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NEW VARIETY RELEASE

Registration of a high-yielding, high citronellol: geraniol ratio and unique sesquiterpene (6, 9- guaiadiene)-rich variety 'CIM-Bharat' of rose scented geranium (*Pelargonium graveolens* L.)

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Key Words

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Abbreviations

BST: Bench Scale Trial
C: G: Citronellol: Geraniol
GC-FID: Gas Chromatography-Flame Ionization Detection
GC-MS: Gas Chromatography-Mass Spectrometry
IET: Initial Evaluation Trial
PST: Pilot Scale Trial

ABSTRACT

Rose scented geranium, a South-African aromatic herb, is cultivated in both sub-tropical and temperate countries for its essential oil derived from the aerial parts that conveys a strong rosy-minty odour with a greenish-yellow colour. The oil is a treasure trove of versatile chemicals with extensive applications in the high grade perfumery, pharmaceutical, and aroma industries. The annual rise in its essential oil import for indegenic consumption in the country warrants its genetic enhancement of existing germplasm for increasing per unit area productivity and quality of essential oil. With this backup, our team initiated a clonal selection scheme to develop a genetically improved clone, CIMAP-21A, capable of producing 70-80kg/ha of oil yield in a single harvest compared to other existing cultivars and check variety CIM-Pawan. The new developed variety also has unique chemical compositions to meet the quality standard of the perfumery industry. It has a high Citronellol: Geraniol (C: G) ratio (>3) with the presence and absence of unique sesquiterpene markers i.e., 6,9-guaiadiene (5.4%) and 10-epi-γ-eudesmol, respectively. The unique sturdy dark green stem and leaves are the distinguished morphological features of this strain. The developedclone is ideally suitable for cultivation in the entire North India and Western regions of the country. This strain has now been christened as CIM-Bharat and it is registered here as a new, improved variety of rose-scented geranium for its commercial release.

INTRODUCTION

Rose-scented geranium (*Pelargonium graveolens* L'Herit. ex Aiton), a member of the family Geraniaceae, is a high-value, perennial, and multi-harvest aromatic herb. It originated from South Africa, 'Reunion Island', Madagascar, Egypt, and Morocco. The essential oil extracted from aerial parts of the rose scented geranium finds extensive applications in the pharmaceutical, perfumery, cosmetic, and food industries. The essential oil also has various antimicrobial and insecticidal properties and is substantially used in aroma therapy. In India, rose scented geranium is cultivated as a rainfed perennial plant under different agro-climatic conditions of South India, namely Nilgiri and Pulney hills of Tamil Nadu, and as an annual plant

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in the North Indian Plains. The main components of its essential oil are citronellol and geraniol. The major types of geranium oils available commercially include Bourbon, Chinese, Algerian, Egyptian, and Moroccan. All the oils differ considerably in their composition regarding the major constituents. Against the internal requirement of approximately 200 tonnes per year, India produces less than 20 tonnes of geranium oil annually and meets its requirement mainly by import (Verma *et al*., 2013). The cultivation of this crop can result in a net profit of over Rs.2-2.5 lacs/annum if the genotypes with 70-80 kg/ha oil yield potential are cultivated. Very few commercial cultivars are available in the Indian market, insufficient to fill the gap between demand and supply (Saxena *et al*., 2008; Singh *et al*., 2018). Moreover, the quality of essential oils produced from available cultivars cannot meet the current requirement of the aroma industry. Nowadays, these industries need rose-scented geranium oils with a high C: G ratio and a high proportion of 6,9-guaiadiene content. The present demand necessitates development of improved cultivars with all potential quality factors along with high oil yield. This report presents the outcomes of a dedicated endeavor in the breeding of rose-scented geranium, resulting in the development of a novel high oil-yielding clone with a distinctive chemotype.

