

VARIETY REGISTRATION

Registration of a new variety of basil- CIM Shishir: A multicut, lodging resistant, cold tolerant, high essential oil yielding, linalool rich inter specific hybrid of *Ocimum*

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ABSTRACT

The essential oil of Ocimum (Family: Lamiaceae) is used in flavour, fragrance, food, oral health care products and several other traditional medicines. This paper describes the development of an inter-specific hybrid variety of Ocimum that contains a desired ratio of linalool and camphor in its essential oil. The variety also have high herb (600 ql/ha) and essential oil yield (250 kg/ha) with 70-75 % (-) linalool. Intensive breeding techniques like inter-specific hybridization and selection process were applied to develop such a variety of Ocimum. This linalool-rich genotype will be a cheap source of linalool for the perfumery and flavour industry. Therefore, development of this improved variety with higher essential oil, high linalool content, with multiple cutting option without much effect of environment/temperature would be helpful to overcome low essential oil. The need of winter tolerant high-yielding genotype is of high importance for future as this will add to the income of farmers as well as various industries that are dependent on bioactive constituent linalool.

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INTRODUCTION

Ocimum (Family: Lamiaceae) is a genus of about 35 species of annual and perennial aromatic herbs and shrubs. Most species are native to the tropical and warm regions of the old world, including India. The essential oil of Indian basil obtained via hydro- or steam distillation of leaves or whole aerial biomass of the plant are used to flavour foods,

dental and oral products, fragrances, and traditional medicines. The global perfume market including essential oil of *Ocimum* has been forecasted to reach to a value of approximately US\$45.6 billion by 2020. The *Ocimum* essential oil has also been shown to contain biologically active constituents that have insecticidal, nematocidal, fungistatic or antimicrobial properties.

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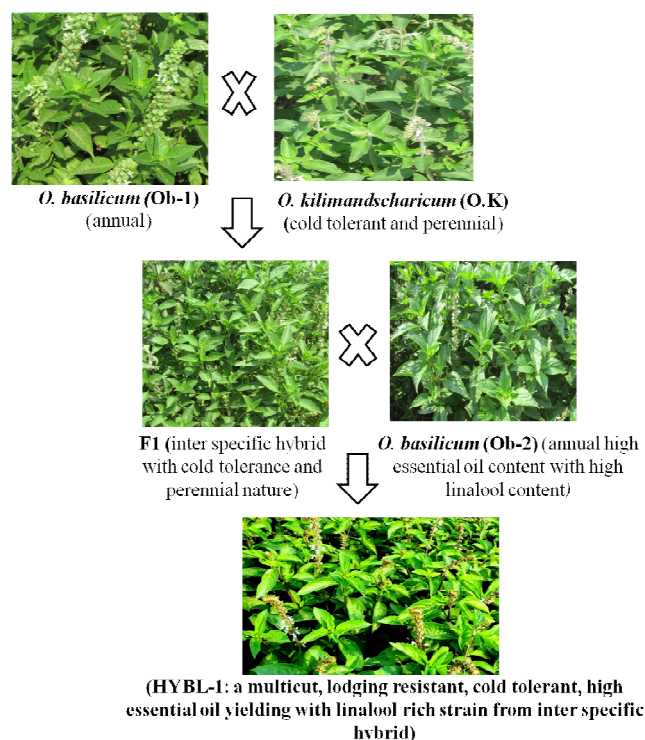
At CSIR-CIMAP, Lucknow U.P. (India) availability of 80 genetic stocks of nine *Ocimum* species namely, *Ocimum sanctum* (Krishna and Shyam tulsi), *O. filamentosum* Forssk, *O. kilimandscharicum*, *O. canum*, *O. gratissimum*, *O. africanum*, *O. viride*, *O. tenuiflorum* and *O. basilicum* (Sweet and French basil) offers many possibilities to develop high yielding cultivars/ varieties for high herb and oil yielding chemotypes with high value chemical constituents like linalool. Linalool rich chemotypes will provide additional income to farmers. Keeping in mind the importance of linalool of basil, there is a need to develop a better plant type having high essential oil yielding traits with better linalool content. Previously, CSIR CIMAP developed a variety CIM Surabhi for linalool rich essential oil but its essential oil yield is not able to fulfil high demand of linalool. This old variety is also cultivated as annual crops means multi cuttings are not possible. Another reason is low temperature during winters also effect the growth of this variety. Therefore, production of a variety with high essential oil having high linalool content with multi cutting potential without adverse effects of environment/ temperature would be helpful to overcome such limitation of vareity CIM-Surabhi. The need of winter tolerant high yielding genotype is utmost important for future as this will add to the income of farmers as well as various industries that are dependent upon the bioactive constituents of *Ocimum* species. High essential oil having high linalool content would also be helpful to formulate value added industrial products. Hence, a planned breeding and selection process was undertaken at CSIR- CIMAP, Lucknow to develop such a strain/variety.

