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Exploration and Characterization of Wild *Cymbopogon martinii* (Roxb) Wats. Var. Sofia

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Abstract

Cymbopogon martinii is commercially cultivated for its essential oil having importance in medicinal and aromatic industries. Oil exhibit antimicrobial, antifungal, antiviral, antihelmintic, antioxidant and chemo preventive drugs. *Cymbopogon martinii* growing wildly was explored from Devarayana Durga hills, Tumkur, Karnataka, India. Plant was characterized based on morphological, cytogenetics and anatomical features by (SEM and Microscopic studies). Flow cytometry analysis, DNA barcoding and essential oil studies were carried out to confirm the ploidy and species specificity. Plant was authenticated as *Cymbopogon martinii* (Roxb.) Wats. variety Sofia from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Ministry of AYUSH, under acc. no. RRCBI-1052. The herbarium (AC-25/2021) and seeds were deposited at ICAR - National Bureau of Plant Genetic Resources (NBPGR), Pusa, New Delhi. Wild plant showed differences in morphology with cultivar varieties generated by CSIR - CIMAP showing the adaptation towards environmental factors. Flow cytometry analysis confirmed species to be tetraploid using hexaploidy control (*C. citratus*). DNA barcoding sequence of *rbcL*, *matK* and ITS 2 spacer regions exhibited close similarities towards *C. martinii* upon NCBI BLAST search and the sequence was deposited in Gen Bank under Acc. no. MT905074. The major constituents of essential oil were found to be Alpha-bisabolol oxide (34.981%), Geranyl geraniol (25.22%), D-limonene (10.092%), Camphene (7.255%), Borneol (4.463%), alpha Pinene (2.04%), Linalool (1.573%), Geraniol (1.364%) and alpha Bisabolol (1.232%) upon GC-MS along with other compounds confirming to Sofia variety. Hence, conservation of explored wild *C. martini* would help in developing new breederlines possessing potential bioactive compounds with economic value for future.

Keywords : Wild *Cymbopogon martinii*, Exploration, DNA barcoding, Essential oil, Terpenoid compounds

Cymbopogons are one of the important members of the grass family *Poaceae* under the tribe *Andropogoneae*, hybridized and cultivated for its essential oil properties. They possess great value in the field of medicine from ancient periods and aroma industries (Promila, 2018). Taxonomically 180 species of Cymbopogons are known till today (Zahra *et al.,* 2020). They have been classified majorly into five series: *Schoenanthi*, *Rusae, Citrati, Proceri* and *Refracti* (Thara Saraswathi *et al*., 2016;). Fifty two species are seen in Africa, 45 in the Indian sub-continent, six each in Australia and South America, four in Europe and two in North America and remaining distributed in South East Asia (Vinutha *et al*., 2016). The wild Cymbopogons are distributed all over India and their distribution pattern has been elaborated by many workers. Cymbopogons are taxonomically determined *via* morphology, anatomy, physical characteristics of essential oils, presence of key secondary metabolic compounds and by molecular marker studies (Ashok *et al*., 2017).

Cymbopogon martinii (Roxb.) is a perennial herb, native to India; belongs to Ruseae series and possess two varieties, Motia - Palmarosa grass (2n=20) and Sofia - Ginger grass (2n=40) (Sree Latha *et al.,* 2017). The plant is commonly known as Rosha grass and is mainly grown for its essential oil. In India, the plant grows under diverse ecological conditions ranging from planar regions to hilly regions (Sangwan *et al*., 2001). It is largely cultivated in Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Uttar Pradesh for its rich geraniol content. Some of the cultivar varieties cultivated by Central Institute

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of Medicinal and Aromatic Plants (CIMAP) are Vaishnavi, Trishna, Tripta and PRC-1 (Padalia *et al*., 2011). It is also cultivated in Brazil, Madagascar and Indonesia (Verma *et al*., 2010). *C. martinii* is a highly cross-pollinated crop, hence a lot of variations are observed with yield of essential oils in the species (Smitha *et al*., 2018). The cultivars of *C. martinii* differ in essential oil content and its constituents at intra and inter-species levels. Morphological differences among them are often blurred, particularly at the intra-species level. The essential oil of *C. martinii* is commercially utilized in manufacturing of soaps, perfumery, cosmetics, medicine and in aroma industries (Verma *et al*., 2010; Ram Suresh *et al.*, 2014 and Promila, 2018). Since 2018, India's overall export value of Palmarosa oil has increased by 61.75 per cent, with the shipments worth 2.969 USD million (Google Wikipedia). The essential oil rich in volatile compounds also possess potential ability towards antimicrobial, antifungal, antiviral, antihelmintic, antioxidant and cytotoxic properties (Padalia *et al*., 2011; Promila 2018 and Bruna *et al*., 2014). Exploration of wild species provide insight on production of novel secondary metabolites which can be utilized for the benefit of mankind. Methods molecular markers, DNA barcoding studies helps in identification of wild species and to distinguish between them (Adhikari *et al*., 2015 and Osathanunkul *et al*., 2016). The present work on *C. martinii* variety Sofia was undertaken to explore the wild genotype species and to understand the underlying concept of essential oil biosynthesis.

Material and Methods

Collection and Identification of the Plant

The wild genotype of *Cymbopogon martinii* growing in rocky hills of Devarayana Durga (water scarce region), Tumkur, Karnataka was collected (Fig. 1) and maintained in the department garden of Microbiology and Biotechnology, Bangalore University for further studies. Sample of the soil was collected from the rhizosphere region of the plant (eight different spots from the area of collection); mixed together and air dried at room temperature, sieved to remove pebbles and particles. Soil sample was subjected to Macronutrients and Micronutrients (S, N, K, P, Fe, Si, Cu, Bo, Mn and Zn) analysis in Environmental Health and Safety Research and Developmental Centre (EHSRDC), Bengaluru, Karnataka. The plant was identified and authenticated from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Ministry of AYUSH, Bengaluru and the accession no. was procured. The herbarium and seeds were deposited at ICAR - National Bureau of Plant Genetic Resources (NBPGR), Pusa, New Delhi.

