

Morphological Characterization of Different *Ocimum* spp. Germplasm Lines under Semi-Arid Region of Haryana

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ABSTRACT

Knowledge about the genetic variation among the various plant traits enables the plant breeders to effectively utilize the available germplasm lines for the development of elite genotypes. The experimental material comprising of 40 germplasm lines of Tulsi (*Ocimum* spp.) received from National Bureau of Plant Genetic Resources, New Delhi were characterized for different morphological traits viz. leaf colour, leaf shape, leaf pubescence, stem colour, stem pubescence, petiole colour, flower colour, calyx colour, calyx pubescence, seed colour, plant height, inflorescence length and fresh herbage yield. The investigation was carried out in the Research Area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Kharif* 2022 and 2023. Among the various categories of different traits/descriptors, the maximum number of germplasm lines were observed for leaf lamina colour-green (25 germplasm lines), leaf shape-elliptical (40), stem colour-green (28), stem pubescence-pubescent (13), seed colour-dull black (35). Plant height ranged from 70 cm (EC 388895) to 119 cm (IC 381185) and inflorescence length from 9.3 cm (IC 75730 and IC 381185) to 24 cm (IC 387838 and EC 469904). Maximum fresh herbage yield per plant was found for the genotype EC 388890 (1250 g) and minimum for IC 387838 (290 g). The study revealed that sufficient genetic variability was present among different germplasm lines. This genetic variability may further be exploited for crop improvement programme to develop improved varieties using appropriate breeding methodology.

Keywords: Tulsi germplasm, morphological characterization, genetic variability

Introduction

The genus *Ocimum* is represented by 66 species across the world. *Ocimum* is a versatile aromatic and most important genus of the family Lamiaceae due to its immense use in traditional system of medicine, perfumery and pharmaceutical industry (Simpson and Conner, 1986). All the aromatic plants belonging to the genus *Ocimum* are collectively called Basil. In India, so far about 9 species of *Ocimum* (*Ocimum tenuiflorum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum* L., *O. micranthum* L., *O. campechianum* L., *O. americanum* L., *O. minimum* L. and *O. citriodorum* L.) have been reported of which the last three are exotic species (Balyan and Pushpangadan, 1988). The *O. basilicum* is known by various names such as 'Sweet Basil', 'Common Basil' or 'French Basil'. The *O. canum* species with a peculiar mint smell is known as 'Mint Basil'. The camphor containing species *O. kilimandscharicum* is commonly called 'Camphor Basil'. The species *O. canum* having borneol smell is known as 'Hoasy Basil' and the species *O. gratissimum* with high contents of eugenol is known as 'Spice Basil'. Hindus worship the plants of *O. sanctum* hence it is popularly known as 'Sacred

Basil' or 'Holy Basil'. The genus *Ocimum* exhibits a range of chromosome numbers, including various haploid chromosome numbers (12, 13, 16, 20, 24, 32, 36 and 38) in addition to the basic chromosome number. According to Carovic et al., (2010), the basic chromosome number for *Ocimum* species is x=12. Moreover, *O. basilicum* and *O. americanum* are known to be tetraploid (2n=4x=48) and hexaploid (2n=6x=72), respectively (Sobti and Pushpangadan, 1979).

The Ocimum species showcase remarkable morphological diversity owing to significant variations in their leaf size, shape, colour and pubescence, which have evolved over the centuries of cultivation. They are highly branched and can grow to a height ranging from 60-150 cm. Their stems and twigs are quadrangular in shape. The leaves are simple and have petioles and appear in a range of shapes from elliptical to ovate and have either entire or serrated margins. The leaves of these plants also bear sessile glands which secrete strongly scented volatile oils with aromatic flavours. The plants have small flowers that are white or purple in colour. These flowers are hermaphrodite and zygomorphic in nature. They are arranged in whorls on racemose inflorescence. The flowers have didynamous stamens and a style with bifid stigma. After the successful process of entomophilous pollination, the corolla naturally detaches and gives way to the development of four round seeds inside the bilabiate calyx. The seed's shape varies from elliptical to globose and becomes mucilaginous when wetted (Pushpangadan and Bradu, 1995).

