

Registration of variety: CIM-Snigddha: A methyl cinnamate rich and high essential oil yielding variety of French basil (*Ocimum basilicum*)

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Abstract

*Basil belongs to the family "Lamiaceae". The essential oil of basil is extensively used in flavour, fragrance, food, oral health and traditional medicines. Methyl cinnamate – one of the important constituent of essential oil of *Ocimum basilicum* is widely used in aroma, pharmaceutical and cosmetic industries. A new strain of *O. basilicum* (OBH-3 now christened as CIM-Snigddha) has been developed by CSIR-CIMAP through intensive breeding efforts for improved herb and essential oil yield coupled with high methyl cinnamate content (78.7%). The variety has consistently recorded a higher biomass and oil yield with high methyl cinnamate content in the field evaluation trials. The potential average herb and oil yield of this new variety is 221 q/ha and 190 kg/ha, respectively. The variety matures within a short duration of 80-90 days and hence fits very well into crop rotation/intercropping cycle between wheat and paddy along with other vegetable crops. The distinct leaf morphology and high tolerance to cold conditions are the two important economic features of this variety that also satisfy DUS criteria.*

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Introduction

Basil (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 is used in food flavouring, fragrances, social/religious rituals and traditional house hold medicines. The essential oil has also

been shown to contain biologically active constituents that have insecticidal, nematocidal, fungistatic or antimicrobial properties.

At CSIR-CIMAP, Lucknow U.P. (India) availability of 189 genetic stocks of nine *Ocimum* species namely, *Ocimum sanctum* (Krishna and Shyam tulsi), *O. kilimandscharicum*, *O. canum*, *O. gratissimum*, *O. africanum*, *O. viridis*, *O. tenuiflorum* and *O. basilicum* (Sweet and French basil) has opened up many possibilities to develop high yielding cultivars/varieties up chemotypes for high herb and oil yield and for oil with high value chemical constituents like linalool. Essential oils with new aromas like that of Methyl, cinnamate,

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Ocimine, eugenol etc in desired combinations are being used today for various innovative cosmetic and perfumery products.

For example India imported methyl cinnamate worth US\$ 679,195. China is the largest supplier of methyl cinnamate accounting for imports worth US\$ 518,267 followed by Germany and United States which exported methyl cinnamate worth US\$ 137,535 and US\$ 13,636 respectively. Average price of methyl cinnamate per unit is US\$ 8.27 and average value per shipment is 5,348\$. India also exported methyl cinnamate worth US\$ 36,509. Pakistan is the largest buyer of methyl cinnamate accounting for exports worth US\$ 24,611 followed by Turkey and Spain which imported methyl cinnamate worth US\$ 5,303 and US\$ 4,730 respectively. Average price of methyl cinnamate per unit is US\$ 10.17 and average value per shipment is 1,660 in November, 2016.

Keeping in mind the importance of basil, a need to develop a better plant type having high essential oil yielding traits with better methyl cinnamate content was felt. Hence, a planned breeding and selection process was undertaken at CSIR- CIMAP, Lucknow to develop such a variety of *Ocimum basilicum*.

ORIGIN OF THE VARIETY

The *Ocimum* germplasm collection and evaluation programme was started in 2002 at CSIR CIMAP, Lucknow which resulted in a collection of 189 genetic stocks belonging to 9 ocimum species. Subsequent to this an exhaustive breeding work was taken up and number of elite lines were developed which provided the basic materials for further characterization under a CSIR network project (BSC 0203). Using half sib selection in an open population of *O. basilicum* followed by selection for high oil and high methyl cinnamate content, 16 genotypes were selected in. These 16 genotypes (OBH-1 to OBH -16) were placed in an Initial Evaluation Trial with one local check (IET Ent-17, RBD, Rep. 3, plot size, =1.5 m²). Based on the result of IET, 9 best performing lines namely, OBH-1, OBH-2, OBH-3, OBH-6, OBH-7, OBH-10, OBH-12, OBH-13 and OBH-14 were advanced further on the basis of higher herb and oil yields with higher methyl cinnamate content) These 9 lines were placed in a Bench Scale Trial (Bench Scale Trial {Entries = 10 (9 + one check), RBD, Reps-3, Plot size = 20 m²) The best four performing genotypes namely, OBH-2, OBH-3 and OBH-9 along with one check were then placed in a Pilot Scale Trial (PST,

