



Variety Registration Report

Registration of new variety CIM-Atishrestha: High-oil and high-patchoulol-yielding variety of patchouli for Southern India

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Abstract

Patchouli (*Pogostemon cablin* Benth.), a perennial aromatic shrub belonging to the family Lamiaceae, is an industrially important crop valued for its essential oil, which is widely utilized in the perfumery, cosmetic, and pharmaceutical industries. Despite its high economic potential, the genetic improvement of patchouli has been limited due to narrow genetic variability and the vegetative mode of propagation. Besides the availability of several exotic cultivars from Singapore and Java for cultivation in India, the patchouli varieties CIM-Samarth, CIM-Shrestha, and CIM-Utkrisht developed by CSIR-CIMAP are also popular. Addressing the pressing need for high-oil-yielding and superior-quality cultivars, a new clone, CIM-P22, registered as the new variety CIM-Atishrestha, has been developed through mutation breeding. It produces a dry leaf yield ranging from 4.00 to 4.80 tonnes per hectare per harvest. The oil content, determined by the Clevenger apparatus, varies between 2.80 and 3.50%, while on a dry weight basis (30 kg sample), it ranges from 2.20 to 2.60%. The oil yield on a dry weight basis is about 88.0 to 125.0 kg per hectare. The patchouli alcohol content, which is the key quality parameter, ranges from 45 to 60% by the Clevenger method, and 35 to 40% in a 30 kg dry weight sample.

Keywords: Atishrestha, Oil, Patchouli, Patchouli alcohol content, Variety.

Introduction

Patchouli (*Pogostemon cablin* Benth.) is a medium-sized aromatic shrub belonging to the family Lamiaceae. Its oil is mainly used for perfumery, cosmetics, and pharmaceutical industries (Fatima et al., 2023). It is mainly grown in Indonesia, Malaysia, China, and Brazil, which collectively account for 90% of the world's total oil production of the world (van Beek & Joulain, 2018). In India, it is mainly grown in Karnataka, Madhya Pradesh, Orissa, Tamil Nadu, Kerala and Assam, with Karnataka being the major producer. The demand for patchouli oil in India is around 300 tonnes per annum and is valued at around 33 crores INR. However, domestically only ~50 tonnes are being produced (Verma et al., 2024). However, the major demand for oil in India is met by imports from Indonesia (Kumar et al., 2024).

Due to a lack of genetic variability and a vegetative mode of reproduction, limited genetic improvement and hybridization programs have restricted the breeding of new high-essential-oil-producing cultivars in India (Kumar et al., 2024). The CSIR-Central Institute of Medicinal and Aromatic Plants (India) has released CIM-Samarth, CIM-Shrestha, and CIM-Utkrisht through breeding efforts (Singh et al., 2019). Although these varieties produce high-quality essential oil, considerable scope for genetic improvement in patchouli is possible. Hence, there is an urgent and immediate need to develop a variety with higher oil content and superior quality (patchoulol content).

In this context, a novel clone designated as CIM-P22, subsequently registered as CIM-Atishrestha, has been developed at CSIR-CIMAP through mutation breeding.

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The variety combines desirable agronomic performance with enhanced essential oil yield and superior chemical quality attributes. CIM-Atishrestha represents an advancement in patchouli improvement efforts aimed at achieving self-reliance in aromatic crop production. Further on, it shall be able to generate higher income for farmers through a distinct, uniform, high-oil-yielding and stable variety suited for Southern Indian agro-climatic conditions.

Materials and Methods

Initially, in the year 2020, 1000 fresh cuttings of the commercial patchouli cultivar CIM-Shrestha were irradiated using Cobalt-60 (^{60}C) gamma chamber ICAR-IIHR, Hessaraghatta, Bengaluru, India, with different doses of gamma rays (20, 30, 40, 50, and 60 Gy) and an exposure time of 10 minutes per dose. However, the cuttings treated with the doses 20 and 30 Gy survived. In the M1V1 generation, identified plants with bud mutations and chimeras. Subsequently, only the plants that survived were further multiplied, resulting in the M1V2 generation. From these, 200 plants were raised and evaluated in the field to detect their stability. Of the 200 plants, 21 morphologically superior plants were selected and subjected for preliminary yield trial (2020-21). Further, 18 out of 21 mutants were identified based on their superiority for morphological and yield traits along with high essential oil content. They were evaluated in the initial evaluation trial (2021-2022) following a Randomized Complete Block Design (RCBD), which aided in the identification of seven superior mutants. Subsequently, evaluation of these mutants in the advanced varietal trial over two years (2022-23 and 2023-24) identified CIM-P22 as the highest essential oil yielder with a superior level of patchouli alcohol over other mutants and the check variety. Therefore, in the

