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**NEW VARIETY RELEASE**

# CIM-SUVAAS: A new source of chavibetol, multi-cut, high-essential oil yielding inter-specific hybrid of *O. basilicum* and *O. kilimandscharicum*

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*Specific environments*

## ABSTRACT

*Ocimum (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. Keeping in mind the importance of chavibetol from a new source of basil, there is a need to develop a better plant type having high essential oil-yielding traits with better chavibetol content. Previously, CSIR-CIMAP developed several varieties, containing chavibetol-rich essential oil, but the essential oil yield could not fulfill the high demand for chavibetol-rich essential oil. Therefore, producing a variety having high essential oil with high chavibetol content with multi-cutting without effects of environment/temperature is helpful. A high-yielding chavibetol-rich genotype is of utmost importance, as this will add to the income of farmers and various industries dependent upon the bioactive constituents of Ocimum species. Essential oil with a new aroma like chavibetol and high essential oil is suitable for various innovative cosmetic products/ perfumery industries. It is also used in chewing gum, mouthwash, mouth freshener and aromatherapy for traditional and therapeutic purposes. High essential oil content would also help to formulate value-added industrial products. Piper betel is the primary source of the unique aroma chemical chavibetol, but the cultivation of betel crops is complicated on a large scale. Piper betle crop require specific environments/conditions for good growth and development. Therefore, the newly developed CIM-Suvaas variety of basil essential oil will be a cheaper source of important aroma compound chavibetol. This is also the first report that shows the presence of chavibetol in basil oil.*



## INTRODUCTION

The genus *Ocimum* (Lamiaceae) contains about 35 species of annual and perennial aromatic herbs and shrubs, most of which 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 to flavor foods, dental and oral products, fragrances, and traditional medicines. The global perfume market,

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including Ocimum's essential oil, has been forecast to reach a value of approximately US$ 45.6 billion by 2018, driven primarily by growth expected in the underpenetrated emerging markets and innovative product launches. The essential oil also contains biologically active constituents with insecticidal, nematicidal, fungistatic or antimicrobial properties.

*Piper betle* is glossy, heart-shaped leaves have tremendous potential as source for herbal drugs. The active constituents of this crop include allyl pyrocatechol, chavibetol, eugenol and hydroxychavicol, which have many therapeutic uses; Heart-shaped *Piper betel* leaves are magnificent reservoirs of phenolic compounds with antiproliferative, antimutagenic, antibacterial, and antioxidant properties. Widely consumed in South Asian countries, its leaf contains a multitude of phenolics such as hydroxychavicol, eugenol, chavibetol, and piperols. Chavibetol is an organic compound of the phenylpropanoid class and is one of the primary constituents of the essential oil from the leaves of the betel plant (*Piper betle*) with a spicy odor.

*Ocimum* has been found to be a new source of chavibetol and eugenol, magnificent reservoirs of phenolic compounds, which are convincing data underscore the remarkable chemotherapeutic and chemopreventive potential of their leaves and essential oil against various cancer types. Herein, we provide an overall perspective on the cancer-fighting benefits of the phenolic phytochemicals like chavibetol in the *Ocimum* leaves and its chavibetol rich essential oil and a comprehensive overview of the mechanisms responsive to dose-driven ROS-mediated signaling cascades conscripted by bioactive phenolics to confer chemotherapeutic and chemopreventive advantages. Essential oil with a new aroma, like chavibetol with high essential oil is suitable for various innovative cosmetic products/ perfumery industries are required. It is also used in chewing gum, mouthwash, mouth freshener and aromatherapy for traditional and therapeutic purposes. Chavibetol-rich chemotypes of *Ocimum* will provide additional income to farmers. This genotype will be a cheap source of chavibetol for the medicinal, cosmetic, perfumery and flavor industry. Keeping in mind the importance of chavibetol from a new source of basil, there is a need to develop a better plant type having high essential oil-yielding traits with better chavibetol content.

