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*Original Research Article*

# Quantification of ursolic acid, correlations and contribution by other traits towards accumulation of ursolic acid in six *Ocimum* species

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## ABSTRACT

Sixteen accessions belonging to six species of *Ocimum* from Uttar Pradesh (12), Andhra Predesh (3) and Marashtra (1) states of India were screened for ursolic acid and higher oil yields. A considerable amount of genetic variability in morphometric traits was recorded in all accessions. It was realized that both the estimate of heritability broad sense in percent (^$h^2$ %) and the corresponding genetic advance (GA) were high for oil yield (98.80 and 120.35) and herb yield/plant (93.52 and 89.27). However, high genetic heritability and moderate genetic advance expressed by days to flower 50% (95.13 and 49.07) followed by plant height (93.54 and 52.17) and ursolic acid yield (99.99 and 78.14), respectively. The ursolic acid expressed ^$h^2$ % and low GA (99.99 and 0.362) followed by oil content, leaf/stem ratio and ursolic acid content (99.99, 0.362). Correlation coefficients among the ten traits indicated that leaf/stem ratio was positively highly and significantly correlated with oil content (0.743**, 0.710**) and oil yield (0.700**, 0.676**). Leaf/stem ratio was also positively and significantly correlated with ursolic acid yield (0.551*, 0.536*). The herb yield was highly positive and significantly correlated with oil yield (0.790**, 0.772**). The oil content also had a high and significant correlation with oil yield (0.877**, 0.867**). In addition, similar to the aforementioned correlations, the ursolic acid was significantly and positively correlated with ursolic acid yield (0.966**, 0.965**) at both genotypic and phenotypic level. The path coefficient revealed that the highest direct contribution to ursolic acid was made by ursolic acid content (0.904) and herb yield (0.264). The oil content had the maximum positive indirect effect (0.471) to the ursolic acid content. The residual effect value was found to be 0.0248. *Ocimum* accessions CIM Ayu followed by Sel-1, CIM Jyoti, CIM Snigdha and CIM Shurabhi may be exploited for commercial cultivation.

(*):-p<0.05 and (**):-p<0.01 level of significance



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## 1. Introduction

*Ocimum* (fam. Lamiaceae) is a genus of about 35 species of annual and perennial aromatic herbs and shrubs. Most species of this genus are native to the tropical and warm temperate regions of the old world, including India (Sobti, et al., 1982; Lal, 2014). The dry herb (leaves) and leaf tea of *Ocimum* along with their essential oils and chemical derivatives (eugenol, methyl eugenol, linalool, methyl chevicol, germacrene A and D, elemicin, β-elmene, (*Z*)-ocimine, etc.) are exported to European countries in sizable quantity every year which are worth 5,000 tons. *Ocimum* has several medicinal properties involving its remedial

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properties against colds and coughs, indigestion, stomach pain and diarrhoea. Nausea, ulcers, ringworm and asthma can also be effectively treated with *Ocimum*. Many people believe that it can lower blood sugar and also increase lactation. *Ocimum* is a rich source of carbohydrates, fibre, phosphorous, calcium, protein, iron, β-carotene, vitamin B1 and vitamin B2 and in aromatic oils (Sobti, et al., 1982; Reuveni et al., 1984). Besides several compounds, considerable amounts of ursolic acid (a ubiquitous triterpenoid) are also produced in *Ocimum*. During the last decade, a remarkable number of reports have been published on ursolic acid, reflecting tremendous interest and progress in our understanding of this compound (Zupancic, 2001; Liu, 2005; Švccová and Ncugcbaucrová, 2010). These reports deal with the isolation and purification of this tritepernoid from various plants and herbs. In addition, the chemical modifications to make more effective and aqua soluble derivatives, the pharmacological research on their beneficial effects, the toxicity studies, and the clinical use of these triterpenoids toward various diseases including anticancer chemotherapies have been pointed out in detail in the literature (Reuveni et al., 1984; Zupancic, 2001; Silva et al., 2003; RazborŠek et al., 2008).

Ursolic acid, a pentacyclic triterpene acid, has been isolated from many kinds of medicinal plants, such as *Ocimum sanctum*, *Rosmarinns officinalis*, *Glechoma hederaceae*, *Melaleuca leucadendron* and *Eriobotrya japonica* (Anandjiwala et al., 2006). This acid has been reported to exhibit antitumor and antioxidant activities (Zupancic, 2001; Silva et al., 2003 ; RazborŠek et al., 2008). Ursolic acid may also play an important role in regulating the apoptosis induced by high glucose presumably through scavenging of ROS (reactive oxygen species) in a distinct medium. It has been found recently that ursolic acid treatment affects growth and apoptosis in cancer cells (Huang et al., 1994). The *Ocimum* oil is used as an anti-perspirant agent and a fly and mosquito repellent. CSIR-CIMAP, Lucknow is actively involved in genetic enhancement of the *Ocimum* species following different breeding approach for better plant type having high yield of herb, essential oil and ursolic acid characters combined with a consistent high yield of phenyl propanoid eugenol and other economic important chemical constituents.

