Research Article

Impact on Variation in Genome Content of Wild *Cymbopogon flexuosus* (Nees ex Steud) Collected from Different Ecological Locations of Karnataka, India

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ABSTRACT

The present work explores the variation in genome content of wild *Cymbopogon flexuosus* from different locations in Karnataka. Collected plant samples showed genotypic and phenotypic variations. The samples of wild *Cymbopogon flexuosus* (E1 and E2) were collected from Bannerghatta hills, Bangalore and Charmadi Ghat, Dakshina Kannada district, respectively. The locations for plant collection were selected based on ecological differences such as altitude, temperature, precipitation and soil type. The soil analysis revealed variation in macronutrient and micronutrient content and composition. The studies on E1 and E2 showed significant differences concerning morphological characteristics such as plant height, leaf length, inflorescence colour and essential oil content. Flow cytometry studies showed differences in genome size between E1 (2718 Mbp) and E2 (2211Mbp), even though their ploidy status was similar. Due to environmental variations in these ecotypes, the plants could be further explored for crop improvement as they continue to evolve in the wild and develop special traits like pest resistance and drought tolerance.

Keywords: Wild *Cymbopogon flexuosus*, Morphology, Essential oil, Genome content, Ploidy status.

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INTRODUCTION

Natural diversity has led to enormous variety in wild plant species, and most genetic variants are found in domesticated plants. These plants show phenotypic differences in wild or cultivated plants due to single-gene (monogenic) allelic variants.[1] The natural variability present in cultivated plants has been exploited since their domestication thousands of years ago by genetically manipulating developmental traits and physiological traits associated with adaptation to agriculture. Analysis of the natural variability of wild species has begun to elucidate the molecular basis of the phenotypic differences related to plant adaptation to different environmental conditions and to determine the ecological and evolutionary processes that support this variability.[2]

Monocots are diverse and show striking variation at many levels, ranging from gene sequence to the number of chromosomes per genome and genome size.[3] Identifying genetically diverse species has proven to be challenging due to the unavailability of information related to genomic diversity. The assessment of genetic diversity is crucial for the conservation and management of these species.[4] Studies on genetic diversity are a significant step in developing plant breeding programmes.[5] A better understanding of the genetic relationship could be established between the wild and cultivated plant species and, therefore, could utilize the genetic resources of a crop's wild relatives with their primary gene pool.[6]

Cymbopogon is an important genus of plants in the family Poaceae, producing essential oil.[7] Cymbopogon has about 140 species worldwide, of which 45 species

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have been recorded in India. The genus Cymbopogon is abundant in the tropics and subtropics of Asia, Africa, and America, with a regular distribution from mountains and grasslands to arid zones.[8]

Wild genotypes of Cymbopogons show a wide range of variation in morphological and anatomical traits,[9] essential oil composition[10] and cytogenetic aspects.[11-12] Essential oils are complex aromatic compounds containing terpenoids, phenols, alkaloids, and tannins.[13] Lemongrass (*Cymbopogon flexuous*) and Palmarosa (*Cymbopogon martinii*) are commercially important species widely grown for their essential oil. The essential oils are used in perfumes, soaps, cosmetics, toiletry, tobacco, and other industrial products.[14]

Knowledge of the mechanisms that cause changes in GC content and their ecological consequences are fundamental to understanding the evolution of the genome. GC content studies have a long tradition in prokaryotic biology and systematics. Flow cytometry measures the DNA content of cells and reveals information about the cellular position in the cell cycle and the ploidy of a given cell population. DNA content is generally expressed as a DNA index, the amount of DNA in the test cell population in normal diploid cells.[15] Flow cytometry facilitates rapid screening of genome size in different populations of the same species and on multiple individuals per population, facilitating the detection of any polyploidy or hybridization events.[16]

Flow Cytometry has several advantages over conventional microscopic techniques in determining the state of eukaryotic cells' cell cycle. It is a rapid, accurate, and convenient technique; observing mature hybrids' morphological characteristics is possible by performing DNA content measurements at the early stages of seedling development. FCM is also helpful in identifying Intra and interspecific hybrids in hybridization breeding.[17] It is an essential criterion in the delimitation of taxa and in predicting lifestyles such as thermotolerance, growth rate or aerobiosis.[18]

The present study was conducted to establish the diverse nature of the plant species concerning the environment.