Fig. 1 : Habitat and habit of wild *Cymbopogon martinii* growing in Devarayana Durga hills, Tumkur, Karnataka

Morphological Studies

The plant was recognized (8 samples), carefully chosen and poised based on the ecological habitat (morphology) and its aroma value. The morphological characters such as length and thickness of culm, length and breadth of leaf, texture and color of the leaf, angle of the leaf with culm, length of ligule, length of leaf sheath, length of inflorescence, nature of branching, length of spatheole, shape of the bisexual spikelet was studied.

Leaf Anatomy

The anatomy of leaf (20 numbers) was studied by taking transverse section of fresh leaf lamina. The section was subjected to freshly prepared Schiff's reagent and incubated for 30 min at room temperature. Sections were then washed thrice for 10 min each in freshly prepared 0.5 per cent (w/v) Sodium metabisulphite in 0.1 per cent HCl and viewed under 10X and 40X magnification (Thara Saraswathi *et al.,* 2016).

The epidermis of the fresh leaves was scraped with sterile blade, stained with safranin and examined for stomatal structure, silica bodies, hooks, epidermal cells and lamina using standard methods described by Ellis (1976). The stomatal index was calculated using the formula,

$$(S/E+S) \times 100$$

Where, S: Number of stomata in an area (under the Microscope)

E: Number of epidermal cells in the same area

Scanning Electron Microscopy (SEM) Analysis

The foliar epidermal micro-morphological characters of the ecotype undertaken for study was analyzed using SEM. The foliar epidermal structural variations in both upper and lower surfaces of the leaf were observed. For Scanning Electron Microscopy (SEM) mature undamaged dried leaves of preserved grass specimens were used for study. Two pieces of leaf were taken and mounted on stubs with double coated scotch tape. One piece of leaf was mounted on stub from lower side in order to expose the upper surface and other piece was staked on stubs from upper side in order to expose lower surface in stubs. The specimens were sputter-coated with Platinum and then observed under SEM, JEOL JSM-6490F Field Emission Scanning Electron Microscope (JEOL Ltd., Japan). Each specimen was analyzed for features of stomata size, macro-hairs, micro-hairs, prickles, papillae and hooks.

Cytogenetic Studies

The root tips of wild *C. martinii* were collected between 12:30 to 2:30 pm. The plant was watered two hours before collecting the root tips to make the cells turgid and pre-treated with 0.002 M of 8-hydroxy quinoline for $2^{1}/_{2}$ hours at 15 ^{0}C. After thorough washing with running water for 30 min the roots were fixed in modified Carnoys I fluid (3:1, absolute alcohol: propionic acid) for 24 hours at 15 ^{0}C and finally the root tips were transferred to 70 per cent ethanol and squash preparations were made (Sree Latha *et al.,* 2017).

Flow Cytometry Analysis

Young leaf tissue (50 mg) was taken for sample preparation, one ml of buffer solution is added to petri dish containing the plant tissue and chopped using sharp razor blade for 60s at 4 ^{o}C. The resulting homogenate was filtered through 40 µm nylon filter to remove large debris. Nuclei are stained with 50 µg/ml propidium iodide (PI) (Choudhury *et al*., 2013) and 50 µg/ml RNase added to nuclear suspension to prevent staining of double-stranded RNA. Samples were incubated on ice and analysed within 10 min. prior to analysis using the instrument - Cytomics FC500 Flow cytometer, Beckman Coulter, USA with analysis software flow Jo V10.0.7 and was verified for linearity and resolution, and setup for doublet discrimination function using the DNA QC Particles kit from BD Biosciences. The genome content is calculated using the formula:

DNA content,

$$\text{Sample 2C DNA content} = \frac{\text{Sample P1 Peak mean} \times \text{Std. 2C DNA content (pg DNA)}}{\text{Standard P1 Peak mean}}$$

For Genome size calculation, 1 pg DNA = 0.978×10^{9} bp

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DNA Barcoding and Phylogenetic Analysis

Total genomic DNA was isolated from the plant sample using CTAB method. DNA was amplified using universally available plant specific oligo primers (*rbcL*, *matK* and ITS2 spacer region) (Ashok *et al*., 2017). 50 µl of PCR reaction mixture contained 50 ng of gDNA, 100 ng of each forward and reverse primers, two µl of 10 mM dNTPs mix, five µl of 10X Taq Polymerase buffer, 3U of Taq polymerase enzyme and made up with PCR grade water. The PCR program was as follows: an initial denaturation at 94 ^{0}C for five min, followed by 35 cycles at 94 ^{0}C for one min, annealing temperature standardized at 60 ^{0}C, extension temperature at 72^{0}C for two min and final extension was at 72 ^{0}C for 10 min. PCR product was run on one per cent agarose gel in 1X TAE buffer and the products were purified using Nucleo-pore, Genetix Biotech PCR clean up kit and purified fragments were sequenced. The sequenced data was edited using Bio edit tool. The experiment was repeated thrice for validation of reproducibility of the barcode sequence. Annotated sequence was subjected to BLAST (NCBI domain) for verification. Sequence thus obtained was submitted to Gen Bank of NCBI for accession number. Clustal W analysis was performed using updated version of MEGA 6.0 software and phylogenetic tree was constructed using Neighbour Joining method.

Essential Oil Studies

Extraction : Fresh leaves (100g) were cut into 3-5 inches length, washed and subjected to hydro distillation using Clevenger's apparatus for 4h at 45-50 ^{0}C (Clevenger, 1928). Anhydrous Sodium sulphate was added to the oil collected to remove moisture content. The oil yield was calculated based on the fresh weight of the leaves (v/w). Further the oil was subjected to GC-MS analysis.

$$\text{Percentage pf essential oil} = \frac{\text{Essential oil weight}}{\text{Fresh sample weight - air - dray sample factor}} \times 100$$

$$\text{Air - dray sample factor} = \frac{100 - \text{Moisture content}}{100}$$

Analysis (GC-MS)

Gas Chromatography - Mass Spectrometry analysis of essential oil was carried out *via* general acquisition on Shimadzu Gas Chromatography-QP2010S using HP-1 (methyl silicone) column (Rxi-5Sil MS) 60 m length, 250 µm thickness with Helium as carrier gas with a flow rate of 1.0 ml / min. Temperature was adjusted from 5 ^{0}C rising to 300 ^{0}C at a rate of 4 ^{0}C/ min., respectively with a hold for two min per ml with 1:50 split ratio. Injector temperature was 5 ^{0}C and the detector temperature was 300 ^{0}C. Identification of the compounds was done by comparing the relative retention indices of the peaks with reference to homologous series of n-alkanes, literature data, 3±10 the mass spectra of the compounds with those reported and by peak enrichment on co-injection with standard samples (Aldrich and Fluka), NIST mass spectral library ver. 11 and WILEY 18 search. The relative components were calculated based on the GC peak areas.