Sixteen accessions relating to six species of *Ocimum* from Uttar Pradesh (12), Andhra Predesh (3) and Marashtra (1) states of India were screened for ursolic acid and higher oil yields at CSIR-CIMAP, Lucknow U.P. (India). The available sixteen genetic stocks belong to six *Ocimum* species namely, *Ocimum sanctum* Krishna and Shyam tulsi, *O. basilicum*, *O. kilimandscharicum*, *O. africanum*, *O. gratissimum*, *Ocimum viride* including seven varieties: CIM Ayu, CIM Angana, CIM Kanchan, CIM Jyoti, CIM Shurabhi, CIM Sharda, and CIM Snigdha. In future, there will be a possibility to develop varieties for high herb, high ursolic and oil yield with high specific needs chemical components like eugenol, methyl eugenol, methyl cinnamate, germacrene A and D, linalool, elemicin, β-elmene, (*Z*)-ocimine etc. content for value addition in herbal products. In our research programme on *Ocimum* improvement, sixteen numbers of varieties/accessions were studied to sort out promising varieties/accessions for high ursolic acid and oil yield of better quality chemotypes. Due to a priori knowledge of the range of variability, correlations and character contributions towards ursolic acid yield having great significance for further breeding, all these varieties/accessions were studied for high ursolic acid and oil yield of better quality.

## 2. Experimental

### 2.1. Plant materials

The genus *Ocimum* contains about 35 species of herbs and shrubs from the tropical regions of Asia and Europe. The essential oil of *Ocimum* extracted via steam distribution from the whole herb is used to flavour foods, dental and oral products, in fragrances and in traditional rituals and medicines (Guenther, 1949; Simon et al., 1990). Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nematicidal and fungistatic (Reuveni et al., 1984; Simon et al., 1990). These properties are related to predominate essential oil constitutes such as methyl chavicol, eugenol, linalool, camphor and methyl cinnamate. Two minor components of the essential oil of sweet basils (*Ocimum basilicum*: Juvocimene I and II) have been reported as potent juvenile hormone analogs. *Ocimum* has also been proved to be a valuable source of anti-carcinogenic agents. The herbal medicinal plants, e.g., *Ocimum* species have been similarly prescribed from the ancient times by the Assyrians (4000 B.C.), Sumerians (3500 B.C.), Indians (3500 B.C.), Chinese (3000 B.C.) and Egyptians (2500 B.C.). These great usages which were temporarily subdued under the impact of modern medicine have resulted in a comeback and an `herbal renaissance' of blooming across the world (Guenther, 1949; Simon et al., 1990). Sweet basil (*Ocimum basilicum* L.) a common garden herb is cultivated in the United States for culinary purposes as a fresh herb and as a dried spice. The oils of commerce are known as European, French, Egyptian, Revnion or Comoro and to a lesser extent Bulgarian and Java basil essences (Heath, 1981). There are several types of basil oil in the international trade markets, each derived principally from different cultivars and chemotypes of sweet basil.

A large number of genetic stocks of *Ocimum* were assembled from various sources from different places of India (Fig. 1.). Out of 125 genetic stocks, a new set having sixteen genetic stocks of *Ocimum* from Uttar Pradesh (12), Andhra Pradesh (3) and Maharashtra (1) were included in this study.

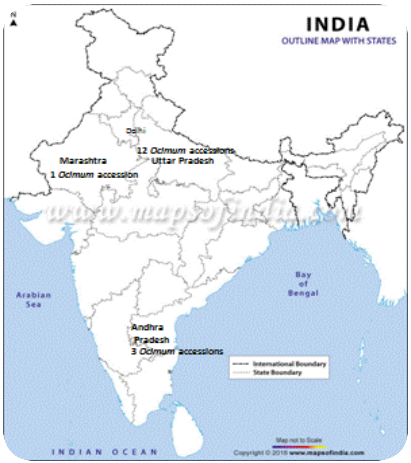


**Fig. 1.** The geographical map of India with sampling area.

Sixteen varieties/accessions were screened for high ursolic acid and oil yield of better quality chemotypes (Table 1, Fig. 2 and Fig. 3). The varieties/accessions were grown in a randomized block design with three replications at the research farm of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, U.P. India PIN-226 015 in the two consecutive years 2014-2015 and 2015-2016 under normal fertility regime (80:40:40) kg N, $P_2O_5$ and $K_2O$/ha., respectively with plot sizes of 50 m$^2$. The crop was harvested after 90 days after planting of crop. The experimental site at the institute research farm was located at 26°. 50' N latitude and 80°.50' E longitude, and 120 m above the mean sea level. The climate is semiarid to subtropical in nature.