Previously, CSIR-CIMAP, India has developed a number of varieties, but not able to fulfill the high demand for chavibetol. Therefore, producing a variety of essential oil with high chavibetol content environment/temperature tolerate would be helpful to overcome the essential oil need. The need for a high-yielding chavibetol-rich genotype is of utmost importance for future as this will add to the income of farmers and various industries dependent upon the bioactive constituents of *Ocimum* species (Dhawan *et al*., 2018). High essential oil with high chavibetol content would also help to formulate value-added industrial products. Piper betle is a chief source of the unique aroma chemical chavibetol, but the cultivation of betel crops is problematic on a large scale as this crop requires specific environment/condition for its good growth and development. Therefore this new basil variety rich in the essential oil containing chavibetol will be a first report from India. Hence, the breeding and selection process in planned way was undertaken at CSIR- CIMAP, Lucknow, to develop such a strain.

***Origin/breeding of the variety***

In the *Ocimum* genetic stocks and evaluation programme, using interspecific hybridization between *O. basilicum* (Ob-1) × *O. kilimandscharicum* (OK) developed during the year 2011-12 followed by selection for high oil and high chavibetol content multi-cutting inter-specific hybrid and was multiplied during 2012-2013 and compared with betel leaf oil in 2013-2014. Hybrid 107 is a non-producing seed; the amphidiploid line 106 h4-1 (producing fertile seeds) also developed using colchicine treatment (Dhawan *et al*., 2016).

## YEAR WISE CROSSING PLAN (2011-2018)

CIM Suvvas: A multi-cut, chavibetol rich, high essential oil yielding strain from interspecific hybrid were initially compared with betel leaf essential oil for chavibetol by GC/MS as below:

