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NEW VARIETY RELEASE

Development of high-oil and high-citronellal *Eucalyptus citriodora* clone CIM-NANDI

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ABSTRACT

Eucalyptus citriodora (E.C.) is an important aromatic tree species in nature. It has wide applications in the cosmetic and pharmaceutical areas. The essential oil of the E.C. mainly consists of citronellal (80%); the world annual production of E.C. is around 250 tons and is produced primarily in Brazil and China; however, the estimated requirement is >1000 tons/ year. So, it is essential to increase oil production to meet the demands of the industries. However, at this point, not a single high-yielding E.C. variety is available for cultivation from CSIR-CIMAP, and the general local cultivar is low yielding. Growing available local cultivars makes producing more oil difficult without the additional burden on primary agriculture arable land. We have developedhigh-oil and high-citronellal Eucalyptus citriodora clone CIM – Nandi to pursue this goal.

INTRODUCTION

Eucalyptus citriodora (E.C.) is an important aromatic tree species endemic to north-eastern Australia. It generally grows to 50-60 m in height, commonly known as lemon-scented or spotted gum (Ayinde, 2016). It has smooth white to pink bark, narrowly lance-shaped to curved adult leaves, flower buds in groups of three, and white flowers and urn-shaped or barrel-shaped fruit. The essential oil of the lemon-scented gum mainly consists of citronellal (80%), produced primarily in Brazil and China (Boland *et al*., 1991). Unrefined oil from the lemon *Eucalyptus* tree is used in the perfumery, and a refined form of this oil is used as insect repellents, especially against mosquitoes. Essential oil refined from the tree leaves can contain up to 98% citronellal content. The smell of the essential oil can vary but mainly includes a strong odor comparable to citronella oil, with a slight note of lemon scent. The oil of this crop acts as a good antiseptic and fumigant. The leaves are used to treat fever by keeping the leaves under the sheets of fever patients and inhaling the steam from boiled leaves for cold and various pulmonary problems, ulcers, wounds, and other skin ailments.

E.C. is distributed naturally, only on the central and northern coasts of Queensland, Australia, but fares well in much of Africa, Brazil, California, Hawaii, India, and even Portugal (Ayinde, 2016). In India, the crop is under commercial cultivation in Karnataka, Andhra Pradesh, Tamil Nadu, Assam, and Maharashtra. One of the critical aspects of this crop is that it can be successfully grown in poor soils and ravine areas. The crop is tolerant to clay, drought, gravel, laterites, light frosts, podzols, poor soil, and slopes but is very susceptible to waterlogging.

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The world essential oil production of E.C. is 250 tons/year. However, the estimated requirement is >1000 tons/year (Essential Oils Market Report Spring 2018). So, it is essential to increase oil production to meet the demands of the industries. Growing available local cultivars makes it very difficult to produce more oil without the additional burden on main agriculture arable land. Hence, the planned breeding and selection process was undertaken at CSIR-CIMAP, Research Centre, Bengaluru, to develop the *Eucalyptus citriodora* strain.

Origen of variety CIM-Nandi

In CSIR-CIMAP, Research Centre, Bengaluru campus, around 114 germplasms of *Eucalyptus* trees were planted and maintained. In 1986, one of the *Eucalyptus* clones was selected and planted on 1 acre, giving about 1.5 % oil content with 63.9% citronellal at the laboratory level. In recent years demand for E.C. oil has increased; based on the increasing demand for E.C. oil, we have planned to identify high oil and citronellal-rich clones. As a first step, we distilled individual trees conserved in CSIR-CIMAP, Research Center - Bengaluru in 2016. The oil content of these trees was ranged between 0.80-3.10%, and its citronellal content ranged between 46.5-85.6% as compared to existing local checks with 1.5% oil and 63.9% citronellal content, respectively.

Further, seeds of high oil and citronellal-rich trees were collected individually and sown for the next level of evaluation in poly-covers. The seedlings (2 months old) of the selected clones and local cultivars were transplanted to the main field in a row trial (10 plants per clone). After nine months, the top portion was nipped to grow more side branches so plants would not grow at a greater height, making harvesting easy. The selected clone -5 (CIM-Nandi) was planted along with a check variety for Initial screening (10 plants each), and a pilot scale evaluation was carried out in the year 2017-2018; 2018-2019; 2019-20. The CIM-Nandi clone was superior to the total oil and citronella content, and the herbage yield was on par with the check. The CIM-Nandi always maintained its superiority over the existing check.