MATERIALS AND METHODS

Collection of Plant Sample

Two genotypes of Cymbopogons were collected from wild conditions with varied Eco-climatic zones such as Bannerghatta Forest, Bangalore (E1) and Charmadi ghat, D.K. (E2). For authentication, the plants collected were submitted to ICAR- National Bureau of Plant Genetic Resource (NBPGR), New Delhi. The reference Standard plant *Cymbopogon flexuosus* (Krishna variety) was collected from CIMAP, Bangalore.

Ecology and soil studies

The soil samples were collected from the plants' rhizosphere (4 different spots from the plot of plant growth), and the soil samples were mixed. The soil was air-dried at room temperature and sieved to remove pebbles and particles. The soil sample was analyzed for Macronutrients and Micronutrients like S, N, K, P, Fe, Si, Cu, Bo, Mn and Zn according to standard operating procedures at EHSC (Environmental Health and Safety Company), Bangalore.

Morphological Studies

The morphology of the plants E1 and E2 were studied based on the plants' appearance, height, leaf size, leaf colour, stem colour and inflorescence.

Essential Oil Studies

Hydrodistillation using a Clevenger's apparatus extracted the essential oil from the fresh leaves. The extraction was carried out for four hours. The essential oil collected was dried over anhydrous sodium sulphate to remove moisture and stored in sealed vials at 4°C until further studies. The essential oils were subjected to GC-MS analysis for compound identification. The column used was Agilent DB 5MS (60 m × 0.25 mm). The MS transfer line temperature was adjusted to 300°C, ion source temperature to 160°C and scans to 50 to 600 Da. The temperature was programmed to an initial 60°C hold for 2 min, Ramp at 5°C/min to 140°C, hold for 5 min and then Ramp at 5°C/min to 300°C hold for 5 min. InjAauto 280°C. The flow rate was adjusted to 1ml/min, and the carrier gas used was helium. The software used was GCMS solution, and the libraries used were NIST 11 and WILEY 8. The name and molecular mass of the components in the essential oil were determined.

Flow Cytometry studies

Preparation of sample

About 50 mg of young leaf tissue from E1, E2, reference Std. and Control (*Allium cepa*) were used for sample preparation. The individual plant (leaf) samples were taken, and 1 ml of buffer was added to each petri dish. The samples were cut with a sharp razor blade for approx. 60 sec at 4°C. 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 a nylon filter of 40 μm to remove large debris. Nuclei were stained with

50 µg/ml propidium iodide (PI), and 50 µg/ml, RNase was added to the nuclear suspension to prevent double-stranded RNA staining. Samples were incubated on ice and analysed within 10 min.[17-18] Before analysis, the Instrument MoFLo XDP Cell Sorter and Analyser were configured with 488 nm operating with Summit TM Software (5.1) and was verified for linearity, resolution and setup for doublet discrimination function. The DNA content and Genome size were calculated using the formulae,

- **For DNA content**

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

- **For calculation of Genome size**

$$1 \text{ pg DNA} = 0.978 \times 10^9 \text{ bp}$$

RESULTS

Identification of plant samples

The plant samples (E1 and E2), and seeds deposited at NBPGR, New Delhi, were identified as *Cymbopogon flexuosus*. (Accession nos. AC26/2021- E1 and AC27/2021- E2).