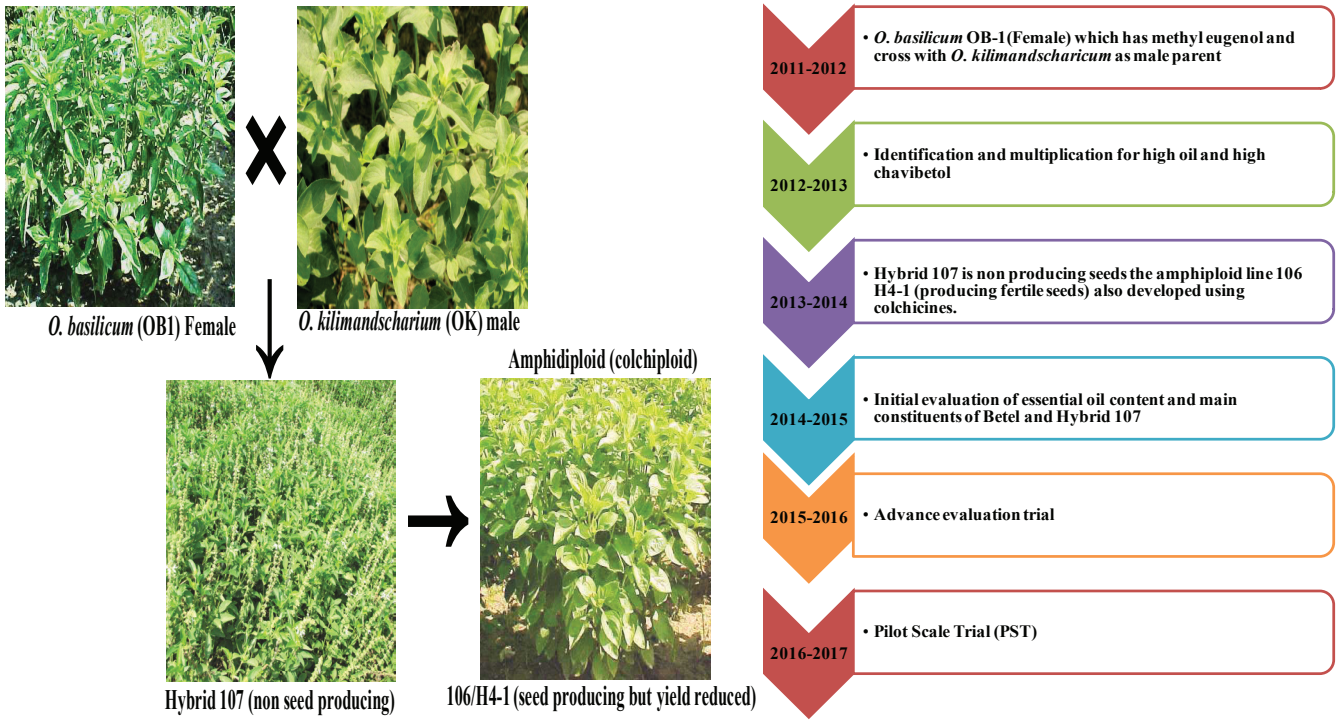



**Table 1: Initial evaluation of essential oil content and main constituents of betel and CIM-Suvvas (2014-2015)**

| Entries | Essential oil content (%) | Chavibetol | Chavibetol acetate | β- caryophyllene | Camphor |
|---|---|---|---|---|---|
| 107 | 0.70 | 25.9 | - | 4.789 | 6.933 |
| Paan/ Betel leaf | 0.50 | 25.812 | 25.649 | 2.314 | - |

As mentioned above, since hybrid 107 is non-producing seeds, then amphiploid line h4-1/ 106 (producing fertile seeds) also developed using colchicines. For the evaluation of essential oil, the 107 lines were placed in a Bench Scale Trial (Bench Scale trial {Entries = 7 (6 + one check), RBD, Reps-3, Plot size = 3 $m^2$) during 2015-16.

The superior two lines, viz., line 107 and 106/ H4-1, were placed in a Pilot Scale Trial (PST, plot size 30 $m^2$) during 2016-17. The top highest-yielding genotype 107 always maintained its superiority in high essential oil and chavibetol content over the check (Table 1; Fig 1). The elite strain, 107 is now proposed to be released as an improved variety for commercial cultivation.

## STATEMENT OF DISTINCTION/ BREEDER'S CLAIM:

Strain 107 is a tall (125 cm), multi-cut, with very medium broad, long with light green leaves and light brown stem of inter-specific hybrid. The inflorescence colour is light green with white patels. The strain has the following DUS (distinctiveness, uniformity and stability) characteristics.

1. The strain is morphologically distinct from other *Ocimum basilicum* varieties and identifiable by its tall, medium broad, long leaves. The inflorescence colour is light green with white petals (Fig. 2).
2. It has a light brown stem.
3. Essential oil extracted from this hybrid strain contains higher chavibetol content (15.50-25.9%) with a low amount of camphor (6.90 %).
5. Strain is also found suitable for cultivating throughout the year with multi-cutting up to 3 harvests, including rain-fed conditions or as a 75-80 day crop only.