Initial Screening of Eucalyptus citriodora clones

Initial screening of E.C. accessions were started in 2015-2016; 114 accessions available in the CSIR-CIMAP, Research Centre Bengaluru were distilled and analyzed for essential oil content. Based on oil content, only twenty accessions were analyzed for essential chemical constituents using gas-chromatography (G.C.). The selected clones were redistilled for confirmation, and all the clones showed consistent results. Among the selected 20 clones, only T5 (CIM-Nandi) and checks were taken for further evaluation trials (Table 1).

Table 1: Clones selected for G.C. analysis after first round of distillation among the 114 accessions.

SL. No	Tree no	% of oil	Citronellal %
1	1	1.85±0.03	81.20±0.65
2	3	1.30±0.05	73.50±1.18
3	**5**	**3.10±0.06**	**82.90**±1.15
4	7	1.40±0.1	64.80±0.57
5	24	1.90±0.02	77.70±1.03
6	27	1.82±0.01	78.10±1.15
7	30	1.55±0.05	74.10±0.57
8	33	2.25±0.02	77.90±1.01
9	49	1.50±0.10	74.80±0.96
10	55	1.15±0.16	71.80±1.15
11	57	1.40±0.21	56.20±1.15
12	60	1.70±0.11	77.80±0.96
13	62	1.90±0.15	70.90±0.83
14	64	1.50±0.05	61.40±0.60
15	68	2.15±0.10	82.80±0.54
16	70	2.10±0.11	71.50±0.54
17	71	1.50±0.10	58.50±1.00
18	73	2.25±0.07	67.90±0.57
19	74	1.50±0.05	78.80±0.11
20	77	2.00±0.05	46.40±0.48
21	Control	1.52±0.04	65.56±1.65

Initialscreening of Eucalyptus citriodora clones

The selected clone (ECT-5) was evaluated along with checks variety in the initial screening trial in 2016-18. The seedlings were planted in a row trial with a plot size of 10m x 10m with a spacing of 1m x 1m and three harvests were recorded (Table 2). It shows the superiority of clone T-5 (CIM-Nandi) over the check.

Table 2: Performance of *Eucalyptus citriodora* in initial screening (1.0 m x 1.0 m plant to plant and row to row distance).

	CIM-Nandi								Check (Local)							
	Herb yield per plant (Kg per plant)				Oil Content (%)				Herb yield per plant (Kg per plant)				Oil Content (%)			
Harvest	**I**	**II**	**III**	**Mean**	**I**	**II**	**III**	**Mean**	**I**	**II**	**III**	**Mean**	**I**	**II**	**III**	**Mean**
1	1.45	5.26	4.90	3.87	2.90	3.00	3.10	3.00	1.50	5.45	5.40	4.12	1.50	1.45	1.50	1.48
2	1.50	5.45	5.50	4.15	3.12	3.00	3.00	3.04	1.60	5.10	4.80	3.83	1.40	1.25	1.45	1.37
3	2.00	5.11	6.00	4.37	3.00	2.95	2.90	2.95	2.45	5.00	6.00	4.48	1.60	1.50	1.60	1.57
4	1.90	5.02	5.15	4.02	2.89	2.78	3.10	2.92	2.20	5.45	5.58	4.41	1.80	1.60	1.70	1.70
5	2.15	4.98	5.50	4.21	3.10	3.10	2.80	3.00	1.80	5.20	5.78	4.26	1.70	1.52	1.50	1.57
6	2.00	4.58	5.80	4.13	3.00	2.89	3.10	3.00	2.50	4.90	6.00	4.47	1.50	1.40	1.60	1.50
7	2.25	5.50	4.90	4.22	2.98	3.00	3.00	2.99	2.50	4.75	5.90	4.38	1.60	1.50	1.45	1.52
8	2.00	5.12	4.80	3.97	2.50	3.12	3.10	2.91	3.00	4.50	4.80	4.10	1.50	1.58	1.20	1.43
9	2.45	4.89	5.50	4.28	3.15	3.15	2.89	3.06	1.80	5.50	5.00	4.10	1.10	1.78	1.45	1.44
10	1.98	5.15	5.70	4.28	3.00	3.00	2.90	2.97	1.70	5.20	4.90	3.93	1.50	1.60	1.47	1.52
Mean	**1.968**	**5.105**	**5.375**	**4.150**	**2.964**	**2.999**	**2.989**	**2.984**	**2.105**	**5.105**	**5.416**	**4.209**	**1.520**	**1.518**	**1.492**	**1.510**
SE	**0.096**	**0.084**	**0.131**	**0.049**	**0.058**	**0.035**	**0.035**	**0.015**	**0.157**	**0.103**	**0.159**	**0.072**	**0.059**	**0.044**	**0.042**	**0.029**
t-cal	**0.67**				**44.76**				**0.67**				**44.76**			
t-tab (5%)	**2.10**				**2.10**				**2.10**				**2.10**			

