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Efficacy of trait selection in *Ocimum gratissimum* L. against differential plant population density stress

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Abstract
Ocimum gratissimum L. (Lamiaceae) is a valuable medicinal and aromatic plant which has a number of important pharmacological properties. The present investigation aims to assess the variability available within the population corresponding to twenty three characters at four levels of plant spacing. Significant differences among the treatment levels were observed for most of the traits in the population of *O. gratissimum* evaluated in the study. Higher observations for all the genetic parameters were recorded for total number of branches, stem diameter(cm), live plants, total plants/bed, dead plants, mortality(%), herb weight for oil, recovery(%) herb oil, recovery(%) herb + flower, herb_Germacrene-D(%), herb_z-b-Ocimene(%) and inflo_Germacrene-D(%) revealing the importance of these traits in phenotypic selection against varying plant densities. Out of all the levels of plant spacing treatment, 0.50m×0.50m came out to be best suited for optimum production of eugenol as well as germacrene-D in both herb as well as inflorescence.

Keywords: Density stress, essential oil, eugenol, germacrene-D, ocimene, *Ocimum gratissimum*

Introduction
Ocimum gratissimum L. (2n=40), Lamiaceae commonly called clove basil is an erect aromatic shrub native to India [9]. The common uses of the plant are in traditional medicine to cure different diseases, e.g. upper respiratory tract infections [5], diarrhoea [3], headache [4], ophthalmia, otitis and skin diseases [7], pneumonia [22], and cough. *O gratissimum* is vernacularly called Ram tulsi (Hindi) or Nimma tulasi (Kannada) which is distributed and cultivated in the tropical regions of India. This species performs well as a commercial source of eugenol for India, and several breeding programs have been carried out for this natural product [17].
Sobti *et al.* [23] reported that the essential oil of *O. gratissimum* cultivated in India contained about 60-80% eugenol, whereas the improved commercial populations estimated to have 55-80% eugenol [24]. There are four reported chemotypes of *O. gratissimum* that are rich in thymol (33-44%), eugenol (55-62%), citral (57-67%) and ethyal cinnamate (50-67%) [2, 10, 12]. Eugenol has anesthetic, hypothermic, myorelaxant and anticonvulsant properties (13) and 1,8-cineole presents stimulant activity [28].
The plant attains a height of about 1-2 m during full bloom, is branched, produces woody stem at the base (Fig. 1-2). Leaves are oppositely arranged in branches, ovate in shape. Being a less demanding and hardy plant, it thrives in difficult and undulated terrain also. Generally, it grows as a weed on roadside, hillocks, pastures, wastelands etc. Sometimes it is also used as a hedge plant and also finds a place as an ornamental and aromatic plant in household gardens. Once planted *Ocimum gratissimum* offers multiple harvests in a year unlike the other species like holy and sweet basil. Clove basil is actually regarded as a cheaper source for a natural product called eugenol when compared to the tree species *Syzygium aromaticum* and *Cinnamomum verum*. Moreover, these tree species need to be cultivated in coastal areas for best secondary metabolite profiles while *O. gratissimum* has an advantage of being perennial and can be cultivated from arid to tropical areas.

Fig 1: Field view of *Ocimum gratissimum* L.

Fig 2: *Ocimum gratissimum* L. in flowering stage.

Naturally growing *Ocimum gratissimum* were collected from the Doon valley and were seed propagated in field condition at Centre for Aromatic Plants at Dehradun, Uttarakhand (Fig. 3). The seeds were germinated and the seedlings were transplanted in field condition to explore the variability available for the twenty three characters in the population of *Ocimum gratissimum* at four levels of plant spacing.

Materials and Methods

Plant material and field experiment: The seeds of *Ocimum gratissimum* were collected from the Doon valley of Uttarakhand and were germinated to produce seedlings in the last week of July, 2022. Thirty days old seedlings were transplanted during last week of August, 2022 in RBD in four different spacing *viz*. 1m×1m, 0.50m×0.50m, 0.70 m×0.40 m and 0.90 m×0.50 m (rr-pp) in six replicates at Centre for Aromatic Plants (CAP), Industrial Area, Selaqui, Uttarakhand-248011, India. Normal agricultural practices were performed all-round the growing season and the data for twenty-three morphometric and chemometric observation were recorded during flowering i.e. the last week of November, 2022.

The experimental area is located at N30.21.812' latitude and E77.51' longitude having an altitude 487 m at the foot of the Himalayas. During summers, the temperature ranges between 36 °C and 16.7 °C and in winters, the temperature lies between 23.4 °C and 5.2 °C with an average rainfall of 2073.3mm annually. Maximum rainfall occurs between June and September; however in December and January the region also receives winter rainfall.

Fig 3. *Ocimum gratissimum* L. (a) in bud stage (b) in flowering stage (c)e & (d) flowers

The freshly harvested aerial herbage (500 g) in triplicate was hydrodistilled for 2h in a Clevenger type apparatus. The distillate (essential oil) was recovered and dried using anhydrous sodium sulphate. The dried essential oils were stored in glass vials at 4-8 °C temperature for further analysis.

