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Variations in morphology, phenology and essential oil composition of sweet basil (*Ocimum basilicum* L.) germplasm accessions

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Abstract

Morphology, phenology and essential oil composition variations were analysed in germplasm accessions of sweet basil (*Ocimum basilicum* L.), an important medicinal and aromatic crop. Significant variations were observed for morphological and essential oil traits. All the fourteen accessions studied could be characterised as ten morphotypes ObC-A through ObC-J. Chemotypic indexing resulted in assigning three chemotypes namely, linalool, methyl chavicol and methyl cinnamate types on the basis of major oil component. Linalool chemotype could also be subgrouped into four subtypes as significant differences were observed in minor components too. Three phenological types were also defined based on genotype performance during different seasons.

Key words: essential oil, morphology, *Ocimum basilicum*, phenology, sweet basil.

Introduction

Sweet basil (*Ocimum basilicum* L. family Lamiaceae) is a widely cultivated species in tropical and warm temperate regions of the world. The plant is an aromatic herb/undershrub used in spices, perfumery, cosmetics, insect repellants, traditional medicines etc. and is also grown for ornamental purposes (Jain & Jain 1973; Dube *et al.* 1989). The plant also has been reported to possess antiseptic and antineoplastic properties (Fatope & Takeda 1988; Darokar *et al.* 1998). The plant is mainly autogamous, but high degree of cross pollination (up to 25%) has also been reported in some cultivars (Tesi *et al.* 1991; Nation *et al.* 1992). The plant possesses wide range of genetic variability as observed through differences in plant morphology and oil composition (Anon. 1966; Darrah 1974; Grayer *et al.* 1996). Genetic diversity is an important parameter utilised for crop improvement either by selection or breeding. The nature and extent of genetic variability is of great relevance to make strategic assessment of future approaches in genetic improvement (Simmonds 1962), in monitoring germplasm during maintenance (Moore & Collins 1993), and its utilization and management (Dwivedi *et al.* 1995, 1997). The present study aims at characterizing genetic variations at morphotypic, chemotypic and phenological levels that will in turn be useful in taking optimum advantage of the conserved germplasm and its proper management.

Materials and methods

Plant material

Seeds of fourteen accessions of sweet basil

(*Ocimum basilicum* L.) were collected from different regions and also from the National Gene Bank for Medicinal and Aromatics Plants (NGBMAP), CIMAP, Lucknow (India). Seeds were sown in autoclaved clay saucers of size 37.5 cm diameter and 10 cm depth in a mixture of sand, soil and farmyard manure in the ratio of 1 : 2 : 1 and germinated under glasshouse conditions during the months of December and April of 1997-1999 for two successive generations prior to their transplanting in the field. The seedlings having 6 - 12 cm height were transplanted in well fertilized plots with a spacing of 50 cm between and within rows in a randomised complete block design (RCBD) with atleast three row replications of seven plants each for every accession. Observations were recorded on five plants in each replication for the duration of flower initiation, mid-flowering, full bloom, early seeding and late seeding, and for nine morphological traits viz. plant height, length of internode (stem), leaf area, leaf shape/surface, leaf colour, stem colour, inflorescence length, length of internode (penduncle), flower colour at the time of flower intiation and full bloom.

Essential oil extraction and analysis

Oil was extracted from 100 g freshly chopped green biomass sampled from plants of different accessions through hydrodistillation technique using a Clevenger type apparatus (Clevenger 1928). Gas liquid chromatography (Hewlett Packard-5890; Series II) of the essential oil samples was performed for determining the essential oil composition based on differential melting points of oil constituents. Oil sample of 0.1 µl was injected into the gas liquid chromatography column of dimension 15 m x 0.53 mm. N_2 was used as a carrier gas at 2ml min^{-1} flow rate. The oven temperature was programmed from 60°C to 220°C @ 6°C min^{-1} with 2 min and 5 min initial and final hold, respectively. Injector and detector temperatures were maintained at 200°C and 220°C, respectively. The retention time of individual standard component was used for identification of their respective peaks. Area percentage calculation under the peaks representing the percent of that oil constituent was recorded and used for germplasm characterization.