Table 3: Mean performance of selected clone and check in pilot scale trail (2018-2020)

Traits	**Herb yield (kg)**				**Oil yield (ml)**		
Harvest	I	II	III	Mean	II	III	Mean
CIM-Nandi	19.68	51.00	53.75	41.47±10.92	910	900	905±5.00
Check	21.00	51.00	54.16	42.05±10.56	480	460	470±10.00

Pilot scale evaluation of the selected clone ECT-5 and check variety (2018-2020)

A pilot Scale trial was conducted in 2018-2020, including clone ECT-5 and check variety. Both the clones were evaluated in a plot size of 20m x 20m with a spacing of 1m x 1m. The superior clone ECT-5(CIM-Nandi) always maintained its superiority over the existing check variety for oil content and citronellal content (Table 3). Since clone ECT-5 has performed well in herb yield, oil yield and oil quality were identified for its formal release as a variety.

Statement of distinction (DUS criterion)

Clone CIM-Nandi is vigorous, and leaves are light green; seedling stage leaves show more hairiness. In later stages of plant growth, the hairiness character will disappear because of this variety's distinguishing morphological features (Table 4).

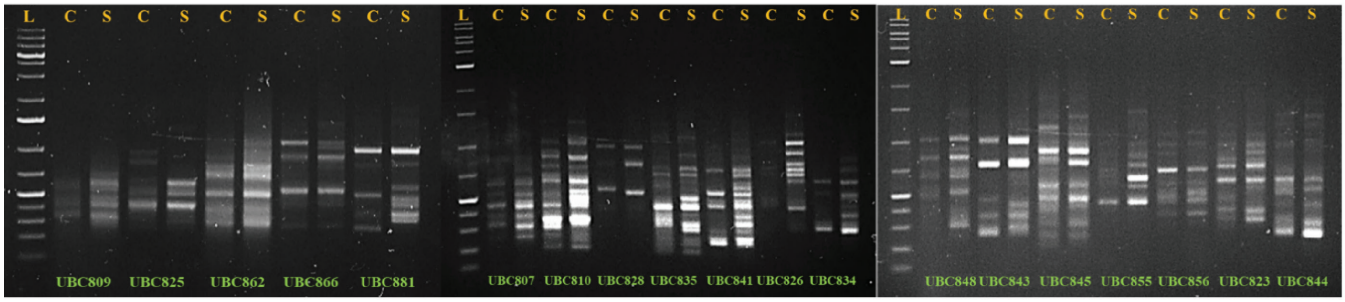

Figure 1: ISSR markers molecular profile of CIM-Nandi clone and check (C: check, S: CIM-Nandi)