Ecology and soil studies

The collection site (Figure 1, 2) of wild *C. flexuosus* (E1), Bannerghatta hills, near Bannerghatta National Park, Bangalore, is about 980 m above sea level (12.770132°N and 77.567758°E). The annual temperature is about 26-29°C, with a yearly rainfall of around 700 mm. Precipitation falls over 8 months (April-November), with the maximum rain (50%) between August and October. January-March is the peak of dry months with precipitation from 0.3 to 46 mm. The Park is a tropical dry forest belonging to the Eastern ghat consisting of southern tropical dry deciduous forest, dry deciduous scrub forest and southern moist mixed forest.[19] The geology of the park and the surrounding regions show that the rocks belong to the most ancient formation, from cryptocrystalline to large granites and complex gneisses. The rocks are light to dark grey or whitish muscovite granite-gneiss or biotite granite-gneiss, and their structure, texture, and appearance vary considerably from place to place. The soil in the upper regions is red and gravelly, usually deep or shallow, mixed with metamorphic rock forms on undulating grounds. The soil of the valleys is sandy and is formed by smaller particles of decomposed rocks washed out and deposited during rains. The soil is shallow at the top of the hill and deep in the valleys and lowlands.[20]

Collection site E2 (Figure 3, 4), foothills of Charmadi Ghat (Dharmasthala region), DK, District showed latitude and longitude of 13.05708°N 75.42791°E. The foothills of Charmadi Ghat belong to Western Ghat. It is a semi-evergreen forest located at an altitude of 240 to 430 m above sea level, with a steep slope in some areas, representing the rainforest of the Western Ghats. The annual temperature is around 26.9-28°C. The soil is lateritic to sandy loamy. The forest receives 3800 mm of precipitation per year, mainly during the southwest monsoon season, i.e. from June to November, with a long dry season of about 6 months.

The soil analysis from the collection site of E1 and E2 was performed for micro and macronutrient analysis by Standard Operating Procedures (SOP) as adopted by Environmental Health and Safety Research and Development (EHSRD) Bangalore and represented in Table 1.

Morphological Studies

The ecotypes of *C. flexuosus* (E1 and E2) expressed variation in morphological characters. E1 showed tall, erect nature with 623±61.15 cm height during the flowering stage and possessed a slender and long inflorescence. The culm was erect, smooth, solid and glabrous at nodes. The leaf colour was dark green, with leaf blades linear tapering at both ends, growing up to 50.67±6.028 cm, having a width of about 1-1.5 cm. The leaf sheath was loose and long, becomes leathery and falls off from culms at maturity. The leaf sheath near the culms is purplish. The inflorescence is reddish-

Table 1: Macronutrients and micronutrients from soil samples of E1 and E2.

Sl. No.	Macronutrients	E1	E2
1.	Available nitrogen as N	1933.03 kg/ha	366.91 kg/ha
2.	Available phosphorous as P_2O_5	463.33kg/ha	70.66 kg/ha
3.	Available potassium as K	470.3kg/ha	237.44 kg/ha
4.	Available sulphur as SO_4	0.79mg/100g	0.11 mg/100g
	Micronutrients		
5.	Boron	1.6mg/kg	2.2 mg/kg
6	Iron	98.53mg/km	183.82 mg/kg
7.	Zinc	26.6mg/kg	16.7 mg/kg
8.	Copper	5.6mg/kg	8.0 mg/kg
9.	Manganese	233.3mg/kg	335.2 mg/kg
10.	Silica	1711.8mg/kg	mg/kg

Bannerghatta hills

Figure 1: Habitat of wild *C. flexuosus*- E1.

Figure 2: Habitat of E1.

Charmadi Ghat, Dakshina Kannada

Figure 3: Habitat of E2 - wild *C. flexuosus*.

brown with many branches. The branches end in a spatheole subtending a pair of racemes. The primary branches of the panicle are arranged spirally, supported by a subtending spathe that also has a prophyll. The inflorescence is supported by the stalk, also known as the raceme base. The raceme is compressed linear and

Figure 4: Habit of E2.

expanded at the tip. The lower pair of spikelets in a semi-sessile raceme are homologous to males; the spikelet stalk is not swollen but very short, and all other pairs in both hands are heterogamous. Spikelet-hermaphrodite has a length of 5-5.5 mm. The upper floret is bisexual; the lower lemma is narrow, transparent, and split to the middle, with an awn in the axil, paleo is absent, and the awn is 18 mm long. There are two styles, and the Stigma is plumose and reddish. The florets are reduced to a lanceolate-acute hyaline scale 3mm long with stamens 3 and anthers 2mm long.

