

ORIGINAL RESEARCH ARTICLE FOR VARIETY REGISTRATION

A linalool rich variety-CIM-Sukhda of *Ocimum basilicum*

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ABSTRACT

Ocimum basilicum L. is an essential oil and aroma producing crop of India. Due to tremendous human demand for new natural flavors, many *Ocimum basilicum* cultivars have been bred during its long history of cultivation in the country. In pursuance of this goal a linalool rich inter-specific hybrid was developed in this study by open pollination of *Ocimum basilicum* with *Ocimum africanum*. Through these continuous breeding efforts new variety CIM-Sukhda was developed. This variety provides higher yield of oil than the check variety CIM-Surbhi and is highly suitable for cultivation in South Indian climate. Introduction of CIM-Sukhda for cultivation will enable marginal farmers to get additional income as a subsidiary crop.

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INTRODUCTION

The genus *Ocimum* belonging to family Lamiaceae, possesses a high level of morphological as well as chemical variability caused by polyploidy and inter- and intra-specific hybridization (Kumar et al., 2016). The species of this genus represent important economic and medicinal herb/shrub that are widely distributed in tropical, subtropical, and warm temperate regions of the world. The economic importance of the genus is mainly due to their aromatic essential oil which is produced in highly specialized structures known as pectate glandular trichomes located on both sides of the leaves, and stored in the sac surrounding these glands. *Ocimum basilicum* L. (Ob) is an erect, sweet-scented, predominantly herbaceous annual plant, native to tropical regions in the Central Africa to South East Asia. Further, *O. africanum* is an inter-specific hybrid of *O. basilicum*

L. and *O. americanum* L (Suddee et al., 2005). Due to tremendous human demand for new natural flavors and aromas, many *O. basilicum* cultivars have been bred during the long history of its cultivation in India. It is in this direction that a linalool rich inter-specific hybrid was developed in this study through hybridization breeding efforts between *O. basilicum* with *O. africanum*.

Linalool (3, 7-dimethyl-1, 6-octadien-3-ol) is an acyclic monoterpene tertiary alcohol and also one of the major floral scents in nature. In perfumery, linalool is one of the most widely used fragrant ingredient that constitutes the perfumes' top notes. Around 60–90% of cosmetic products use linalool of synthetic origin (Verma, 2016). It is also added to household detergents, waxes and processed food and beverages as a fragrance and flavouring agent. In industry, it is also an important intermediate in the chemical synthesis of vitamin E, vitamin A,

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farnesol, ionones, and citronellol. Besides, linalool is also used as an insecticide for pet ectoparasite control. The annual worldwide consumption of linalool exceeds 1000 metric tons.

Keeping in mind the importance of linalool in perfumery and cosmetics industries, a need to develop a better strain of *Ocimum* with high linalool content and adaptability for South Indian conditions was felt. Hence, the planned breeding and selection process was undertaken at CSIR-CIMAP, Research Centre, Bengaluru to develop such a strain of *Ocimum*. Details of this varietal development programme are summarized here.

Origin of the variety CIM-Sukhda

The *Ocimum* germplasm collection and evaluation programme was started in 2013 at CSIR-CIMAP, Research Centre, Bengaluru with 92 genetic stocks of *Ocimum* species. During evaluation of this genetic stock of *Ocimum basilicum*, *Ocimum africanum* and *Ocimum americanum*, one plant with distinct morphology in comparison to the other three species was observed and harvested separately. Some portion of the plants was also distilled for ascertaining its essential oil quality. Phylogeny study of this variant by ISSR and RAPD molecular markers confirmed its true hybrid nature (Fig. 1 and Fig.2). GC-MS essential oil profiles (Table 1) also indicated it to be a linalool rich strain.