Table 4: Description of the new clone CIM-Nandi

Attributes	CIM-Nandi	Check
100 seed weight (g)	Mean: 0.725± 0.005 Range: 0.720-0.731	Mean: 0.715±0.015 Range: 0.70-0.730
Leaf Length (cm)	21.78±0.66 21.12-22.44	21.65±1.00 20.65-22.65
Leaf Width (cm)	3.65±0.45 3.20-4.10	3.60±0.4 3.20-4.00
Petiole length (cm)	2.50±0.50 2.00-3.00	2.60±0.50 2.20-3.00
Herb yield (kg/plant/harvest	5.375±0.131 6.00-7.00	5.46±0.159 6.00-7.00
Oil content (%) (Clevenger)	2.99±0.015 2.78-3.15	1.50±0.029 1.20-1.70
Oil content (%) (30 kg capacity unit)	1.80-2.20	0.80-1.00
Oil yield (kg/t/harvest) (estimated)	14.50-16.00	7.00-8.00
Citronellal (%) (Clevenger)	88.00 ±1.31 82.50-90.60	67.00±2.271 63.00-77.94
Citronellal (%) (30 kg capacity unit)	80.17±3.38 76.89- 83.45	69.45±5.30 64.15-74.75
Citronellol (%) (30 kg capacity unit)	2.15-4.15	4.56-7.62

Table 5: Physical parameters of CIM-Nandi

Parameters	ISO 3044:2020(E) CIM-ECT-5	ISO 3044:2020(E)	BISof CIM-ECT-5	BIS
Optical rotation	+1.68° (20 °C)	−1° to +3° (20 °C)	+1.74° (27 °C)	- 2° to + 6°
Refractive index	1.4535 (20 °C)	1.450 to 1.460 (20 °C)	1.4509 (27 °C)	1.447 0 to 1.4570 (27 °C)
Miscibility in 80% ethanol	1.2 volumes (25.8 °C)	It shall not be necessary to use more than four volumes of ethanol 70% (volume fraction) to obtain a clear solution with one volume of essential oil		The material shall be soluble in two volumes of ethyl alcohol (80 percent by volume)
Miscibility in 70% ethanol	2 volumes (28.7 °C)			
Relative Density	0.8673 (25.6 °C)	0.860 to 0.880 (20 °C)	0.8673 (25.6 °C)	0.8500–0.8700 (27 °C)

Table 6: Chemical profile of CIM-ECT-5 clone by GC-MS analysis

Compound	RI (Experimental)	R.I. (Literature)	Content (%)		
			CIM-ECT-5	ISO 3044:2020(E)	BIS
α-Pinene	933	932	0.20		
β-Pinene	977	974	0.85		
Myrcene	991	988	0.15		
Limonene	1028	1024	0.15		
1,8-Cineole	1030	1026	0.64	0.5-3.0	

cis-β-Ocimene	1036	1032	0.13		
γ-Terpinene	1057	1054	0.22		
Linalool	1101	1095	0.53		
Isopulegol	1144	1145	5.85	5.0-10.0	
Citronellal	1157	1148	78.85	75.0-85.0	Min. 70.0
neoiso-Isopulegol	1168	1167	0.37		
Citronellol	1229	1223	6.63	3.0-10.0	
Geraniol	1255	1249	0.38		
Citronellyl acetate	1355	1350	1.00	0.1-2.0	
trans-Jasmone	1390	1390	0.07		
β-Caryophyllene	1416	1417	1.27		
Germacrene D	1479	1480	0.30		
Bicyclogermacrene	1493	1500	0.32		

Note: Essential oil distilled in 30 kg capacity distillation unit

Molecular/chemical profile of the developed clone

The ISSR molecular profile of CIM-Nandi and Check was presented in Figure 1. We can clearly distinguish the local cultivar and CIM-Nandi clone based on the molecular profile. Similarly, the chemical profile and physical parameters of CIM-Nandi was mentioned in Figure 2 and Table 5 and 6.

Recommended productions ecology

The new clone is suitable for cultivation in rainfed and irrigated conditions and low fertility land. New clones can be planted throughout the year if an irrigation facility is available, ideal for high/low fertility land.