The data were analysed for the analysis of variance (ANOVA) by the standard statistical procedure for randomised complete block design (Federer 1955) for calculating mean values, standard error of means and critical difference (CD) at 0.05 and 0.01 probability levels for each quantitative trait. Based on the significance of variation in their morphological/essential oil characteristics, the plants were characterised as morphotype or chemotype.

Results and discussion

Plant morphology

Significant morphological variations were observed for all the traits studied (Table 1). Plant height ranged from 41 cm (Ob-P) to 79 cm (Ob-N) and the stem internodal length ranged from 1.45 cm (Ob-P) to 4.80 cm (OC-19). Significant differences in leaf and inflorescence morphology were also observed (Fig. 1 a & b). A great degree of variation was observed in leaf size with leaf area varying from 2.6 cm^2 (Ob-P) to 9.4 cm^2 (OC-19). Leaf surface could be differentiated into smooth and wrinkled and leaf shape showed variation from almost flat to spoon shaped or with curved margin. The inflorescence (penduncle) length ranged from 6.8 cm (Ob-P) to 17.0 cm (OC-23, OC-12, V_1M_0, OC-16 and OC-10) and flower whorls were either densely or sparsely arranged on it with peduncle internodal length ranging from 0.4 cm (V_2M_2 and Ob-P) to 1.3 cm (V_1M_0). Variability of stem, leaf and flower colour was also observed among the germplasm due to the presence of variable degree of purplish anthocyanin pigmentation.

All the fourteen accessions were categorised as ten morphotypes that were easily distinguishable based on their morphological features (Table 2). Except for the morphotypes ObC-A and ObC-I, which had 3 accessions each (OC-23, OC-12, V_1M_0 and OC-10, OC-24, OC-25, respectively), all the other morphotypes included one accession each. Darrah (1974) reported eight

Table 1. Variations in plant morphology in *Ocimum basilicum* germplasm accessions

Accession No.	Quantitative trait					Qualitative trait			
	Plant height (cm)	Length of internode (stem) (cm)	Leaf area (cm^2)	Inflorescence (peduncle) length (cm)	Length of internode (peduncle) (cm)	Stem colour	Leaf colour	Leaf shape/surface	Flower colour
OC-23	66.7	4.1	5.9	16.0	1.1	Green	Bright green	Spoon shaped, dorsally concaved with wrinkles	White
OC-12	63.3	4.0	6.0	16.0	1.0	Green	Bright green	Spoon shaped, dorsally concaved with wrinkles	White
V_1M_0	62.0	4.1	5.8	17.0	1.2	Green	Bright green	Spoon shaped, dorsally concaved with wrinkles	White
OC-61	50.0	3.0	6.7	10.0	0.7	Green	Green	Almost flat with little or no wrinkles	White
OC-62	55.0	2.8	5.4	12.0	1.0	Purplish green	Bright green	Dorsally concaved, smooth	Purplish white
OC-16	55.0	1.9	3.0	16.0	0.6	Green	Green	Almost flat, smooth	White
V_2M_1	66.0	1.9	6.5	15.0	0.5	Green	Bright green	Almost flat, smooth	White
V_2M_2	52.0	2.0	4.9	14.0	0.4	Purplish green	Dark green	Almost flat, smooth	Purplish white
Ob-P	43.7	1.6	2.7	7.0	0.4	Green	Green	Almost flat, smooth	White
OC-19	75.0	4.0	9.2	16.0	0.7	Green	Bright green	Dorsally concaved with wrinkles, margins downwardly curved	White
OC-10	54.7	3.2	2.9	15.3	1.0	Purplish green	Green	Almost flat, smooth	Purplish white
OC-24	56.7	2.7	3.1	15.0	0.8	Purplish green	Green	Almost flat, smooth	Purplish white
OC-25	53.7	2.9	3.0	14.7	0.9	Purplish green	Green	Almost flat, smooth	Purplish white
Ob-N	62.0	2.8	5.5	12.0	0.6	Purplish green	Bright green	Almost flat, smooth	Purplish white
SEM	2.37	0.17	0.25	0.94	0.03				
CD (P=0.05)	6.98	0.50	0.72	2.74	0.10				
CD (P=0.01)	9.12	0.66	0.95	3.62	0.13				