MATERIALS AND METHODS

Origin/breeding of the variety

Using the available *Ocimum* genetic stocks, an inter specific hybridization programe was launched between *O. basilicum* Ob-1(annual) × *O. kilimandscharicum* OK (cold tolerant and perennial) × *O. basilicum* Ob-2 (annual high essential oil content with high linalool content). This was followed by selection for high oil and high linalool content, cold tolerant and perennial/multi cutting inter specific hybrid.

Crossing Plan:



RESULTS AND DISCUSSION

Field Evaluation trials

This HYBL-1: A multicut, linalool rich, high essential oil yielding strain from inter specific hybrid were placed in an Initial Evaluation Trial with 16 other lines with three its parents (Ob-1, Ob-2 and O.K.) and check variety CIM Surabhi (IET Ent-20, RBD, Rep. 3, plot size, =1 m²). In the IET 5 best promising lines namely, HYBL-1, Ob-1, H-2, Ob-2, H-1 + check variety CIM Surabhi (selected on the basis of herb and essential oil yield with high linalool type) were placed in a Bench Scale Trial (Bench Scale trial {Entries = 6 (5 + one check), RBD, Reps-3, Plot size = 3 m²). The Three lines namely, HYBL-1, H-1, and Variety CIM Surabhi were then placed in a Pilot Scale Trial (PST, plot size 28.8 m² two years) for two consecutive years. The top highest yielding genotype, HYBL-1, always maintained its superiority and all desired characters as mentioned above over the check and others for high oil and high linalool content (Table 1-3; Figures 1-4). The elite strain (HYBL-1) is now proposed to be released as an improved variety for commercial cultivation.

Table 1. Initial evaluation trial of *Ocimum* (Entries 20 × Replications 3; Plot size = 1m²; 2014-2015)

S.No.	Entries	Plant Height (cm)**	Branches/plant**	Fresh herb/plant (kg/plot)**	Oil content (%) **	Oil yield (gm/plot)**	(-) Linalool content (%)	Methyl Chevicol content (%)	Camphor Content (%)	Eugenol content (%)
1.	H1-1	108.33	5.67	0.817	0.83	6.79	4.36	5.18	14.06	20.35
2.	HYBL-1	128.33	13.00	1.50	0.95	14.67	79.38	5.45	6.58	0.40
3.	Oc-3	106.67	8.00	1.13	0.48	5.27	2.413	1.62	2.35	55.21
4.	Oc-4	96.00	7.67	1.02	0.59	5.88	40.53	48.71	0.37	0.05
5.	H2	98.33	6.66	1.02	0.73	7.58	58.42	7.35	14.34	1.54
6.	Oc-5	120.00	11.33	1.05	0.49	4.72	9.51	1.51	15.29	19.47
7.	Ob-1	103.33	7.00	0.917	0.92	8.44	32.78	5.27	1.33	23.60
8.	Ob-6	88.33	8.00	0.783	0.43	3.37	5.37	69.25	0.67	1.40
9.	O.K.	116.67	11.67	0.783	0.45	3.51	4.36	71.60	55.62	2.83
10.	Oc-7	100.00	6.67	0.750	0.52	3.87	5.09	2.27	0.57	0.04
11.	Oc-8	91.67	6	0.767	0.87	6.65	1.66	2.84	55.09	3.36
12.	H1 (4n)	111.67	9.33	0.767	0.47	3.58	60.40	8.99	10.68	1.35
13.	Ob-2	87.67	6.67	0.833	0.82	6.83	61.97	2.34	22.59	0.69
14.	Oc-7	121.83	8.33	1.17	0.48	5.50	41.00	45.90	0.43	0.05
15.	Oc-8	115.33	8.00	0.767	0.77	5.89	30.57	0.52	0.17	20.99
16.	Oc-9	117.00	8.33	0.733	0.49	3.56	32.07	0.60	0.014	23.60
17.	Oc-10	108.67	6.33	0.800	0.48	3.89	41.30	0.54	0.015	15.32
18.	Oc-11	91.67	7.67	0.767	0.63	4.87	32.93	0.53	0.022	14.33
19.	H1	118.67	8.33	0.767	0.47	2.98	61.44	9.70	10.60	1.63
20.	Surabhi	93.33	7.33	0.283	0.67	2.84	72.74	0.50	0.00	8.47
CD (5%)	-	11.58	2.07	0.297	0.12	2.77	1.33	0.82	0.35	0.72
CD (1%)	-	15.48	2.77	0.397	0.16	3.71	1.78	1.097	0.46	0.96

