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The diversity in holy basil (*Ocimum tenuiflorum*) germplasm from India

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ABSTRACT

A total of one hundred and nine accessions of cultivated holy basil (*Ocimum tenuiflorum* L.) germplasm, representing different phyto-geographical regions of India were investigated for morphological characterization. Data were recorded on 32 descriptor traits (both qualitative and quantitative) using the minimal descriptors developed by the ICAR-National Bureau of Plant Genetic Resources, with minor modifications. Analysis of the data was carried out using Ward's Minimum Variance method and categorized into seven major clusters. PCA analysis revealed that the first six principal components (Eigen value greater than 1), are contributing 72.33% of the total variance which were mostly influenced by mature leaf length, leaf width, leaf petiole length, plant height, seed length, seed width, days to flower initiation, essential oil percentage, seed length/width ratio, leaf length/width ratio, number of primary branches and fresh herbage yield. All the accessions showed high degree of variation, indicating rich morphological diversity within the population.

Key words: Accessions, Diversity, India, Minimal descriptors, Morphological characterization, Principal components

Ocimum tenuiflorum L. (syn. *O. sanctum* L.), commonly known as "Tulsi" (in Hindi) and "Holy basil" (in English), is considered as the most sacred herb among the Indians.The genus *Ocimum* (family: Lamiaceae) includes about 50-150 species from the tropical regions of Asia, Africa, Central and South America (Bailey 1924; Darrah 1980; Gupta *et al*. 2002 and Devi 2001). In India, it is represented mainly by nine species (*Ocimum tenuiflorum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum*, *O. micranthum* L., *O. campechianum* L., *O. americanum* L., *O. minimum* L. and *O. citriodorum* L.), of which last three are exotic but cultivated from long time (Willis 1919).The chromosome number of *O. tenuiflorum*is reported 2*n* = 36, which is the lowest among the members of *Ocimum* genus (Carovic-Stanko 2010).

Holy basil is a native of India and has a wide range of distribution covering the entire Indian sub-continent ascending to 1800m in the Himalayas and as far as in the Andaman Nicobar Islands (Kirtikar and Basu 1984). In ancient Indian scriptures, it is valued as an important medicinal plant (Singh *et al.* 2002) and grown in temples and courtyards for religious and medicinal purposes in India for over 3000 years; while the Chinese people use it in the natural herbal preparations as medicines (Sirkar 1989; Monga 2011). Globally it is distributed in Asia, Australia, West Africa and some of the Arab countries (Pistrick *et al.* 2001). Based on phylogenetic studies, morphological characterization and the study of variation patterns in different accessions of *Ocimum* are relevant even in the present era of molecular systematics (Scotland *et al*. 2003; Smith and Turner 2005; Lee 2006; Bruce *et al*. 2007). Evidence from phytogeography and molecular studies have indicated the north-central region in India as the center of origin (Bast *et al.* 2014; Mishra *et al.* 2014). Based on the leaf characters, mainly of two morphotypes cultivated in India with light green-leaves (Rama tulsi) and purple (Shyamatulsi) have been recognized for economic value (Kothari *et al.* 2005; Malav *et al.* 2015; Saran *et al*. 2017). Many of the *Ocimum* spp. including *O. tenuiflorum* are economically cultivated due to their high valued essential oils (Janmoni and Mohamed 2013). It is widely used in traditional, Ayurvedic, Greek, Roman and Unani systems of medicine for the treatment of various diseases (Vishwabhan 2011) due to its ethano-pharmacological properties (Joseph and Vrundha 2013; Gupta *et al*. 2002; Pattanayak *et al*. 2010; Pingale *et al*. 2012).

Despite the ancient history of cultivation of the holy basil in the Indian sub-continent and availability of rich diversity, meager efforts have been made for characterizing

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the morphological diversity. In an earlier study carried out by Malav *et al.* (2015), variation in different genotypes has been expressed mainly were studied from different regions of northern part of India only. Therefore, the present study was undertaken to characterize the accessions of *O. tenuiflorum* augmented from different phyto-geographical regions of India facilitating its use in genetic resource management and utilization through the identification of morphological traits of economic importance.

MATERIALS AND METHODS

Augmenting the germplasm and raising material

A total of 109 germplasm accessions of *O. tenuiflorum* were used in the present study, assembled from different sources, from National Gene bank (83 accessions) and collected during the multi-crop exploration programme (26 accessions) representing different phyto-geographical regions, which covers 20 states of India . The selected accessions were physically examined for seed health before undertaking experimental work.