pilot-scale evaluation (2024-25), the clone CIM-P22 was further tested for herb yield, oil content, and patchouli alcohol along with a check variety, CIM-Shrestha. A graphical representation of the above-described method is presented in Figure 1.

Oil extraction by Clevenger apparatus

The patchouli oil is extracted from the 10 to 15 days shade-dried leaf material by hydro-distillation using a Clevenger apparatus. About 100 g of shade-dried leaf material was filled in a 2000 mL flask in the ratio of 1:3 (w/v) and the flask was heated at a temperature of 50 to 60°C for 8 hours. The collected oil sample was dried by adding anhydrous sodium sulphate and kept at 4°C until further GC analysis. The 3 number of replications used per sample for distillation.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC)

Essential oils were analysed in an Agilent 7890B gas chromatograph fitted with a flame ionization detector. Separation was performed using a DB-5 capillary column (30 m length $\times$ 0.25 mm i.d., film thickness 0.25 μm) and the oven temperature was programmed with an initial temperature of 60°C and raised to 240°C at 3°C/min, held at 240°C for 2 minutes. H_2 was used as a carrier gas and the flow rate was 1-mL/min. A split/splitless injector was used with a split ratio of 1:35 at 250°C. FID detector was maintained at 280°C throughout the analysis.

Gas chromatography-mass spectrometry (GC-MS)

For the GC-MS analysis, a Shimadzu Nexis GC-2030 instrument hyphenated with a Shimadzu QP2020 NX mass spectrometer detector was used. Compounds were separated on an SH-I-5Sil MS (30 m $\times$ 0.25 mm i.d.; film

Induced variability in Patchouli

Doses : Gamma rays 20, 30 40 Gy
No. of cuttings: 250 fresh cuttings



CIM-Shrestha



Mutant

Figure 1: Graphical representation new variety development

Table 1: Performance of selected patchouli mutants during primary evaluation (Year 2020-2021)