Table 1: Mean performance of elite selections in Initial Evaluation Trial for the three economic traits in *O. basilicum*

| Entries | IET**, E=17, RBD, Reps-3, plot size=1.5 m ² | | |
|---------|--|-----------------|--------------------|
| | Fresh herb yield/plot (g) | Oil Content (%) | Oil yield/plot (g) |
| OBH-1 | 384.79 | 0.55 | 2.11 |
| OBH-2 | 645.69 | 0.52 | 3.34 |
| OBH-3 | 759.39 | 0.90 | 6.83 |
| OBH-4 | 692.39 | 0.55 | 3.81 |
| OBH-5 | 391.25 | 0.43 | 1.69 |
| OBH-6 | 544.33 | 0.52 | 2.48 |
| OBH-7 | 344.03 | 0.61 | 2.36 |
| OBH-8 | 356.98 | 0.62 | 2.20 |
| OBH-9 | 347.38 | 0.53 | 1.85 |
| OBH-10 | 523.99 | 0.52 | 2.70 |
| OBH-11 | 523.77 | 0.53 | 1.73 |
| OBH-12 | 475.12 | 0.53 | 2.53 |
| OBH-13 | 590.90 | 0.60 | 3.55 |
| OBH-14 | 705.07 | 0.55 | 3.88 |
| OBH-15 | 301.83 | 0.53 | 1.66 |
| OBH-16 | 383.70 | 0.58 | 2.29 |
| Check | 256.18 | 0.57 | 1.45 |
| CD 5% | 53.60 | 0.07 | 0.60 |
| CD 1% | 72.50 | 0.10 | 0.80 |

**.-P<0.01

Table 2: Mean performance of selected entries in IET with respect to essential oil composition

| Entries | IET**, E=17, RBD, Reps-3, plot size=1.5 m ² | | |
|---------|--|--------------|---------------------|
| | Methyl cinnamate (e+z) (%) | Linalool (%) | Methyl chevicol (%) |
| OBH-1 | 73.72 | 0.37 | 1.86 |
| OBH-2 | 64.67 | 8.63 | 2.97 |
| OBH-3 | 77.50 | 2.00 | 8.85 |
| OBH-4 | 42.73 | 0.36 | 3.69 |
| OBH-5 | 48.57 | 6.91 | 1.80 |
| OBH-6 | 60.03 | 0.02 | 3.02 |
| OBH-7 | 29.24 | 4.70 | 6.26 |
| OBH-8 | 44.18 | 0.85 | 18.82 |
| OBH-9 | 57.18 | 0.64 | 8.82 |
| OBH-10 | 45.94 | 0.47 | 0.52 |
| OBH-11 | 51.20 | 1.23 | 22.79 |
| OBH-12 | 47.72 | 0.85 | 1.42 |
| OBH-13 | 75.07 | 8.41 | 0.51 |
| OBH-14 | 60.60 | 0.13 | 0.64 |
| OBH-15 | 59.53 | 0.13 | 1.07 |
| OBH-16 | 54.83 | 0.22 | 2.07 |
| Check | 56.43 | 11.17 | 13.09 |
| CD 5% | 2.3 | 0.40 | 4.0 |
| CD 1% | 3.1 | 0.50 | 1.54 |

**-P<0.01

Table 3: Mean performance of elite selections in Bench Scale Evaluation Trial for the three economic traits in *O. basilicum*

| Entries | BST**, E=10, RBD, Reps-3, plot size=20.00m ² | | |
|---------|---|-----------------|---------------------|
| | Fresh herb yield/plot (kg) | Oil Content (%) | Oil yield/plot (Kg) |
| OBH-1 | 40.35 | 0.53 | 0.217 |
| OBH-2 | 42.30 | 0.57 | 0.242 |
| OBH-3 | 52.25 | 0.90 | 0.470 |
| OBH-6 | 36.35 | 0.52 | 0.187 |
| OBH-7 | 23.17 | 0.60 | 0.139 |
| OBH-10 | 33.67 | 0.52 | 0.173 |
| OBH-12 | 29.57 | 0.58 | 0.171 |
| OBH-13 | 36.42 | 0.59 | 0.215 |
| OBH-14 | 45.70 | 0.55 | 0.254 |
| Check | 23.66 | 0.55 | 0.130 |
| CD 5% | 7.3 | 0.07 | 0.06 |
| CD 1% | 10.0 | 0.10 | 0.08 |