The seeds were treated overnight with 0.1% KNO_3 solution before sowing.The treated seeds were sown in the nursery during the second week of July at ICAR-NBPGR, Experimental farm at Issapur, New Delhi (located at latitude of 28° 34' N, longitude of 76° 51' E and an altitude of 220 m above MSL) and the seedlings were transplanted in experimental plots after 30 days using augmented block design. The study was undertaken for the two consecutive years 2018 and 2019 using prominent checks IC-75730 (green type) and IC-381185 (black type).

The data on vegetative and reproductive parts were recorded for the entire growth period as per minimal descriptors. The seed measurement data were recorded using a stereoscopic microscope (model number SMZ-1500 Nikon), herbarium specimens of variability depicting accessions were deposited in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi and hydro-distillation of essential oil extractionwas carried out using clevenger-type apparatus.

Recording of morphological data

Characterization data were recorded for 32 traits during the vegetative and reproductive growth period of the plant.The abbreviated form of each character is asqualitative [plant habit (Hab), leaf lamina colour (LLC), leaf shape (LS), leaf surface pubescence (LSP), leaf

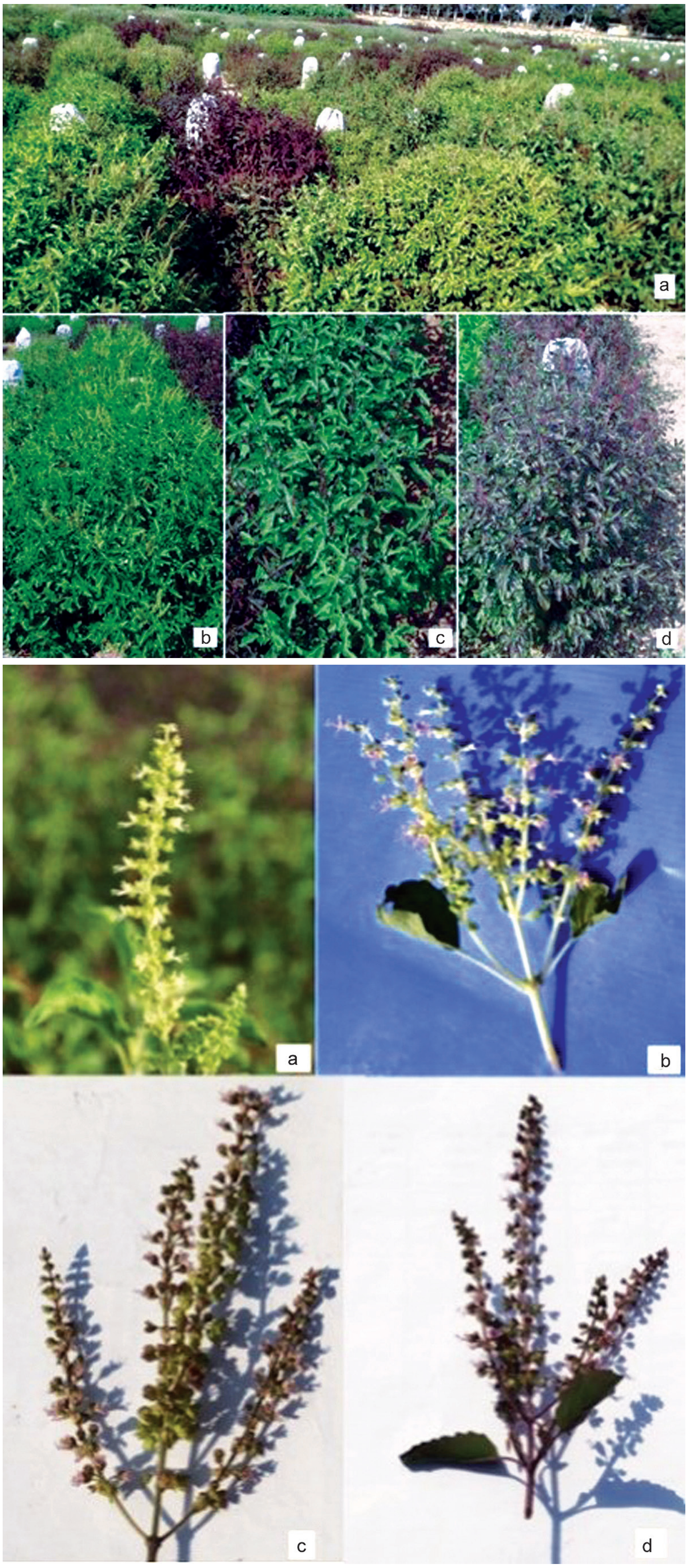

Fig 1 (a) Field view of germplasm at Issapur farm, (b) green type, (c) intermediate type and (d) black type.