Results and Discussion

Collection and Identification of the Plant

The morphology, anatomy (SEM and Microscopic) and cytogenetics studies on the plant confirmed it as *Cymbopogon martinii* (Roxb.) Wats. variety Sofia. The plant was found growing in rocky hills (under water stressed condition) with limited soil availability. The soil color was found to be reddish brown having fine texture containing nitrogen (4030.38 kg/ha), potassium (716.8 kg/ha), iron (122.28 ppm), manganese (157.6 mg/kg) and silica (675.7 mg/kg) in higher percentages (Table 1). The P^H of the soil was found to be 6.8. Plant was authenticated under the Accession no. SMPU/RARIMD/BNG/2019-20/352/RRCBI-1052 from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Ministry of AYUSH, Bengaluru, Herbarium sheet and seeds were deposited under the accession number **AC-25/2021** in ICAR-National Bureau of Plant Genetic Resources, Pusa, New Delhi.

Morphological Studies

The morphological features were studied under following parameters: The plant showed bushy

TABLE 1

List of macro and micronutrients analyzed for the soil sample collected from Devarayana Durga hills, Tumkur, Karnataka

Parameter	Quantity
Macronutrients:	
Available nitrogen as N	4030.38 kg/ha
Available phosphorous as P_2O_5	285.46 kg/ha
Available potassium as K	716.8 kg/ha
Available sulphur as SO_4	0.20 mg/100g
Micronutrients:	
Boron	2.63 mg/100g
Iron	122.28 ppm
Zinc	60.9 mg/kg
Copper	10.8 mg/kg
Manganese	157.6 mg/kg
Silica	675.7 mg/kg

appearance, having erect culms with length of 140 - 175 cm. The tillers were 16 - 21 in numbers and each tiller unbranched. The leaves were light green in color with dark green purplish pinch at the base. Each leaf showed 54 - 65 cm length with width of 0.5 - 1 cm. the leaf was linear lanceolate in shape, coarsely scab rid along margin, cordate at base, amplexicaul and make obtuse angle with the culm. The ligule was membranous, chartaceous, 3 - 4 mm long. The leaf sheath was shorter than the internode and exposed at the nodal region. The inflorescence showed purplish green color with purplish red margins, linear - oblong, false decompounded panicle, upto 35 cm long, moderately dense; spathe three cm long, spatheole 15-18 mm long, elliptic acute, orange to bright red at maturity, racemes 10 - 12 mm long, sessile and pedicelled, pedicel spikelet hairy, swollen, adnate to rachis, sessile spikelet 2 - 3 mm long, elliptic oblong, glumes two in numbers, upper and lower, lower glume two mm length with deep median groove, two nerved, broadly winged, awn 16-18 mm long, upper glume four mm length, hairy with 7-9 nerves. Flowering period between September - November.

Leaf Anatomy

The anatomical features of wild *C. martinii* leaf studied showed following parameters: Lamina flat, tiny vascular bundles arranged in abaxial row. Keel prominent, dome like abaxially with 25 - 32 bundles. Xylem and phloem distinguishable, metaxylem absent, median bundle distinctly large and anchor shaped, colorless sclerenchyma cells in girder projecting abaxially from the keel extending towards upper lamina. The bulliform cells were absent. The leaf margin showed numerous prickles (Fig. 2a). Free hand sections of the leaf observed under light microscope revealed presence of oil glands. The Schiff's reagent stained the aldehyde groups in the oil as purplish-pink, revealing the accumulation of oil glands around bundle sheath cells (Fig. 2b). Epidermal cell walls straight, epidermal hair unicellular or multicellular, without glandular hairs. Scanning electron microscopic observation revealed the epidermal surface containing large waxy coat, prickles clinging towards epidermis, interspersed with silica depositions. Stomata are paracytic and elongated with raised rim and long narrow aperture).

Microscopic observation of epidermal cells showed the presence of short and long cells. The short cells (14-16 µm) of coastal region alternated with silica bodies in uninterrupted rows compared to short cells of intercostal region. The silica bodies showed characteristic dump-bell shape. The long cells (33-40 µm) possessed thin walls, prickles absent, micro hairs present. Short cells are wider and more inflated than long cells. Long cell tapering at acute apex making the tip pointing towards leaf apex. Stomata were observed in rows, subsidiary cells were triangular showing low dome shape (Fig. 2b). The number of stomata counted in the microscopic field was 0.0940 mm^2 and the Stomatal Index was found to be 16.19088 ± 1.863806 (Table 2).

Scanning Electron Microscopy (SEM) Analysis

Significant variation was found in leaf epidermal cell morphology in terms of number, size and shape. Subsidiary cells were found to be triangular in shape expressing low to high domed structures. During the study five different types of trichomes were identified such as, macro hairs, micro-hairs, hooks, prickles and papillae. Macro-hairs, Micro-hairs,

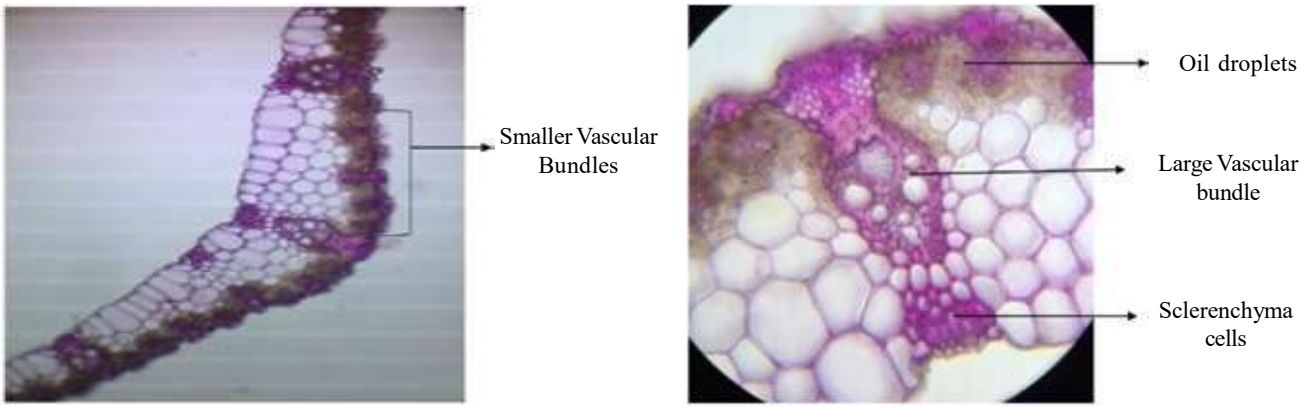

Fig 2: a. T.S of leaf showing arrangement of vascular bundle (VB): large 3 VB intermediated with 6-7 smaller VB (10X). Deposition of oil droplets around the vascular bundles when subjected to Shiff's stain (40 X)