Plants are the primary source of secondary metabolites and oils with therapeutic potential due to which people have relied on plant-based medicines for health care since the dawn of civilization. The therapeutic properties of Ocimum have been acknowledged since ancient times, not only in India but also in the ancient civilizations of China, West Asia, Europe and Africa. This recognition has elevated the status of *Ocimum* spp. to a highly valued medicinal and aromatic crop plants. Tulsi is a valuable natural source of various essential oils and fragrant compounds that hold great economic and medicinal significance. Its essential oils contain several notable chemicals including eugenol, methyl eugenol, thymol, linalool, methyl chavicol, camphor, citral, elimicin, sesquiterpene alcohols, linalyl acetate, geraniol, and methyl cinnamate (Khosla et al., 2000). The medicinal herb O. basilicum has generally been used to cure renal problems, warts, worms, cough, diarrhoea and headaches. Its oil is directly applied to the skin for treating acne and externally it can be used as an ointment for bug bites (Javanmardi et al., 2002). Its

seeds are used in treatment of dysentery and chronic diarrhoea (Gangrade et al., 2000). Oil of *O. gratissimum* has been recommended for use in biological mosquito control because of its ability to repel insects. Leaves of *O. canum* are used to cure a variety of eye conditions, bronchitis as well as parasitic skin conditions (Naithani and Kakkar, 2002).

Materials and Methods

Experimental material: The study was carried out in the research area of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant breeding, CCS Haryana Agricultural University, Hisar in the *Kharif* season of the years 2022 and 2023. The experimental material was comprised of 40 genotypes of Tulsi (*Ocimum* spp.). Among these, 38 genotypes were received from ICAR- National Bureau of Plant Genetic Resources (NBPGR), New Delhi and 2 genotypes from the germplasm pool maintained at the Medicinal, Aromatic and Potential Crops Section, which were characterized for morphological traits. The species to which each of the studied genotypes belongs is presented in Table 1.

Field layout of experiment: To conduct the experiment, all the 40 genotypes of Tulsi were grown in the Research Area of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Kharif* 2022 and 2023. Hisar is located in semi-arid sub-tropical region at 29°-10°N latitude and 75°-46°E longitude with elevation of 215.52 m above mean sea level.

Nursery preparation and Transplanting: To raise the healthy plants, nursery was prepared by sowing the seeds of all the 40 different genotypes on the raised beds separately. Light irrigation was given to the young seedlings as per requirement for good growth. Twenty days old young healthy seedlings of all the 40 different genotypes were transplanted in the Randomized Block Design (RBD) with three replications. One seedling per hill was transplanted at 10 cm spacing between the plants in two rows each of 3 m length with a row to row distance of 30 cm. A light irrigation was applied immediately after transplanting. All the required cultural practices were followed to raise the good Tulsi crop.

Recording of observations: The observations for all the 40 genotypes were recorded at appropriate plant growth stage i.e. from flowering to reproductive stage of the crop for the following 10 different qualitative traits: Stem colour, Stem pubescence, Leaf shape, Leaf colour, Leaf pubescence, Petiole colour, Flower colour, Calyx colour, Calyx pubescence and Seed colour. All the morphological observations were recorded at flowering stage. Under natural field conditions, the data for the different traits were recorded on five randomly selected plants from each genotype in each replication.

Results

The 40 genotypes of Tulsi (*Ocimum* spp.) taken for the present investigation was characterized for 10 different qualitative traits mentioned as below:

Stem colour

Based on stem colour, five different groups *viz.* dark green, green, light green, purple, and light purple (Figure 1) were observed among 40 different genotypes of Tulsi and all the genotypes were categorized accordingly (Table 2, Figure11a). Four genotypes exhibited dark green stem colour, 28 genotypes green stem colour whereas light green stem colour appeared only in one genotype. Purple stem colour was observed in five genotypes and two genotypes displayed light purple stem colour.

Stem pubescence

On the basis of the presence or absence of the hairs on the stem, Tulsi genotypes were classified as sparse pubescent and non-pubescent (Figure 2). Five genotypes were observed to have sparse pubescence whereas the remaining 35 genotypes had no pubescence (Figure 11b).

Leaf shape

Based on the leaf shape, Tulsi genotypes may be characterized into three groups such as elliptical, subovate and ovate. But, in the present investigation only elliptical leaf shape (Figure 3) was observed for all the 40 genotypes (Figure 11c). None of the genotypes showed sub-ovate and ovate leaf shape.