Fig 2 Variation ininflorescence and seed colour.

margin (LM), stem colour (SC), stem pubescence (SP), calyx colour (CC), calyx pubescence (CP), corolla colour (CoC), anther filament colour (AFC), petiole colour (PC), seed shape (SS) and seed colour (SeedS)] and quantitative [leaf lamina length (LL), leaf lamina width (LW), leaf length/width ratio (LWR), leaf petiole length (LPL), plant height (Pht), inflorescence length (IL), number of branches/plant (NBP), days to flower initiation (DFI), fresh herb yield per plant (YFH), seed length (SL), seed width (SW), seed length/width ratio (SLWR), 100 seed weight (TW) and essential oil% (EO)]. For the morphological characterization of significant traits, minimal descriptors developed by ICAR-NBPGR were used with slight modifications (Singh *et al*. 2003). All qualitative and quantitative data were recorded at the peak of the vegetative and flowering stage. On average, data on 10 plants were recorded and the average value of each record was used for analysis. For the recording of leaf colour and that of the inflorescence/flower colour, the RHS colourchart was used (Royal Horticultural Society 1986).

For statistical analysis, IBM SPSS (Version 20) (Statistical Package for the Social Sciences) statistical software package was used. Ordination of the accessions was done by using Principal Component Analysis (PCA). A hierarchical clustering technique of Ward's Minimum Variance (Ward 1963) was used to classify the accessions into distinct clusters.

RESULTS AND DISCUSSION

Data analysis of morphological diversity and characterization

Cluster analysis of 109 genotypes of *O. tenuiflorum* based on morphological (quantitative) traits was done using the ward's minimum variance technique which is considered to be most suitable for classifying the accessions in distinct and well-defined clusters. The dendrogram indicated that all the genotypes had been categorized into seven major clusters while the clusters represented the genotypes from different phyto-geographical regions. The application of cluster analysis techniques for obtaining results from the analysis of the quantitative traits of the populations allowed us to establish seven different clusters (Fig 3). Cluster 1 included 17 accessions which is represented by a large inflorescence with the maximum length in accession KP/PKM-2017/12 and also by having the minimum number of primary branches and early flowering. Cluster 2 included 22 accessions represented bythe maximum leaf length-width ratio with the maximum ratio in accession IC-281334 (3.34). Cluster 3 included 9 accessions represented by lowest inflorescence length and maximum fresh herbage yield/plant in which the maximum value was recorded in accession IC-583299 (1690 g). Cluster 4 included 17 accessions represented by maximum plant height with maximum in accession IC-599368 (109 cm) and with the maximum number of primary branches with maximum branches in accession IC-626374 (21.50), highest seed length width ratio with the highest ratio in accession IC- 599368 (1.49) and by maximum test weight with maximum test weight in accession IC-381185 (40.08 mg). Cluster 5 included 14 accessions represented by lower leaf length, lower leaf width, lower leaf petiole length, lower plant height, maximum days to flower initiation with maximum DFI in accession IC-589206 (69 days) and minimum seed test weight. Cluster 6 included 8 accessions represented by maximum leaf length in PKM-2017/9 (11.75 cm), maximum leaf width in IC-599317 (4.67 cm), maximum leaf petiole length with maximum petiole length in accession IC-599317 (4.16 cm), minimum herbage yield/plant, minimum seed length and lowest seed width. Cluster 7 included 22 accessions represented by minimum leaf length width ratio, maximum seed length, maximum seed width and minimum seed length width ratio. Cluster 2 and 7 represented maximum of 44 accessions, mainly from Uttar Pradesh and Odisha.