Initial evaluation of segregating materials

During Kharif-2015 season, 1000 F₂ plants were planted for evaluation and allowed for open

pollination. 450 half sib progenies were then selected based on higher herb yield and oil content. These plants constitute the population for next generation. In Summer-2016, Cycle-2 (C-2) populations were evaluated for herb yield, oil yield and linalool content. Phenotypically superior 250 plants were selected based on the oil yield. The plants with oil content more than 0.4 % and linalool content more than 75 % were then selected to constitute the base population for next C-3 cycle. C-3 population was evaluated in Kharif-2016 for herb yield, oil yield and linalool content. Based on these results 110 plants with oil content > 0.5% and linalool content 74.00- 79.00 % were selected to constitute C-4 base population for evaluation.

Initial varietal evaluation trial (IVT)

Morphologically similar looking plants were harvested and bulked. These seeds were used for the Initial Varietal Trial (IVT) with popular check variety CIM-Surbhi in bed size of 3.6 m × 3.6 m with 15 replication (45 cm × 45cm) in Kharif 2017 season. The data pertaining to IVT is presented in Table 2. Line CIM-Sukhda yielded higher herb yield and linalool content than the check variety CIM-Surbhi (Fig.3).

Bench scale trial

In summer- 2018 a Bench Scale Trail (BST) with bed size of 200 m² (45×45cm) was conducted. The data is presented in Table 3 and Table 4. When CIM-Sukhda was distilled in 30 kg capacity unit, its oil recovery was found to be around 0.3 %.

Table 1: GC-MS profile of CIM-Sukhda

Compound (%)	<i>O. basilicum</i> (Ob)	<i>O. africanum</i> (Of)	CIM-sukhda
Methyl chavicol	47.21	9.98	--
Linalool	27.52	1.70	73.95
Citral	--	26.61	3.69
β-citral	--	21.08	3.00
Eucalyptol	1.38	--	2.52
Eugenol	3.42	--	--
Methyl Eugenol	2.82	--	--
cis-α-Bisabolene	--	2.36	3.57
γ-cadinene	3.14	--	--
cis-9-Hexadecenoic acid, trimethylsilyl ester	--	4.33	--
trans-(2-Ethylcyclopentyl)methanol	--	2.94	--
β-elemene	1.19	--	1.74
β-cubebene	--	--	1.45

Table 2: Mean performance of CIM-Sukhda and check variety CIM-Surbhi for oil yield, herb yield and linalool content in Initial Varietal Trial (IVT; plot size 3.6 x 3.6 m²)