1. Analysis of essential oils using Gas chromatography and gas chromatography–mass spectrometry analyses

GC analyses were carried out by an Agilent Technology 8890 gas chromatograph with 7693A autosampler data handling system equipped with a split/splitless injector and fitted with FID using N2 as the carrier gas. The column was HP-5 capillary column (30 m × 0.32 mm × 0.25 µm film thickness) and temperature program was used as follows: initial temperature of 60 °C (hold: 2 min) programmed at a rate of 3 °C/min to a final temperature of 240 °C (hold: 5 min). Temperatures of the injector and detector were maintained at 210 & 250 °C respectively. The injection volume was 0.5 µL.

The gas chromatography-mass spectrometry (GC-MS) analyses of the oils were performed with a Agilent Technology 8890 gas chromatograph with PAL RSI 85 Autosampler equipped with a split/splitless injector (split ratio 1:50) data handling system. The column was HP-5MS UI capillary columns (30 m × 0.25 mm × 0.25 µm film thickness). Helium was the carrier gas at a flow rate 0.80 mL/min. The GC was interfaced with (Agilent GC-MS/MS_7010 B system) mass detector operating in the EI+ mode. Temperature program used was the same as described above for GC analyses. The temperatures of the injector, transfer line and ion source were maintained at 280 °C. Mass spectra was taken over m/z 40-450 amu that revealed the total ion current, using an ionizing voltage of 70 eV. Identification of compounds: The identification of constituents was performed by matching their recorded mass spectra with installed MS library (NIST 2.3 and Wiley FFNSC) and available literature [1].

2. Statistical analysis

The mean data for twenty-three traits of four spacing treatments were offered to statistical analysis using statistical software ver 0.3 based on Singh and Choudhary [25] and Panse and Sukhatme [19] for ANOVA and correlation analysis.

Results and Discussion

ANOVA for all the twenty-three traits, evaluated during the 1st-year trial, has been presented in Table 1. A perusal of the table revealed highly significant (p<0.01) differences among the treatment means for almost all the traits except total number of inflorescence which was found to be significant at 5% level of significance. However, no significant treatment differences were observed for the two traits *viz.* days to 25% flowering and days to 50% flowering.

Variability is the most important characteristic feature of any population. Estimation of variability is an important prerequisite for realizing the response to selection as the progress in the breeding depends upon its amount, nature and magnitude. In the present investigation, the variability available for the twenty three traits in the population of *O. gratissimum* was analysed at four levels of plant spacing (shift it to introduction last para). Therefore, the effect of plant population densities on the genetic variability of traits was assessed in the present study (Table 2). Estimates of genetic parameters for all the 23 morphological traits in *O. gratissimum* revealed high PCV as well as GCV for the total number of branches, inflorescence length (cm), stem diameter (cm), live plants, total plants/bed, dead plants, mortality (%), herb weight for oil (g), recovery (%) herb oil, recovery (%) herb+flower, herb_germacrene-D (%), herb_z-b-Ocimene (%) and inflo_germacrene-D (%). High values for PCV and GCV indicate low environmental influence for these traits, which in turn account for their effective selection in improvement programs. A moderate amount of genetic variability for the traits *viz.* plant height (cm), the total number of inflorescence, leaf length (cm), leaf width (cm), inflo_z-b-Ocimene (%) and herb +flower for oil (g) implicates that there is considerable scope for improving these traits in a desirable direction through a selection programme against density

stresses. A similar trend for variability in morphological traits was observed by Gupta [11] and Panwar, *et al.* [20].

Heritability (broad sense) refers to the proportion of total variation that may be transmitted down to the next generation; consequently, high heritability accounts for the repeatability of performance for the selected attributes. In *O. gratissimum*, amongst the traits studied, moderate to high values for broad sense heritability was observed for all the traits except days to 25% flowering and days to 50% flowering indicating a higher selection efficacy of these traits in genetic improvement due to less influence from the environment, as reported by Khodadadi *et al.* [16], Sumathi *et al.* [26] and Asaigbe *et al.* [3].

The most heritable trait in the *O. gratissimum* was observed to be the herb weight for oil (g) followed by total plants/bed, live plants, inflo_z-b-Ocimene (%), inflo_Germacrene-D (%), recovery (%) herb+flower, herb_Germacrene-D (%), dead plants, herb_Eugenol (%) and herb_z-b-Ocimene (%) respectively. These results are in agreement with the findings of Pilania *et al.* [21] and Thoppil and Jose [27] who studied broad-sense heritability in some quantitative and qualitative traits with oil content.