Origin of the Variety

A mixed plant population having natural variants collected from western India and breeding lines developed by tissue culture techniques at CSIR-CIMAP, Lucknow and maintained at CSIR-CIMAP, Lucknow, and CSIR-CIMAP, Purara was used as the parental base population to initiate a breeding program. Under the clonal breeding program of rose-scented geranium, 600-700 individual plant populations generated from rooted cutting (15-20cm) of genetic stocks of the base population were primarily screened for vigour, morphology, herb and oil yield with C: G ratio as well as 6,9- guanidine content. After rigorous screening for five years, 26 individual plant populations were identified for promising yield and quality parameters. A breeding program for rose-scented geranium was established using a diverse plant population composed of natural variants from western India and breeding lines that were developed through tissue culture techniques at CSIR-CIMAP in Lucknow. This population was maintained across two locations: CSIR-CIMAP Lucknow and CSIR-CIMAP Purara. The focus of the breeding effort was clonal improvement. Initially, between 600 to 700 individual plant populations were generated from rooted cuttings (measuring 15-20cm) sourced from the genetic stocks of the base population. These populations underwent a comprehensive evaluation process encompassing factors such as vigor, morphology, herb and oil yield, the ratio of carbon to glucose (C:G ratio), and the presence of 6,9-guanidine.

After a diligent and meticulous screening process spannining five years, a subset of 26 individual plant populations emerged as standout candidates, demonstrating significant promise in terms of both yield and quality traits. This selection of populations holds the potential to serve as a valuable foundation for subsequent stages of the breeding program, aiming to cultivate enhanced varieties of rose-scented geranium with improved attributes

Initially, the evaluation process involved subjecting the 26 identified individual plant populations to an Initial Evaluation Trial (IET) over the course of one year (2018-2019). This trial utilized a randomized complete block design (RCBD) with three replications, and a reference variety known as CIM-Pawan was included for benchmarking purposes. The evaluation primarily focused on factors related to yield and yield-contributing traits, and the results were summarized in Table 1.

Following the IET, four particularly promising individual plant populations were chosen based on their oil yield surpassing 49 kg/ha, in addition to displaying encouraging traits related to yield. These selected populations then underwent Bench Scale Trials (BSTs) using an RCBD with four replications and a plot area of 6.25 sqm. These trials took place over two consecutive years, 2019-20 and 2020-21, and the outcomes were recorded in Tables 2.1 and 2.2. The reference variety CIM-Pawan was also included in the BSTs to enable comparative analysis.

From the BST, a standout strain/clone named CIMAP21A emerged, consistently outperforming the check variety and other promising strains across attributes such as vigorous growth, elevated herb and oil yield of superior quality. This exceptional strain was then progressed to the Pilot Scale Trial (PST) stage. In PST, CIMAP-21A was cultivated alongside the check variety CIM-Pawan in larger

Table 1: Yield attributes of twenty-six selected individual plant clones in IET (2018-19)

Population	Plant height (cm)	Canopy (cm)	Leaf Stem Ratio	Herb yield (g plant^{-1}) (single harvest-15th April to 15th May)	Essential oil content (%) (Clevenger level)	Expected essential oil yield (kg ha^{-1}) (based on a single harvest)
CIMAP-1	111	240	1.43	1070	0.07	26.66
CIMAP-2	117	220	1.09	2278	0.05	40.55
CIMAP-3	110	222	1.05	1995	0.05	35.51
CIMAP-4	106	215	1.14	1590	0.06	33.96
CIMAP-5	105	165	1.42	1626	0.07	40.52
CIMAP-6	120	173	1.11	1354	0.05	24.10
CIMAP-7	112	210	1.40	1471	0.07	36.66
CIMAP-8	131	187	**0.98**	1920	**0.03**	20.51
CIMAP-9	118	265	1.13	**2445**	0.05	43.52
CIMAP-10	116	168	1.08	1231	0.05	21.91
CIMAP-11	**136**	225	1.15	2296	0.05	40.87
CIMAP-12	110	178	1.06	1256	0.04	17.89
CIMAP-13	102	172	1.07	1491	0.04	21.23
CIMAP-14	114	230	1.45	1587	0.07	39.55
CIMAP-15	86	185	1.48	1383	0.10	49.23
CIMAP-16	81	240	1.22	1068	0.08	30.42
CIMAP-17	102	**285**	1.47	1205	0.07	30.03
CIMAP-18	77	185	1.82	1110	0.13	51.37
CIMAP-19	84	245	1.24	1490	0.08	42.44
CIMAP-20	91	235	1.53	1191	0.10	42.40
CIMAP-21A	98	250	1.92	1490	**0.17**	**90.17**
CIMAP-22	46	**115**	**1.99**	**180**	**0.17**	**10.89**
CIMAP-23	41	125	1.93	292	0.13	13.51
CIMAP-24	84	220	1.88	1482	0.10	52.76
CIMAP-25	73	175	1.86	1617	0.08	46.05
CIMAP-26	**71**	165	1.87	842	0.09	26.98
CIM-Pawan	73.4	160.45	0.85	811	0.18	51.98
S.Em.±	3.544	7.501	0.053	52.133	0.003	1.336
CD (1%)	9.383	19.862	0.140	138.048	0.008	3.537
CD (5%)	7.066	14.956	0.106	103.953	0.006	2.663