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	Alpha patchoulene (%)	Alpha bulnesene (%)	Alpha guaiene (%)	Seychellene (%)	Patchouli alcohol (%)
1	P2	46.00	14.60	58.90	6.87	5.85	218.20	41.40	2.50	5.15	11.38	8.79	5.76	47.83
2	P16	48.40	13.40	69.60	6.60	5.01	390.80	77.30	2.50	5.39	9.54	7.39	5.26	51.91
3	P6	42.00	16.20	62.80	5.85	4.67	466.20	96.20	2.45	4.56	9.61	7.25	5.25	51.20
4	P7	38.60	17.80	58.80	6.32	5.00	372.60	73.10	1.64	4.81	10.77	8.00	5.45	50.63
5	P20	48.60	14.00	59.60	6.35	5.01	374.60	78.70	2.50	4.64	9.53	7.35	5.28	51.36
6	P22	50.00	19.20	66.20	6.64	5.39	491.00	78.00	3.25	4.33	9.27	6.63	4.93	54.04
7	P23	40.40	14.00	56.30	5.70	4.28	367.80	76.60	3.00	5.50	11.45	8.70	5.90	48.17
8	P24	54.20	15.40	67.30	6.24	4.77	459.60	89.40	2.45	4.74	7.94	5.97	4.61	55.70
9	P25	49.80	17.00	71.20	6.63	4.85	600.20	117.80	2.30	4.41	8.81	6.69	5.13	54.81
10	P26	47.80	15.60	59.20	5.22	3.95	276.60	63.50	1.35	5.80	9.84	8.06	5.56	50.94
11	P27	52.20	15.60	71.70	5.80	4.33	425.80	98.80	3.05	3.96	8.13	6.19	4.65	56.43
12	P29	46.40	14.60	63.00	4.95	3.40	333.00	76.30	2.80	4.79	10.14	8.04	5.53	48.70
13	P30	42.80	16.00	67.80	6.17	4.54	563.60	106.00	2.50	4.43	9.70	7.02	5.00	53.67
14	P31	45.40	15.00	65.40	6.09	4.97	394.20	76.20	2.25	3.96	7.95	6.24	4.49	55.99
15	P32	61.80	15.40	72.60	7.08	5.51	606.00	114.70	2.82	5.29	10.83	8.80	6.01	48.02
16	P33	51.00	15.20	56.10	4.77	3.73	337.40	67.30	2.25	3.91	8.30	6.01	4.50	56.81
17	P34	43.20	16.80	65.80	6.41	4.89	470.70	98.70	2.53	5.30	9.14	6.99	5.15	52.50
18	P35	47.00	15.00	59.00	5.81	4.43	395.20	89.60	2.30	5.43	10.83	9.26	6.26	44.61
19	P36	40.40	13.00	60.20	6.34	4.61	312.40	67.00	2.50	4.95	9.68	7.71	5.54	52.70
20	P37	41.80	15.40	56.40	6.31	4.85	322.30	68.20	2.25	4.98	9.95	7.80	5.63	51.84
21	P39	50.12	16.52	55.26	6.34	4.87	450.25	100.25	3.25	5.31	13.53	8.63	5.48	44.05
22	Parent	48.00	22.40	72.30	6.27	4.93	551.40	100.90	2.50	5.76	14.74	9.83	5.99	41.53
SE		5.31	2.04	5.80	0.58	0.56	0.45	101.84	18.54	177.64	0.36	0.23	0.11	0.88
SD		23.20	9.40	17.34	2.31	2.45	1.90	387.80	76.40	833.20	1.68	1.10	0.50	4.14
t (21) df		41.54**	36.26**	51.24**	49.30**	39.20**	25.62**	19.01**	21.33**	42.1*	27.75*	33.14*	51.76*	57.77*

* $p < 0.05$, ** $p < 0.01$

Note: The crop was harvested 4.5 months after transplanting. Fresh leaves were dried for approximately 10–15 days before subjecting them to distillation. The distillation process was carried out at a temperature of 55–60 °C.

Table 2: Performance of selected patchouli mutants and check variety in initial variety trial

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	Caryophyllene (%)	α -Guaianene (%)	Seychellene (%)	α -Patchoulene (%)	α -Bulnesene (%)	Pogostol (%)	Patchouli alcohol (%)
1	P2	72.10	21.50	69.35	5.66	4.07	158.00	56.00	2.30	3.56	8.57	5.76	5.12	11.47	3.45	47.70
2	P4	66.80	35.20	71.80	6.13	4.69	404.80	106.00	2.40	3.99	9.41	6.25	5.93	12.39	3.68	44.38
3	P5	67.90	22.70	81.15	6.29	4.72	173.10	97.00	2.59	4.24	10.92	6.66	6.54	14.08	3.56	39.42
4	P6	71.60	22.80	69.55	5.76	4.04	110.00	60.00	2.62	4.00	9.64	6.24	5.48	12.73	4.12	43.33
5	P7	64.70	21.80	67.80	5.62	4.03	332.00	60.00	2.27	3.50	8.66	5.72	5.08	11.29	3.45	48.67
6	P16	69.20	22.10	72.30	5.43	3.87	194.00	68.00	2.96	3.74	8.89	5.94	5.61	11.63	4.25	47.26
7	P20	71.50	23.30	74.55	5.94	4.19	406.00	100.00	2.25	3.67	9.17	5.89	5.33	12.34	3.89	46.17
8	P22	71.90	35.60	78.40	6.43	5.21	450.40	118.00	2.95	5.08	8.48	5.74	5.01	11.23	3.56	48.67
9	P23	71.70	21.40	73.90	5.52	3.99	486.00	61.50	2.85	4.01	9.82	6.24	6.10	12.97	3.75	43.25
10	P24	76.90	22.00	82.25	6.18	4.45	346.00	98.00	2.61	4.02	9.45	6.35	5.53	12.88	3.48	44.75
11	P25	66.80	19.70	66.50	5.53	4.10	150.00	49.00	2.55	4.19	9.89	6.51	5.72	13.22	3.78	42.58
12	P26	80.40	23.60	74.60	5.85	4.30	340.00	88.00	2.33	3.54	8.28	5.60	5.02	10.99	4.52	46.95
13	P29	72.90	23.50	77.10	5.79	4.20	170.00	68.00	2.13	3.43	8.45	5.65	5.51	11.91	4.12	49.24
14	P31	72.40	19.40	68.95	5.80	4.20	296.00	61.00	2.60	3.73	8.61	5.95	5.26	11.86	3.75	45.73
15	P32	68.40	22.30	76.05	6.10	4.76	338.30	103.00	2.47	3.75	9.00	5.93	5.69	12.25	3.76	45.98
16	P34	88.10	23.50	72.80	6.02	4.48	320.00	82.50	3.25	4.14	10.64	6.49	6.48	13.96	3.48	41.20
17	P36	68.30	16.50	58.20	5.34	3.88	185.00	41.00	2.65	4.06	10.30	6.56	6.59	13.28	4.23	43.44
18	P39	69.30	19.10	66.30	5.55	3.98	236.00	47.00	2.94	3.56	8.75	5.75	5.16	12.16	3.65	48.37
19	Parent	72.00	18.60	59.25	7.37	5.73	278.00	83.00	2.24	3.96	9.72	6.29	6.15	12.61	3.58	44.20
CD (5%)		9.12	6.44	6.95	0.52	0.51	249.33	27.41	0.37	0.23	0.68	0.31	0.35	1.08	.65	2.59
CD (1%)		12.37	8.72	9.42	0.71	0.69	202.36	37.15	0.50	0.32	0.93	0.42	0.47	1.47	.84	3.54
CV (%)		6.21	14.14	4.75	4.36	5.74	26.14	18.63	6.95	10.75	13.83	8.85	10.88	16.51	9.52	7.93