The observations were recorded on ten economic traits, namely days to flower 50%, plant height (cm), primary branches/plant, leaf/stem ratio, fresh herb yield (q/ha), oil content (%) and oil yield (Kg/ha), oleanolic acid content (%), ursolic acid content in (%) and ursolic acid yield (kg/ha) (calculated value on herb yield and ursolic acid content). Oil content was estimated by hydrodistillation of fresh herb of each varieties/accessions for two hours in Clevenger's apparatus (Clevenger, 1928).

## 2.2. Analysis of oleonolic acid and ursolic acid

### 2.2.1. Chromatographic system

The gradient liquid chromatographic system (model LC-10A series; Shimadzu, Tokyo, Japan) consisted of two LC-10AD pumps controlled by a CMB-10A interface module, a model 7725i manual injector valve (Rheodyne) equipped with a 20 mL sample loop, and a multi-dimensional UV-VIS detector (model SPD-10A). Data were collected and analysed using a class LC-10. Work station equipped with an HP-DeskJet printer. The method involves the use of a Waters Spherisorb S10 ODS2 column (250 × 4.6 mm, I.D., 10 microm) and binary gradient mobile phase profile. The various other aspects of analysis viz. extraction efficiency, peak purity and similarity were validated using a photodiode array detector, and a mobile phase consisting of 0.1% TFA in water: acetonitrile (85:15, v/v). The mobile phase was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min. The flow-rate was adjusted to 1 mL min$^{-1}$ with a run time of 25 minutes. The injection volume was adjusted to 10 µL and detection was subsequently made at 215 nm. Linearity was observed over the range of 10-250 µg mL$^{-1}$ with correlation coefficients of 0.9987 and 0.9977. Detection limits were 0.2402, 0.1073 ng mL$^{-1}$ and quantitation limits were 0.8007, 0.7460 ng mL$^{-1}$ (Gnoatto et al., 2005).

### 2.2.2. Preparation of sample solutions

An accurately weighed sample of dry leaves was refluxed for two hours in a mixture of methanol