E2 is semi-erect grass about 629.67±54.61 cm in height during the flowering stage, having a bent inflorescence. The stem is erect, smooth, solid and glaborous at the nodes. The leaf colour is light green. The leaf blades are linear, tapering at both ends, growing up to 58.67±4.19 cm in length and having a 1.5-1.75 cm width. The leaf sheath is long and deep purplish near the culms. The inflorescence is greenish-purple in colour, with many branches ending in a boat-shaped spatheole. The primary branches are arranged in a tire with three rays in each tire. The inflorescence is arranged alternately along the zig-zag axis. The raceme is compressed linear and expanded at the tip. The lower pair of spikelets in a semi-sessile raceme is homologous to males. The stalk of the spikelet on the stalk is not swollen but very short. All other pairs in both hands are heterogamous. Spikelets-hermaphrodites have a length of 5.5-5.75 mm. The upper floret is bisexual; the lower lemma is narrow, transparent, and split to the middle, in the axil with an awn, paleo is absent, and the awn is 20 mm long. There are two styles and one Stigma, which are plumose and purple. The florets are reduced to lanceolate-acute hyaline. The stamens are purplish with purple anthers. The difference in the morphology of the two ecotypes is tabulated in Table 2 and Figure 5-8.

Table 2: Morphological characters of Ecotypes (E1 and E2).

Sl. No.	Parameter	E1- wild *C. flexuosus*	E2- *wild C. flexuosus*
1	Habit	Tall erect	Semi erect
2	Tiller no.	22-26	26-30
3	Leaf colour	Dark green	Light green
4	Stem colour	Purple	Purple
5	Matured plant height (cm)	623±61.14	629.67±54.60
6	Suppressed internodes (no.)	6-8	7-9
7	Leaf length (cm)	50.67±6.03	58.68±4.19
8	Leaf venation	Parallel	Parallel
9	Inflorescence colour	Reddish	Greenish purple
10	Ligule (mm)	0.40±0.10	0.47±0.06
11	Auricle (mm)	0.63±0.06	0.67±0.06
12	Awn (mm)	18.67±0.58	20.0±2.0
13	Anther colour	Reddish yellow	Purple
14	Stigma colour	Reddish	Purple

Data represent the mean ± SD of three determinants.

Figure 5: Morphological differences in the inflorescence of E1 and E2.

A- Slender and long inflorescence (E1), **B**-Bushy and stout inflorescence (E2).

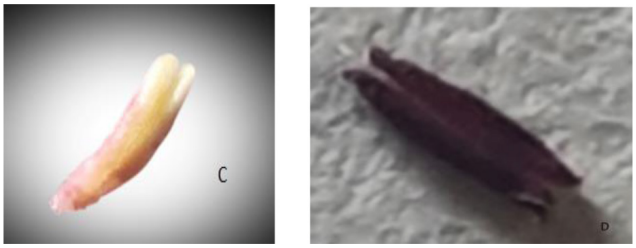

Figure 6: Microscopic view of Anther.

C- Yellow anther of E1, **D**- Purple anther of E2.

Essential oil studies

The major compounds identified in E1 were Elemicin (33%), δ-gurjunene (11.134%), β-bourbonene (4.257%), and β-caryophyllene (6.803%) and Limonene (4.405%) of the total essential oil that contained about forty-eight compounds.[21] The major compounds identified in E2 were Citral A and B (53.927% and 28.1%), Neryl acetate (5.608%) and Caryophyllene oxide (1.661%) of seventy-four compounds identified (Figure 9, 10).