Parent: CIM-Shrestha

Table 3: Performance of CIM-P-22 (CIM-Atishrestha) and check variety of patchouli in advance varietal trials (AVT) (Year 2022-23): Harvest-I

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	Caryophyllene (%)	α -Guaiane (%)	Seychellene (%)	α -Patchoulene (%)	α -Bulnesene (%)	Pogostol (%)	Patchouli alcohol (%)
1	P16	55.33	15.28	66.83	7.00	5.11	307.22	60.00	2.63	3.19	7.71	5.32	5.03	10.29	3.58	51.42
2	P22	60.40	17.00	59.35	8.12	6.22	428.00	85.00	3.10	3.33	8.02	5.49	4.89	10.94	3.78	50.89
3	P31	64.17	18.44	68.06	7.16	5.34	315.56	73.89	2.60	3.50	8.26	5.67	5.03	11.16	3.45	49.26
4	P34	51.33	12.39	59.19	6.97	4.68	179.44	36.11	2.13	3.48	8.27	5.62	5.81	11.26	4.12	49.46
5	P36	60.17	17.44	72.56	7.04	5.35	310.56	44.44	2.50	3.24	8.14	5.42	4.90	11.70	3.58	49.08
6	P39	50.50	16.42	51.21	8.08	5.88	383.33	81.67	2.87	3.65	9.00	5.87	5.27	12.06	4.56	46.50
7	Parent	64.33	17.67	80.17	6.56	4.85	476.67	86.67	2.33	4.27	11.34	7.12	6.83	13.41	3.56	41.73
CD (5%)		3.00	2.41	4.93	0.35	0.34	113.97	11.51	0.15	0.48	1.62	0.80	0.93	1.30	0.45	4.33
CD (1%)		4.82	3.37	6.91	0.48	0.47	159.77	16.14	0.20	0.72	2.45	1.22	1.41	1.97	0.79	6.56
CV (%)		5.27	8.26	4.23	2.67	3.54	18.67	9.68	3.16	10.35	14.25	10.62	13.16	8.62	8.56	6.85

Table 4: Performance of CIM-P-22 (CIM-Atishrestha) and check variety of Patchouli in advance varietal trials (AVT) (Year 2022-23): Harvest-II