**=P<0.01

Table 2. Advance evaluation trial of *Ocimum* (Entries 6 × Replications 3); Plot size = 3.0m²; 2015-2016)

S.No.	Entries	Plant Height (cm)**	Branches/plant**	Fresh herb/plant (kg)/plant*	Oil content (%)**	Oil yield (g/plot)**	(-) Linalool content (%)	Methyl Chevicol content (%)	Camphor content (%)	Eugenol content (%)
1.	HYBL-1	129.00	15.00	5.40	0.72	38.72	74.511	5.311	9.520	0.331
2.	Ob-1	107.67	7.67	2.72	0.58	15.86	15.678	9.406	2.019	50.244
3.	H2	108.33	7.00	4.07	0.55	22.33	58.273	7.397	14.527	1.560
4.	Ob-2	90.00	7.33	3.47	0.60	20.82	60.708	2.467	21.313	0.683
5.	H1	110.67	7.67	4.85	0.65	31.52	52.710	8.438	10.805	1.522
6.	Surabhi	91.00	8.00	3.07	0.62	18.90	71.567	1.417	0.00	8.057
CD (5%)	-	6.56	1.40	0.24	0.06	2.81	1.331	0.795	0.745	0.329
CD (1%)	-	9.33	1.99	0.37	0.09	3.99	1.893	1.130	1.058	0.467

**=P<0.01

Table 3. Pilot scale evaluation trial of *Ocimum* (Entries 3 x Replications 3; Plot size = 28.8m²)

S.No.	Entries	Plant Height (cm)**	Branches/plant**	Fresh herb/plant (ql)/ha**	Oil content (%) **+	Oil yield (kg/plot)**	(-) Linalool content (%)	Methyl Chevicol content (%)	Camphor content (%)	Eugenol content (%)
1.	HYBL-1	128.33	14.17	789.05	0.39	312.71	73.59	6.03	9.03	0.35
2.	H1	111.67	7.67	446.69	0.17	74.71	40.43	8.57	10.39	1.42
3.	Surabhi	91.20	5.00	324.70	0.35	114.75	69.65	1.30	0.00	7.96

**=P<0.01; + Bulk distillation Statement of distinction/ Breeder's claim

Table 4. Description of the strain.

Attributes	CIM Shishir	Check variety CIM Surabhi
Growth habit	Tall Bushy	Semi spreading
Days to harvest after transplanting	Three cuttings/year with the interval of 90 days	80-85
Days to flower (50 %)	120	70-80
Plant height (cm)	125-130	80-85
Colour of leaf	medium green	Light green
Leaf surface	Undulated surface	Plain
Length of leaf (cm)	9.30-9.50	6.20-6.30
Width of leaf (cm)	4.50-5.20	3.40-3.50
Fresh herb yield (ql/ha)	600.00	221.30
Oil content in fresh herb (%)	0.80	0.75
In Clevenger		
Oil content in fresh herb (%)*	0.39	0.35
Bulk distillation		
Potential oil yield (kg/ha)**	250	115
Linalool content (%)	70-75	65-70
Camphor content (%)	9-10	0.00
Methyl chavicol content (%)	24.21	0.44

*- Bulk distillation

+ Based on oil content data obtained through Clevenger apparatus;

- Estimate based on 28.80 m² plot size.Table 5. Recommended cultivation schedules for the variety CIM Shishir**