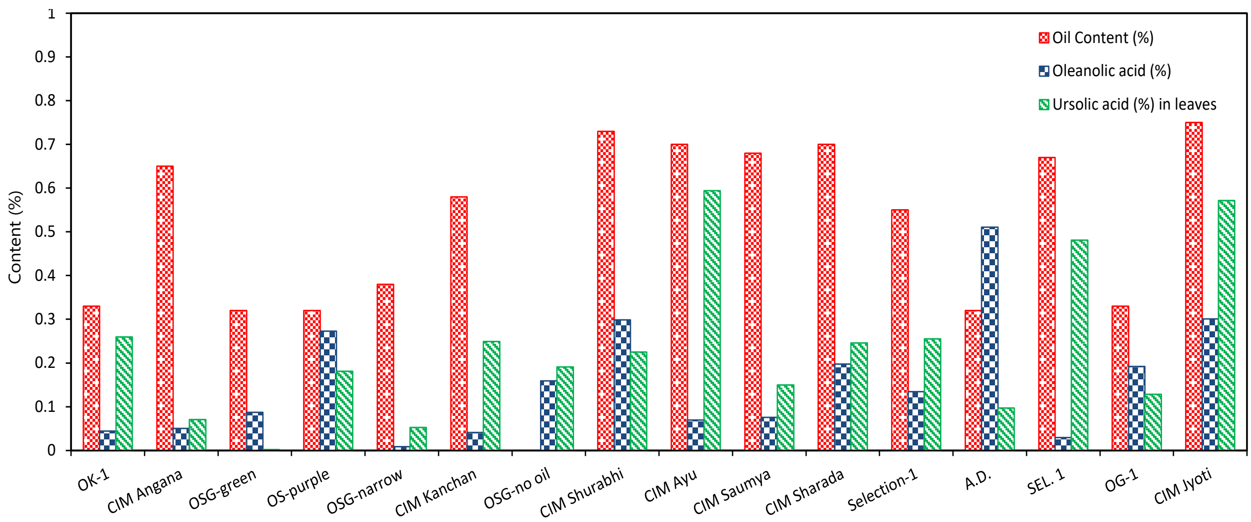


**Fig. 2.** Essential oil, oleanolic acid and ursolic acid content in Ocimum.

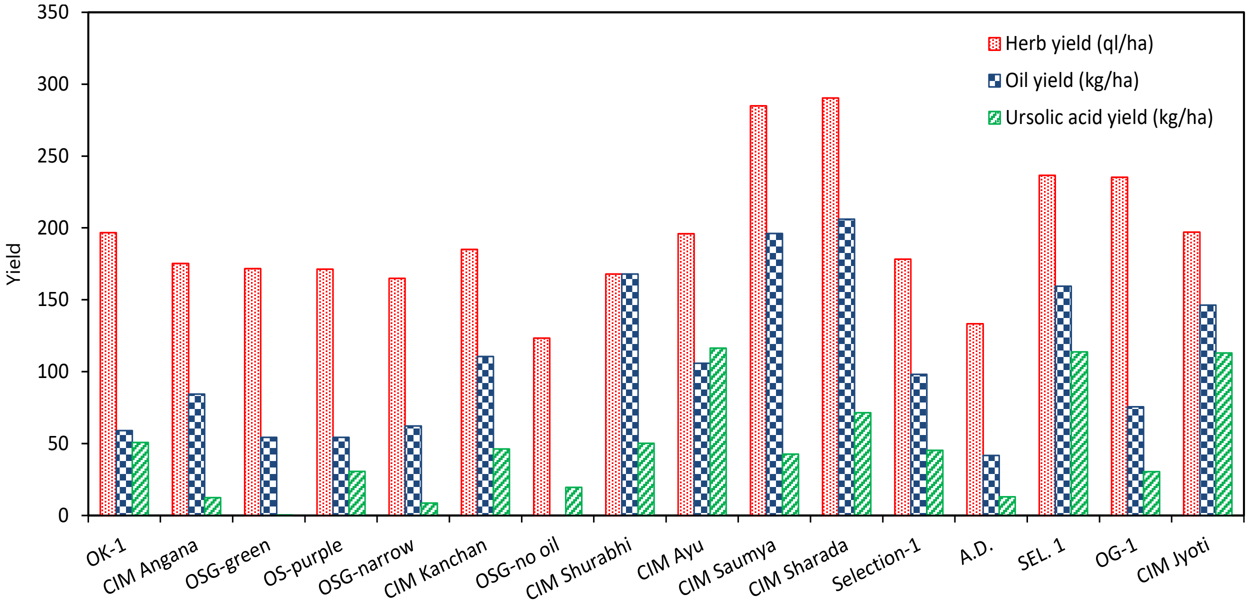


**Fig. 3.** Essential oil, oleanolic acid and ursolic acid content in Ocimum.

(90%) and water (10%) filtered, cooled, diluted with water and extracted with methanol. Methanol layer was evaporated and the resulting residue was reconstituted in 1 mL of HPLC grade methanol.

2.3. Statistical analyses

Statistical analyses were done using the Statistical Software 4.0 version, available in the Division of Genetics and Plant Breeding of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India, that is based on the standard methods in Panse and Sukhatme (1967) along with Singh and Chaudhary (1979). The pooled mean values of two years for the all ten characters were subjected to ANOVA, correlation and path coefficient analyses (Dewey and Lu, 1959; Lal et al., 2001).

## 3. Results and Discussion

Variation among the pooled mean over two years of sixteen diverse genetic accessions/varieties was highly significant ($p<0.01$) for the all ten traits. The results from the analysis of variance, means, standard errors and critical difference (C.D.) revealed highly significant differences among the accessions for all the ten studied characters accounting for the existence of considerable genetic variability among the accessions of *Ocimum* (Table 1 and Table 4 Fig. 1-4). The statistical and genetic parameters for the heritable and non-heritable components of variation were computed. The sampling variance was moderate to high for nearly all the traits examined as genotypic coefficient of variation (GCV) 22.22 to 80.22% and phenotypic coefficient of variation (PCV) 24.75 to 101.90%. The heritable portion of phenotypic variance reflected by the size of $\sigma^2g$ relative to $\sigma^2p$ expressed as $^\wedge h^2$ ($_{BS}$) was generally very large (93.52 to 99.99) for the eight characters except for primary branches/plant (79.39) and oleanolic acid content (61.98) that showed moderate heritability. In other words, all characters except primary branches/plant and oleanolic acid content were apparently only minimally influenced by environment factors (Table 4). Silva et al. (2008) also reported high variation in ursolic acid content in the eight *Ocimum* species in north eastern Brazil.

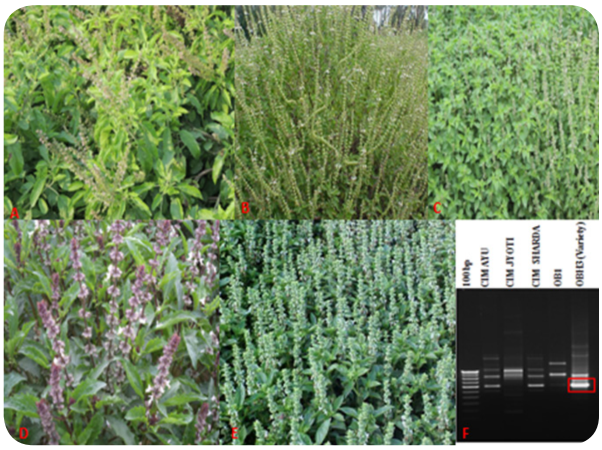


**Fig. 4.** High ursolic yielder varieties A- CIM Ayu, B- Sel-1 (OB-1), C- CIM Jyoti, D- CIM Snigdha (OBH-3), E- High (-) linalool rich essential oil (78.70%) and medium ursolic yielder variety CIM Shurabhi and their F-SCoT molecular marker profile.