**-P<0.01

Table 4: Mean performance of selected entries with respect to essential oil composition

| Entries | BST**, E=10, RBD, Reps-3, plot size=20.00m ² | | |
|---------|---|--------------|---------------------|
| | Methyl cinnamate (e+z) content (%) | Linalool (%) | Methyl chevicol (%) |
| OBH-1 | 75.73 | 8.433 | 0.450 |
| OBH-2 | 64.57 | 9.233 | 4.167 |
| OBH-3 | 77.50 | 1.85 | 9.433 |
| OBH-4 | 62.67 | 0.026 | 4.333 |
| OBH-7 | 32.00 | 4.85 | 7.333 |
| OBH-10 | 45.33 | 0.463 | 0.653 |
| OBH-12 | 47.20 | 0.850 | 1.300 |
| OBH-13 | 40.42 | 0.383 | 2.067 |
| OBH-14 | 60.53 | 0.167 | 0.833 |
| Check | 56.50 | 11.20 | 13.10 |
| CD 5% | 4.40 | 0.40 | 1.50 |
| CD 1% | 6.02 | 0.50 | 2.10 |

**-P<0.01

Table 5: Mean performance of elite selections in Pilot Scale Trial for the three economic traits in *O. basilicum*

| Entries | PST, Entries=5, plot size = 50.00m ² | | | | | | | |
|---------|---|--------------------------|-----------------|---------------------|-------------------|------------------------------------|--------------|---------------------|
| | Fresh herb yield/plot (kg) | Fresh herb yield/ha (ql) | Oil Content (%) | Oil yield/plot (Kg) | Oil yield/ha (Kg) | Methyl cinnamate content (e+z) (%) | Linalool (%) | Methyl chevicol (%) |
| OBH-2 | 105.30 | 210.60 | 0.60 | 0.632 | 126.40 | 64.11 | 8.33 | 0.39 |
| OBH-3 | 110.70 | 221.40 | 0.90 | 0.996 | 199.20 | 77.46 | 1.95 | 9.10 |
| OBH-9 | 95.70 | 191.40 | 0.57 | 0.545 | 109.00 | 62.00 | 0.02 | 4.00 |
| Check | 90.80 | 181.60 | 0.55 | 0.499 | 99.88 | 52.25 | 0.03 | 4.50 |

plot size 50 m²) along with check. The highest yielding genotype, OBH-3 that always maintained its superiority over the check and others for high oil and high methyl cinnamate content (Table 1-7; Figures 1-3) is now christened as a new variety CIM-Snigddha. The elite variety is now proposed to be registered as an improved cultivar for its commercial cultivation.

STATEMENT OF DISTINCTION/ BREEDER'S CLAIM

Strain OBH-3 is a tall (100.90cm) genotype with dark green leaves and light green colour stem. 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 broad, medium green leaves (Figure 1).



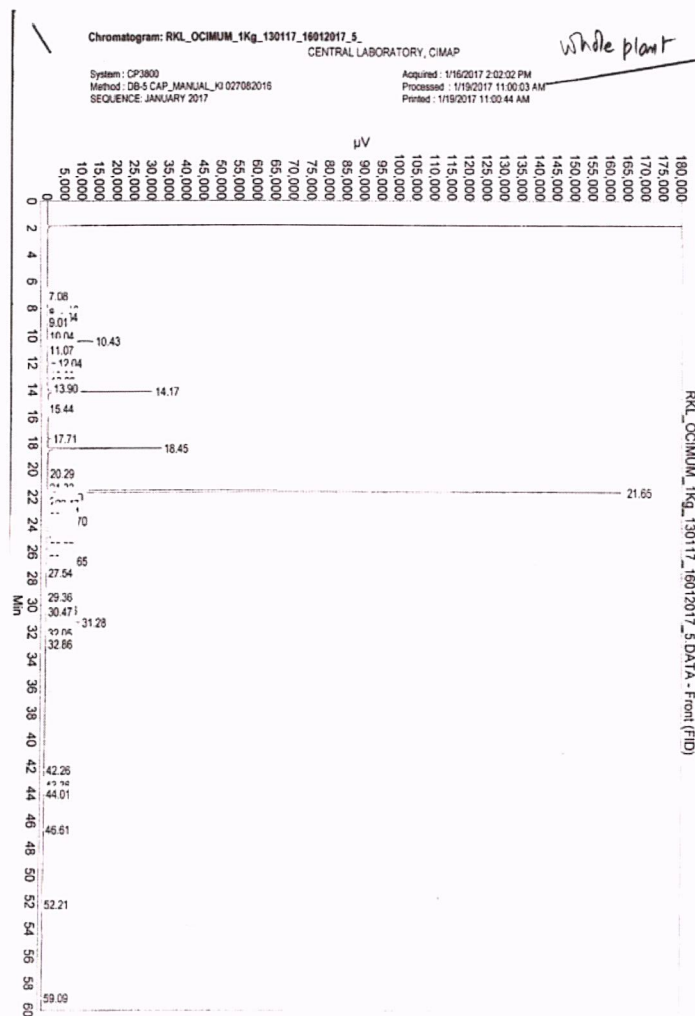
Fig. 1: Field view of the high oil and methyl cinnamate rich chemotype CIM-Snigddha of *O. basilicum*