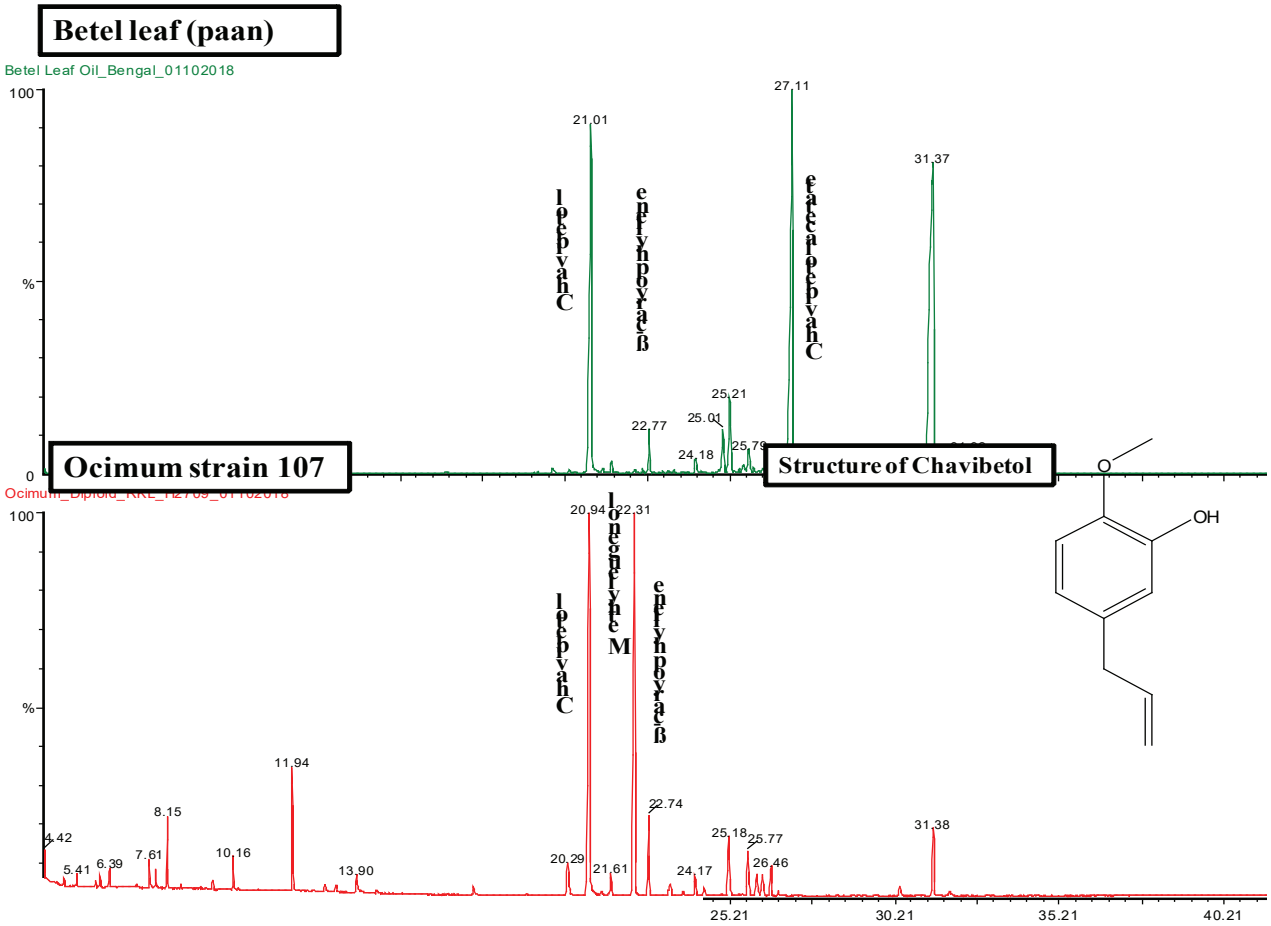