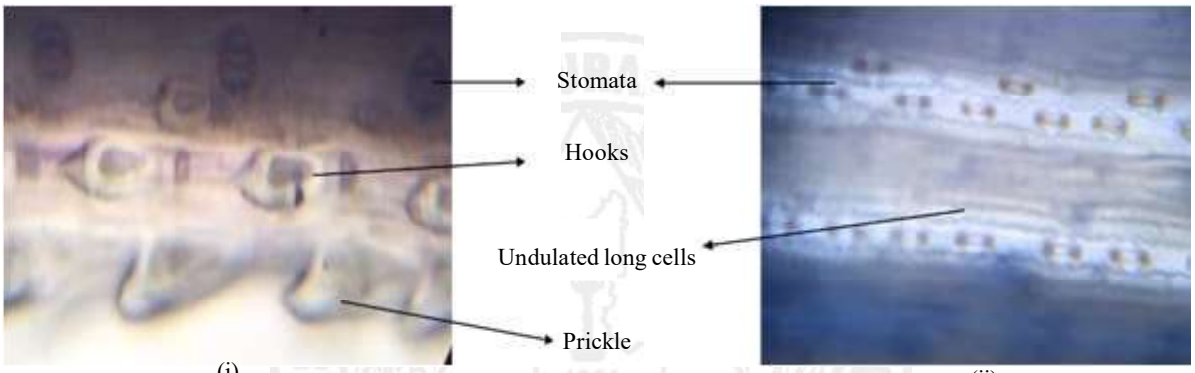

Fig 2b: i) Leaf peel of upper epidermis stained with safrannin showing blunt edged prickles; arrangement of hooks with alternate stomata
ii) Leaf stained with lactophenol cotton blue showing elongated cells with wavy walls along with stomata running parallel along the veins

TABLE 2

Calculation of stomatal index for the stomata present on the dorsal surface of the leaf along with epidermal cells and paracytic stomatal count

Plant	Type of stomata	Stomata No.	Epidermal cells	Stomatal index
		18	114	13.6363
		22	109	16.7938
		23	102	18.4
wild *Cymbopogon martini* (leaf)	Paracytic			
		25	116	17.7304
		19	113	14.3939
	Average ± Standard deviation	21.4 ± 2.57682	110.8 ± 4.955805	16.19088 ± 1.863806

and prickles were very commonly observed and occurred on upper surface of the leaf. Hooks and papillae were less or commonly observed, dumb bell-shaped silica bodies were observed during the study (Fig. 3). Long-cells similar in shape coastally and intercostal (narrow). Mid - intercostal long - cells were rectangular having undulating walls.

Cytogenetic Studies

The cytogenetic studies made in wild *C. martinii* revealed the species belonging to tetraploid race with chromosome number 2n=40. Karyo morphological studies showed the root tip cells containing 2n=40 chromosomes, irrespective of B-chromosomes in their germ line. The chromosomes were ranged according to its size length (medium to small). It consisted of 17 metacentric and three submetacentric haploid set of chromosomes.

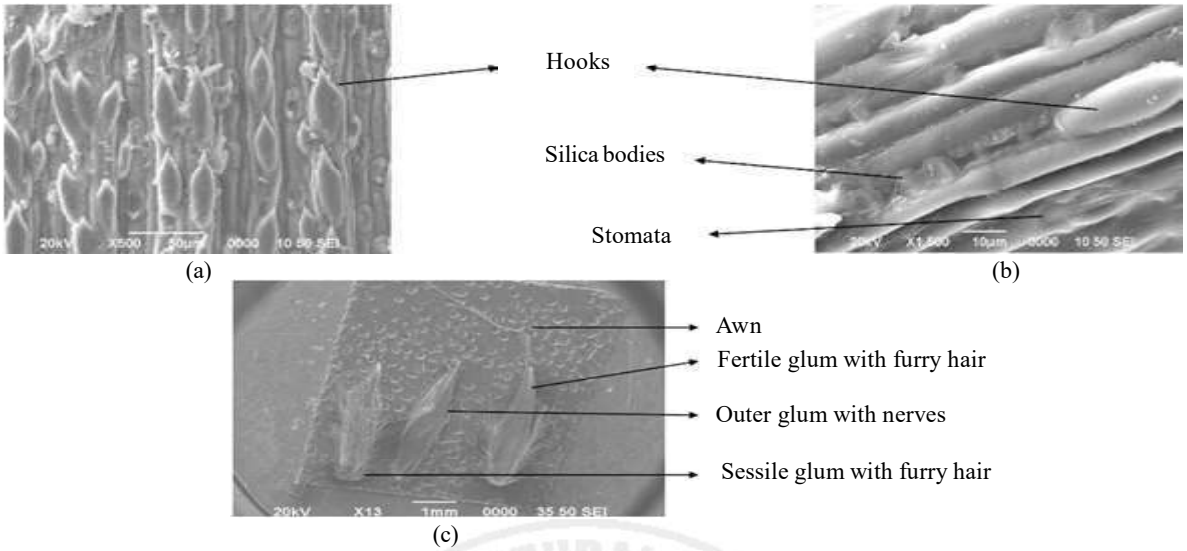

Fig. 3: a) SEM images of the epidermal layer of the plant leaf showing prickles along veins on the leaves; dumble shaped silica bodies between the midrib region of the veins and stomatal arrangement beside the veins. b) SEM image of inflorescence showing fertile and sessile glumes covered with furry hairs and awns

Flow Cytometric Analysis

2C DNA content of wild *C. martinii* was estimated to be 1.86 pg over the control *C. citratus* (hexaploid) with 2C DNA of 3.49 pg. The nuclear DNA genome size at G1 cell cycle phase was found to be 97862 MI. Based on the histogram alignment and the 2C DNA content the species was found to be tetraploid. The ratio of peak means (*C martinii*: *C. citratus*) were calculated and from the calculation the species undertaken for study belong to tetraploid race (Table 3 and Fig. 4).