Qualitative traits: In the studied accessions,leaf lamina and leaf petiole colour varied from green, purple green to purple. Leaf shape was elliptical with dentate margin and smooth upper leaf surface while glandular hairs were

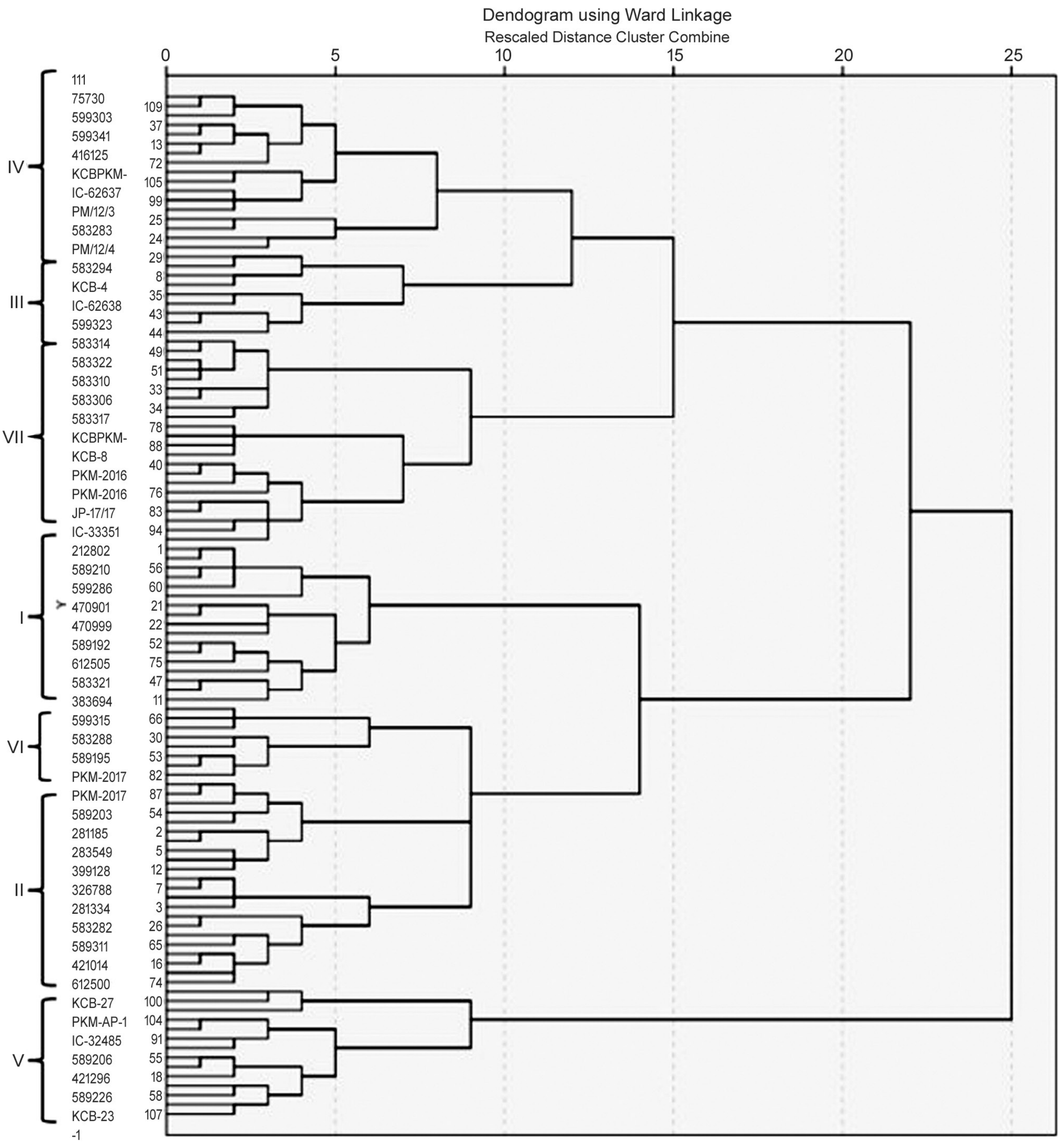

Fig 3 Dendrogram generated based on quantitative morphological traits of 109 accessions of *O. tenuiflorum.*

present on the lower surface in all the accessions. The veins on the upper lamina were more pronounced in younger leaves. Pubescence character was variable from glabrous to pubescent. The younger parts were more pubescent. Calyx colour varied from green, purple green to purple with small glandular hairs. Corolla and anther filament colour varied from white, purple white to purple. Leaves showed curly serrate appearance at the time of 25-30 days after sowing. Leaf tip shape varied from blunt to acute (mostly acute). Leaf base was oblique to equal in all accessions. Glandular hairs present on stem surface, stem colour varied from green to purple in the vegetative stage, but after maturation became dark woody in appearance. Seeds were broad-ellipsoid with brown to brownish black colour.