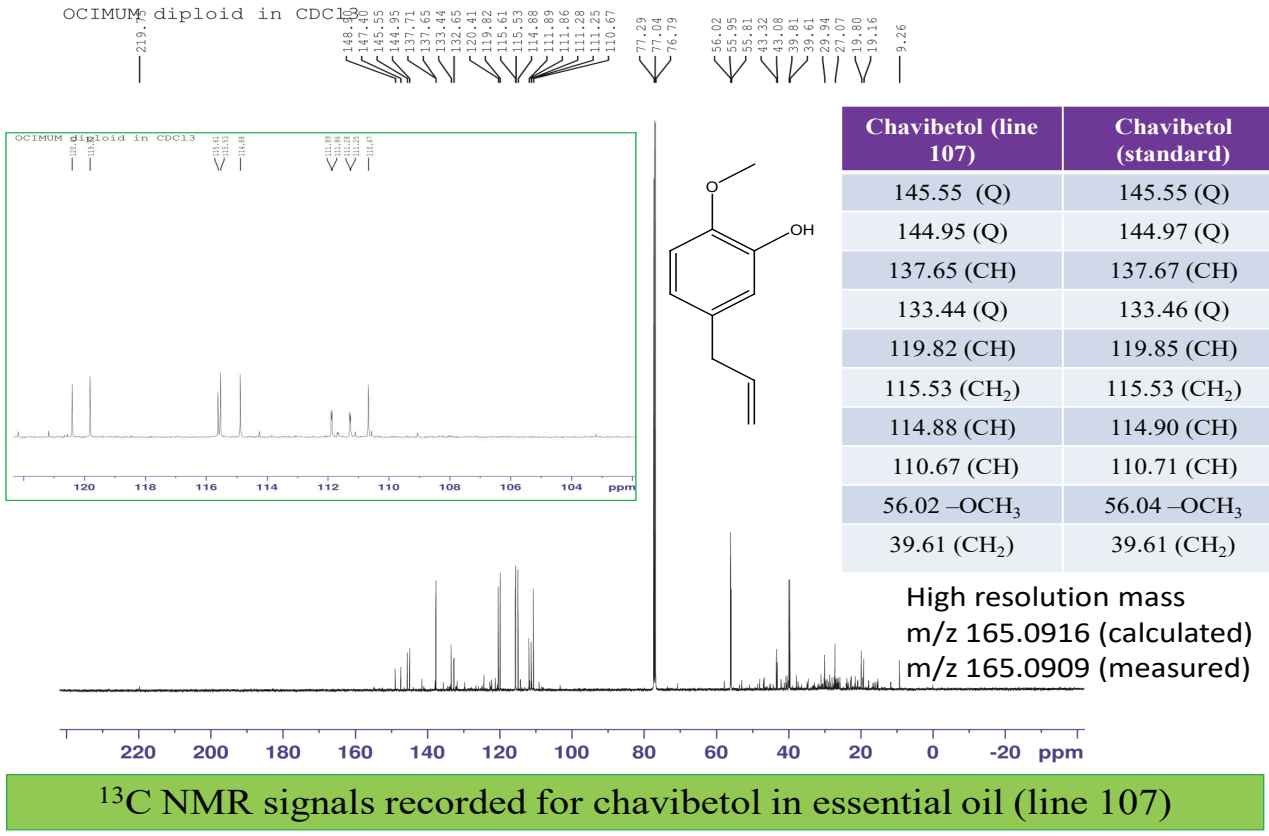