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	Caryophyllene (%)	α -Guaiane (%)	Seychellene (%)	α -Patchoulene (%)	α -Bulnesene (%)	Pogostol (%)	Patchouli alcohol (%)
1	P16	62.78	16.11	63.50	7.23	5.43	349.44	71.11	1.78	3.71	9.08	6.03	5.42	11.87	3.23	47.42
2	P22	62.94	15.33	48.28	8.55	6.66	484.44	84.44	2.90	3.55	8.47	5.75	5.53	11.28	3.89	48.53
3	P31	58.61	12.89	49.22	6.81	4.84	171.67	39.44	2.55	3.92	10.05	6.68	6.01	12.49	3.65	45.21
4	P34	70.33	15.83	75.67	7.43	5.64	641.67	101.33	2.50	3.49	8.59	5.95	5.30	11.26	3.75	48.15
5	P36	65.39	15.00	55.17	10.06	7.75	530.00	108.89	2.80	3.80	10.29	6.55	6.70	13.52	3.45	43.99
6	P39	67.94	14.17	60.31	7.19	5.24	363.89	78.89	2.80	3.62	8.61	5.92	6.17	11.19	4.27	46.32
7	Parent	56.17	10.83	53.25	8.24	6.49	241.67	50.00	2.25	4.26	11.02	6.91	6.29	13.44	3.48	40.90
CD (5%)		5.70	2.28	4.44	0.65	0.71	208.35	28.17	0.12	0.34	1.32	0.59	0.68	1.33	0.41	3.52
CD (1%)		7.99	3.19	6.23	0.91	1.00	292.09	39.49	0.17	0.52	1.99	0.89	1.03	2.01	0.72	5.34
CV (%)		5.05	8.94	4.31	4.61	6.68	29.46	20.75	2.75	6.98	10.64	7.18	8.76	8.35	7.56	5.88

Table 5: Performance of CIM-P-22 (CIM-Atishrestha) and check variety of patchouli in advance varietal trials (AVT) (Year 2023-24): Harvest-I

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	β -caryophyllene (%)	α -Guaiene (%)	Seychellene (%)	α -Patchoulene (%)	α -Bulnesene (%)	Pogostol (%)	Patchouli alcohol (%)
1	P16	90.10	6.10	74.00	6.07	4.67	862.00	177.20	2.65	3.36	9.53	5.28	3.40	13.89	3.83	47.63
2	P22	81.50	7.90	76.70	6.18	5.09	950.00	232.75	3.69	3.17	8.98	5.11	3.31	12.89	4.01	49.99
3	P31	78.95	4.80	53.35	7.58	6.21	581.40	121.20	2.43	3.27	9.16	5.42	3.42	13.25	3.67	49.33
4	P34	70.40	9.70	69.60	7.37	6.17	980.00	225.00	2.92	3.56	9.84	5.52	3.64	14.19	3.66	46.77
5	P36	92.40	6.90	64.30	6.82	5.27	846.00	194.00	1.67	3.46	9.38	5.40	3.53	13.62	3.87	46.44
6	P39	74.60	4.00	58.95	7.45	5.86	306.60	67.20	3.10	2.53	6.95	4.31	2.63	10.21	4.37	58.01
7	Parent	70.90	6.00	70.80	6.24	5.15	655.00	142.00	2.22	3.64	10.74	5.79	3.82	15.51	3.32	44.09
CD (5%)		5.88	13.28	3.69	3.94	4.80	17.72	11.61	10.02	0.48	1.51	0.61	0.49	2.12	0.43	5.84
CD (1%)		8.16	1.43	4.31	0.48	0.47	220.17	31.18	0.46	0.73	2.29	0.93	0.75	3.21	0.65	8.85
CV (%)		11.32	1.98	5.99	0.66	0.65	18.65	16.35	0.64	11.25	12.53	8.89	11.16	12.14	8.56	9.14

Table 6: Performance of CIM-P-22 (CIM-Atishrestha) and check variety of patchouli in advance varietal trials (AVT) (Year 2023-24): Harvest-II