The critical perusal of results indicated that high heritability estimate ($^\wedge h^2$ $_{BS}$) with the corresponding high genetic advance (GA) is more reliable for selection than that with low heritability estimate and low genetic advance. The estimate of heritability broad sense in percent ($^\wedge h^2$ $_{BS}$ %) and corresponding genetic advance (GA), both were high for herb yield (93.52 and 89.27) and oil yield (98.80 and 120.35) followed

**Table 1**
Mean performance of *Ocimum* accessions/varieties and their origin.

| No. | *Ocimum* accessions | Common name | Botanical name | Origin | Characters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Days to flower (50%) | Plant height (cm) | Primary branches/plant | Leaf/stem (ratio) | Herb yield (ql/ha) | Oil content (%) | Oil yield (kg/ha) | Oleanolic acid (%) | Ursolic acid (%) in leaves | Ursolic acid yield (kg/ha) |
| 1 | OK-1 | Camphor tulsi | *O. kilimandscharicum* | CIMAP, Lucknow (U.P.) | 103.33 | 176.67 | 4.00 | 0.38 | 196.67 | 0.33 | 59.00 | 0.0441 | 0.2596 | 50.70 |
| 2 | CIM Angana | Shyam tulsi | *O. sanctum* | CIMAP, Lucknow (U.P.) | 113.33 | 120.00 | 5.33 | 0.58 | 175.33 | 0.65 | 84.33 | 0.0503 | 0.0708 | 12.44 |
| 3 | OSG-green | Krishna tulsi | *O. sanctum* | Asthi, Lucknow (U.P.) | 116.67 | 116.67 | 6.67 | 0.57 | 171.67 | 0.32 | 54.33 | 0.0871 | 0.0019 | 0.33 |
| 4 | OS-purple | Purple tulsi | *O. sanctum* | Nasik (Maharashtra) | 96.67 | 120.00 | 4.67 | 0.47 | 171.33 | 0.32 | 54.22 | 0.2726 | 0.1809 | 30.66 |
| 5 | OSG-narrow | Green tulsi | *O. sanctum* | Rameshwerum (A.P.) | 63.33 | 93.33 | 6.67 | 0.45 | 165.00 | 0.38 | 62.20 | 0.0084 | 0.0525 | 8.59 |
| 6 | CIM Kanchan | Holy basil | *O. sanctum* | CIMAP, Hyderabad (A.P.) | 116.67 | 106.67 | 5.00 | 0.62 | 185.00 | 0.58 | 110.57 | 0.0409 | 0.2491 | 46.19 |
| 7 | OSG-no oil | Green tulsi | *O. sanctum* | Tinnevely (A.P.) | 66.67 | 66.67 | 4.00 | 0.40 | 123.33 | 0.00 | 0.00 | 0.1591 | 0.1911 | 19.57 |
| 8 | CIM Shurabhi | Sweet basil | *O. basilicum* | CIMAP, Lucknow (U.P.) | 76.67 | 83.33 | 4.67 | 0.70 | 168.00 | 0.73 | 168.00 | 0.2985 | 0.2250 | 50.12 |
| 9 | CIM Ayu | Krishna tulsi | *O. sanctum* | CIMAP, Lucknow (U.P.) | 110.00 | 118.00 | 4.33 | 0.52 | 196.00 | 0.70 | 105.88 | 0.0696 | 0.5939 | 116.43 |
| 10 | CIM Sharada | Zanzibar basil | *O. basilicum* | CIMAP, Lucknow (U.P.) | 80.00 | 96.67 | 5.33 | 0.52 | 285.00 | 0.68 | 196.23 | 0.0758 | 0.1498 | 42.63 |
| 11 | CIM Snigdha | French basil | *O. basilicum* | CIMAP, Lucknow (U.P.) | 81.67 | 83.33 | 6.33 | 0.63 | 290.43 | 0.70 | 206.05 | 0.1978 | 0.2458 | 71.37 |
| 12 | Selection-1 | Viride | *O. viride* | CIMAP, Lucknow (U.P.) | 128.33 | 125.00 | 7.67 | 0.40 | 178.33 | 0.55 | 98.08 | 0.1345 | 0.2554 | 45.35 |
| 13 | A.D. | Colchiploid | A.D. of $F_1$ | CIMAP, Lucknow (U.P.) | 126.67 | 126.67 | 4.00 | 0.38 | 133.33 | 0.32 | 41.67 | 0.5104 | 0.0972 | 12.82 |
| 14 | SEL. 1 | Hybrid | $F_1$ | CIMAP, Lucknow (U.P.) | 123.33 | 126.67 | 6.33 | 0.73 | 236.67 | 0.67 | 159.47 | 0.0294 | 0.4812 | 113.76 |
| 15 | OG-1 | Van tulsi | *O. gratissium* | CIMAP, Lucknow (U.P.) | 148.33 | 141.67 | 7.67 | 0.35 | 235.35 | 0.33 | 75.50 | 0.1920 | 0.1283 | 30.33 |
| 16 | CIM Jyoti | Lemon tulsi | *O. africanum* | CIMAP, Lucknow (U.P.) | 75.00 | 78.33 | 4.67 | 0.72 | 197.00 | 0.75 | 146.33 | 0.3009 | 0.5764 | 113.04 |
| | $CD_{5\%}$ | - | - | - | 9.34 | 11.85 | 1.03 | 0.05 | 20.32 | 0.06 | 10.85 | 0.1616 | 0.0025 | 0.466 |
| | $CD_{1\%}$ | - | - | - | 12.71 | 15.97 | 1.39 | 0.07 | 27.59 | 0.08 | 14.63 | 0.2179 | 0.0033 | 0.629 |
| | Range | - | - | - | 63.33-148.33 | 66.67-176.67 | 4.00-7.67 | 0.35-0.73 | 123.33-290.43 | 0.00-0.75 | 0.00-206.05 | 0.0084-0.5104 | 0.0019-0.5939 | 0.33-116.43 |