where values mentioned in bold fonts indicate the maximum and minimum range of particular traits.

plots spanning 100 sqm, commencing from the year 2021-22.

The pilot Scale Trial was conducted across multiple locations to ascertain the stability of the elite strain/clone in terms of yield-contributing traits, as documented in Table 3. The results of this multi-location trial consistently demonstrated CIMAP-21A's superiority over the check variety in all the assessed yield parameters. As a culmination of this successful breeding and evaluation process, the elite strain/clone was bestowed the name 'CIM-BHARAT'. This variety is now poised for widespread commercial release owing to its proven performance and suitability for large-scale cultivation.

RESULTS

Table 1 provides an overview of the yield performance exhibited by 26 carefully selected clones or plant populations (designated as CIMAP-1 to CIMAP-26) of rose-scented geranium. This performance is juxtaposed against the benchmark variety CIM-Pawan, serving as a reference point. As a result of the Initial Evaluation Trial (IET) data, four individual plant populations or clones (CIMAP-15, CIMAP-18, CIMAP-21A, and CIMAP-24) were identified as promising candidates due to their oil yield surpassing 49 kg/ha and displaying encouraging attributes related to yield. These selected candidates were subsequently advanced for further assessment in bench and pilot scale trials, as documented in Tables 2 and 3. Throughout these trials, CIM-Pawan was employed as a comparative benchmark.

In terms of oil yield (measured in kg/ha) and other traits contributing to yield, it was consistently

Table 2.1: Yield attributes of four promising clones under BST-I (2019-20)

Clones	Leaf Stem Ratio					Fresh biomass (q ha^{-1}) (On a single harvest-15th April to 15th May)					Essential oil content (%) (Clevenger level)					Essential Oil yield (kg ha^{-1}) (Based on a single harvest)				
	R_1	R_2	R_3	R_4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean
CIMAP-15	1.52	1.49	1.38	1.42	1.45	439.03	482.94	473.69	452.90	462.14	0.10	0.10	0.10	0.10	0.10	39.07	42.98	42.16	40.31	41.13
CIMAP-18	1.86	1.82	1.74	1.69	1.78	348.02	364	371.10	337.36	355.12	0.13	0.13	0.14	0.12	0.13	40.27	42.11	46.24	36.03	41.09
CIMAP-21A	1.79	1.93	1.84	1.96	**1.88**	516.68	478.88	494	526.76	**504.08**	0.16	0.17	0.17	0.18	**0.17**	73.58	72.45	74.74	84.39	**76.27**
CIMAP-24	1.89	1.75	1.80	1.92	1.84	523.48	513.46	475.89	490.92	500.94	0.10	0.10	0.10	0.10	0.10	46.59	45.70	42.35	43.69	44.58
CIM-Pawan	0.88	0.86	0.92	0.94	0.90	356.76	334.57	324.33	349.94	341.40	0.19	0.17	0.18	0.18	0.18	60.33	50.62	51.96	56.06	54.69
S.Em.±	0.036					10.311					0.0034					1.198				
CD @ 1%	0.156					44.550					0.014					5.177				
CD @ 5%	0.111					31.775					0.010					3.693				

Values mentioned in bold fonts indicate the maximum and minimum range of particular traits

Table 2.2: Yield attributes of four promising clones under BST-II (2020-21)