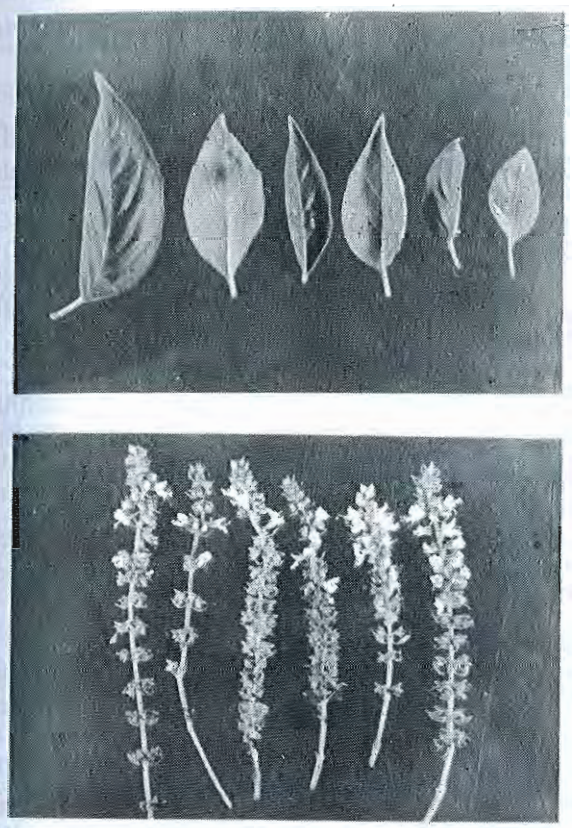

Fig. 1. Variability in *Ocimum basilicum* germplasm accessions a. leaf morphology b. inflorescence morphology

morphotypic variants in *O. basilicum* from wide germplasm collections. The variants mostly varied in leaf and inflorescence morphology and also had variation in aroma and flavour. Later, Darrah (1980) grouped *O. basilicum* germplasm into four broad categories based on plant height, bushiness and leaf size. Borodkin & Girenko (1982) observed variation in habit, foliar and floral characters in 80 varieties of *O. basilicum* and grouped them into 8 groups, which were further classified into three types on the basis of anthocyanin pigmenation. Intraspecific diversity in any crop is primarily recognized as variation in plant morphology. These morhotypic variations can be used for characterizing genetic diversity, although indirectly, and have previously been successfully employed in cotton (Singh & Gupta 1968), rice (Maurya & Singh 1977), pearl millet (Singh *et al.* 1981), faba bean (Khare & Singh 1992), periwinkle (Dwivedi *et al.* 2000) and in several other crops.

Phenology

The accessions could be grouped into three types based on their relative performance, (i) those which grew and flowered best in summer (OC-12, OC-16, OC-19, OC-23, OC-61, OC-62, V_1M_0, V_2M_1, V_2M_2 and Ob-P); (ii) those which grew and flowered best in rainy season (Ob-N) and (iii) those which grew and flowered best both in summer and rainy seasons (OC-10, OC-24 and OC-25).