Sl. No	Mutants	Plant height (cm)	Primary branches	Canopy cover (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Oil yield (%)	β -caryophyllene (%)	α -Guaiene (%)	Seychellene (%)	α -Patchoulene (%)	α -Bulnesene (%)	Pogostol (%)	Patchouli alcohol (%)
1	P16	58.75	15.60	59.50	6.00	5.62	546.50	163.95	2.52	3.42	9.96	5.52	3.57	14.25	3.55	46.15
2	P22	59.80	13.30	58.40	4.66	3.11	682.00	204.6	3.33	3.12	8.59	5.08	3.14	12.64	3.84	51.12
3	P31	83.70	17.30	76.10	5.21	3.24	706.30	211.89	2.53	3.23	9.50	5.24	3.43	13.57	4.07	47.49
4	P34	63.60	14.60	57.60	5.54	5.60	503.00	150.90	2.63	3.56	9.84	5.52	3.64	14.19	3.66	46.77
5	P36	77.40	17.60	70.10	5.10	4.39	719.00	215.70	2.95	3.48	9.41	5.32	3.42	13.77	3.72	47.40
6	P39	62.00	9.60	53.80	4.91	3.17	464.50	139.35	3.08	2.53	6.95	4.31	2.63	10.21	4.37	58.01
7	Parent	69.16	6.16	70.30	6.10	5.22	548.50	164.55	2.21	3.37	9.86	5.37	3.54	14.26	3.59	44.93
CD (5%)		8.24	1.32	4.26	0.50	0.69	193.13	57.94	0.10	10.68	11.78	8.08	10.57	11.05	7.71	9.16
CD (1%)		11.44	1.84	5.91	0.70	0.95	268.06	80.42	0.14	0.45	1.41	0.55	0.46	1.92	0.39	5.85
CV (%)		6.99	5.40	3.77	5.30	8.70	18.61	18.61	2.01	0.69	2.14	0.83	0.70	2.91	0.58	8.87

Table 7: Pilot scale trial 2024-25; Plot size = 200 m²

Sl. No	Parameters	CIM-P22 (CIM-Atishrestha)	CIM-Shrestha	CIM-Samarth	CIM-Utkrisht	BIS accepted range
1	Dry wt. (t/ha/harvest)	4.00–4.80	4.0–4.70	5.5–6.00	6.23–7.50	-
2	Oil content (dry weight) % (Clevenger basis)	2.8.0–3.50	2.10–2.60	1.4–1.60	1.4–1.5	-
3	Oil content (dry weight) % (30 kg)	2.20–2.60	1.80–2.20	0.8–1.00	0.7–9.00	-
4	Oil yield on dry weight (Kg/ha)	88.0–125.0	72.0–107.0	55–60	80–90	-
5	Patchouli alcohol (%) (Clevenger basis)	45.00–60.00	40.00–45.00	30.50–36.50	25.00–30.00	25.00-35.00%
6	Patchouli alcohol (%) (30 kg)	35–40	25–30	20.00–25.00	20.50–26.00	25.00-35.00%
7	Pogostol (%)	3.2–3.8	3.0–3.5	3.10–3.50	1.00–1.50	
8	Optical rotation (°C)	-56.198–56.199	-58.196–58.212	-58.155–58.251	-13.50–14.12	-66 to -40
9	Specific gravity	0.9598–0.9645	0.9626–0.9635	0.9442–0.9453	0.9701–0.9712	0.9480 to 0.9710
10	Refractive index	1.5074–1.5144	1.5086–1.5125	1.512–1.542	1.5015–1.5025	1.5020 to 1.5120

Note: Herb yield and oil yield per ha per harvest are estimated data

thickness 0.25 µm) capillary column. Helium was used as a carrier gas maintained at a flow rate of 1-mL/min and a split ratio of 1:35. The column oven temperature was programmed with an initial temperature of 60°C and increased at the rate of 3°C/min to 240°C. The GC inlet temperature and the GC-MS interface temperature were set at 280°C. Electron ionization was achieved at 70 eV with a source temperature of 230°C and a quadrupole analyser was used for the separation of ions.

Compounds were identified by matching the mass spectral data with the NIST library (Version 2.0) and further confirmed by comparing the peak retention times, elution order, and relative retention index (RRI) (Adams, 2007).

Results and Discussion

Preliminary trial (2020-2021)

In this trial, 21 mutant clones of the M1V2 generation were evaluated for yield and biochemical traits under field conditions (Table 1). The clones were planted in progeny rows as single-row plots. Based on herb yield, oil content, and patchoulol concentration, 18 mutants were selected for the next cycle of evaluation. The oil content ranged from 2.5 to 3.5%, and the patchouli alcohol content ranged from 45 to 60%, as compared to the check variety CIM-Shrestha, which had an oil content of 2.0 to 2.5% and patchouli alcohol content of 35 to 45%. These 18 mutants were re-evaluated in the initial evaluation trial for patchouli alcohol, oil content, and other agronomic traits along with the check variety CIM-Shrestha.