Clones	Leaf Stem Ratio					Fresh biomass (q ha^{-1}) (On a single harvest-15th April to 15th May)					Essential oil content (%) (Clevenger level)					Essential Oil yield (kg ha^{-1}) (Based on a single harvest)				
	R_1	R_2	R_3	R_{4w}	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean
CIMAP-15	1.52	1.49	1.38	1.42	1.45	439.03	482.94	473.69	452.90	462.14	0.10	0.10	0.10	0.10	0.10	39.07	42.98	42.16	40.31	41.13
CIMAP-18	1.86	1.82	1.74	1.69	1.78	348.02	364	371.10	337.36	355.12	0.13	0.13	0.14	0.12	0.13	40.27	42.11	46.24	36.03	41.09
CIMAP-21A	1.79	1.93	1.84	1.96	**1.88**	516.68	478.88	494	526.76	**504.08**	0.16	0.17	0.17	0.18	**0.17**	73.58	72.45	74.74	84.39	**76.27**
CIMAP-24	1.89	1.75	1.80	1.92	1.84	523.48	513.46	475.89	490.92	500.94	0.10	0.10	0.10	0.10	0.10	46.59	45.70	42.35	43.69	44.58
CIM-Pawan	0.88	0.86	0.92	0.94	0.90	356.76	334.57	324.33	349.94	341.40	0.19	0.17	0.18	0.18	0.18	60.33	50.62	51.96	56.06	54.69
S.Em.±	0.036					10.311					0.0034					1.198				
CD @ 1%	0.156					44.550					0.014					5.177				
CD @ 5%	0.111					31.775					0.010					3.693				

Values mentioned in bold fonts indicate the maximum and minimum range of particular traits

Table 3: Pilot Scale trails of 'CIMAP-21A' and 'CIM-Pawan' at multi locations in India

Clones	Leaf Stem Ratio					Fresh biomass (q ha^{-1}) (On a single harvest-15th April to 15th May)					Essential oil content (%) (Clevenger level)					Essential Oil yield (kg ha^{-1}) (Based on a single harvest)				
	R_1	R_2	R_3	R_4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean
CIMAP-15	1.48	1.35	1.46	1.39	1.42	471.37	428.52	462.35	442.05	451.07	0.10	0.10	0.10	0.10	0.10	41.95	38.14	41.15	39.34	40.15
CIMAP-18	1.66	1.83	1.79	1.72	1.75	328.93	361.82	354.90	339.32	346.24	0.13	0.11	0.12	0.12	0.12	38.06	35.42	37.90	36.24	36.98
CIMAP-21A	1.92	1.75	1.89	1.80	**1.84**	467.55	514.31	482.32	504.46	**492.16**	0.15	0.17	0.16	0.16	**0.16**	62.42	77.82	68.68	71.84	**70.08**
CIMAP-24	1.71	1.88	1.85	1.76	1.80	464.64	511.10	501.32	479.31	489.09	0.10	0.10	0.10	0.10	0.10	41.35	45.49	44.62	42.66	43.53
CIM-Pawan	0.84	0.92	0.86	0.90	0.88	349.63	317.84	342.93	327.88	334.57	0.17	0.19	0.18	0.18	0.18	52.90	53.75	54.94	52.53	53.60
S.Em.±	0.035					9.728					0.0034					1.113				
CD @ 1%	0.153					42.022					0.015					4.808				
CD @ 5%	0.109					29.975					0.010					3.429				

Values mentioned in bold fonts indicate the overall mean and mean of particular traits at the Lucknow location

noted that CIMAP-21A demonstrated superior performance in comparison to both the benchmark and other clones within the Bench Scale Trials (BSTs). This exceptional performance led to the progression of CIMAP-21A to the multi-location trials, where its stability in relation to oil yield and other yield-contributing traits was evaluated in comparison to the benchmark variety (Fig. 1, 2). Across these multi-location trials, CIMAP-21A consistently maintained its superiority and stability in the traits under scrutiny.where, values mentioned in bold fonts indicate the maximum and minimum range of particular traits.