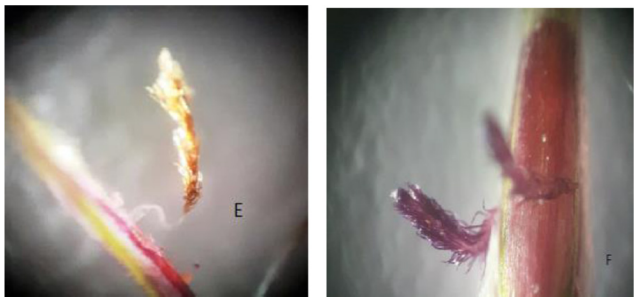

Figure 7: Microscopic view of Stigma.

E- Yellow Stigma of E1, **F**-Purple Stigma of E2.

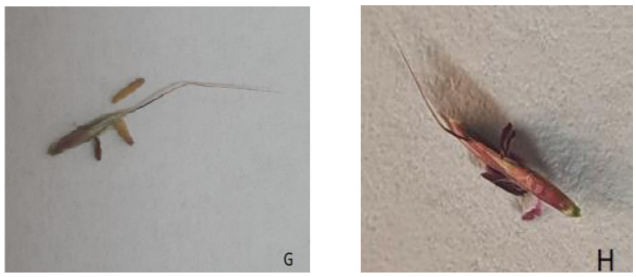

Figure 8: Sessile spikelet.

G- spikelet of E1, **H**- spikelet of E2.

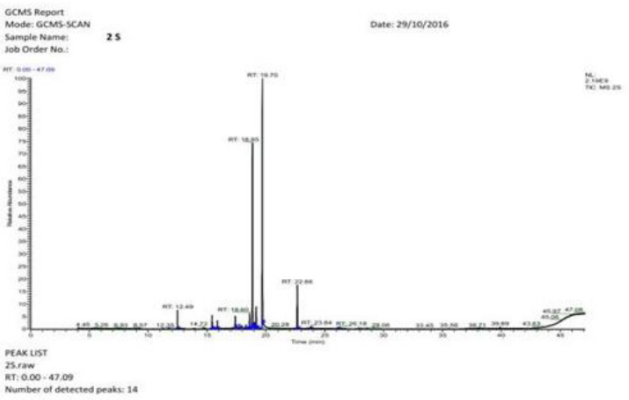

Figure 9: Chromatogram of the essential oil of E1.

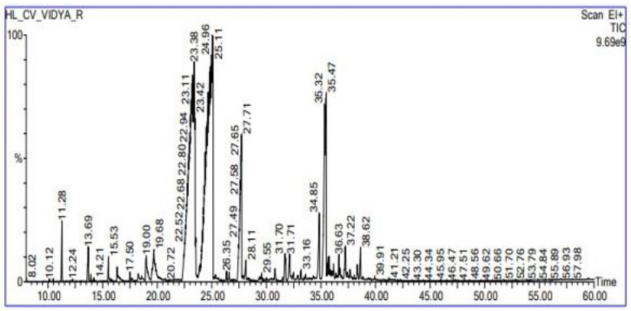

Figure 10: Chromatogram of the essential oil of E2.

Flow Cytometric studies

The results (Figure 11-15) of Flow cytometry analysis revealed the difference in genome size of E1 and E2.

Allium cepa (control) and cultivar *C. flexuosus* -Krishna variety (standard) was used. The samples' genome size and ploidy status were determined and tabulated in Table 3.

Flow Cytometric studies have revealed a significant difference in the genome size and have indicated significant differences at cellular and tissue level, influencing phenotypic and ecological behaviour. Since the genome size of E1 is larger than E2, the plants are shorter in height. The plants were scarcely distributed in and around the hilly region of Bannerghatta. Whereas E2 was distributed lavishly around the foothills of Charmadi Ghat and the plants were taller, which could be due to lower GC content. The difference in morphological characters is a clear indication attributed to the amount of GC content in plant samples, the height, distribution and phenotypic features. The decrease in the genome content of the cultivar variety is probably due to domestication.

Table 3: Size of genome and status of ploidy in E1 and E2.