DNA Barcoding and Phylogenetic Analysis

All the three primers (ITS2, *matk* and *rbc*L) successfully amplified the barcode region of interest. fragment. The ITS2 spacer region amplified a partial fragment with a length of 531bp, likewise *matK* and *rbc*L loci of Chloroplast region successfully amplified a complete length of 868bp and 864bp, respectively. The sequence was deposited in GenBank NCBI under accession no. MT905074.

Out of three *rbcL* showed complete 100 per cent homology, whereas *matK* revealed 0.15 per cent and

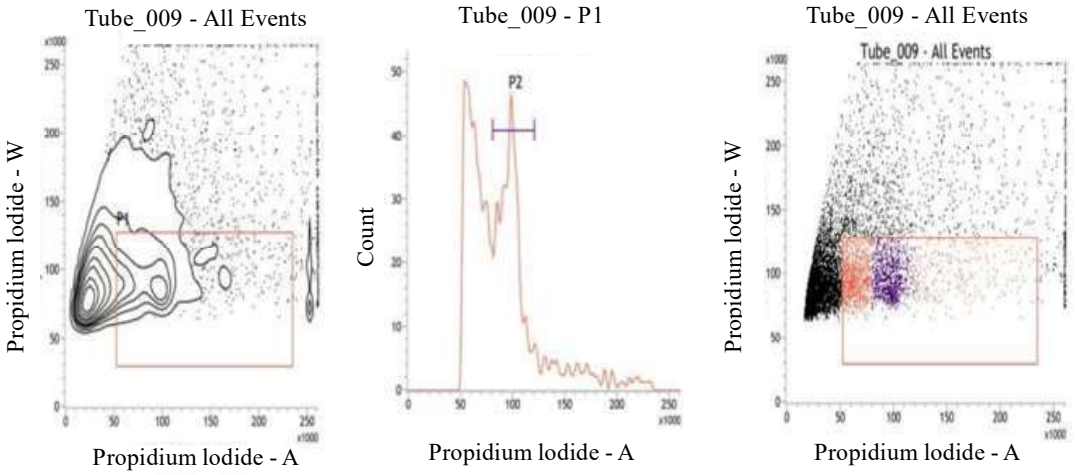

Fig. 4: Histogram showing Relative DNA content of the sample

TABLE 3
Total 2C DNA content from flow cytometric analysis

Sample	M1 Mean	2C DNA Content (pg)
Control (*CRBC*)	122891	2.33
Wild *C. martinii*	97862	1.86

ITS 2 spacer with 0.68 per cent sequencing diversity. Percentage identity was heigh at 98.19 per cent for ITS2 region comparing to other two. The phylogenetic tree of all the three sequences constructed separately by neighbor - joining tree showed close similarity towards *C. martinii* (Fig. 5).

Essential Oil Studies

Essential oil from wild *C. martinii* showed golden yellow color with dense woody-spicy note and emitted turpentine like aroma with an oil yield of 120 µl/100g. GC-MS analysis of the oil led to the identification of 67 compounds. The oil was dominated by cyclic and acyclic oxygenated sesquiterpenoids (36.516%); oxygenated monoterpenes (31.262%) and monoterpene hydrocarbons (26.362%) along with traces of other compounds. The compounds identified in higher percentages were Geranyl geraniol (25.22%), D-limonene (10.092%), Camphene (7.255%), Borneol (4.463%), alpha Pinene (2.04%), Linalool (1.573%), Geraniol (1.364%) alpha Bisabolol (1.232%) and alpha Bisabol oxide (34.981%) (Table 4). Biosynthesis of these terpenoid compounds takes place by the involvement of two biochemical pathways localized in two different cellular compartments for the formation of isopentenyl diphosphate (IPP): *viz.,* 1-deoxy-D-xylulose-5-phosphate (DOXP) or methyl-erythritol-4-phosphate (MEP) occurring in plastids producing monoterpenes, diterpenes,

