extinct. Several species may have contributed to the domesticated plant's genome based on its ploidy in few cases. Other related species to the immediate wild relative may also be part of a vast gene pool that can provide a reservoir of genetic diversity for the crop. The genetic diversity scenario presented for the Cymbopogon species and variants is valuable for conserving their germplasm and beneficial for breeding new or novel varieties/chemotypes of Cymbopogons.

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