Species	Control (*Allium cepa*)	Standard (Cultivar *C. flexuosus*)	E1 (Wild *C. flexuosus*)	E2 (Wild *C. flexuosus*)
2C DNA content (pg)	33.5	1.508	2.78	2.26
Genome size (Mbp)	32,763	1,475	2,718	2,211
Ploidy status	Diploid	Diploid	Tetraploid	Tetraploid

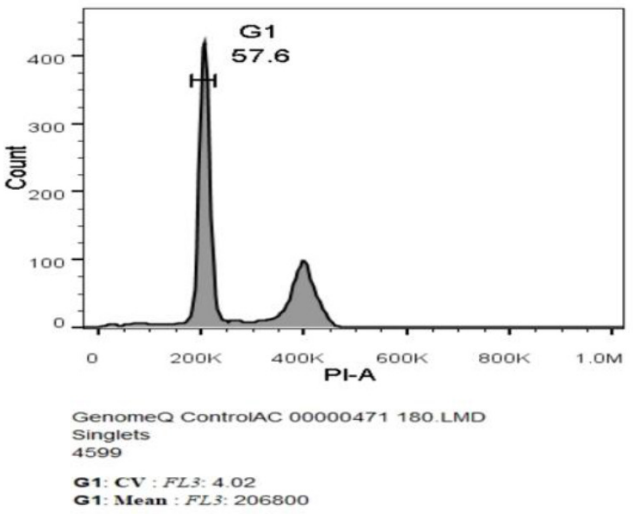

Figure 11: Histogram of a number of nuclei in control (*A. cepa* nuclei).

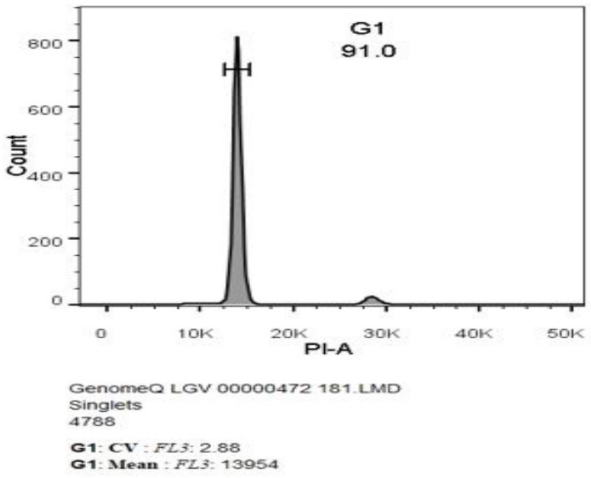

Figure 12: Histogram of a number of nuclei in standard (*C. flexuosus*).

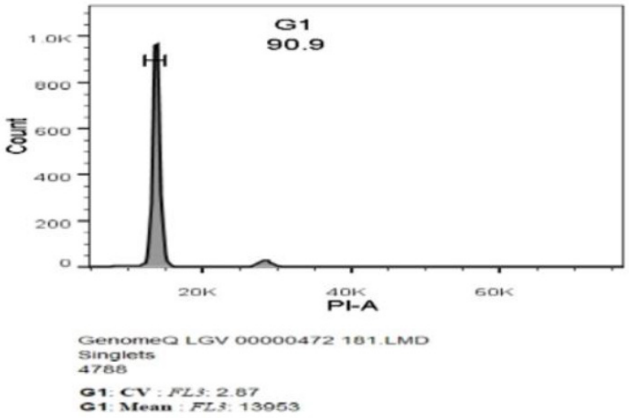

Figure 13: Histogram of a number of nuclei isolated from E1.