Heritability alone is not a reliable indicator of genetic improvement, hence estimates of heritability should be combined with genetic advance expressed as a percent of the mean for a deeper understanding of genetic improvement [14]. All the traits exhibited high heritability combined with high genetic advance except for inflorescence length (cm), total no. of inflorescence, herb_Eugenol (%) and inflo_Eugenol (%) indicating the traits could respond effectively to selection in all the treatment levels due to the preponderance of fixable additive gene action. Moderate heritability with moderate genetic advance was observed for total no. of inflorescence whereas, high heritability with moderate genetic advance as per cent of mean was observed for herb_Eugenol (%) and inflo_Eugenol (%). Low values of genetic advance for these traits indicated non-additive gene effect.

Among all the traits studied, the total number of branches, stem diameter (cm), live plants, total plants/bed, dead plants, mortality (%), herb weight for oil (g), recovery (%) herb oil, recovery (%) herb+flower, herb_Germacrene-D (%), herb_z-b-Ocimene (%) and inflo_Germacrene-D (%), recorded a high amount of genetic variability along with heritability(BS) and genetic advance. Therefore, there could be greater scope for improving these traits by simple phenotypic selection against all the spacings. Selection would be more meaningful if the structure of yield is probed through its components because the polygenic nature of yield eludes the breeder of the selection schemes that tend to select for yield *per se*. This is biometrically achieved by estimating the correlation coefficients (Tables 3-4). Direct selection of genotypes for yield *per se* is not feasible due to its complex nature, therefore identification of yield attributing traits has become highly necessary [15]. The estimates of correlation coefficients mostly indicate the inter-relationships of the traits [30].

Out of all the twenty three yield attributing traits, plant height (cm), total number of branches, inflorescence length (cm), leaf width (cm), stem diameter (cm), mortality (%), herb weight for oil (g) and herb_z-b-Ocimene(%) exhibited strong positive correlation at both phenotypic as well as genotypic levels, indicating a great influence of these traits on final yield herb with inflorescence for essential oil while number of live plants and total plants/bed exhibited strong negative genotypic as well as phenotypic correlations. The traits such as days to 50% flowering, recovery (%) herb oil and herb_Eugenol (%) have shown significant genotypic correlation with yield but non significant phenotypic correlation. These types of trait associations can be important when practising indirect selection under productivity target breeding programmes for crop improvement under density stress tolerance. Similar kinds of genetic relationships were reported by Ibrahim *et al.* [13] in *O. basilicum* and other crops by Baslma [4] and Mijic *et al.* [18].

Table 1: Analysis of variance for morphological traits evaluated in *Ocimum gratissimum*

S. No.	Traits	Codes	Replications (MS) df=5	Treatments (MS) df=3	Error(MS) df=15
1.	Plant height (cm)	T1	62.815	1259.469**	26.127
2.	25% flowering (days)	T2	12.767	5.000	7.500
3.	50% flowering (days)	T3	8.367	13.611	12.478
4.	Total Branches	T4	70.525	1576.362**	135.125
5.	Inflorescence length (cm)	T5	3.890	32.034**	4.270
6.	Total no. Inflorescence	T6	242.667	738.833*	195.867
7.	Leaf length (cm)	T7	1.883	33.847**	3.113
8.	Leaf width (cm)	T8	0.789	5.391**	0.525
9.	Stem diameter (cm)	T9	0.046	1.189**	0.065
10.	Number of live plants	T10	7.874	3240.203**	3.679
11.	Number of plants/bed	T11	1.074	3582.264**	2.416
12.	Number of dead plants	T12	0.842	522.042**	2.842
13.	Mortality (%)	T13	9.853	848.661**	16.368
14.	Herb weight for oil (g)	T14	4.056	68205.568**	7.879
15.	Recovery (%) herb oil	T15	0.002	0.129**	0.002
16.	Recovery (%) herb+flower	T16	0.002	0.178**	0.001
17.	Herb_Eugenol (%)	T17	1.754	214.844**	1.242
18.	Herb_Germacrene-D (%)	T18	0.388	32.552**	0.175
19.	Herb_z-b-Ocimene (%)	T19	0.370	57.839**	0.438
20.	Inflo_Eugenol (%)	T20	0.232	102.475**	0.900
21.	Inflo_Germacrene-D (%)	T21	0.130	16.996**	0.065
22.	Inflo_z-b-Ocimene (%)	T22	0.243	35.179**	0.132
23.	Herb +flower for oil (g)	T23	582.100	19858.778**	632.344

*= p <0.05, **= p <0.01 respectively.