Leaf colour

All the 40 Tulsi genotypes were categorized into four different groups (dark green, green, light green and purple) based on the colour of their leaves (Figure 4). Dark green leaf colour was observed for 8 genotypes, green leaf colour for 25 genotypes, light green leaf colour for 6 genotypes whereas only one genotype exhibited purple leaf colour (Figure 11d).

Leaf pubescence

On the basis of the presence or absence of the hairs on the leaf surface, Tulsi genotypes can be classified as sparse pubescent, dense pubescent and non-pubescent (Figure 5). In the present investigation, only two genotypes were observed to have sparse pubescence. Dense pubescent was not observed in any of the genotype whereas 38 genotypes showed non-pubescent characteristic for leaf pubescence (Figure 11e)



Petiole colour

Fourty Tulsi genotypes were classified into three different groups based on their petiole colour namely green, purple and purple green (Figure 6). Thirty-six genotypes exhibited green petiole colour, one purple petiole colour whereas three genotypes displayed purple green petiole colour (Figure 11f).

Flower colour

On the basis of flower colour, Tulsi genotypes were classified into three categories which included white, light purple and purple (Figure 7). Among the 40 genotypes, 27 genotypes exhibited white flower colour, 8 genotypes light purple flower colour and purple flower colour expressed in the remaining 5 genotypes (Figure 11g).

Calyx colour

Five categories of calyx colour (Figure 8) *viz.* green (25 genotypes), light green (2 genotypes), purple (6 genotypes) and dark purple (1 genotype) were observed for the 40 Tulsi genotypes studied for the present investigation (Figure 11h).

Calyx pubescence

Tulsi genotypes can be classified as sparse pubescent, dense pubescent and non-pubescent based on the presence or absence of the hairs on the calyx. In the present investigation, 13 genotypes were observed to have sparse pubescence, whereas 27 genotypes showed non-pubescent characteristic for calyx pubescence (Figure 9). None of the genotypes exhibited dense pubescence on calyx (Figure 11i).

Seed colour

Four categories of seed colour (Figure 10) viz. black (1 genotype), dull black (35 genotypes), brownish black (1 genotype) and brown (3 genotypes) were observed for the 40 Tulsi genotypes studied in the present investigation (Table 1, Figure 11j).

The maximum number of germplasm lines observed leaf lamina colour-green (25 germplasm lines), non pubescent leaf (38), leaf shape-elliptical (40), stem colour-green (28), stem pubescence-pubescent (5), calyx colour-green (25), calyx pubescence-pubescent (13), petiole colour-green (36), flower colour-white (27) and seed colour-dull black (35).

Discussion

Knowledge about the genetic variation of the various plant characters enables the plant breeders to effectively utilize the available germplasm lines for the development of elite genotypes (Gowda et al., 2019; Singh et al., 2020; Arya et al., 2024). Morphological and cytological studies have helped in resolving the identity issues in many genera (Paton and Putievsky, 1996).

The experimental material of the present investigation comprising of 40 genotypes of Tulsi (*Ocimum* spp.) were characterized for 10 qualitative traits *viz*. stem colour, stem pubescence, leaf shape, leaf colour, leaf pubescence, petiole colour, flower colour, calyx colour, calyx pubescence and seed colour.

On the basis of stem colour, all the 40 Tulsi genotypes were categorized into five groups: green (28 genotypes), purple (5), dark green (4), light purple (2) and light green stem colour (1). Based on presence or absence of minute hairs on the surface of the stem, two categories were formed: sparse pubescence (5 genotypes) and nonpubescent (35). Regarding leaf shape, elliptical leaf shape was observed in all the 40 genotypes of Tulsi. Based on leaf colour, all the Tulsi genotypes were classified into four group: green (25 genotypes), dark green (8), light green (6) and purple leaf colour (1). On the basis of leaf pubescence, two categories were observed: sparse pubescent (2 genotypes) and non-pubescent (38). Genotypes were classified into three groups on the basis of petiole colour: green (36 genotypes), purple green (3) and purple petiole colour (1). Based on flower colour, three categories were formed viz. white (27 genotypes), light purple (8) and purple (5). Based on the colour of the calyx, the genotypes were classified into five groups: green (25 genotypes), purple (6), light purple (6), light green (2) and dark purple calyx colour (1). On the basis



Figure 1. Classification of 40 Tulsi genotypes on the basis of stem colour.