Table 6: Description and average performance data of CIM-Snigddha

| Attributes | CIM-OBH-3 | Check |
|-------------------------------------|-----------|----------------|
| Growth habit | Bushy | Semi spreading |
| Days to harvest after transplanting | 70 | 100 |
| Days to flower (50 %) | 60 | 90 |
| Plant height (cm) | 100 | 120 |
| Colour of leaf | Darkgreen | Light green |
| Inflorescence colour | Purple | Light green |
| Flower colour | White | Off white |
| Length of leaf (cm) | 6.20-6.30 | 5.50-5.60 |
| Width of leaf (cm) | 3.40-3.50 | 1.50-1.55 |
| Fresh herb yield (ql/ha) | 221 | 145 |
| Oil content in fresh herb (%)* | 0.90 | 0.50 |
| Potential oil yield (kg/ha)** | 190 | 99 |
| Methyl cinnamate (e+z) content (%) | 78.7 | 52 |
| Linalool content (%) | 1.95 | 0.03 |
| Methyl chavicol content (%) | 9.10 | 4.50 |
| Oleanolic acid (%) | 0.198 | 0.065 |
| Ursolic acid (%) in leaves | 0.246 | 0.124 |
| Rotation at 19.5° C | + 1.200 | +1.00 |
| Refractive Index at 20°C | 1.5538 | 1.100 |

*- Based on oil content data obtained through from Clevenger apparatus;

**-. Estimate based on 50 m² plot size.



Chromatogram: RKL_OCIMUM_1Kg_130117_16012017_5
CENTRAL LABORATORY, CIMAP

System: CP3800
Method: DB-5 CAP_MANUAL_KJ_027082016
SEQUENCE: JANUARY 2017

Acquired: 1/15/2017 2:02:02 PM
Processed: 1/19/2017 11:00:03 AM
Printed: 1/19/2017 11:00:44 AM

Peak results:

| Index | Time | Area | Area % | Retention Index |
|-------|-------|---------|--------|-----------------|
| 1 | 6.43 | 2.7 | 0.012 | 870.15 |
| 2 | 6.53 | 5.7 | 0.024 | 874.13 |
| 3 | 8.06 | 11.7 | 0.049 | 875.18 |
| 4 | 8.88 | 18.7 | 0.077 | 889.17 |
| 5 | 7.08 | 2.8 | 0.012 | 899.44 |
| 6 | 8.06 | 10.6 | 0.043 | 1059.92 |
| 7 | 8.12 | 137.7 | 0.580 | 1059.11 |
| 8 | 8.23 | 3.2 | 0.013 | 1059.13 |
| 9 | 8.64 | 133.9 | 0.544 | 1059.93 |
| 10 | 8.81 | 3.3 | 0.013 | 1059.98 |
| 11 | 10.04 | 8.1 | 0.034 | 1059.72 |
| 12 | 10.43 | 127.3 | 0.519 | 1059.72 |
| 13 | 10.87 | 6.5 | 0.026 | 1059.07 |
| 14 | 11.07 | 6.9 | 0.028 | 1114.21 |
| 15 | 12.04 | 129.5 | 0.529 | 1114.21 |
| 16 | 12.81 | 7.8 | 0.032 | 1181.87 |
| 17 | 12.88 | 16.5 | 0.068 | 1181.89 |
| 18 | 13.28 | 22.7 | 0.093 | 1174.21 |
| 19 | 13.90 | 70.6 | 0.287 | 1187.52 |
| 20 | 14.17 | 150.6 | 0.603 | 1186.47 |
| 21 | 15.44 | 6.8 | 0.027 | 1225.51 |
| 22 | 15.44 | 0.0 | 0.004 | 1225.51 |
| 23 | 17.71 | 56.4 | 0.227 | 1280.14 |
| 24 | 18.45 | 106.4 | 0.411 | 1280.45 |
| 25 | 20.29 | 10.1 | 0.040 | 1345.30 |
| 26 | 21.65 | 13.0 | 0.051 | 1372.11 |
| 27 | 21.65 | 35034.5 | 79.662 | 1380.25 |
| 28 | 21.91 | 34.6 | 0.137 | 1380.87 |
| 29 | 21.92 | 155.7 | 0.616 | 1380.86 |
| 30 | 22.65 | 1.5 | 0.006 | 1386.21 |
| 31 | 22.67 | 114.8 | 0.453 | 1459.96 |
| 32 | 23.01 | 102.8 | 0.401 | 1474.62 |
| 33 | 23.15 | 9.0 | 0.035 | 1479.38 |
| 34 | 23.35 | 7.0 | 0.028 | 1479.57 |
| 35 | 23.72 | 229.2 | 0.904 | 1479.47 |
| 36 | 23.72 | 47.2 | 0.187 | 1479.72 |
| 37 | 23.87 | 4.6 | 0.018 | 1479.52 |
| 38 | 24.06 | 14.0 | 0.055 | 1481.80 |
| 39 | 24.32 | 12.2 | 0.048 | 1488.41 |
| 40 | 24.56 | 18.0 | 0.070 | 1494.54 |
| 41 | 24.63 | 43.4 | 0.169 | 1497.06 |
| 42 | 24.64 | 2.2 | 0.009 | 1497.90 |
| 43 | 25.12 | 4.7 | 0.018 | 1498.54 |
| 44 | 25.38 | 65.2 | 0.255 | 1479.88 |
| 45 | 25.47 | 22.0 | 0.086 | 1480.67 |
| 46 | 25.88 | 26.3 | 0.103 | 1491.41 |
| 47 | 26.10 | 15.0 | 0.057 | 1494.29 |
| 48 | 26.38 | 56.3 | 0.222 | 1501.08 |
| 49 | 26.47 | 7.7 | 0.030 | 1500.24 |
| 50 | 26.65 | 264.8 | 1.034 | 1500.96 |
| 51 | 26.66 | 9.0 | 0.035 | 1516.60 |
| 52 | 27.24 | 22.0 | 0.087 | 1519.30 |
| 53 | 27.21 | 6.1 | 0.024 | 1524.51 |
| 54 | 27.54 | 3.5 | 0.013 | 1537.80 |
| 55 | 28.05 | 2.7 | 0.011 | 1571.37 |
| 56 | 28.35 | 8.2 | 0.032 | 1575.21 |
| 57 | 28.38 | 2.8 | 0.011 | 1581.68 |
| 58 | 30.35 | 31.8 | 0.125 | 1708.54 |
| 59 | 30.47 | 1.6 | 0.006 | 1712.13 |
| 60 | 31.28 | 184.3 | 0.728 | 1530.78 |
| 61 | 31.58 | 12.8 | 0.050 | 1552.14 |
| 62 | 31.77 | 24.0 | 0.095 | 1558.45 |
| 63 | 32.05 | 3.8 | 0.015 | 1558.51 |
| 64 | 32.80 | 4.8 | 0.019 | 1575.21 |
| 65 | 32.80 | 9.7 | 0.038 | 1580.22 |
| 66 | 43.23 | 15.0 | 0.059 | 1906.57 |
| 67 | 43.36 | 2.7 | 0.011 | 2000.56 |

1,8-Cineole
(L) B-Cineole
linalyl
Camphor

Methyl cinnamate

(Z) -

(E) - 78.7%

Methyl cinnamate

Cp-v (oxidized)?

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Fig. 2: Chromatogram of the essential oil of new variety CIM-Snigddha

Table 7: Recommended cultivation schedules for the variety of CIM Snigddha

| S. No. | Practices/ | Suitable time |
|--------|------------------------|--|
| 1. | Nursery sowing | March first week |
| 2. | Transplanting in field | Last week of March (20-30 days after sowing) |
| 3. | Harvesting for oil | First week of June (80-85 days after transplanting) |
| 4. | Harvesting for seed | Last week of November to first week of December (120 days after transplanting) |

Year of development: 2012-2016; Proposed year of release: 2017;
Breeder seeds up to March = 100 gm

- It has light green stem
- Essential oil extracted from this strain contain higher amount (77%) of methyl cinnamate (e+z) content. (Fig 2)

- Strain is also suitable for rain-fed cultivation (March-December)
- The others distinguishing morphological features of this variety and recommended agronomic practices are given below in Table 6 & 7.

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