Chemotypic profiling

Highly significant variations in the oil content and essential oil composition were recorded in the present study (Table 3). Oil content (v/w) in the flowering tops on fresh weight basis (FWB) varied from 0.25% (Ob-P) to 0.70% (OC-10). Of the five major oil constituents recorded, linalool content ranged from 14.18% (OC-10) to 75.60% (V_2M_2)and methyl chavicol content ranged from 0.11% (V_2M_2) to as high as 76.60% (OC-10). Methyl cinnamate was maximum (53.20%) in Ob-N and it was less than 1.0% in all other accessions. Eugenol content in the germplasm varied from 1.88% (OC-24) to 22.65% (OC-61) and 1-8 cineole ranged from 1.41% (OC-24) to 19.07% (V_1M_0). Based on the oil profile data analysis, three chemotypes namely, *Linalool* type, *Methyl chavicol* type and *Methyl cinnamate* type were defined (Table 4). *Linalool* type was assigned to those plants that yielded oil rich in linalool (45-75%). Simliarly, *Methyl chavicol* types and *Methyl cinnamate* types were assigned to those plants that yielded oil rich in methyl chavicol (65-78%) and methyl cinnamate (45-53%), respectively. The present investigation also revealed significant variation in oil content and oil compsition of *O. basilicum* (Table 3). Of the fourteen accessions, ten accessions were characterized as *Linalool* types and one accession (Ob-N) and three accessions (OC-10, OC-24 and OC-25) were characterized as *Methyl cinnamate* and *Methyl chavicol* types, respectively.

Large variations in oil composition of *O. basilicum* (7 chemotypes) have been reported earlier and

Table 2. Morphotypes of *Ocimum basilicum* as defined through differences in their morphological characteristics

Morphotype	Accession(s)	Characteristics
ObC-A	OC-23, OC-12, V_1M_0	Medium stature plant with spoon shaped, curled medium sized, bright green leaves. Inflorescence long with white flower petals and sparsely arranged flower whorls.
ObC-B	OC-61	Medium stature plant with flat, smooth, medium sized dull green leaves. Inflorescence of medium length with white flower petals and densely arranged flower whorls.
ObC-C sized,	OC-62	Medium stature plant with dorsally concaved, smooth, medium bright low green leaves. Inflorescence of medium length with purplish white flower petals and sparsely arranged flower whorls.
ObC-D	OC-16	Medium stature plant with flat, smooth, medium sized bright green leaves. Inflorescence long with white flower petals and very densely arranged flower whorls
ObC-E	V_2M_1	Medium stature bushy plant with flat, smooth, medium sized bright green leaves. Inflorescence long with white flower petals and very densely arranged flower whorls.
ObC-F	V_2M_2	Medium stature bushy plant with almost flat, smooth, medium sized dark green leaves. Inflorescence of medium length with purplish white flower petals and very densely arranged flower whorls.
ObC-G	Ob-P	Dwarf bushy plant with almost flat, smooth, green leaves. Inflorescence of short length with white flower petals and very densely arranged flower whorls.
ObC-H	OC-19	Tall plant with dorsally concaved (margin downwardly curved) or flat, large sized bright green leaves. Inflorescence long with white flower petals and densely arranged flower whorls.
ObC-I	OC-10, OC-24, OC-25	Medium stature bushy plant with flat, small sized dark green leaves. Inflorescence of medium length with purplish white flower petals and densely arranged flower whorls.
ObC-J	Ob-N	Tall plant with spoon shaped, curled/flat, medium sized bright light green leaves. Inflorescence of medium length with purplish white flower petals and densely arranged flower whorls.

Plant height: Dwarf-35-50 cm, Medium-55-70 cm, Tall- >70 cm

Inflorescence length : Short- 6-10 cm, Medium-10-14 cm, Long >14 cm

Flower arrangement (penduncle internodal length): Very dense- 0.4-0.6 cm, dense 0.7-0.9 cm, sparse >0.9 cm

Table 3. Variation in essential oil content and composition among accessions of *Ocimum basilicum*