The essential oil compositions of CIMAP-21A and check CIM-Pawan were also characterized using GC-FID and GC-MS. Altogether, 69 compounds were identified from the proposed clone. The combined percentages of these compounds ranged from 96.4% to 98.5% with an average of 97.4% present in the essential oil. The proportion of economically important chemical compositions of geranium essential oils extracted from CIMAP-21A and other released cultivars/varieties along with check CIM-Pawan are summarized in Table 4. The essential oil of CIMAP-21A constituted 34.1% of citronellol and 6.6% of geraniol content, which showed a resemblance with China-type rose-scented geranium oil and has a high C: G ratio (>3) compared to other released varieties as shown in Table 4. The oil of clone CIMAP-21A also produced a unique sesquiterpene 6,9-Guaiadiene (5.4%) which did not produce in any available released cultivars and check variety and made it a unique chemotype (Table 4). Also, 10-*epi*-γ-eudesmol was not present in the oil of clone CIMAP-21A in contrast to other released cultivars and check variety. Representative GC-MS chromatogram of clone CIMAP-21A and mass library for 6,9- Guanidine is depicted in Figure

Figure 1: Close-up photographs of CIMAP-21A with check variety CIM-Pawan

Figure 2: Field view of proposed clone CIMAP-21A at different locations

Table 4: Essential oil composition of rose-scented geranium clone CIMAP-21A & check CIM-Pawan

	Essential Oil Yield (kg ha^{-1})				Total Oil Yield (kg ha^{-1}) [H_1+H_2]	
	CIM-Pawan		CIMAP-21A		CIM-Pawan	CIMAP-21A
	H_1	H_2	H_1	H_2		
L_1	61.50	15.28	66.90	56.60	76.78	123.50
L_2	67.52	16.43	69.87	59.05	83.94	128.93
L_3	61.75	15.98	71.50	53.25	77.73	124.75
L_4	57.29	14.79	66.04	56.59	72.08	122.63
L_5	61.80	15.12	67.60	50.97	76.92	118.57
L_6	65.75	15.95	68.56	58.76	81.69	127.32
L_7	**69.77**	**17.25**	**75.18**	**63.78**	**87.01**	**138.96**
Mean	63.59	15.44	70.27	56.20	**79.03**	**126.48**
S.Em.±	2.157	0.552	2.166	1.996	2.747	4.383
CD @ 1%	6.207	1.589	6.232	5.745	7.905	12.614
CD @ 5%	4.531	1.160	4.550	4.194	5.771	9.209

[a] L_1: Satara district (MH); L_2: Ratnagiri district (MH); L_3: Bhandara district (MH); L_4: Jalgaon district (MH); L_5: Ahmednagar district; L_6: Barabanki district (UP); L_7: Lucknow district (UP).[b] H_1: First harvesting; H_2: Second harvesting.[c] Planting time: First fortnights of December 2021; First harvesting time (H-1): Second fortnights of April 2022 (130-140 Days after planting); Second harvesting time (H-2): First fortnights of August 2022 (90-100 Days after first harvest).