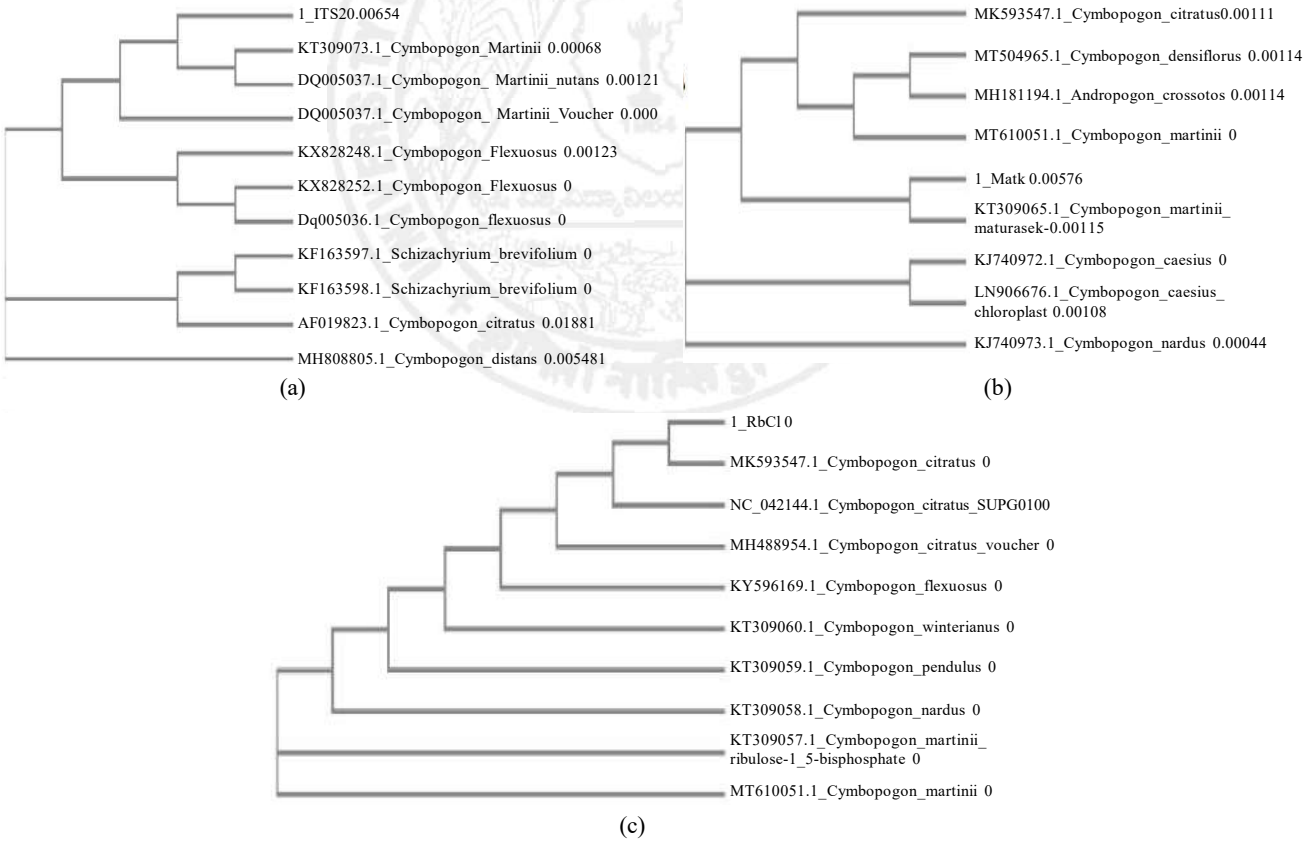

Fig. 5: Phylogenetic tree drawn using Neighbour Joining method for all the three amplified primers: a) ITS2 spacer region sequence showing close vicinity to *C. martinii* sequence; b) *matK* sequence showing close similarity towards *C. martinii*; c) *rbcL* sequence showing close relatedness towards *C. martinii*

TABLE 4

Compounds of the essential oil of wild *C. martinii* upon GC-MS analysis

Compound	Area %	Retention time (RT)	Molecular weight
Unsaturated aliphatic hydrocarbons			
1-Pentadecyne	0.014	23.455	208
4-nonanone	0.088	15.542	142
(E)-2-undecen-1-ol	0.035	16.677	170
Cyclo hexane-1-ethanol	0.018	39.607	168
3,5,9-trimethyl-deca-2,4,8-trien-1-ol	0.027	40.007	194
Allylidene cyclo hexane	0.020	18.068	122
2-pentacosanone	0.368	23.020	366
3,4-dimethyl-6-ethyl phenol	0.044	21.049	254
Santolina triene	0.014	20.654	136
Benzofuran	0.178	18.268	152
Monoterpene hydrocarbons			
Piperitone	0.053	24.175	152
Thujone	0.082	18.578	152
Alpha pinene	**2.041**	**10.215**	**136**
Camphene	**7.255**	**11.520**	**136**
Beta-pinene	0.393	12.320	136
D-limonene	**10.092**	**14.086**	**136**
Beta- ocimene	0.846	14.141	136
E, Z-allo ocimene	0.184	16.892	136
Beta phellandrene	0.311	12.055	136
3-carene	0.684	14.376	136
Trans-Pinane	0.054	15.717	138
Iso pulegol	0.050	19.003	154
Borneol	**4.501**	**19.818**	**154**
Bornyl chloride	0.016	21.544	172
Longipinane	0.044	39.902	206
Oxygenated monoterpenes			
Geraniol	**1.469**	**22.589**	**154**
Cis- carveol	0.19	20.479	152
Trans- carveol	0.045	21.869	152
Alpha-terpineol	0.998	20.424	154
Camphor	0.471	19.543	152
Apo camphor	0.145	17.743	138
Iso borneol	0.078	19.208	154
Longi borneol	0.319	30.783	222
Limonene oxide (E)	0.038	18.113	152
(Z)-Iso geraniol	0.015	17.527	154
Menthe-1,8-dien-7-ol	0.018	25.095	150
cis- para-2 Menthenol	0.015	17.462	154
Linalool	**1.573**	**16.567**	**154**
Eucalyptol	0.028	14.511	154
Longifolene	0.105	38.021	222
Geranyl geraniol	**25.222**	**37.711**	**290**
E-Geranyl linalool	0.013	41.918	290
Neryl-acetate	0.678	27.381	210
Phenylpropanoids compounds			
Elemicin	0.020	37.846	208
Iso elemicin	0.565	33.910	208
Sesquiterpene hydrocarbons			
Carvone	0.276	23.755	150
Burbonene	0.017		
Beta-bourbonene	0.039	26.091	204
Beta-elemene	0.071	34.735	204
Alpha-bergamotene	0.096	27.612	204
Caryophyllene	0.489	27.872	204
Beta-sesquiphellandrene	0.024	28.257	204
gamma Cadinene	0.560	31.709	204
Globulol	0.146	32.324	311
Alpha-bisabolol	**1.232**	**36.746**	**222**
Patchoulane	0.124	36.966	206
Santalene	0.023	39.837	204
Santalol	0.016	41.548	220
Naphthalenone	0.056	20.023	208
Iso bornyl methacrylate	0.019	40.457	220
Oxygenated sesquiterpene hydrocarbons			
Caryophyllene oxide	0.015	33.660	220
(E)-Germacrene	0.267	30.343	204
Verbenol	0.218	29.637	152
Delta-cadinol	0.035	36.351	222
Alpha- bisabolol oxide	**34.981**	**36.181**	**238**
Boronal	0.038	38.461	206
Elemol	0.159	32.699	222

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isoprene units and Acetate - Mevalonate pathway (MVA) occurring in cytoplasm leading to formation of sesquiterpenes. IPP is the structural unit of all the isoprenoids synthesized in plants. Geranyl diphos phate (GPP) is the universal precursor of monoterpenes and is synthesized by the condensation of IPP and its isomer dimethylallyl diphosphate (DMAPP) by geranyl diphosphate synthase (GPPS). GPP undergoes further isomerization, acetylation, deacetylation, cyclization and dehydrogenation to form various monoterpene and terpenoid compounds in the presence of Terpene synthases (GES) and Terpene cyclase (TEC) enzymes (Fig. 6). GPP initiates the formation of Farnesyl diphosphate (FPP) a precursor molecule for synthesis of sesquiterpenoid compounds is formed by the addition of IPP to GPP in the presence of enzyme Farnesyl diphosphate synthase (FPPS) (Fig. 7). A diterpene precursor molecule Geranylgeranyl diphosphate (GGPP) is obtained by the addition of IPP for FPP with the enzyme Geranylgeranyl diphosphate synthase (GGPPS). Biosynthesis of these compounds are shown in a form of pathways using the evidences from KEGG and MatCyc tools.