Accession No.	Oil content (%)	Linalool (%)	Methyl chavicol (%)	Methyl cinnamate (%)	Eugenol (%)	1-8-cineole (%)
OC-23	0.48	53.42	00.89	00.20	10.64	15.82
OC-12	0.45	60.38	00.70	00.13	09.30	14.98
V_1M_0	0.43	52.49	00.65	00.18	08.75	16.95
OC-61	0.33	54.20	00.36	00.15	20.54	03.29
OC-62	0.33	67.77	00.25	00.16	11.08	07.93
OC-16	0.38	51.18	00.60	00.18	09.88	12.28
V_2M_1	0.35	66.23	00.39	00.15	13.95	06.67
V_2M_2	0.35	73.66	00.18	00.20	07.48	06.58
Ob-P	0.25	47.51	00.67	00.22	08.87	06.15
OC-19	0.35	55.67	00.77	00.14	08.58	16.65
OC-10	0.70	16.70	76.19	00.14	03.56	03.03
OC-24	0.68	20.25	65.75	00.11	02.83	02.49
OC-25	0.68	19.70	68.23	00.12	03.35	03.31
Ob-N	0.60	16.53	4.83	51.59	05.63	05.05
SEM	0.01	01.99	00.91	01.16	00.85	00.55
CD(P=0.05)	0.03	05.79	02.67	05.47	02.46	01.59
CD(P=0.01)	0.04	07.65	03.51	07.24	03.25	02.10

Table 4. Chemotypes of *Ocimum basilicum*

Chemotype	Accession (s)	Oil constituents (v/v)	
		Major	Minor
Linalool type	OC-23,OC-12, V_1M_0, OC-61, OC-62, OC-16, V_2M_1, V_2M_2, OC-19 and Ob-P	Linalool (45-75%) 1,8 - cineole (2-20%); Eugenol (5-22%)	Methyl chavicol (<1.5%); Methyl cinnamate (<1.0%)
Subtypes			
a) Hyper linalool subtype	OC-62, V_2M_1 and V_2M_2	Linalool (>65%), Eugenol (6-13%) and 1, 8-cineole (5-9%)	"
b) High linalool + eugenol subtype	OC-61	Linalool (52-56%)+ Eugenol (18-22%)	"
c) High linalool + 1, 8-cineole subtype	OC-12, OC-16,OV-19, OV-23 and V_1M_0	Linalool (50-65%)+1, 8- cineole (10-20%)	"
d) Linalool subtype	Ob-P	Linalool (45-55%)+ Eugenol and 1, 8- cineole (<10%)	"
Methyl chavicol type	OC-10, OC-24 and OC-25	Methyl chavicol (65-78%); Linalool (13-22%)	Eugenol (1-5%); 1, 8 cineole (1-5%)
Methyl cinnamate type	Ob-N	Methyl cinnamate (45-53%); Linalool (14-19%)	Methyl chavicol (4-6%) Eugenol (5-7%); 1, 8 cineole (4-6%)

have been utilised as a chemical index of fine classification (Vimalan *et al.* 1990). Lawrence (1992) made extensive studies on basil and on the basis of chemical composition and morphological variation, he recognized four distinct chemotypes of *O. basilicum* along with numerous subtypes. The present investigation also led to the identificaiton of four subtypes of linalool chemotype namely, hyper linalool, high linalool + eugenol, high linalool +1, 8- cineole and linalool subtypes, based on differences in eugenol and 1, 8- cineole concentration among the accessions belonging to this chemotype (Table 4). High linalool + 1, 8- cineole subtype included maximum number of accessions (OC-12, OC-16, OC-19, OC-23 and V_1M_0) followed by hyper linalool subtype with three accessions (OC-62,V_2M_1 and V_2M_2). High linalool + eugenol and linalool sub types included one accession each (OC-61 and Ob-P, respectively). The variations in essential oil composition and quantity are due to genetic factors and also depend on certain environmental factors that influence genetic experession. Several other studies have also depicted similar variations in major oil constituents of *Ocimum* species (Fleisher 1981; Vernin *et al.* 1984; Tesi *et al.* 1991). Grayer *et al.* (1996) recognized five basic essential oil profiles in *O. basilicum*. The genotypes, characterised as distinctive chemotypes could be hybridized in different patterns to develop chemotypes for their specific industrial applications. Several secondary metabolites are produced in plants that have no apparent role within the primary production system of plants. However, these secondary compounds are responsible for wide chemical diversity seen in plants. Their role in evolution system has also been predicted by providing relative fitness i.e., the capacity to outperform its competitors within the ecosystem (Swanson 1995).

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