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Genetic diversity and chemotype selection in genus *Ocimum*

Smita Singh[a,b,*], R.K. Lal[b], Ranjana Maurya[c], C.S. Chanotiya[c]

[a] *Academy of Scientific and Innovation Research (AcSIR), India*
[b] *CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Department of Genetics and Plant Breeding, India*
[c] *Analytical Chemistry Department, CSIR-CIMAP, Lucknow, India*

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A B S T R A C T

The present study was conducted to examin the genetic diversity and clustring pattern among twenty five accessions of basil. The highly significant ANOVA results indicated the presence of high amount of genetic variability which was also confirmed by the wide range of D^2-values (0.074-212.97). The all accessions, grouped into seven divers clusters (I–VII). The intra-cluster divergence was maximum within cluster-VII (D^2 = 43.32), the inter-cluster divergence was highest between clusters-II and VI (D^2 = 155.59). The high genetic divergences among the accession were also confirmed by spatial distribution analysis. The contribution of the character towords the genetic diversity was highest for oil content 56.09% followed by plant height 18.94% and the lowest rank was recorded for the number of branches 7.55%. The highly divergent accessions namely, G-4, G-7, G-9, G-11, G-18 and G-25 may be exploited for further crop improvement for the development of high essential oil yielding *Ocimum* cultivars of better quality.

1. Introduction

Ocimum (fam. Lamiaceae) is a genus of about 200 species of annual and perennial herbs and shrubs. Most of the species are native to the tropical and warm temperate regions of the world, including India. The annual export of dry leaves, its products, essential oil and its derivatives/chemical constituents of *Ocimum* are worth 5000 tons (Bhasin, 2012; Lal, 2014). The dry herb (leaves), *Ocimum* leaf tea, essential oil and its chemical derivatives (eugenol, methyl–eugenol, linalool, methyl chevicol, germacrene A and D, elemicin, ß-elmene, (Z)-ocimine) (Labra et al., 2004; Lal et al., 2003; Lal, 2014; Zheljazkov et al., 2008) are exported to European countries in sizable quantity every year. *Ocimum* has several medicinal propertie as well as it is also rich in carbohydrates, fiber, phosphorous, calcium, protein, iron, beta-carotene, vitamins B1 and B2 and in aromatic oils (Costa et al., 2015; Costa et al., 2016; Ismaile, 2006; Lal, 2012). It is good for colds and coughs, indigestion, stomach pain and diarrhoea. Nausea, ulcers, ringworm and asthma (Ojo et al., 2012). It is said to lower blood sugar and increase lactation. The oil is used as anti-perspirant and as fly and mosquito repellent (Anonymous, 1973; Da Costa et al., 2014; Verma et al., 2011). Several name derivations and beliefs are associated with basil, but the common name basil is probably derived from the Greek words *basileus* meaning "king" or *basilikon* meaning "royal".

The study of genetic variability for diverse morpho-economic traits in the *Ocimum* genetic stocks is a prelude to potential crop improvement, to keeping in the mind above high importance, the genetic divergence among them was quantified by multivariate analysis with objective: to assess the proximity of accessions each other and classify them in to different clusters/groups for further utilization in heterosis breeding programme.

2. Materials and methods

Primarily fifty collections were assembled from eight states (Bihar, Chandigarh, Uttar Pradesh, Rajasthan, Uttarakhand, Maharashtra, Andhra Pradesh and Karnataka) of India. Removing of duplicates, twenty five accessions were examined for high herb and essential oil yield with better essential oil quality in the initial evaluation trail (IET, entries = 25; RBD, reps = 3; plot size = 1.5m^2) in field (Table 1). They were grown in the two consecutive years 2012-13 and 2013-14 at the research farm of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, 226 015, U.P. (India). The plants were planted in one row of 3 m long and 50 cm x 30 cm rows to rows/plants to plants. The experimental site of the institute research farm was located at 26.5° N latitude and 80.50°E longitude, and 120 m above mean sea level. The climate was semiarid – subtropical in nature. The plants received normal cultural operations, irrigation, and fertilizer applications (50 kg N + 40 kg P_2O_5 + 40 kg K_2O/ha). These twenty five accessions of *Ocimum* (Fig. 1) were belonging to four *Ocimum* species namely accession of *Ocimum basilicum*-18; *Ocimum sanctum*-6 and *Ocimum*

* Corresponding author.
E-mail address: cimap.smita@gmail.com (S. Singh).