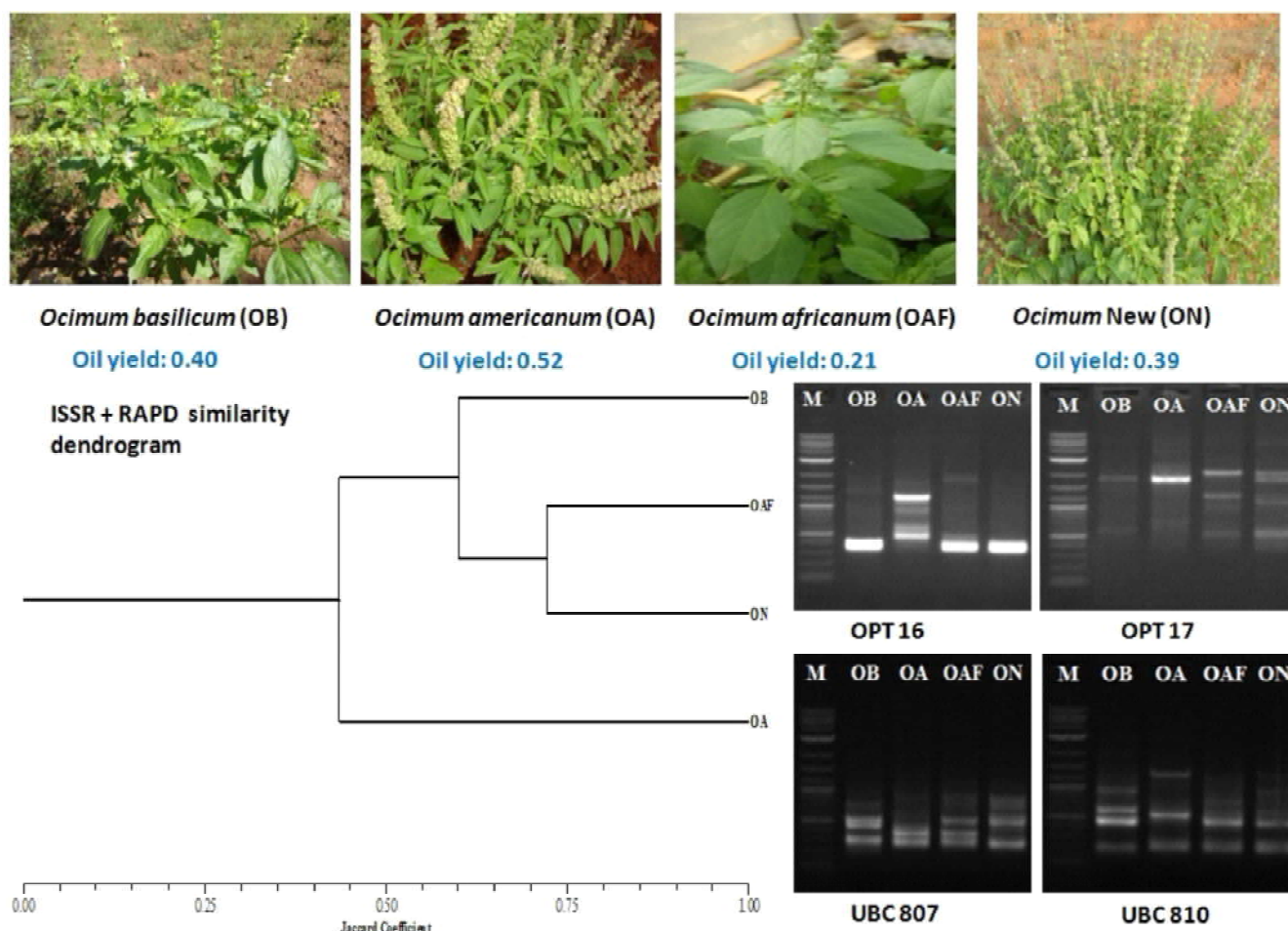
Varieties	Herb yield (g/plant)	Oil yield (%)	Linalool (%)
CIM-Sukhda	283.63 ^a	0.535 ^a	76.67
CIM-Surbhi	136.00	0.274	67.43
CV (%)	18.78	5.95	2.044
SE (±)	6.608	0.0039	0.1944
t-Cal	6.62	17.91	18.44
t-tab (5%)	1.997		
t-tab (1%)	2.64		

Number of sample used for t test analysis N=75; ^asignificantly different at p=0.05

Table 3: Mean performance of CIM-Sukhda in Advance Trial for oil yield, herb yield and linalool content (Gross plot-200 m² Net plot- 158 m²)

Varieties	Herb yield (g/plant)	Herb yield Kg/plot	Herb Yield (q/ha)	Oil content (%)	Oil Yield (Kg/ha)	Linalool (%)	
CIM-Sukhda	417.866 ^a	525.50	262.50	0.52 ^a	104.8	78.22	75.00-80.00
CIM-Surbhi	240	405.00	202.50	0.38	80.60	70.12	69.00-70.22
CV (%)	6.182			3.892		1.662	
SE (±)	2.071			0.0052		0.3368	
t-cal	22.92			21.23		18.98	
t-tab (5%)	1.997						
t-tab (1%)	2.64						

Number of sample used for t test analysis N=75; ^asignificantly different at p=0.05;