$F_1$= *O. basilicum*×*O. Kilimandscharicum*; Ursolic acid (kg/ha) is a calculated value on herb yield

**Table 2**
Correlations among nine economic traits in *Ocimum*.

| **Characters** | **Days to flower (50%)** | **Plant height (cm)** | **Primary branches/plant** | **Leaf/stem (ratio)** | **Herb yield (ql/ha)** | **Oil content (%)** | **Oil yield (kg/ha)** | **Oleanolic acid (%)** | **Ursolic acid (%) in leaves** | **Ursolic acid yield (kg/ha)** |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to flower (50%) | - | 0.730** | 0.387 | -0.250 | 0.045 | -0.041 | -0.185 | 0.022 | -0.048 | -0.033 |
| | | 0.686** | 0.341 | -0.245 | 0.034 | -0.032 | -0.179 | 0.005 | -0.046 | -0.032 |
| Plant height (cm | -0.034 | - | 0.109 | -0.438 | 0.042 | -0.170 | -0.290 | -0.185 | -0.089 | -0.077 |
| | 1.003 | | 0.118 | -0.415 | 0.059 | -0.161 | -0.279 | -0.142 | -0.087 | -0.074 |
| Primary branches/plant | 0.049 | 0.208 | - | -0.049 | 0.376 | 0.107 | 0.182 | -0.375 | -0.262 | -0.136 |
| | 0.985 | 0.797 | | -0.042 | 0.368 | 0.083 | 0.169 | -0.228 | -0.232 | -0.122 |
| Leaf/stem (ratio) | -0.149 | -0.042 | 0.004 | - | 0.300 | 0.743** | 0.700** | -0.112 | 0.448 | 0.551* |
| | 0.968 | 0.994 | 1.009 | | 0.284 | 0.710** | 0.676** | -0.036 | 0.436 | 0.536* |
| Herb yield (ql/ha) | -0.153 | 0.301 | 0.374 | 0.044 | - | 0.556* | 0.790** | -0.311 | 0.210 | 0.429 |
| | 1.255 | 0.668 | 0.882 | 0.991 | | 0.526* | 0.772** | -0.230 | 0.203 | 0.415 |
| Oil content (%) | 0.181 | 0.027 | -0.144 | -0.038 | -0.096 | - | 0.877** | -0.122 | 0.521* | 0.648** |
| | 1.207 | 1.007 | 1.132 | 1.002 | 1.008 | | 0.867** | -0.084 | 0.514* | 0.639** |
| Oil yield (kg/ha) | 0.037 | -0.004 | 0.169 | -0.172 | 0.446 | 0.406 | - | -0.072 | 0.399 | 0.588* |
| | 1.005 | 0.999 | 0.950 | 1.001 | 0.984 | 0.991 | | -0.058 | 0.397 | 0.585* |
| Oleanolic acid (%) | -0.088 | -0.012 | 0.125 | 0.345 | 0.041 | 0.099 | -0.014 | - | -0.032 | -0.078 |
| | 3.585 | 0.987 | 1.153 | 2.379 | 1.028 | 1.123 | 0.983 | | -0.025 | -0.061 |
| Ursolic acid (%) in leaves | 0.463 | 0.065 | 0.275 | 0.082 | -0.145 | 0.266 | 0.155 | 0.054 | - | 0.966** |
| | 1.019 | 1.002 | 1.005 | 0.999 | 1.002 | 0.999 | 0.999 | 1.011 | | 0.965** |
| Ursolic acid yield (kg/ha) | -0.191 | 0.066 | -0.276 | 0.105 | 0.089 | 0.154 | -0.103 | 0.023 | -0.098 | - |
| | 0.991 | 1.002 | 0.992 | 0.999 | 0.999 | 0.999 | 1.000 | 1.002 | 1.000 | |

*-p<0.05; **-p<0.01; values in upper diagonal belongs to genetic ($r_g$) and phenotypic correlations ($r_p$) and below diagonal relates to environmental correlation ($r_e$) and co-heritability (COH in broad scence in broad sense), respectively.