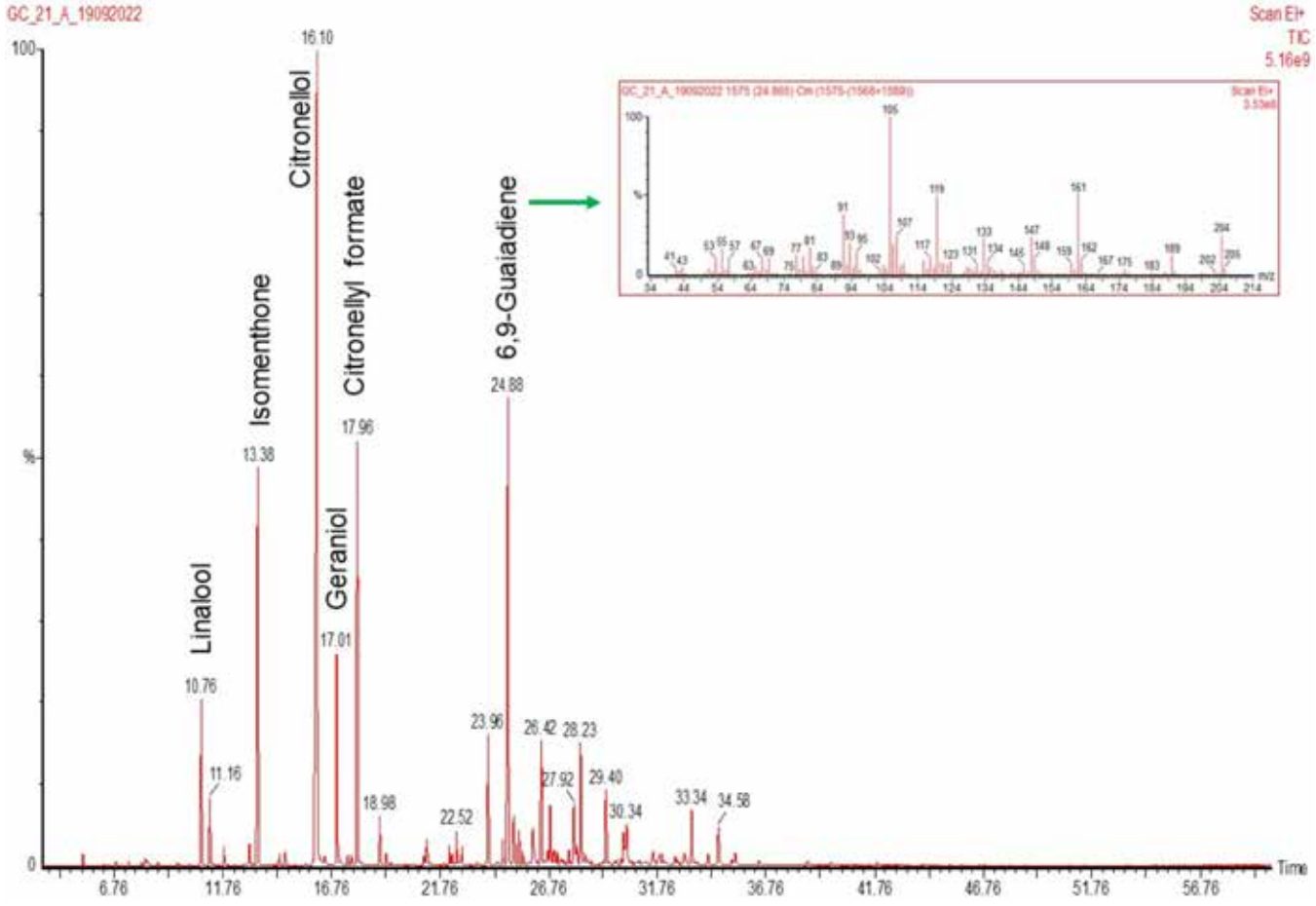