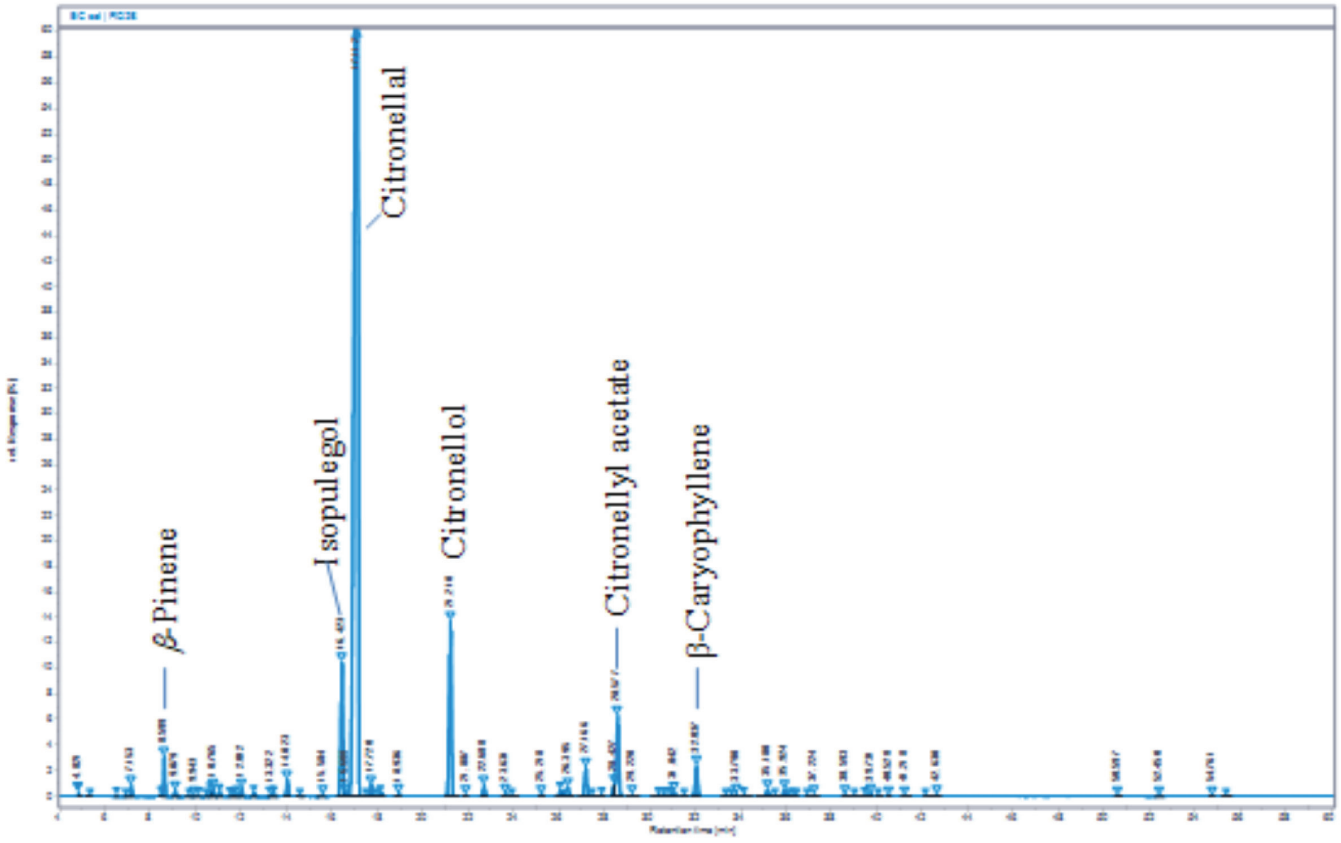

Figure 2: GC-FID Chromatogram of selected clone CIM-Nandi

CIM-Nandi **Local**

Figure 3: General plant view of CIM-Nandi and local cultivar

Specific areas of its adaptation

The new clone is proposed for all India levels (all tropical and subtropical climatic zones of India)

Package of practices along with attainable yield levels

S. No.	**Recommended agronomical Practices**	
1.	Planting	Round the year is the ideal time for planting during the monsoon season.
2.	Seed material	50 g/acre
3.	Spacing	Plant to Plant Spacing-1meter Row Row spacing- 1meter
4	Suitable planting condition conditions	Suitable for irrigated, rain-fed conditions, degraded wasteland high suitable
5	Practice of nipping	Planting after nine months of nipping is recommended to reduce plant height and branching.
6	Harvesting	I harvest: 14 Months II and subsequent: 110-120 days
7	Herb Yield (t/ acre/harvest)	5 to 7
8	Oil yield (kg/ acre/harvest)	80-100

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