Devarayana Durga hill belongs to a southern thorny forest consisting majorly of grassland ecosystem. It covers an area of 42.27 sq. km with latitude 13.375 ^{0}N and longitude 77.213 ^{0}E. Hill is situated with the elevation of 1290 m above sea level. It receives low rainfall of 150 cm throughout the year with an average temperature of 32.9 ^{0}C and covered with various types of medicinal plants along with abundance of *Cymbopogon martinii*.

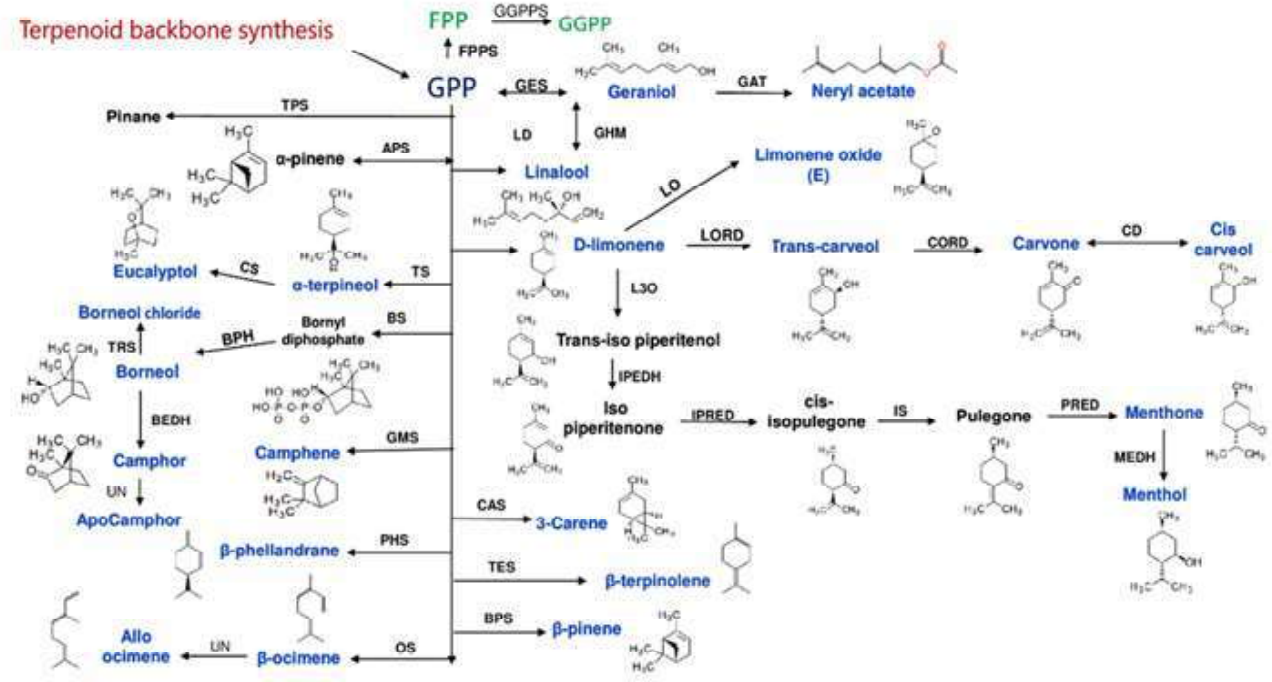

Fig. 6 : Schematic overview of biotransformation of monoterpenoid compounds in wild *C. martinii*. Enzymes are indicated in bold italics and sourced out from *KEGG* and Met Cyc pathway tools. *GPP* geraniol diphosphate a precursor leading to monoterpenes: *LS* limonene synthase, *ORD* oxidoreductase, *GHM* geranyl hydroxy mutase, *TPS* trans pinene synthase, *APS* alpha pinene synthase, *TS* alpha terpineol synthase, *PHS* beta phellandrene synthase, *OS* beta ocimene synthase, *CS* 1,8-cineole synthase, *CMS* camphene synthase, *LD* linalool dehydratase, *GAT* geranyl acetyl transferase, *LO* limonene 1,2 mono oxygenase, *L3O* limonene-3-monooxygenase *LORD* limonene oxidoreductase, *CORD* trans carveol oxidoreductase, *CD* carveol dehydrogenase, *IS* isomerase, *CAS* carene synthase, *TES* terpinolene synthase, *TRS* transferase, *BPS* beta pinene synthase, *GES* geraniol synthase, *BS* bornyl synthase, *BPH* bornyl pyrophosphate hydrolase, *AOXD* asymmetric oxidase, *BEDH* borneol dehydrogenase, *CH* camphor hydroxylase, *IPEDH* iso-piperitenol dehydrogenase, *IPRED* iso-piperitone reductase, *IS* isomerase, *PRED* pulegone reductase, *MEDH* menthol dehydrogenase.