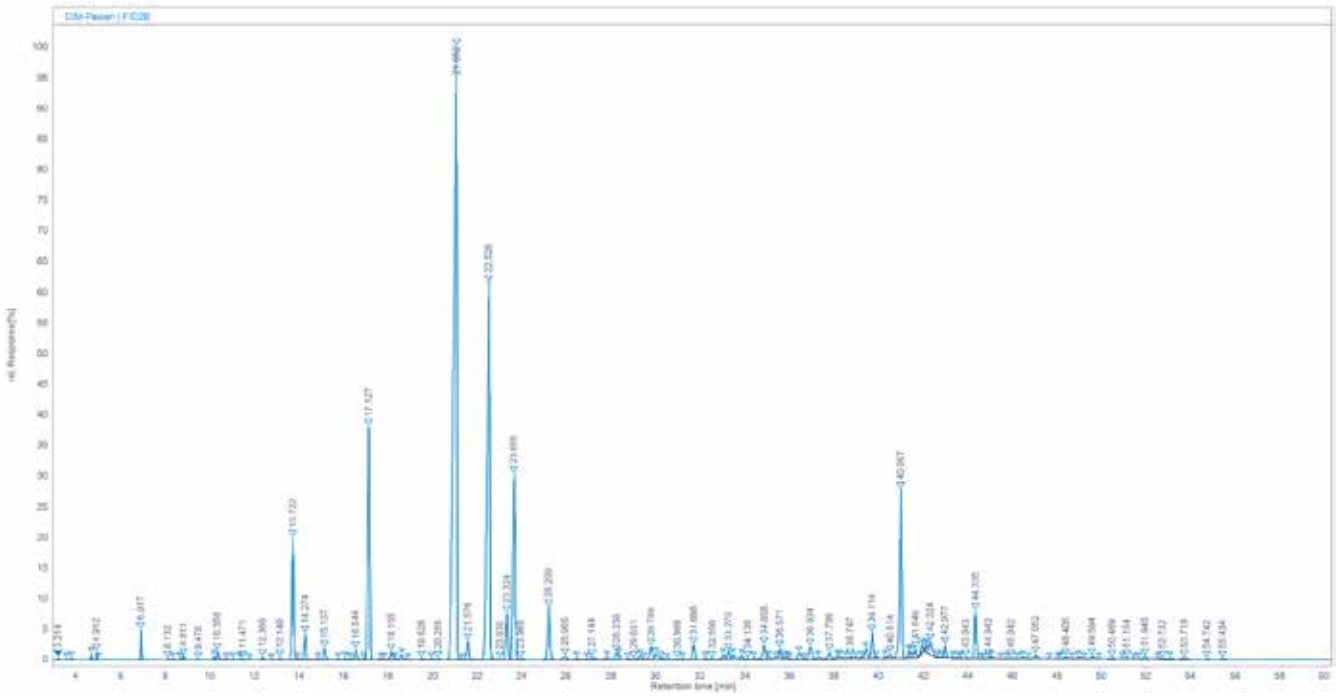