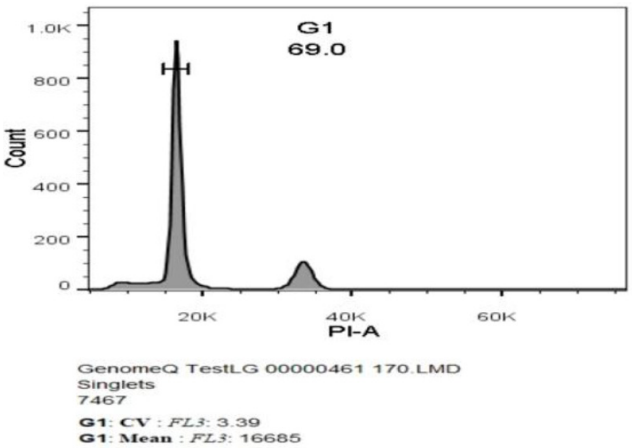

Figure 14: Histogram of a number of nuclei isolated from E2.

DISCUSSION

In the present study, two wild ecotypes (E1 and E2) of *C. flexuosus* collected from two varied ecological locations

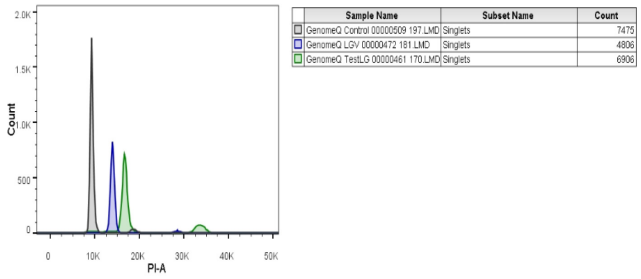

Figure 15: Histogram of E1 and E2 compared with cultivar *C. flexuosus* -Krishna variety.

of Bannerghatta hills and Bengaluru and Charmadi ghat Dakshina Kannada, respectively expressed a significant difference in morphological characters, essential oil content and composition showing variation in ploidy level and also in genome size.

The soil analysis of ecotype (E1) revealed higher concentrations of silica, available nitrogen, phosphorous, potassium and sulphur content, showing the soil formation process due to the weathering of muscovite leading to the presence of high silica content (1711.8mg/kg). The soil analysed from ecotype (E2) showed lower nitrogen, phosphorous and potassium content that can be attributed to the presence of laterite soil type.[22]

There was a difference in morphological characters between the ecotypes E1 and E2. The length of the leaf blade was smaller in E1 than in E2, possibly due to the larger genome size. This observation was much like the findings by Knight,[23] where cell division rate and cell size significantly influence leaf morphology. There was a difference in tiller numbers between E1 and E2, where E2 expressed more tillers than E1. Species with large genomes are found at intermediate altitudes, and species with small genomes tend to occur at both low (sea level) and high (2440m) altitudes.[24] The relationship between genome size and plant growth altitude may depend on factors such as temperature and rainfall.[24] Species with large genomes tend to be excluded from extreme environments with shorter growing seasons.[25] The E2 ecotype that sets its inflorescence during the hottest spring and summer months (April-June) tends to have a smaller genome size than the E1 ecotype, which grows in early spring (January-March) showing a larger genome size.[26] Smaller genome-sized E2 produced more significant quantities of seeds when compared to E1. The smaller genome size also helps to complete the life cycle faster.[27] The inflorescence of E2 was much thicker than that of E1 and bore a large number of seeds.

There was a difference between leaf and leaf mass of the ecotypes E1 and E2, which was reflected in their genome size. E1 with a lower leaf surface area showed a larger genome size, and E2 with a high leaf surface area showed a low genome size. The larger the genome size, the fewer and thicker will be the leaf, showing low specific leaf mass. Species with large genome sizes are limited to less stressful environments with longer growing seasons. Due to ecological constraints, species with larger genome sizes showed a small population size leading to a high probability of extinction.[24]

According to the earlier report by Smarda,[28] the relationship between the GC content and the ecological distribution of monocotyledons, particularly their tolerance to extreme temperatures, is related to the GC content. Higher the GC content, the tolerance is increased with the ability to grow in harsh conditions like seasonal droughts or extreme cold. Grasses show consistently high genomic GC content. GC-rich genes were characterized by fewer or no introns, a much higher GC content in the 5' region of the gene, more methylated CpG dinucleotide in the leading strand, and a higher frequency of regulatory TATA boxes in their promoter regions[29-30] suggests that GC-rich genes promote the plant's response to environmental stress. Certain cultivated monocots species showed significantly lower GC content than wild species of monocots.[31] Significant differences in genome content between cultivar and wild species have been reported.[32]