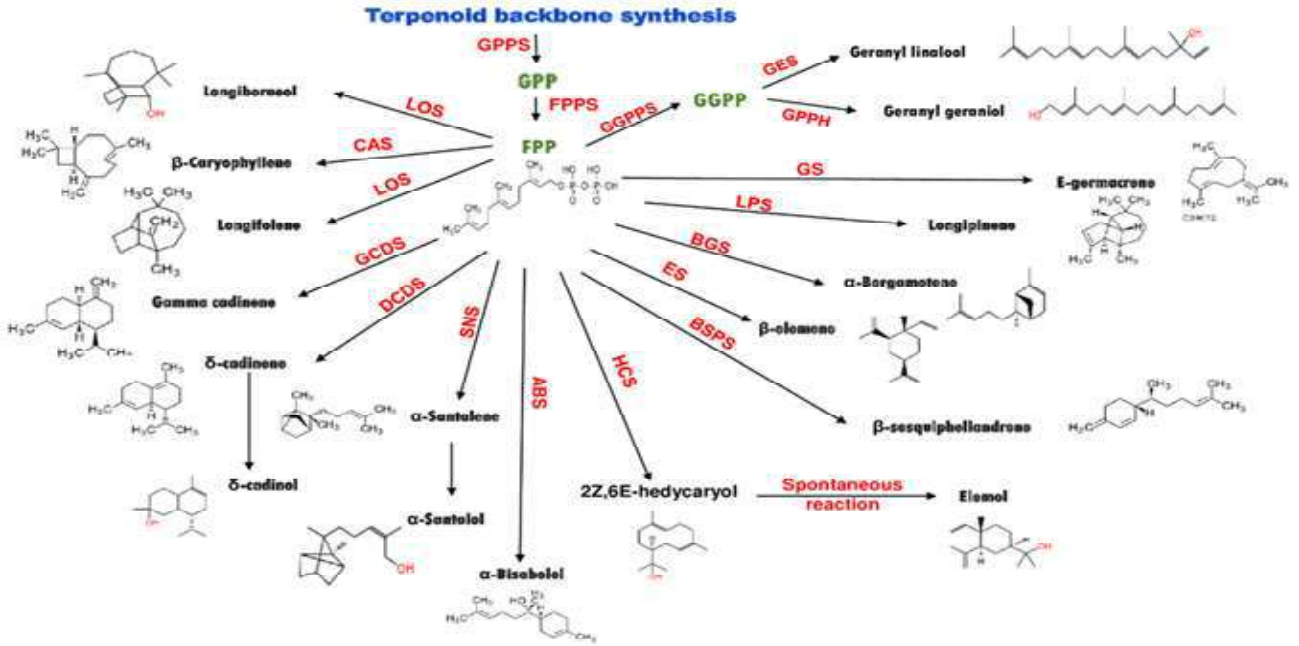

Fig. 7: Schematic overview of biotransformation of diterpenoid and sesquiterpenoid compounds in wild *C. martinii*. GPP geraniol diphosphate gets converted to FPP in the presence of IPP isopentenyl diphosphate, *FPP* Farnasel diphosphate a precursor leading to sesquiterpenes and *GGPP* geranyl geraniol diphosphate precursor of diterpenoid compounds: Enzymes are indicated in bold italics and sourced out from *KEGG* and Met Cyc pathway tools: *LOS* longifolene synthase, *CAS* beta caryophyllene synthase, *GCDS* gamma cadinene synthase, *DCDS* delta cadinene synthase, *SS* alpha santalene synthase, *BSPS* beta sesquiphellandrene synthase, *HCS* 2Z,6E-hedycaryol synthase, *ABS* alpha bisabolol synthase, *ES* elemene synthase, *LPS* longipinene synthase, *GS* germacerene synthase, *GPPH* Geranyl geranyl diphosphohydrolase, *GELS* geranyl linalool synthase, *SNS* á-santalene synthase, *BGS* á-Bergamotene synthase.

The soil was dark reddish brown, having fine particles with dry texture. The hill is recorded to possess 54 per cent of medicinal plants belonging to herbs, shrubs and climbers as per Tumkur taluk forest survey report by (http://www.fao.org/3/aa021e/AA021e03.htm). When compared to the cultivar varieties of CSIR - CIMAP (Mehera *et al.,* 1975; Sharma 1987), the wild *C. martinii* studied was shorter in length (1.7 m) with reduced leaf width as modification for withstanding drought stress along with long-medium compact, compound and erect inflorescence.

The three primers (ITS 2, *rbcL* and *matK*) used for DNA barcoding amplified complete Coding Sequence (CDS) region and showed sequence similarity of 98.19, 100 and 97.3 per cent with respect to cultivar *C. martinii* and phylogenetic analysis showed distance similarity and close vicinity towards *C. martinii.* The ratio of 2C DNA content in wild *C. martinii* showed that the species undertaken for study is a tetraploid belonging to sofia variety.

The essential oil analysis from the plant identified 67 compounds dominated by oxygenated sesquiterpenes and monoterpenes. The production of higher percentages of compounds in wild geno type when compared to the cultivar variety is influenced by the ontogeny, developmental stage and environmental conditions (Ganjewala *et al*., 2010; Padalia *et al*., 2011; Smitha *et al*., 2018 and Smitha *et al.,* 2021). The monoterpenoid compound geraniol (70-90 %) was found as major compound in the cultivars (Smitha *et al*., 2018; Siddiqui *et al*., 2011; Raina *et al*., 2003 and Neetu Jain *et al.,* 2017) whereas the wild type studied showed alpha bisabolol oxide as the major compound with reduced geraniol content (Neetu Jain *et al.,* 2017; Smitha *et al.,* 2018 and Zara *et al*., 2020).

Drought stress reduces transpiration rate and CO_2 intake and increases the supply of NADPH + H^+ thus enhancing the metabolic pathways for essential oil production (Fatima *et al*., 2006; Khalid, 2006 and Aliabadi *et al*., 2009). The environmental stress taken up by the wild plant has led to upregulation of genes resulting in increased number of sesquiterpene compounds (Mahajan *et al*., 2020 and Palesh *et al*., 2020). Higher production of sesquiterpenoid compounds (alpha-bisabolol oxide and alpha-bisabolol) in the essential oil has also been reported in *Matricaria chamomilla* due to environmental stress (Baghalian *et al*., 2008). The essential elements such as nitrogen, phosphorous and iron present in higher percentages in the soil of wild *C. martinii* growth might have triggered the pathway contributing for increased production of terpenoid compounds (Mahajan *et al*., 2020).

The present study on wild *Cymbopogon martinii* var sofia showed finger print compounds in the essential oil leading for the production of monoterpenoid and sesquiterpenoid compounds. Plants are known to offer great promise and find utilization by upregulation of terpenoid compounds beneficial to mankind. Studies on essential oil biosynthesis from the wild species offer strategies for production of novel and economic compounds through metabolic engineering. The germplasm of wild *C. martinii* can find its utilization in broadening genetic base for developing newer commercial varieties.

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