Figure 3. Classification of 40 Tulsi genotypes on the basis of leaf shape.

of presence or absence of minute hairs on the calyx surface, two categories were observed such as nonpubescent (27) and sparsely pubescent (13). Genotypes were classified into four groups based on seed colour viz. dull black (35), black (3), brownish black (1) and brown seed colour (1). The characterization of Tulsi genotypes based on qualitative traits has also been reported by Kumar et al., (2012a), Chhaya et al., (2013), Nassar et al., (2013), Malav et al., (2015) and Kumar et al., (2019). Yaldiz, and Camlica, (2021) also studies the agromorphological and phenotypic variability of sweet basil genotypes for breeding purposes. The morphological characterization in different medicinal plants is carried by different researchers for their proper identification and further utilization in breeding programme (Arya et al., 2024; Singh et al., 2024).

Conclusions

It may be concluded from present study that the sufficient morphological variability was present among different germplasm lines of Tulsi. The presence of genetic variation for different traits enables the plant breeders to utilize the germplasm lines EC 388890, IC 469938, IC 388785 and IC 328582 having maximum fresh herbage yield for the development of elite genotypes.



Figure 2. Classification of 40 Tulsi genotypes on the basis of stem pubescence.



Figure 4. Classification of 40 Tulsi genotypes on the basis of leaf colour.



Figure 5. Classification of 40 Tulsi genotypes on the basis of leaf pubescence.



Figure 7. Classification of 40 Tulsi genotypes on the basis of flower colour.



Figure 9. Classification of 40 Tulsi genotypes on the basis of calyx pubescence.



Figure 6. Classification of 40 Tulsi genotypes on the basis of petiole colour.



Figure 8. Classification of 40 Tulsi genotypes on the basis of calyx colour.



Figure 10. Classification of 40 Tulsi genotypes on the basis of seed colour.





Figure 11. Figures represent morphological descriptors of various qualitative characters in Ocimum spp.

Non-pubescent

Sparse pubescent

Continuing Figure 11





Serial Number	Genotypes	Species	Source	Serial Number	Genotypes	Species	Source
1.	IC 44681	Ocimum basilicum	NBPGR	21.	EC 469904	Ocimum basilicum	NBPGR
2.	IC 387837	Ocimum basilicum	NBPGR	22.	EC 326771	Ocimum basilicum	NBPGR
3.	IC 369247	Ocimum basilicum	NBPGR	23.	EC 388893	Ocimum basilicum	NBPGR
4.	IC 387838	Ocimum basilicum	NBPGR	24.	EC 388887	Ocimum basilicum	NBPGR
5.	IC 388785	Ocimum basilicum	NBPGR	25.	EC 388895	Ocimum basilicum	NBPGR
6.	IC 469938	Ocimum basilicum	NBPGR	26.	EC 388896	Ocimum basilicum	NBPGR
7.	IC 326735	Ocimum basilicum	NBPGR	27.	EC 388782	Ocimum basilicum	NBPGR
8.	IC 312264	Ocimum basilicum	NBPGR	28.	EC 388737	Ocimum basilicum	NBPGR
9.	IC 110207	Ocimum basilicum	NBPGR	29.	EC 388889	Ocimum basilicum	NBPGR
10.	IC 338794	Ocimum basilicum	NBPGR	30.	EC 338772	Ocimum basilicum	NBPGR
11.	IC 336833	Ocimum basilicum	NBPGR	31.	EC 388788	Ocimum basilicum	NBPGR
12.	IC 201223	Ocimum basilicum	NBPGR	32.	EC 388890	Ocimum basilicum	NBPGR
13.	IC 328582	Ocimum basilicum	NBPGR	33.	NSV 38	Ocimum basilicum	NBPGR
14.	IC 338959	Ocimum basilicum	NBPGR	34.	RDV 45	Ocimum basilicum	NBPGR
15.	IC 333833	Ocimum basilicum	NBPGR	35.	Local 1	Ocimum sanctum	HAU
16.	IC 281185	Ocimum basilicum	NBPGR	36.	Local 2	Ocimum tenuiflorum	HAU
17.	IC 381552	Ocimum basilicum	NBPGR	37.	DOS 1	Ocimum tenuiflorum	DMAPR
18.	IC 436153	Ocimum basilicum	NBPGR	38.	IC 75730	Ocimum basilicum	NBPGR
19.	IC 381158	Ocimum basilicum	NBPGR	39.	IC 381185	Ocimum basilicum	NBPGR
20.	IC 326732	Ocimum basilicum	NBPGR	40.	EC 112548	Ocimum basilicum	NBPGR

Table 1. Species and source of 40 Tulsi genotypes used in present study.