Table 2: Genetic parameters for morphological traits in *Ocimum gratissimum*

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23
Genotypic variance	205.558	-0.417	0.189	240.206	4.627	90.494	5.122	0.811	0.187	539.421	596.641	86.533	138.716	11366.280	0.021	0.030	35.601	5.396	9.567	16.929	2.822	5.841	3204.406
GCV (%)	14.397	1.233	0.654	31.672	24.443	14.961	15.444	15.472	31.864	42.984	39.424	153.970	126.050	58.068	35.895	27.713	8.943	24.398	22.481	5.814	21.210	19.426	18.349
Phenotypic variance	231.685	7.083	12.667	375.331	8.897	286.361	8.236	1.336	0.253	543.100	599.057	89.375	155.083	11374.160	0.023	0.030	36.843	5.571	10.005	17.829	2.887	5.973	3836.750
PCV (%)	15.284	5.086	5.359	39.590	33.895	26.614	19.583	19.859	37.014	43.130	39.503	156.477	133.279	58.088	37.686	28.140	9.097	24.790	22.990	5.967	21.453	19.644	20.078
h² (Broad Sense)%	0.887	-0.059	0.015	0.640	0.520	0.316	0.622	0.607	0.741	0.993	0.996	0.968	0.894	0.999	0.907	0.970	0.966	0.969	0.956	0.949	0.977	0.978	0.835
Genetic Advance over mean (%)	27.935	-0.616	0.165	52.194	36.313	17.326	25.091	24.831	56.508	88.246	81.049	312.094	245.578	119.578	70.428	56.225	18.108	49.465	45.287	11.671	43.196	39.574	34.544

Where, T1=Plant height (cm), T2=25% flowering(days), T3=50% flowering(days), T4=Total Branches, T5=Inflorescence length (cm), T6=Total no. Inflorescence, T7=Leaf length(cm), T8=Leaf width(cm), T9=Stem diameter(cm), T10=Live plants, T11=Total plants/bed, T12=Dead plants, T13=Mortality(%), T14=Herb weight for oil(g), T15= Recovery%herb oil, T16=Recovery%herb+flower, T17=Herb_Eugenol(%), T18=Herb_Germacrene-D(%), T19=Herb_z-b-Ocimene(%), T20=Inflo_Eugenol(%), T21= Inflo_Germacrene-D(%), T22=Inflo_z-b-Ocimene(%), T23= Herb+flower for oil (g)

Table 3: Genotypic correlation coefficients among yield and their attributing morphological traits in *Ocimum gratissimum*

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23
T1	1																						
T2	-0.137	1																					
T3	0.815**	-0.945**	1																				
T4	0.987**	-0.323	0.773**	1																			
T5	0.176	-0.113	0.169	0.512*	1																		
T6	-0.477*	0.211	-0.022	-0.298	0.967**	1																	
T7	0.770**	-0.514*	0.487*	0.909**	0.908**	0.352	1																
T8	0.106	-0.387	0.644**	0.125	0.442*	-0.184	0.944**	1															
T9	0.302	-0.749**	0.26	0.451*	0.959**	0.825**	0.849**	0.515*	1														
T10	-0.464*	0.560**	-0.165	-0.508*	-0.574**	-0.663**	-0.724**	-0.524**	-0.927**	1													
T11	-0.044	0.268	-0.600**	-0.144	-0.721**	-0.123	-0.546**	-0.177	-0.955**	0.894**	1												
T12	0.888**	-0.587**	0.219	0.757**	-0.367	-0.851**	0.337	0.727**	-0.136	-0.163	0.298	1											
T13	0.983**	-0.905**	0.287	0.910**	-0.115	-0.670**	0.558**	0.881**	0.107	-0.347	0.108	0.973**	1										
T14	0.730**	-0.371	0.345	0.922**	0.843**	0.226	0.138	0.945**	0.746**	-0.580**	-0.419*	0.311	0.525**	1									
T15	-0.450*	-0.038	-0.638**	-0.293	0.147	-0.135	-0.262	-0.307	-0.418*	0.780**	0.538**	-0.475*	-0.522**	-0.101	1								
T16	0.685**	0.893**	-0.366	-0.714**	0.056	0.866**	-0.375	-0.696**	0.298	-0.31	-0.611**	-0.685**	-0.681**	-0.452*	-0.308	1							
T17	-0.721**	0.12	-0.277	-0.807**	-0.17	0.690**	-0.550**	-0.804**	0.095	-0.18	-0.455*	-0.616**	-0.660**	-0.619**	-0.319	0.982**	1						
T18	0.384	-0.427*	0.218	0.362	-0.38	-0.157	-0.031	0.352	-0.657**	0.645**	0.876**	0.552**	0.455*	0.071	0.477*	-0.925**	-0.834**	1					
T19	0.735**	-0.34	0.533**	0.918**	0.859**	0.219	0.152	0.952**	0.772**	-0.634**	-0.468*	0.317	0.536**	0.902**	-0.162	-0.413*	-0.583**	0.02	1				
T20	-0.814**	0.915**	-0.497*	-0.794**	0.144	0.930**	-0.410*	-0.781**	0.245	-0.157	-0.536**	-0.846**	-0.836**	-0.456*	-0.056	0.974**	0.939**	-0.859**	-0.430*	1			
T21	0.29	-0.115	0.291	0.526**	0.653**	-0.086	0.582**	0.533**	0.165	0.179	0.171	-0.007	0.108	0.675**	0.661**	-0.664**	-0.780**	0.509*	0.627**	-0.492*	1		
T22	0.642**	-0.072	0.142	0.438*	-0.718**	-0.125	-0.049	0.413*	-0.484*	0.122	0.542**	0.931**	0.818**	-0.062	-0.375	-0.601**	-0.460*	0.623**	-0.061	-0.756**	-0.205	1	
T23	0.779**	-0.19	0.520**	0.914**	0.775**	0.291	0.142	0.929**	0.870**	-0.827**	-0.629**	0.38	0.598**	0.955**	-0.436*	-0.251	-0.409*	-0.16	0.973**	-0.329	0.382	-0.004	1