Data on 18 qualitative traits were measured; among them, only eight traits were showing variation within the accessions. The qualitative traits, namely leaf colour, stem colour, petiole colour, calyx colour, corolla colour, anther filament colour and seed colour were used for assessing diversity. Upper leaf lamina colour of 52.3% accessions and

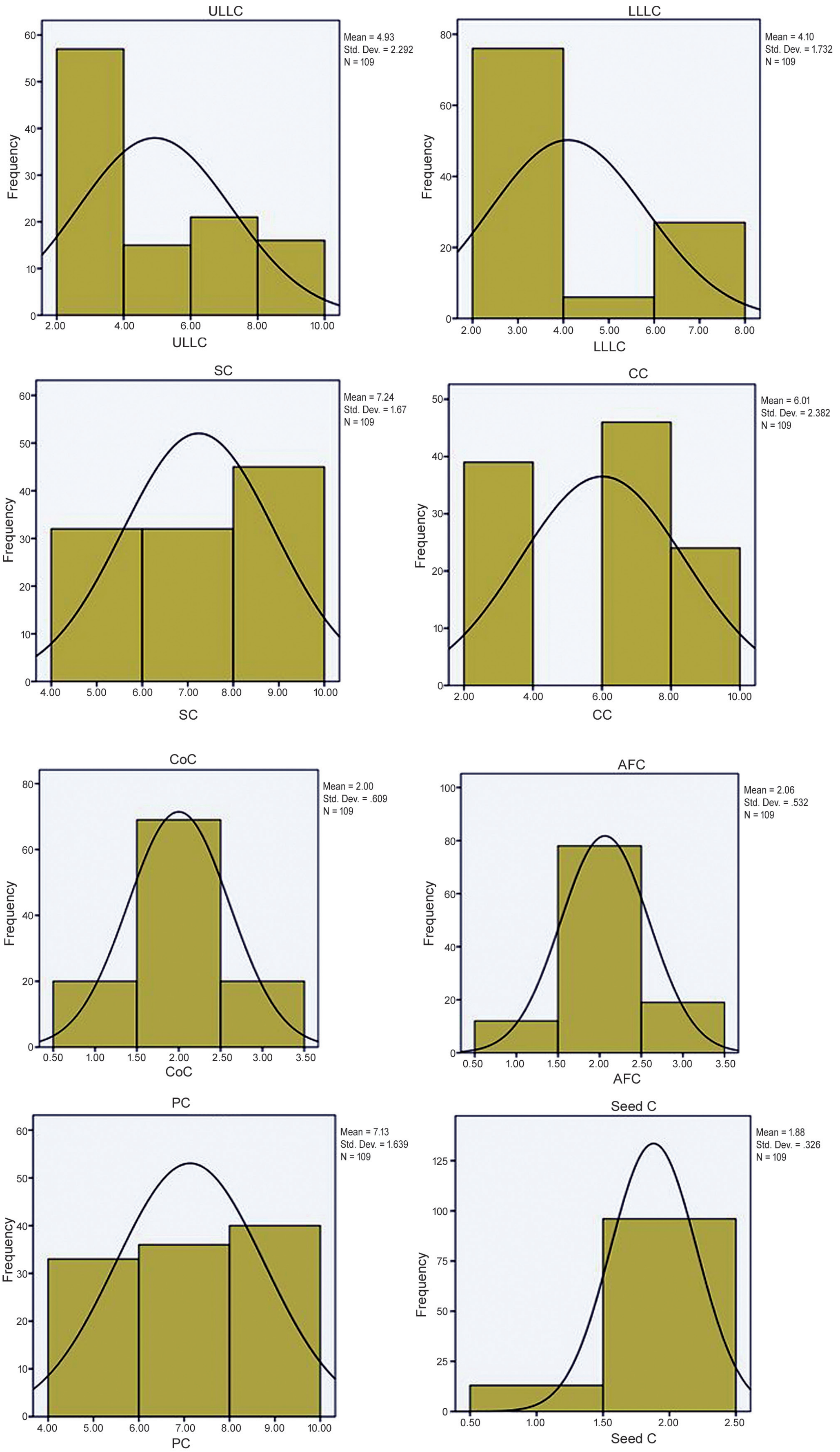

Fig 4 Histogram based on the frequency and percentages of 8 variable qualitative traits.