Characters: Stem colour	No. of Genotypes	Genotypes	
Dark green	4	IC 369247, IC 381552, EC 388887, EC 388895	
Green	28	IC 44681, IC 387837, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388896, EC 388782, EC 388737, EC 388889, EC 33877 IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, Local 1, Local 2	
Light green	1	IC 336833	
Purple	5	EC 388788, EC 388890, IC 75730, IC 381185, DOS 1	
Light purple	2	EC 388893, EC 112548	
Stem pubescence	9		
Sparse pubescent	5	IC 388785 , EC 469904, IC 75730, IC 381185, DOS 1	
Non-pubescent	35	IC 44681, IC 387837, IC 369247, IC 387838, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 326771, EC 388893, EC 388887, EC 388895, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2	
Leaf shape			
Elliptical	40	IC 44681, IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, IC 436153, IC 381158, IC 326732, EC 469904, EC 326771, EC 388893, EC 38887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, RDV 45, NSV 38, Local 1, Local 2, DOS 1, IC 75730, IC 381185, EC 112548	
Sub-ovate	0	-None-	
Ovate	0	-None-	
Leaf colour			
Dark green	8	IC 369247, IC 381552, EC 388893, EC 388887, EC 388895, IC 75730, Local 1, DOS 1	
Green	25	IC 44681, IC 387837, IC 387838, IC 388785, IC 326735, IC 312264, IC 110207, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388896, EC 388782, EC 388737, EC 388889, EC 388788, EC 388890, IC 381158, IC 326732, NSV 38, EC 112548	
Light green	6	IC 469938, IC 338794, EC 338772, IC 436153, RDV 45, Local 2	
Purple	1	IC 381185	
Leaf pubescence			
Sparse pubescent	2	IC 388785, IC 381185	
Dense pubescent	0	-None-	
Non-pubescent	38	IC 44681, IC 387837, IC 369247, IC 387838, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388893, EC 38887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388899, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, IC 75730, DOS 1, Local 1, Local 2	

Table 2. Classification of 40 Tulsi genotypes on the basis of morphological characters.



Continuing Table 2

Characters: Stem colour	No. of Genotypes	Genotypes
Petiole colour		
Green	36	IC 44681, IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Purple	1	IC 381185
Purple green	3	EC 388893, IC 75730, DOS 1
Flower colour		
White	27	IC 44681, IC 387837, IC 387838, IC 388785, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388887, EC 388895, EC 388782, EC 388737, EC 388889, EC 338772, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Light purple	8	IC 369247, IC 469938, IC 326735, IC 381552, EC 388896, EC 388788, EC 388890, IC 436153
Purple	5	IC 336833, EC 388893, IC 75730, IC 381185, DOS 1
Calyx colour		
Green	25	IC 387838, IC 388785, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388887, EC 388895, EC 388782, EC 388737, EC 388889, EC 338772, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Light green	2	IC 44681, IC 387837
Purple	6	EC 388893, EC 388788, EC 388890, IC 436153, IC 75730, DOS 1
Light purple	6	IC 369247, IC 469938, IC 326735, IC 336833, IC 381552, EC 388896
Dark purple	1	IC 381185
Calyx Pubescenc	e	
Sparse pubescent	13	IC 44681, IC 369247, IC 388785, IC 469938, IC 312264, IC 338794, IC 381552, IC 75730, IC 381185, EC 469904, EC 388893, EC 338772, DOS 1
Dense pubescent	0	-None-
Non-pubescent	27	IC 387837, IC 387838, IC 326735, IC 110207, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 436153, IC 381158, IC 326732, EC 326771, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 388788, EC 388788, EC 388890, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Seed colour		
Black	1	IC 44681
Dull black	35	IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388893, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, DOS 1
Brownish black	1	IC 336833
Brown	3	IC 75730, IC 381185, Local 2

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