**Table 3**
Direct (bold) and indirect effects on nine economic traits in *Ocimum*.

| **Characters** | **Days to flower (50%)** | **Plant height (cm)** | **Primary branches/plant** | **Leaf/stem (ratio)** | **Herb yield (ql/ha)** | **Oil content (%)** | **Oil yield (kg/ha)** | **Oleanolic acid (%)** | **Ursolic acid content (%) in leaves** | **Ursolic acid yield ($r_g$)** |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to flower (50%) | **-0.132** | 0.109 | 0.043 | -0.048 | 0.012 | -0.001 | 0.023 | 0.003 | -0.043 | -0.033 |
| Plant height (cm) | -0.0965 | **0.150** | 0.012 | -0.084 | 0.011 | -0.004 | 0.037 | -0.022 | -0.081 | -0.077 |
| Primary branches/plant | -0.051 | 0.016 | **0.112** | -0.009 | 0.099 | 0.002 | -0.023 | -0.045 | -0.237 | -0.136 |
| Leaf/stem (ratio) | 0.033 | -0.066 | -0.005 | **0.191** | 0.079 | 0.016 | -0.088 | -0.014 | 0.405 | 0.551 |
| Herb yield (ql/ha) | -0.006 | 0.006 | 0.042 | 0.057 | **0.264** | 0.012 | -0.099 | -0.037 | 0.190 | 0.429 |
| Oil content (%) | 0.005 | -0.025 | 0.012 | 0.142 | 0.147 | **0.022** | -0.111 | -0.015 | 0.471 | 0.648 |
| Oil yield (kg/ha) | 0.025 | -0.043 | 0.020 | 0.133 | 0.208 | 0.019 | **-0.126** | -0.009 | 0.361 | 0.588 |
| Oleanolic acid (%) | +0.003 | -0.028 | -0.042 | -0.021 | -0.082 | -0.003 | 0.009 | **0.120** | -0.029 | -0.078 |
| Ursolic acid content (%) in leaves | 0.006 | -0.013 | -0.029 | 0.085 | 0.056 | 0.011 | -0.050 | -0.004 | **0.904** | 0.966 |

Residual effects = 0.0248

by plant height (93.54 and 52.17) and ursolic acid yield (99.99 and 78.14), high heritability and moderate genetic advance for days to flower 50 % 95.13 and 49.07), respectively. Therefore, these traits might be highly amenable to direct selection for their genetic improvement over a short span of time. On the other hand, the character leaf/stem ratio (99.99 and 89.27), oil content (97.18 and 0.43) and ursolic acid content (99.99 and 0.362) expressed high heritability and low genetic advance. Other characters expressed medium heritability and low genetic advance were primary branches/plant (79.39 and 1.98) and oleanolic acid content (61.98 and 0.158). Therefore, selection of these characters would be difficult.

In addition to heritability in broad sense $^h^2{}_{BS}$ and genetic advance GA, the correlations among

**Table 4**
Other allied genetic parameters in *Ocimum*.

| No. | Characters | $\sigma^2$g | $\sigma^2$p | $\sigma^2$e | GCV | PCV | CV (%) | SEM | $h^2_{(BS)}$% | GA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Days to flower (50%) | 627.08 | 659.17 | 32.08 | 24.63 | 25.25 | 5.57 | 3.27 | 95.13 | 49.07 |
| 2 | Plant height (cm) | 733.09 | 783.68 | 50.59 | 24.38 | 25.21 | 6.41 | 4.11 | 93.54 | 52.17 |
| 3 | Primary branches/plant | 1.47 | 1.85 | 0.68 | 22.22 | 24.94 | 11.32 | 0.36 | 79.39 | 1.98 |
| 4 | Leaf/stem (ratio) | 0.016 | 0.017 | 0.0009 | 24.17 | 24.86 | 5.83 | 0.02 | 94.50 | 0.25 |
| 5 | Herb yield (ql/ha) | 2147.51 | 2296.35 | 148.84 | 23.93 | 24.75 | 6.30 | 7.04 | 93.52 | 89.27 |
| 6 | Oil content (%) | 0.047 | 0.048 | 0.0014 | 43.21 | 43.83 | 7.36 | 0.02 | 97.18 | 0.43 |
| 7 | Oil yield (kg/ha) | 3496.39 | 3538.84 | 42.45 | 58.26 | 58.61 | 6.42 | 3.76 | 98.80 | 120.35 |
| 8 | Oleanolic acid (%) | 0.015 | 0.025 | 0.0094 | 80.22 | 101.90 | 62.83 | 0.06 | 61.98 | 0.158 |
| 9 | Ursolic acid (%) in leaves | 0.031 | 0.003 | 0.0001 | 74.91 | 74.91 | 0.630 | 0.001 | 99.99 | 0.362 |
| 10 | Ursolic acid yield (kg/ha) | 1438.84 | 1438.91 | 0.078 | 79.40 | 79.41 | 0.586 | 0.162 | 99.99 | 78.14 |