**Figure 1: Phylogeny of developed hybrid using RAPD and ISSR molecular markers**

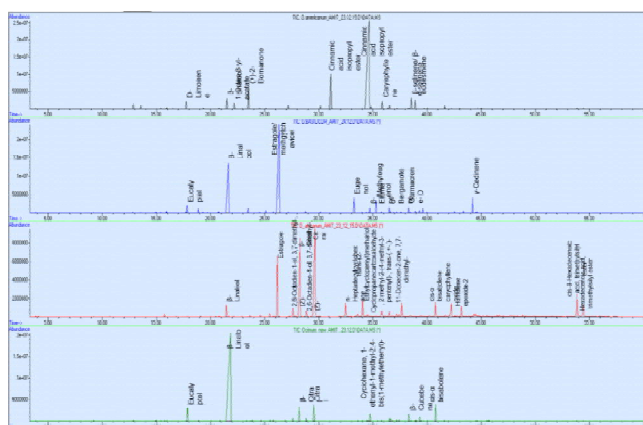


Figure 2: Chromatogram of the essential oil of variety CIM-Sukhda and Parents

Based on the results of all the trials elite strain CIM-Sukhda is now proposed to be released for commercial cultivation.

Statement of distinction

CIM-Sukhda is a highly vigorous variety in terms of its growth (Fig.4). It is characterized by medium dark green leaves and light green stem. Flower colour is white and inflorescence is long

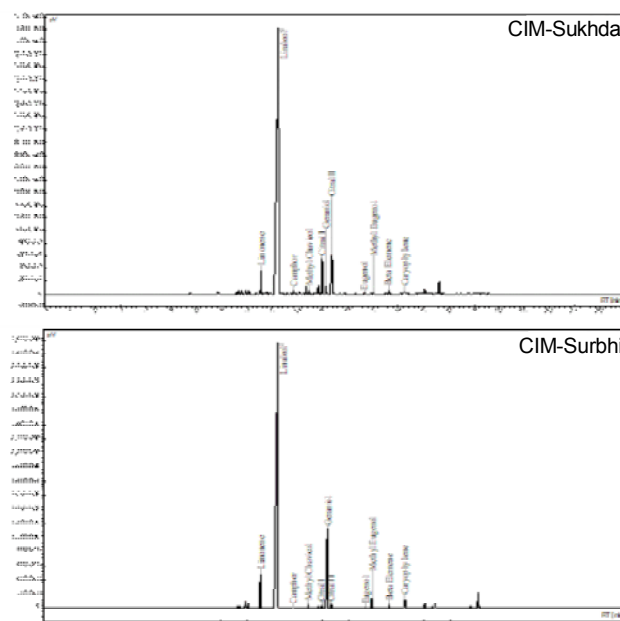


Figure 3: GC profile of CIM-Sukhda and CIM-Surbhi

and pinkish. These are the distinguishing morphological (DUS) features of this variety. The recommended agronomic practices of cultivation of CIM-Sukhda are provided in Table 5.