**Figure 1:** GC-MS Chromatogram of the essential oil of betel leaf and CIM-Suvvas

**Figure 2:** Variety CIM-Suvaas with high essential oil and chevibetal rich inter specific hybrid

**Figure 3:** Field view of check line 106/H4-1




| Chavibetol (line 107) | Chavibetol (standard) |
|---|---|
| 145.55 (Q) | 145.55 (Q) |
| 144.95 (Q) | 144.97 (Q) |
| 137.65 (CH) | 137.67 (CH) |
| 133.44 (Q) | 133.46 (Q) |
| 119.82 (CH) | 119.85 (CH) |
| 115.53 ($CH_2$) | 115.53 ($CH_2$) |
| 114.88 (CH) | 114.90 (CH) |
| 110.67 (CH) | 110.71 (CH) |
| 56.02 –$OCH_3$ | 56.04 –$OCH_3$ |
| 39.61 ($CH_2$) | 39.61 ($CH_2$) |

**Figure 4:** $^{13}C$ NMR signals recorded for chavibetol in essential oil of CIM-Suvaas

The other distinguishing morphological features of this variety are given in following Table-2 below:

**Table 2: Advance evaluation trial (Entries 7 × Replications 3); Plot size = (3.0m2) (2015-2016)**

| S.No. | Entries | *Plant height (cm) | *Branches /plant | *Herb yield/ plot (kg) | *Oil content (%) | *Oil yield (g/plot) | Chavibetol content (%) | Seeds producing status |
|---|---|---|---|---|---|---|---|---|
| 1. | **107** | 127.33 | 13.00 | 1.67 | 0.65 | 10.86 | 25.950 | No seed |
| 2. | **HY1** | 103.33 | 6.67 | 1.05 | 0.73 | 7.58 | - | No seeds producing |
| 3. | **H1-1** | 121.67 | 8.33 | 1.30 | 0.48 | 6.24 | - | No seeds producing |
| 4. | **OB-1** | 96.67 | 7.33 | 0.37 | 0.57 | 2.84 | - | Seed producing |
| 5. | **H2** | 103.33 | 9.33 | 0.82 | 0.62 | 3.58 | - | Seed producing |
| 6. | **OK** | 106.67 | 11.67 | 0.849 | 0.48 | 3.31 | - | Seed producing |
| 7. | **106/H4-1 (Check)** | 101.67 | 7.00 | 0.85 | 0.72 | 8.44 | 16.50 | Seed producing |
| **$CD_{5\%}$** | - | 12.277 | 2.552 | 0.285 | 0.305 | 4.427 | | |
| **$CD_{1\%}$** | - | 17.233 | 3.582 | 0.399 | 0.428 | 6.214 | | |

**=P<0.01

**Table 3: Pilot scale evaluation trial of *Ocimum*; Plot size = (30 m²) (2016-2017)**

| Entries | Plant Height (cm) | Branches/ plant | Fresh herb/ plant (ql)/ha | *Oil content (%) | *Oil yield (kg/plot) | Chavibetol Content (%) | Camphor content (%) |
|---|---|---|---|---|---|---|---|
| **107 (CIM-Suvaas)** | 125.50 | 10 | 600.50 | 0.35 | 210.34 | 25.90 | 9.03 |
| **106/H4-1** | 120.30 | 7 | 400.55 | 0.25 | 99.86 | 16.50 | 10.39 |