*= p <0.05, **= p <0.01 respectively.

Where, T1=Plant height(cm), T2=25% flowering(days), T3=50% flowering(days), T4=Total Branches, T5=Inflorescence length (cm), T6= Total no. Inflorescence, T7=Leaf length(cm), T8=Leaf width(cm), T9=Stem diameter(cm), T10=Live plants, T11=Total plants/bed, T12=Dead plants, T13=Mortality(%), T14=Herb weight for oil(g), T15=Recovery% herb oil, T16=Recovery% herb+flower, T17=Herb_Eugenol (%), T18= Herb_Germacrene-D(%), T19= Herb_z-b-Ocimene(%), T20=Inflo_Eugenol(%), T21= Inflo_Germacrene-D(%), T22=Inflo_z-b-Ocimene(%), T23= Herb +flower for oil (g)

Table 4: Phenotypic correlation coefficients among yield and their attributing morphological traits in *Ocimum gratissimum*

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23
T1	**1**																						
T2	0.167	**1**																					
T3	0.361	0.16	**1**																				
T4	0.867**	0.272	0.257	**1**																			
T5	0.072	0.125	0.25	0.085	**1**																		
T6	-0.208	0.137	-0.218	-0.088	0.176	**1**																	
T7	0.643**	0.136	0.267	0.791**	0.397	0.028	**1**																
T8	0.727	0.232	0.334	0.688**	0.409*	-0.238	0.749**	**1**															
T9	0.299	0.202	0.066	0.432*	0.515*	0.647**	0.597**	0.341	**1**														
T10	-0.432*	-0.099	-0.212	-0.389	-0.417*	-0.349	-0.577**	-0.415*	-0.776**	**1**													
T11	-0.04	-0.066	-0.103	-0.114	-0.523**	-0.560**	-0.419*	-0.12	-0.822**	0.889**	**1**												
T12	0.835**	0.081	0.257	0.590**	-0.255	-0.449*	0.309	0.599**	-0.118	-0.166	0.296	**1**											
T13	0.891**	0.072	0.319	0.669**	-0.083	-0.327	0.509*	0.707**	0.061	-0.34	0.109	0.961**	**1**										
T14	0.689**	0.335	0.4	0.738**	0.614**	0.124	0.818**	0.732**	0.639**	-0.578**	-0.419*	0.305	0.495*	**1**									
T15	-0.409*	0.075	-0.085	-0.239	0.055	-0.086	-0.157	-0.169	-0.392	0.732**	0.521**	-0.420*	-0.423*	-0.099	**1**								
T16	0.621**	-0.238	-0.242	-0.571**	0.044	0.513*	-0.308	-0.569**	0.233	-0.305	-0.603**	-0.655**	-0.613**	-0.444*	-0.288	**1**							
T17	-0.656**	-0.257	-0.335	-0.635**	-0.129	0.392	-0.452*	-0.610**	0.096	-0.176	-0.444*	-0.590**	-0.611**	-0.609**	-0.278	0.955**	**1**						
T18	0.375	0.108	0.17	0.29	-0.279	-0.520**	-0.054	0.222	-0.551**	0.633**	0.859**	0.544**	0.436*	0.072	0.445*	-0.884**	-0.801**	**1**					
T19	0.708**	0.34	0.325	0.753**	0.554**	0.253	0.802**	0.706**	0.701**	-0.608**	-0.452*	0.308	0.497*	0.979**	-0.147	-0.391	-0.554**	0.032	**1**				
T20	-0.767**	-0.163	-0.283	-0.647**	0.126	0.480*	-0.347	-0.607**	0.205	-0.143	-0.526**	-0.830**	-0.801**	-0.445*	-0.06	0.934**	0.890**	-0.841**	-0.406*	**1**			
T21	0.282	0.277	0.201	0.441*	0.468*	-0.005	0.480*	0.471*	0.166	0.18	0.175	0.005	0.116	0.666**	0.637**	-0.645**	-0.750**	0.493*	0.624**	-0.479*	**1**		
T22	0.603**	0.011	0.09	0.339	-0.503*	-0.563**	-0.049	0.284	-0.401	0.12	0.532**	0.903**	0.763**	-0.06	-0.358	-0.573**	-0.444*	0.604**	-0.06	-0.722**	-0.204	**1**	
T23	0.674**	0.297	0.128	0.671**	0.527**	0.304	0.764**	0.696**	0.723**	-0.753**	-0.560**	0.346	0.532**	0.874***	-0.386	-0.214	-0.379	-0.155	0.901**	-0.309	0.376	0.018	**1**

*= p <0.05, **= p <0.01 respectively.