Initial evaluation trial (IET) (2021-22)

The initial evaluation study of patchouli clones involved the examination of eighteen mutant clones along with the parental control variety over the period of 2021-22. Based on oil content, patchoulol, herb yield, and morphological characteristics, among eighteen mutant clones, seven clones were selected (Table 2).

Advanced varietal trial (Year- 2022-23 and 2023-24)

Seven selected mutant clones and the parent variety CIM-Shrestha were evaluated in Advanced Varietal Trials (AVT) during the years 2022–23 and 2023–24 in a randomized complete block design with three replications. The planting was with a spacing of 45 × 45 cm and the total plot size was 3.25 × 3.25 m. Based on the performance, clone CIM-P22 (later renamed as CIM-Atishrestha) across years of the AVT was selected for the plot scale trial. Results of the AVT are presented in Tables 3, 4, 5, and 6.

Pilot scale evaluation (PST)

Furthermore, CIM-P22 along with check varieties CIM-Shrestha, CIM-Samarth and CIM-Utkrisht were evaluated in PST during 2024–25 in a plot size of 200 m². In this trial, the CIM-P22 clone was outperformed compared

Table 8: Description of the new clone CIM-P22 (CIM-Atishrestha)

Sl. No	Parameters	CIM-P22 (CIM-Atishrestha)	CIM-Shrestha
1	Plant height (cm)	60.00–70.00	55.00–72.00
2	Plant canopy (cm ²)	60.00–75.00	55.00–75.00
3	Colour of leaf	Dark Green	Dark Green
4	Dry wt. (t/ha/harvest)	4.00 to 4.80	4.0 to 4.70
5	Oil content (dry weight) % (Clevenger basis)	2.8.0–3.50	2.10–2.60
6	Oil content (dry weight) % (30 kg)	2.20–2.60	1.80–2.20
7	Oil yield on dry weight (Kg/ha)	88.0–125.0	72.0–107.0
8	Patchouli alcohol (%) (Clevenger basis)	45.00–60.00	40.00–45.00
9	Patchouli alcohol (%) (30 kg)	35.00–40.00	25.00–30.00
10	Optical rotation (°C)	-56.198–56.199	-58.196–58.212
10	Specific gravity	0.9598–0.9645	0.9626–0.9635
11	Refractive index	1.5074–1.5144	1.5086–1.5125



Figure 2: General field view of CIM-P22 (CIM-Atishrestha) clone and CIM-Shrestha

to check varieties with respect to oil content and patchouli alcohol (Table 7). The clone CIM-P22 (CIM-Atishrestha) outperformed the check variety CIM-Shrestha in all the trials by at least 20 to 30%. Its mean patchouli alcohol content in all trials varied from 45 to 55%, which was higher than the check variety and well above the minimum 25% prescribed by BSI standards. Based on its superiority over the check variety in all the trials, the CIM-P22 (CIM-Atishrestha) clone was recommended for formal release as a new patchouli variety.

Description of proposed strain

The data pertaining to the morphological traits, chemical properties, and physical parameters of the patchouli

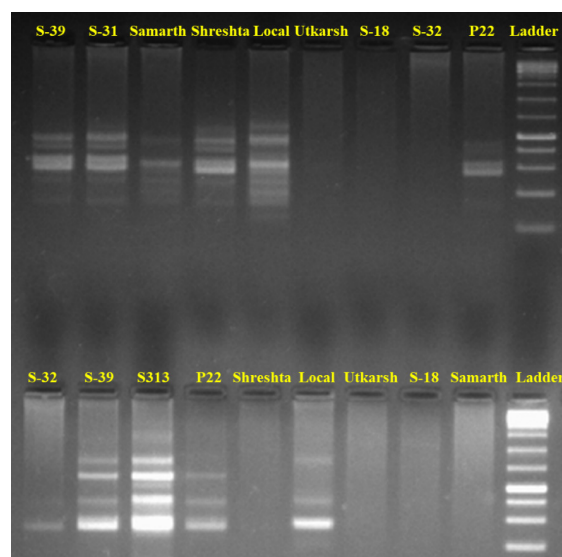


Figure 3: ISSR-marker based profile of CIM-P22 (CIM-Atishrestha) clone and Checks. (Upper marker: UBC 809; Lower marker: UBC 825)

variety CIM-Shrestha and new clone CIM-P22(CIM-Atishrestha) are mentioned in Table 8.