S.No.	Practices	Suitable time
1.	Planting materials	Rooted plants by vegetative cuttings
2.	Transplanting in field	Last week of March
3.	Harvesting for oil for the first cutting	First week of June (90 days interval three cuttings/years after transplanting)

**Figure 1: Strain HYBL-1: high oil and linalool rich inter specific hybrid**



Figure 3: Strain HYBL-1 third cutting in operation in pilot scale trial



Figure 4: Check variety CIM Surabhi of *O. basilicum*

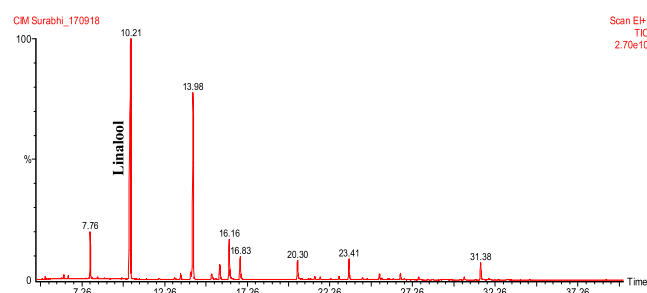


Figure 5: GC-MS Chromatogram of essential oil of the check variety CIM Surabhi of *O. basilicum*

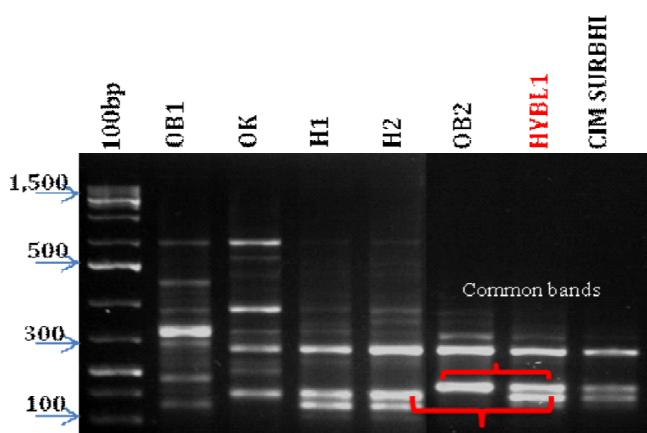


Figure 6: Representative SCoT profile shows variations at genetic level. The figure shows DNA profile with SCoT 11 primer with different *Ocimum* genotypes with check variety CIM Surabhi.

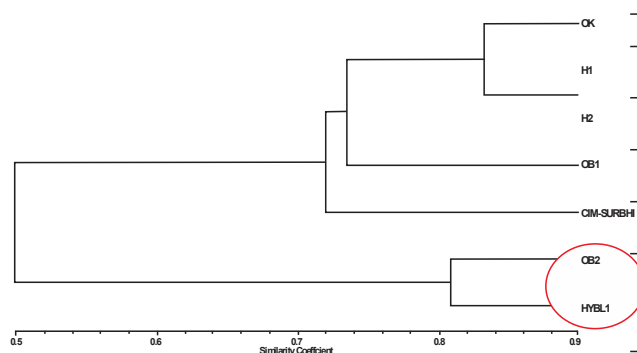


Figure 7: Dendrogram showing the genetic relationships among different genotypes of *Ocimum*, HYBL1 and checked variety using SCoT analysis.

Strain HYBL-1 is a perennial, tall (128cm), multi cut, cold tolerant, lodging resistant with very broad, long, undulated surface, medium green leaves and yellowish green stem in color of inter specific hybrid (Table 4; Figure 5-7). The strain has the following DUS (distinctiveness, uniformity and stability) characteristics.

1. The strain is morphologically distinct from other *Ocimum basilicum* varieties and clearly identifiable by its very tall, very broad, long, undulated surface, medium dark green with robust growth (Figure 1).
2. It has yellowish green stem
3. The strain has a unique feature and advantage of better survival in winter season in comparison to other *O. basilicum* varieties.
4. Essential oil extracted from this hybrid strain contain higher amount of linalool content (70-75 %) with low amount of camphor (9.03 %).
5. Strain is also suitable for cultivating in whole year with multicutting upto 3 harvest including rain-fed conditions.

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