Where, T1=Plant height(cm), T2=25% flowering(days), T3=50% flowering(days), T4=Total Branches, T5=Inflorescence length(cm), T6=Total no. Inflorescence, T7=Leaf length(cm), T8= Leaf width(cm), T9=Stem diameter(cm), T10= Live plants, T11=Total plants/bed, T12=Dead plants, T13= Mortality(%), T14=Herb weight for oil(g), T15= Recovery % herb oil, T16=Recovery % herb+flower, T17= Herb_Eugenol (%), T18= Herb_Germacrene-D(%), T19= Herb_z-b-Ocimene(%), T20=Inflo_Eugenol(%), T21=Inflo_Germacrene-D(%), T22=Inflo_z-b-Ocimene(%), T23=Herb +flower for oil (g)

Table 5: The treatment means ($\bar{x}$) of eugenol and germacrene-D over four spacings of *Ocimum gratissimum* L.

Spacing	T17	T18	T20	T21
1m×1m	75.4	6.978	75.765	5.625
0.50m×0.50m	64.487	11.39	70.372	9.183
0.60m×0.50m	61.717	11.603	65.68	7.693
0.60m×0.70m	65.285	8.113	71.238	9.178
Mean	66.722	9.521	70.764	7.92
C.V.	1.670	4.391	1.341	3.224
S.E.	0.455	0.171	0.387	0.104
C.D. 5%	1.371	0.515	1.168	0.314
C.D. 1%	1.896	0.711	1.615	0.434
Range Lowest	61.717	6.978	65.68	5.625
Range Highest	75.4	11.603	75.765	9.183

Where, T17= Herb_Eugenol (%), T18= Herb_Germacrene-D (%), T20= Inflo_Eugenol (%), T21= Inflo_Germacrene-D (%)

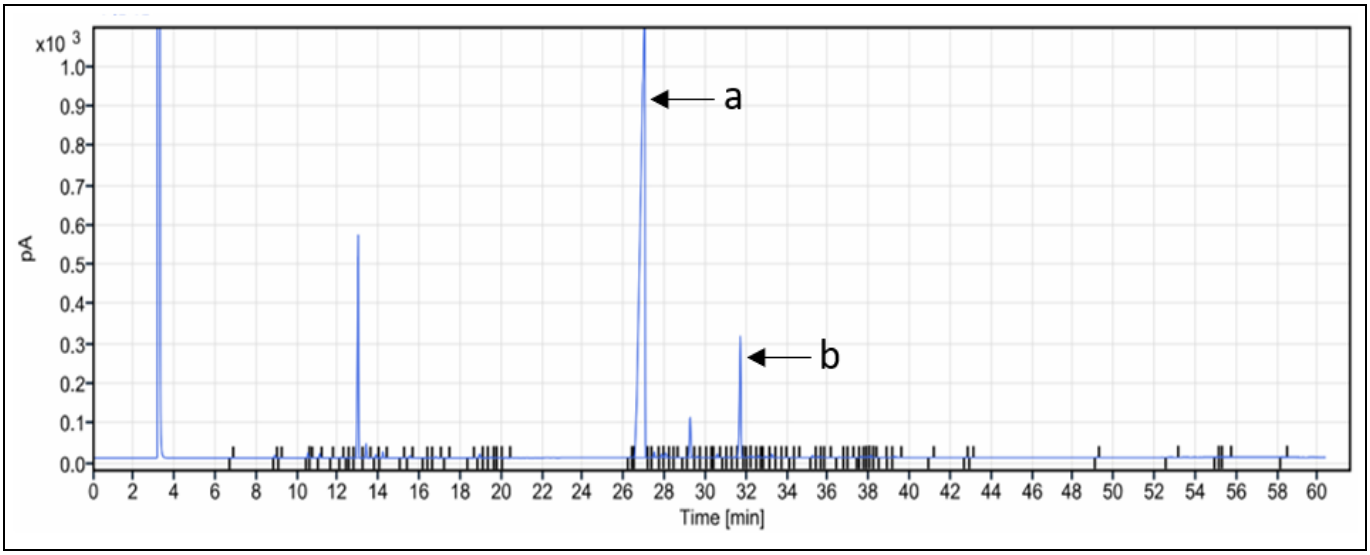

Fig 4: G.C.M.S chromatogram representing the presence of (a) Eugenol (b) Germacrene-D in the essential oil extracted from herb and inflorescence of *O. gratissimum* in 0.50m×0.50m spacing.