Statement of distinction (DUS criterion)

New mutant clone CIM-P22 is distinct from CIM-Shrestha with respect to oil content and patchouli alcohol concentration. CIM-P22 plants are moderately tall with a well-spread canopy and dark green foliage. CIM-P22 exhibits superior essential oil characteristics, with patchouli alcohol content ranging from 45.0 to 60.0% (Clevenger basis), as against 40.0 to 45.0% in CIM-Shrestha. The optical rotation (-56.198–56.199°C) and specific gravity (0.9598–0.9645) of CIM-P22 oil indicate higher purity and stability relative to CIM-Shrestha (-58.196–58.212°C; 0.9626–0.9635). Thus, CIM-P22 (CIM-

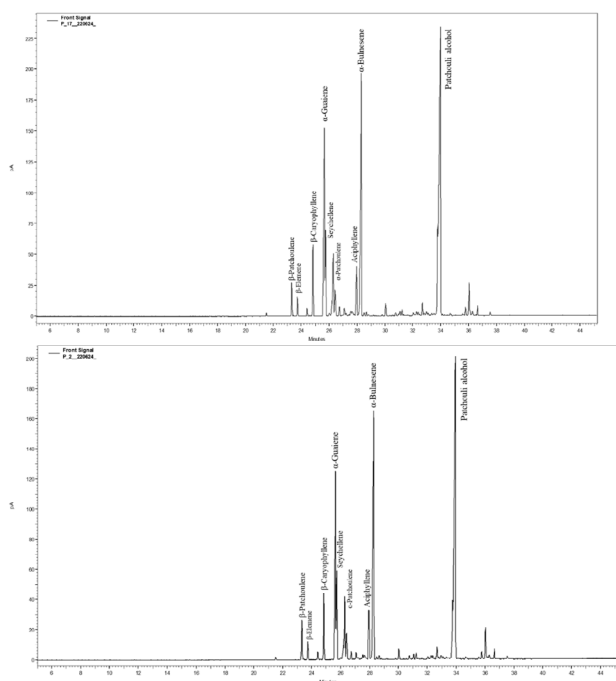


Figure 4: Representative chemical profile of CIM-P22 clone (CIM-Atishrestha) and CIM-Shrestha (Parent)

Atishrestha) is distinct from CIM-Shrestha primarily on higher oil recovery efficiency, elevated patchouli alcohol content, and favorable physicochemical oil traits, which clearly satisfy the DUS criterion of distinctness. Morphological, molecular and biochemical variation are shown in Table 8 and Figures 2, 3 and 4. The performance of the new variety across multiple generations of vegetative propagation and rigorous multi-year field trials spanning 2020-2025 confirmed the stability. Important traits like oil content, herb yield and patchouli alcohol showed no significant variations across the generations.

Farmers benefit from the new variety

CIM-Atishrestha, yielding more oil (16–23 kg/ha) than the available existing varieties, delivers direct farmer benefits through 20 to 30% superior performance over CIM-Shrestha. This increases the net income of farmers from ₹1.5 lakh/ha to over ₹1.8–2 lakh/ha at current prices (₹10,000/kg oil). With respect to Indonesian standards (SNI 06-2385-2006: ≥30% patchouli alcohol; typical varieties 29–36%), CIM-Atishrestha (35–40%). While vegetative propagation ensures trait fidelity, long-term stability requires monitoring for off-types in commercial multiplication.

Author Contribution

Channayya Hiremath conceptualized and supervised the study and was primarily responsible for breeding, genetic improvement, data preparation, and proposal development. Dinesh A. Nagegowda carried out molecular analyses. V. Sundaresan and Sanjay Kumar

contributed to clone selection and evaluation. Niranjan Kumar, V. S. Pragadheesh, and K. V. N. Satya Srinivas performed chemical analyses and data interpretation. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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