Figure 3: Chromatographic profile (GC-MS: total ion chromatogram) of rose-scented geranium clone proposed for release

CIM-Bharat



CIM-Pawan

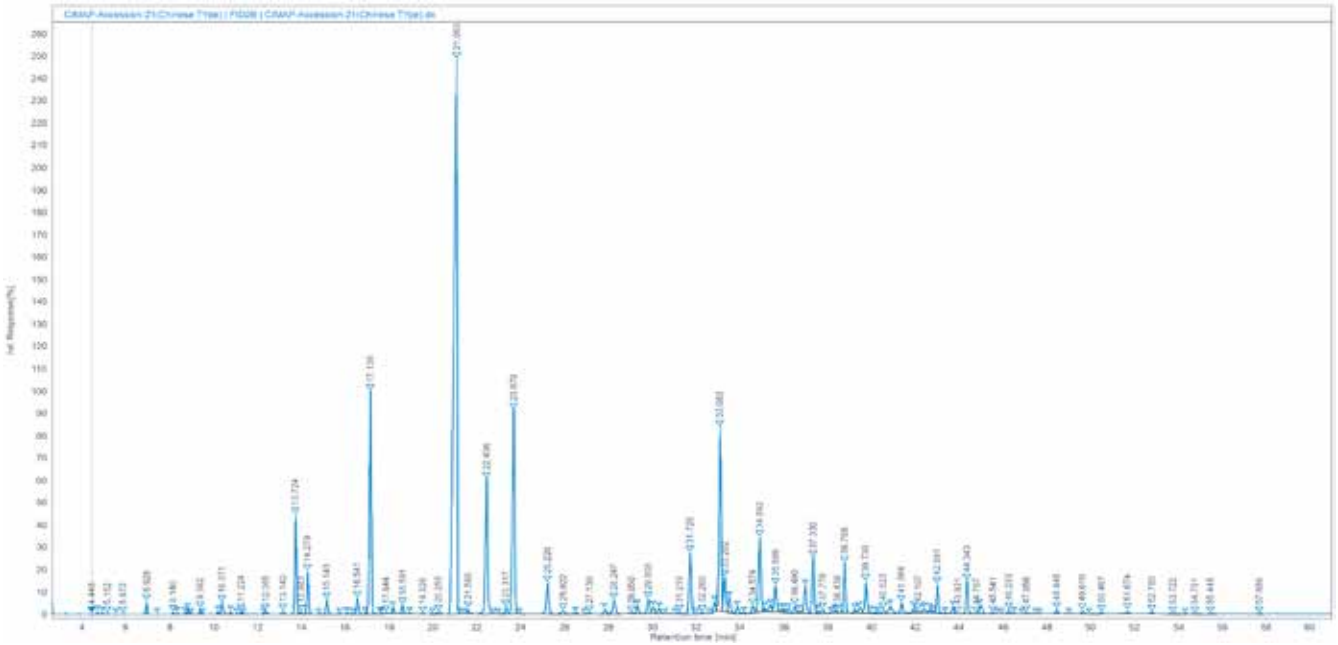


Figure 4: GC chromatogram of clone CIMAP-21A (CIM-Bharat) & CIM-Pawan

3. Also, the GC chromatogram of clone CIMAP-21A and check CIM-Pawan are represented in Figure 4.

Statement of Distinction/Breeder's Claims

The clone CIMAP21A of rose-scented geranium, which is now christened as CIM-Bharat, is a fast-growing upright robust herb with a dark green stem. Compared to check CIM-Pawan, the variety CIM-Bharat has a dark green leaf with a prominently revolute leaf margin, rough, coarse, and hirsute leaf texture, palmatipartite to pinnatisect leaf shape and less prominent venation

Table 5: Comparative description of CIM-Bharat and check CIM-Pawan

Parameters	CI-MAP-21A (Candidate variety)	CIM-Pawan (Check variety)	% superiority over the check
Growth habit	Upright robust	Upright robust	-
Stem	sturdy	soft	-
Stem and leaf colour	Dark green	Light green	-
Herb yield (q/h) (Two cuts)	895.05	520.24	72.04
Essential oil content (%)	0.17	0.18	-
Essential oil yield(kg/h) (Two cuts)	126.48	79.03	60.04
Citronellol (%)	34.1	34.2	-
Geraniol (%)	6.6	17.7	-
C:G ratio	5.1 (>3)	1.93 (0.5-2.0)	164.25
10-epi-γ-eudesmol(%)	nd	6.3	-
6,9-Guaiadiene(%)	5.4	nd	100

Figure 5: Morphological differences between CIM-Bharat and CIM-Pawan

up to the apex, purplish-pink flowers with distinct veins, and the high amount of branches/plant as the distinct morphological features. The variety has 6,9-Guaidiene content, which is one of the important quality factors of the geranium oil and is not found in check CIM-Pawan and other commercial cultivars of rose-scented geranium. Also, CIM-Bharat's C:G ratio is higher (>3) than the existing check variety CIM-Pawan. The other distinguished feature of this variety and check CIM-Pawan are presented in

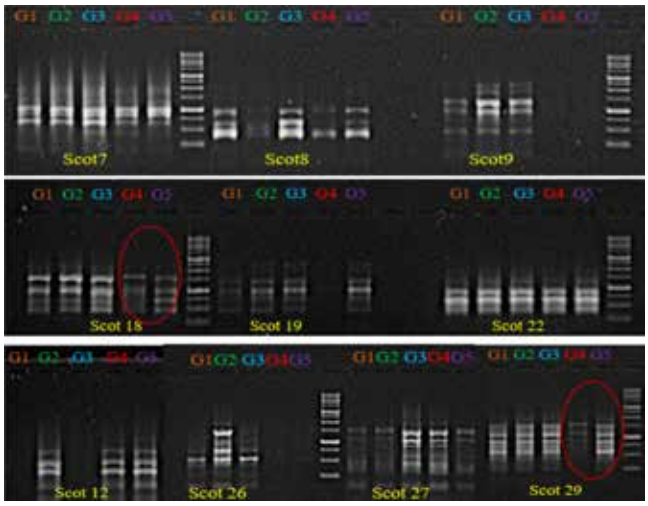


Figure 6: Molecular fingerprint of CIM-Bharat with check CIM-Pawan and other released varieties Where, G1-Bourbon, G2-CIM-Pawan, G3-CIM-Bio G171, G4-Kelkar, G5-CIM-Bharat

Figure 5 & Table 5, and the molecular fingerprint shows the distinction of CIM-Bharat with the check (Fig. 6).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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