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Table 1
Accessions of *Ocimum* and their geographic origin.

S.N0	Code	Accessions	Botanical name	Origin
1	G1	French basil	*Ocimum basilicum*	Chennai, A.P., (India)
2	G2	Vikarsudha	*Ocimum basilicum*	CSIR-CIMAP, Lucknow U.P. (India)
3	G3	Sweet basil	*Ocimum basilicum*	Gandhi Nagar Gujarat (India)
4	G4	French basil	*Ocimum basilicum*	Bangalore, Karnataka (India)
5	G5	French basil	*Ocimum basilicum*	Mangalore, Karnataka (India)
6	G6	French basil	*Ocimum basilicum*	Chandigarh (India)
7	G7	Shaym tulsi (CIM Angana)	*Ocimum sanctum*	CSIR-CIMAP, Lucknow U.P. (India)
8	G8	Sweet basil	*Ocimum basilicum*	Singapore
9	G9	Sweet basil	*Ocimum basilicum*	Singapore
10	G10	Sweet basil (Kushmohak)	*Ocimum basilicum*	CSIR-CIMAP, Lucknow U.P. (India)
11	G11	Sweet basil	*Ocimum basilicum*	Košice, Slovak Republic
12	G12	Krishna tulsi (CIM Ayu)	*Ocimum sanctum*	CSIR-CIMAP, Lucknow U.P. (India)
13	G13	French basil	*Ocimum basilicum*	Mangalore, Karnataka (India)
14	G14	Indian basil	*Ocimum basilicum*	Muzaffarpur, Bihar (India)
15	G15	Indian basil (CIM Saumya)	*Ocimum basilicum*	CSIR-CIMAP, Lucknow U.P. (India)
16	G16	Holi Basil	*Ocimum sanctum*	Udaipur, Rajasthan (India)
17	G17	Zanzibar basil	*Ocimum basilicum*	Tanzania
18	G18	Indian basil	*Ocimum basilicum*	Bareilly, Uttaranchal, (India)
19	G19	Scare Basil (CIM Kanchan)	*Ocimum sanctum*	CSIR-CIMAP, Lucknow U.P. (India)
20	G20	Indian basil	*Ocimum basilicum*	Lucknow, U.P. (India)
21	G21	Indian basil	*Ocimum basilicum*	Lakhimpur (Kheri), U.P. (India)
22	G22	Kapoor/camphor basil	*Ocimum kilimandscharicum*	CSIR-CIMAP, Lucknow U.P. (India)
23	G23	Shaym tulsi	*Ocimum sanctum*	Nasik, Maharashtra (India)
24	G24	Holi Basil	*Ocimum sanctum*	Lucknow, U.P. (India)
25	G25	Indian basil (sel-2)	*Ocimum basilcum*	CSIR-CIMAP, Lucknow U.P. (India)

kilimandscharium (Champhor basil)-1.

2.1. Essential oil analysis

The fresh 100 g aerial part of plant was collected and processed by hydrodistillation for 3 h in a Clevenger apparatus to obtain the essential oil (Clevenger, 1928). Identification of the essential oil composition was analyzed by gas chromatography (GC) and (MS).