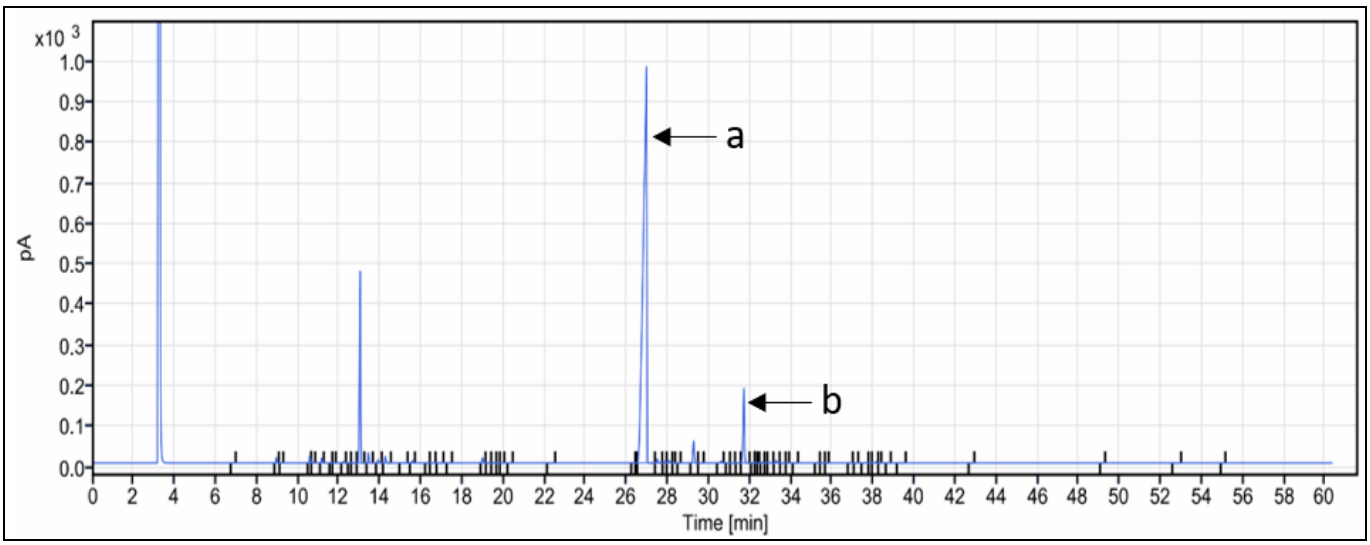

Fig 5: G.C.M.S chromatogram representing the presence of (a) Eugenol (b) Germacrene-D in the essential oil extracted from inflorescence of *O. gratissimum* in 0.50m×0.50m spacing.

Although, eugenol from herb was abundantly produced (75.4%) in spacing 1m×1m the same spacing was poor for germacrene-D (6.98%) in the essential oil extracted for herb (Table 5). However, spacing 0.50m×0.50m was optimum for both – higher production of eugenol (64.49%) and germacrene-D (11.39%) from herb (Fig. 4). Similarly, eugenol from inflorescence was estimated to be highest (75.77%) in spacing 1m×1m whereas, germacrene-D from inflorescence was recorded higher out of all the spacings for 0.50m×0.50m (9.18%) and 0.60m×0.70m (9.18%). Therefore, spacings 0.50m×0.50m and 0.60m×0.70m were found to be optimum for both eugenol from inflorescence i.e. 70.37% and 71.24% respectively, as well as germacrene-D from inflorescence (Fig. 5).

Conclusion

From the foregoing discussion on variability analysis it was deduced that all the three genetic parameters *viz*., variability, heritability and genetic advance were influenced by plant densities. The correlation analysis revealed that the yield trait i.e. herb +flower for oil possessed strong genetic association with the morphological traits. The present study revealed that the five component traits–total number of branches, stem diameter, mortality, herb weight for oil and herb_z-b-ocimene emerged as most important traits for the improvement of oil yield in *O. gratissimum*. Hence, due emphasis should be placed on these traits when breeding for high essential oil yield. Additionally, eugenol percent was observed to significantly increase in 1m×1m spacing treatment whereas germacrene-D percent significantly increased under 50 cm × 50 cm spacing suggesting the influence of plant population densities on chemical constitution of essential oil composition in *O. gratissimum*.

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Reference

1. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry Carol Stream: Allured Publishing Corporation. 2007;456:544-545.
2. Ali ME, Shamsuzzaman LAM. Sci. Res. (Dacca) 5, 91-94; via (1969) C. A. 1968;70:60746c.
3. Asaigbe PI, Ekpenyong UO, Uyoh EA, Brisibe EA.