^$\sigma^2$g, ^$\sigma^2$p, $\sigma^2$e- Variance due to genotype, phenotype and environment; gcv, pcv genetic: and phenotypic coefficient of variation; ^h ($_{BS}$) - heritability in broad sense; GA – Genetic advance, CD – Critical difference, SEM-standard error mean, respectively.

characters also have a direct bearing on success of selection for high yield. The results indicated that the genotypic correlations were higher than phenotypic correlations for all the traits except two traits combination plant height and primary branches/plant and plant height and herb yield (Table 2). Correlation coefficients among the ten traits revealed that days to flower 50% was highly positively and significantly correlated with plant height (0.730**, 0.686**); leaf stem ratio with oil content (0.743**, 0.710**) and oil yield (0.700**, 0.676**). The herb yield was also positively and significantly correlated with oil yield (0.790** and 0.772**); oil content with oil yield (0.877** and 0.967**) and with ursolic acid yield (0.648** and 0.639**), respectively. On the other hand the ursolic acid content showed a positive and highly significant correlation with ursolic acid yield (0.966** and 0.965**) at both genotypic and phenotypic levels 0.5 and 0.01% level of significance. The leaf/stem ratio with ursolic acid yield (0.551*, 0.536*), herb yield with oil content (0.556*, 0.526*) and oil content with ursolic acid content (0.521*, 0.514*) and oil yield with ursolic acid yield (0.588* and 0.585*) were positively correlated with each other at both genetic and phenotypic levels at 0.5% level of significance only. The other traits were weakly correlated each other (Table 2). These traits were also reinforced by high values of co-heritability. High co-heritability value of a character contribution suggests that the increase in one of the character will increase its other co-heritable trait (Lal et al., 2001). Hence, these traits were found as suitable criteria for selection of ursolic acid yield. Silva et al., (2003) also recorded high variations in essential oils of *Ocimum basilicum* L., *O. basilicum* var minimum and *Ocimum basilicum* var *purpurascens* grown in North-eastern Brazil. Likewise, Švccovȧ and Ncugcbaucrovȧ (2010) also studied on 34 cultivars of basil and reported considerable variations for economic and biochemical characters.

The path coefficient results revealed that the highest direct contribution to ursolic acid yield was made by ursolic acid content (0.904), and herb yield (0.264) followed by leaf/stem ratio (0.191), plant height (0.150), oleanolic acid content (0.120), primary branches/plant (0.112) and oil content (0.022). The direct contribution of other two traits days to flower 50% and oil yield to ursolic acid yield was negative but their indirect contribution was invariably large via leaf/stem ratio (0.405), oil content (0.471), oil yield (0.361) and ursolic acid content although residual effect was very low 0.0248 (Table 3). These traits were also reinforced by high values of co-heritability (Table 2 and Table 3). Therefore, the choice of most economic traits ursolic acid content and herb yield may be used as a best selection criterion for improvement of ursolic acid yield. Ursolic acid, oil content followed by oil yield and leaf stem ratio might be a better choice for selection in *Ocimum* crop.

Ursolic acid yield is a very important/effective trait in the *Ocimum* (Gird et al., 2015; Richard et al., 2016; Misra et al., 2017). Moreover, high ursolic acid yield coupled with high essential oil yield with desired oil quality will be of great importance in national and international trade. Thus, in the selection for high ursolic acid yielding genotype, the other trait like high essential oil yield of better quality parameters should also be considered in the *Ocimum* crop improvement program. Among the *Ocimum* accessions/varieties, variety CIM Ayu was the highest ursolic acid yielder with oil yield followed by Sel-1, CIM Jyoti and CIM Snigdha, respectively. These accessions/varieties may be exploited for commercial cultivation.

## 4. Concluding remarks

Genetic improvement and development of high yielding varieties are dependent upon genetic variability. We examined genetic variation for ten traits of sixteen accessions/varieties in order to understand genetic variability, genetic, correlations and character contribution towards ursolic acid yield in *Ocimum*. Genotypic and phenotypic coefficient of variations had the largest values for essential oil yield followed by herb yield and ursolic acid yield. The path coefficient under study revealed that the highest direct contribution to ursolic acid yield was made by ursolic acid content (0.904) and herb yield (0.264) followed by leaf/stem ratio (0.191) and plant height (0.150). All these traits expressed high heritability ($^h^2_{BS}$) and acceptable genetic advance with positive correlations. Oil content and ursolic acid yield followed by ursolic acid content and ursolic acid yield were highly significant and showed positive correlations with each other at both genetic and phenotypic levels. Among the *Ocimum* accessions/varieties, variety CIM Ayu was the highest ursolic acid yielder with oil yield followed by Sel-1, CIM Jyoti, CIM Snigdha and CIM Shurabhi. Therefore, these *Ocimum* accessions/varieties could be exploited for cultivation.

## Conflict of interest

The authors declare that there is no conflict of interest.

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