2.2. Statistical analysis

The Pooled mean data of two years were statistically analyzed on the five morpho-economic traits like plant height (cm), primary branches/plant, fresh herb yield/plot (gm), oil content (%) and oil yield/plot (gm) using D^2 statistical analysis (Mahalanobis, 1936). We have used two following methods for clustering a) Tocher method (Rao, 1952) and b) Canonical root method (Mahalanobis, 1936). Based on the degree of divergent 25 genotype were grouped in to 7 clusters namely cluster I to VII such that the genotype within the cluster had smaller D^2-value among themselves that these between clusters (Table 2). Thus intra cluster value for divergence for each of the clusters were observed to lesser than inter cluster values of divergence in all cases (Table 3) as i) average intra cluster distance For the measure of intra cluster distance the formula is $\Sigma D_i^2/n$, where ΣD_i^2 is the sum of distance between all possible combinations, (n) of the populations included in a cluster. ii) average inter cluster distance. The procedure of calculating the inter cluster distance is first to measure the distance between cluster I and II, between cluster I and III, cluster I and IV, between cluster II and III and cluster II and IV and so on. Likewise the clusters are taken one by one and their distance from other clusters calculated.

The cluster formation was also confirmed by Tocher's methods (Rao, 1952) followed by the spatial distribution of genotypes in a $\lambda 1-\lambda 2$ chart (Fig. 2) based on statistical software ver. 0.3 available at CSIR-CIMAP in the Department of Genetics and Plant Breeding based on (Panse and Sukhatme, 1967; Singh and Chaudhury, 1979).

3. Results

The analysis of variance (ANOVA) from the pooled mean data over two years revealed highly significant differences ($P < 0.01$) for all the five characters studied indicating the presence of considerable amount of genetic variations among the twenty five accessions of *Ocimum* (Figs. 3–6). Individual D^2 values for all the $n(n-1)/2 = 300$ pairs were also calculated for all the 25 accessions. The genetic variability in the accessions was relatively very large, although 40% accessions could be grouped in cluster I and II and additional 32% within cluster III and IV followed by 12% in the cluster V and rest 8% each in cluster VI and VII. The cluster VI and VII were related and distinctly placed from the rest of others cluster were highly divergent. The magnitude and extent of genetic variability among twenty five accessions, between any pair of

Table 2
Cluster distribution of accessions of *Ocimum.*

Cluster No.	Accessions in cluster	Accessions included in clusters
I	5	$\mathbf{G_4}$ (Bangalore, Karnataka (India), $\mathbf{G_{10}}$ (CSIR-CIMAP, Lucknow U.P. (India), $\mathbf{G_{19}}$ (CSIR-CIMAP, Lucknow U.P. (India), $\mathbf{G_{20}}$ (Lucknow, U.P. (India), $\mathbf{G_{24}}$ (Lucknow, U.P. (India)
II	5	$\mathbf{G_5}$ (Mangalore, Karnataka (India), G15 (CSIR-CIMAP, Lucknow U.P. (India), $\mathbf{G_{16}}$ (Udaipur, Rajasthan (India), $\mathbf{G_{21}}$ (Lakhimpur (Kheri), U.P. (India), $\mathbf{G_{23}}$ (Nasik, Maharashtra (India)
III	4	$\mathbf{G_1}$ (Chennai, A.P., (India), $\mathbf{G_6}$ (Chandigarh), $\mathbf{G_8}$ (Singapore), $\mathbf{G_{17}}$ (Tanzania)
IV	4	$\mathbf{G_2}$ (CSIR-CIMAP, Lucknow U.P. (India), $\mathbf{G_{13}}$ (Mangalore, Karnataka (India), $\mathbf{G_{14}}$ (Muzaffarpur, Bihar (India), $\mathbf{G_{22}}$ (CSIR-CIMAP, Lucknow U.P. (India)
V	3	$\mathbf{G_3}$ (Gandhi Nagar Gujarat (India), $\mathbf{G_9}$ (Singapore), $\mathbf{G_{25}}$ (CSIR-CIMAP, Lucknow U.P. (India)
VI	2	$\mathbf{G_7}$ (CSIR-CIMAP, Lucknow U.P. (India), $\mathbf{G_{18}}$ (Bareilly, Uttaranchal, (India)
VII	2	$\mathbf{G_{11}}$ (Košice, Slovak Republic), $\mathbf{G_{12}}$ (CSIR-CIMAP, Lucknow U.P. (India)