Principal component analysis and heritability estimates in different accessions of African basil (*Ocimum gratissimum* L.). Canadian J Plant Breed. 2014;2(2):47-54.
4. Basalma D. The correlation and path analysis of yield and yield components of different winter rapeseed (*Brassica napus* ssp. *Oleifera* L.) cultivars. Res. J Agric. Biol. Sci. 2008;4(2):120-125.
5. Bouquet A. Féticheurs et médecines traditionelles du Congo (Brazzaville), ORSTOM (Office de Ia Recherche Scientifique et Technique Outre-Mer), Paris; c1969, p.143,.
6. Bouquet A, Debray M. Plantes médicinales de Ia Côte d'Ivoire, ORSTOM (Office de la Recherche Scientifique et Technique Outre-Mer), Paris. c1974. p. 97.
7. Dallmeier K, Carlini EA. Anesthetic, hypotermic, myorelaxant and anticonvulsant effects of synthetic eugenol derivatives and natural ana1logues. Pharmacology. 1981;22:113-12.
8. El-Said F, Sofowora EA, Malcolm SA, Hofer A. Planta Med. 1969;17:195-200.
9. Flore du Rwanda, Spermatophytes, (Troupin, G., ed), pp. INRS publication No. 30, Butare (Rwanda). 1985;3:321-327.
10. Gildemeister E, Hoffmann Fr. Die ätherischen Ole, 4. Auflage, Band Akademie Verlag, Berlin. 1961;7:502-505.
11. Gupta SC. Variation in herbage yield, oil yield and major component of various Ocimum species/varieties (chemotypes) harvested at different stages of maturity. Journal of Essential Oil Research. 1996;8(3):275-279.
12. Hegnauer R. Chemotaxonomie derPflanzen, Band Birkhäuser Verlag, BasellStuttgart. 1966;4:314.
13. Ibrahim MM, Aboud KA, Hussein RM. Genetic variability and path coefficient analysis in sweet basil for oil yield and its components under organic agriculture conditions. Journal of American Science. 2011;7(6):150-157.
14. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans 1. Agronomy journal. 1955;47(7):314-318.
15. Jyothsna S, Patro TSSK, Ashok S, Rani YS, Neeraja B. Studies on genetic parameters, character association and path analysis of yield and its components in finger millet (*Eluesine coracana* L. Gaertn). International Journal of Theoretical and Applied Sciences. 2016;8(1):25.
16. Khodadadi M, Fotokian MH, Miransari M. Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. Australian Journal of Crop Science. 2011;5(1):17-24.
17. Khosla MK. Breeding in genus Ocimum: phytochemical studies of essential oils. Indian Perfumer. 1988;32(3):236-247.
18. Mijić A, Liović I, Zdunić Z, Marić S, Marjanović-Jeromela A, Jankulovska M. Quantitative analysis of oil yield and its components in sunflower (*Helianthus annuus* L.). Romanian Agricultural Research. 2009;26:41-46.
19. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. I.C.A.R. New Delhi, second edition. c1967. p. 152-154.
20. Panwar NS, Kumar A, Malik SS, Dwivedi VK, Kumar G, Singh PB. Assessment of Variability Parameters for Agro-morphological and Phyto-chemical Traits in Basil (*Ocimum basilicum* L.) Germplasm. Indian Journal of Plant Genetic Resources. 2011;24(1):91-95.
21. Pilania DS, Pareek SK, Suneja P, Kumar A. Characterization of French basil (*Ocimum basilicum* L.) germplasm for essential oil yield and quality under stress environment. Indian perfumer. 2005;49:49-55.
22. Puyvelde L. van, Ngaboyisonga M, Rwangabo PC, Mukarugambwa SP, Kayonga A, Runyinya-Barabwiriza. Enquetes ethnobotaniques sur la médecine traditionelle rwandaise, Prefecture de Kibuye, Université Nationale du Rwanda, Butare. 1977;1:34.
23. Sobti SN, Pushpagandhan P, Atal CK. Genus Ocimum – A potential source of essential oils. Indian Perfm. 1976;20(1A):59-68.
24. Sobti SN, Pushpagandhan P, Bradu BL, Jain BB. Development of an eugenol containing Ocimum species. Indian Perfum. 1979;23(1):16-20.
25. Singh RK, Chaudhary BD. Biometrical methods in quantitative genetic analysis. Biometrical methods in quantitative genetic analysis. c1977.
26. Sumathi P, Madineni S, Veerabadhiran P. Genetic variability for different biometrical traits in pearl millet genotypes (*Pennisetum glaucum* LR BR.). Electronic journal of plant breeding. 2010;1(4):347-440.
27. Thoppil JE, Jose J. Intraspecific genetic control of major essential oil constituents in *Ocimum basilicum* L. Nucleus-Calcutta-International Journal of Cytology. 1994;37(1):30-33.
28. Umezu T, Sakata A, Ito H. Ambulation-promoting effect of pep1permint oil and identification of its active constituents. Pharmacology Biochemistry and Behavior. 2001;69:383-390.
29. Wong W. Econ. Bot. 1976;30:103-142.
30. Wright S. Systems of mating. I. The biometric relations between parent and offspring. Genetics. 1921;6(2):111.