The content of essential oil in the two ecotypes was found to be very different. The difference in chemical composition may be due to a difference in the ecotype. As a result, they can be considered chemotypes of the same species due to factors such as temperature, soil type, climate, and differences in development and physiology.[33] E1 can be considered an elemicin type as this is the major component and E2 is the citral type.

The nuclear genome is characteristic of a particular organism.[18] Knowing its size is essential for identifying species, verifying their taxonomic status and identifying plant material. Measuring DNA content in cells is a well-acknowledged method for monitoring cell proliferation, cell cycle and DNA ploidy. Ploidy indicates the number of chromosomes in a cell. If DNA replication is abnormal, the cell population may have abnormal DNA content and different ploidies. Variation in DNA content can be linked to many other factors influencing organism form and function. The total DNA content impacts the organism differently than the phenotype or genotype.[34] The DNA content impacts nuclear and cell volume and could affect other morphological features. Species with larger genomes are less likely to generate progenitor species.[35] Ecological limits on the distribution and abundance of species with large genomes have shown

that they are limited to a less stressful environment with a longer growing season.[36] Volatile compounds in plants mediate the relationship of plants with abiotic factors such as light, temperature, drought, etc. e., and biotic factors such as microbial pathogens, herbivores, insects and other beneficial organisms.[37] Isoprene and monotherapy increase the heat resistance of photosynthesis, protect the photosynthetic apparatus and help maintain photosynthetic activity under high-temperature stress (temperature above 40°C) by stabilizing thylakoid membranes and suppressing ROS.[38] Of the 45 species of Cymbopogon found in India, only a few, such as the citronella, Palmarosa and lemongrass species, are cultivated for the commercial production of essential oil for the industry. Conventional breeding efforts have been made to improve varieties belonging to different Cymbopogon species, but these efforts do not include assessing and considering genetic diversity for parent selection. Knowledge of molecular marker-assisted genetic diversity profiles, the morphological and biochemical relationship and differences between Cymbopogon species[39] may offer further advantages of a strategic combination of traits and germplasm diversity.

CONCLUSION AND SUMMARY

In the present work, *C. flexuosus* expressed the difference in morphology, ploidy level and genome size due to differences in the ecological sites. The environmental conditions could further contribute to the origins of chemotypes. Soil analysis revealed a difference in soil composition from the collection sites, and the effect of this difference in the composition could lead to the difference in the volatile compounds. The morphological difference was significant. The importance of this study was to bring out the difference in the ecotypes of Crop wild relatives that are essential in breeding programmes as they are genetically related to cultivated plants. They are unattended by humans and continue to evolve in the wild as they develop traits like drought and pest resistance. Crop wild relatives could be the key to successful crop improvement by supplying genetic variability and beneficial characteristics. Further, more studies must be encouraged as there is a multitude of correlations suggesting that an increase in the genome size affects the phenotype through physical consequences of increased nuclei size and volume, as meagre information is available about variation in gene expression in wild genotypes and their genomic data associated with Cymbopogon.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EHS: Environmental Health and Safety Research and Development; **E1:** ecotype 1; **E2:** ecotype 2; **FCM:** Flow CytoMetry; **D.K:** Dakshina Kannada; **NBPGR:** National Bureau of Plant Genetic Resource; **CIMAP:** Central Institute of Medicinal and Aromatic Plants; **N:** Nitrogen; **S:** Sulphur; **K:** Potassium; **Fe:** Iron; **Si:** silicon; **Cu:** copper; **Bo:** Boran; **Mn:** manganese; **Zn:** zinc; **GC-MS:** Gas Chromatography-Mass Spectroscopy; **GC:** Genome content.

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