*Bulk-distillation

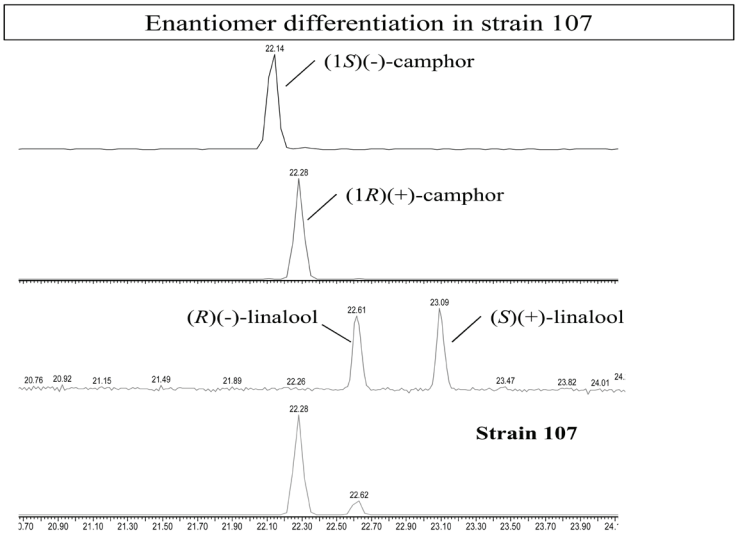
**Table 4: Description of the strain**

| Attributes | CIM-Suvvas | Check 106/H4-1 |
|---|---|---|
| Growth habit | Tall Open | Medium tall |
| Days to harvest after transplanting | Three cuttings/year with the interval of 90 days | 100-120 |
| Days to flower (50%) | 60 | 90 |
| Inflorescence colour | Light green | Light green |
| Flower petals colour | White | White |
| Plant height (cm) | 125-130 | 100-120 |
| Colour of leaf | Light green | Light green |
| Leaf surface | Plain | plain |
| Length of leaf (cm) | 8.30-8.50 | 7.00-7.50 |
| Width of leaf (cm) | 4.00-4.20 | 5.50-.50 |
| Fresh herb yield (ql/ha) | 600.00 | 400.55 |
| Oil content in fresh herb (%) **In clevenger** | 0.65 | 0..55 |
| Mean oil content in fresh herb (%)* **Bulk distillation** | 0.35 | 0.25 |
| Mean potential oil yield (kg/ha)** | 210.34 | 99.86 |
| Superiority over check (%) | 54.10 | - |
| Chavibetol content (%) | 16.00-25.90 | 10.15-19.12 |
| Mean camphor content (%) | 6.933 | 14.92 |
| Refractive index @ 20˚C | 1.4946 minimum<br>1.5014 maximum | |
| Relative density@ 20˚C | 0.9544 minimum<br>0.9601 maximum | |
| Optical Rotation @ 20˚C | +6.0 minimum<br>+9.7 maximum | |

*- Bulk distillation; + Based on oil content data obtained through Clevenger apparatus; **- Estimate based on 30 m² plot size. Calculated as/ha *

**Table 5: Recommended cultivation schedules for the variety CIM-Suvvas**

| S. No. | Agronomical 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) |
| 4. | One crop is also possible | 75-80days with (30×30) cm spacing |




Results

Optically pure/ single enantiomer such as (*R*)(-)-linalool and (1*R*)(+)-camphor were identified in strain 107.

Both these isomers revealed >99% enantiomeric excess.

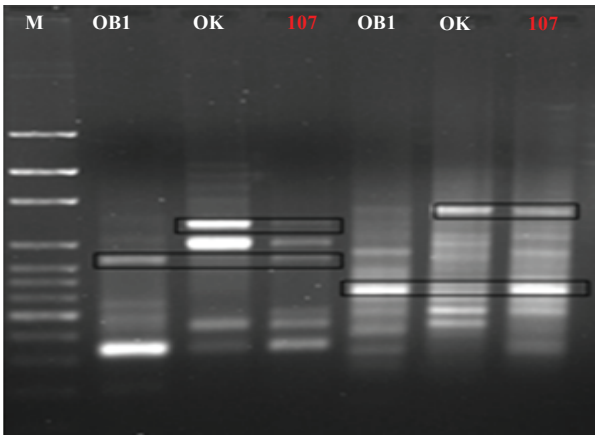



**Figure 5:** Representative ISSR profile shows variations at genetic level. The figure shows DNA profile with ISSR 20 primer with OB1, OK and 107 (CIM-Suvaas) of *Ocimum* genotypes

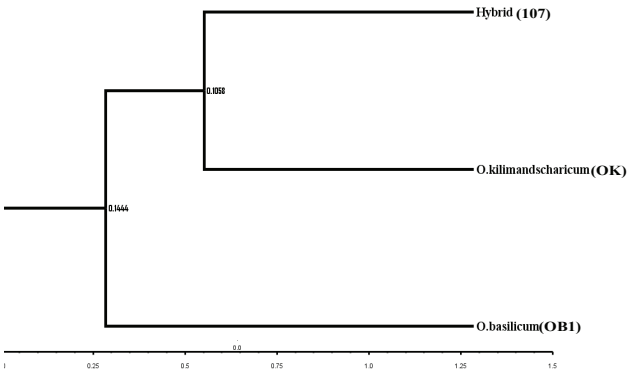



**Figure 6:** Dendrogram showing the genetic relationships among different genotypes of *Ocimum*, using ISSR analysis of OB1, OK and 107 (CIM-Suvaas).

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