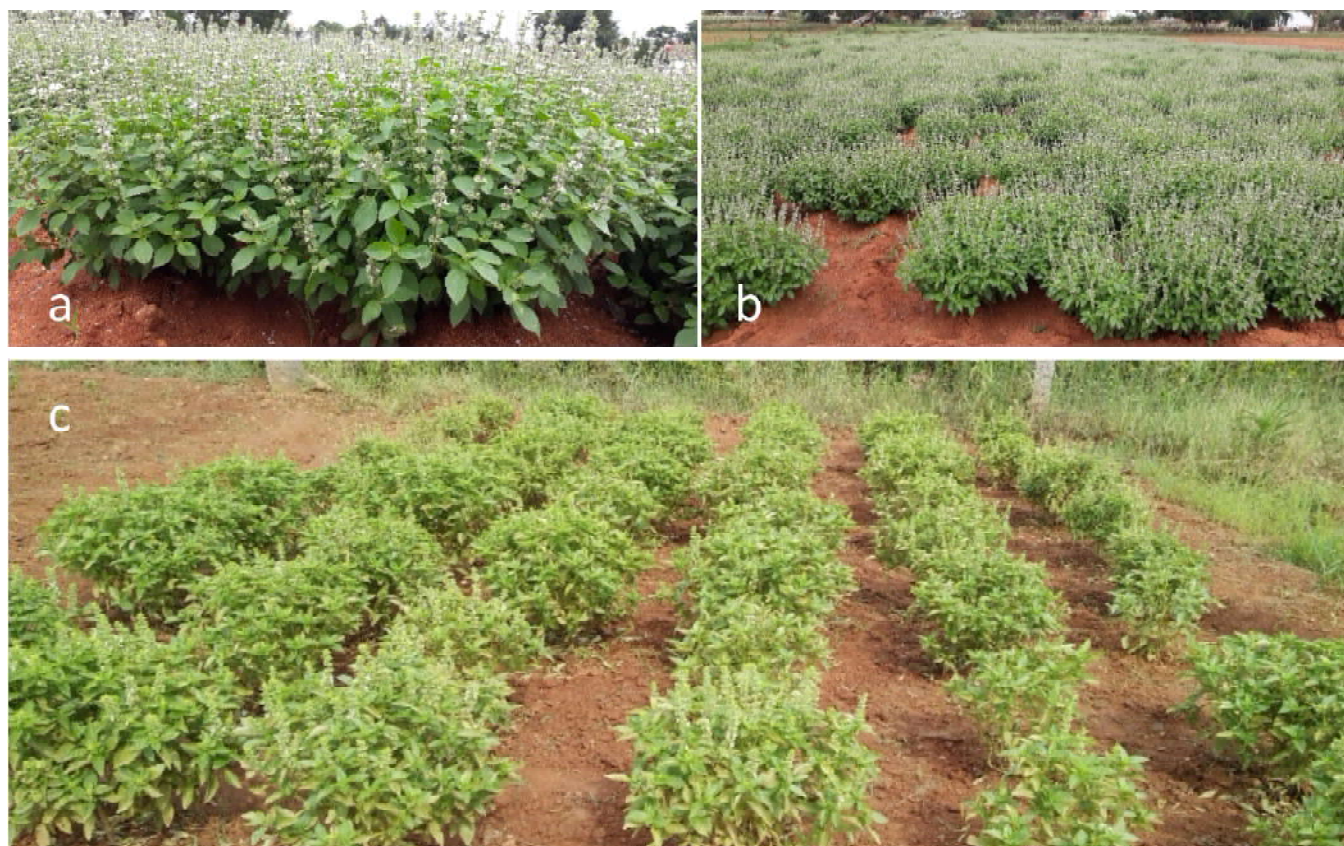


Figure 4: General view of CIM-Sukhda (a and b) and CIM-Surbhi (c)

Table 4: Mean performance of CIM-Sukhda in Bench Scale Trial (BST) for oil yield, herb yield and linalool content (Gross plot-200 m² Net plot- 158 m²)

Varieties	Herb yield (g/plant)	Herb yield Kg/plot	Herb Yield (q/ha)	Oil Content (%)	Oil Yield (Kg/ha)	Linalool (%)
CIM-Sukhda	506.43 ^a	448.50	224.25	0.536 ^a	89.68	76.25
CIM-Surbhi	280.23	298.50	148.50	0.463	59.20	71.22
CV (%)	20.13			7.96		
SE (±)	8.62			0.0032		
t-cal	15.226			13.77		
t-tab (5%)	1.997					
t-tab (1%)	2.64					

Number of sample used for t test analysis N=75; ^asignificantly different at p=0.05

Table 5: Recommended cultivation practice for the variety CIM-Sukhda

Agronomic features	Practices
Suitable sowing time	Kharif and Summer Season
Adaptability	Over the different years of evaluation trials new strain L1 showed less variation in the yield and oil content compared to check variety. All the trials were conducted in Bengaluru condition. The new strain is therefore recommended for South Indian climatic conditions.
Spacing (cm)	45×45
Fertilizer NPK (kg/acre/y)	140:30:30 (Urea applied in two split dose)
Harvesting time	Flowering completion time/ seed setting stage i.e. 90-95 days after transplanting
Seed rate per acre	70-100 gm for nursery preparation

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NOTE

CIM-Sukhda was released for commercial cultivation on the occasion of CSIR-CIMAP Kisan Mela on 31 January, 2019.

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