Table 1 Frequency analysis of 8 variable qualitative traits

Trait	Descriptor	Frequency	Percent	Trait	Descriptor	Frequency	Percent
ULLC	3 Green	57	52.3	CoC	1 White	20	18.3
	5 Dark green	15	13.8		2 Light purple	69	63.3
	7 Purple Green	21	19.3		3 Purple	20	18.3
	9 Purple	16	14.7				
LLLC	3 Green	76	69.7	AFC	1 White	12	11.0
	5 Dark green	6	5.5		2 Light purple	78	71.6
	7 Purple green	27	24.8		3 Purple	19	17.4
SC	5 Green	32	29.4	PC	5 Green	33	30.3
	7 Purple green	32	29.4		7 Purple green	36	33.0
	9 Purple	45	41.3		9 Purple	40	36.7
CC	3 Green	39	35.8	SeedC	1 Brown	13	11.9
	7 Purple green	46	42.2		2 Brownish-black	96	88.1
	9 Purple	24	22.0				

lower leaf lamina colour of 69.7% accessions were found green, while petiole colour of 36.7% accessions and stem colour of 41.3% accessions was observed purple. Green and purple green in the calyx and light purple colour in corolla was observed in most of the accessions. Anther filament colour of 71.6% accessions was observed as light purple and 88.1% accessions had brownish blackseeds. Frequencies and percentages of these qualitative traits are mentioned in Table 1.

Quantitative traits

Minimum leaf length (5.04 cm) and leaf width (2.06 cm) was observed in accession IC-583300 collected from Haridwar, Uttarakhand and maximum leaf length (11.83 cm) and maximum width (3.34 cm) in IC-583279 from Mathura, Uttar Pradesh. Leaf length width ratio ranges from 1.96 to 3.34 with a minimum in IC-583304 from Bijnor, Uttar Pradesh and maximum in IC-281334 from Gonda, Uttar Pradesh. Petiole length varied from 1.58 to 4.16 cm. Plant height varied from 52.84-109.00 cm with the maximum in IC-599368 from Angul, Odisha and minimum in JP-17/7 from Andaman and Nicobar Islands. Inflorescence length varied from 8.85 to 16.55 cm. The number of primary branches varied from 9.34-21.50 with maximum branches/ plant in IC-626374 from Andaman and Nicobar Islands. Days to flower initiation ranging from 43-72 days with early flowering in KP/PKM-2017/12 from South Dinajpur, West Bengal and late flowering in KCB-8 from North Cachar Hills, Assam. Fresh herbage yield per plant was ranging from 360.00 to 1710.00 g with maximum herbage yield in PM-12/6 from Neemuch, Madhya Pradesh and minimum in IC-583300 from Haridwar, Uttarakhand. Seed length ranged from 1055.40 to 1406.50 µm, seed width from 752.06 to 1078.09 µm and seed length width ratio from 1.25 to 1.50. Test weight of 100 seeds varied from 13.10 to 40.90 mg. The essential oil percentage on fresh weight basis varied from 0.05 to 0.37 with the minimum in IC-470931 from S-24 Pargana, West Bengal and maximum in IC-583285 from Hatharas, Uttar Pradesh.

Principal components analysis

The analysis of the grown accessions at full maturity growth stage generated the first six principal components using PCA,which contributed 72.33% of the total variability. The principal component analysis is also validated through the Scree plot (Eigen value more than 1) analysis to determine the number of factors to retain in exploratory factor analysis (FA) or principal components to keep in a principal component analysis (PCA). Each eigen value represents the amount of variance that has been captured by one component in which maximum variability was contributed by the first component at the individual level (22.15%). For quantitative traits, the principal components of the first 14 traits of *O. tenuiflorum* together with eigen vector coefficients, are presented in Table 2. The first principal component was most highly influenced by characteristics pertaining to mature leaf length, width and petiole length. In the second PC, the traits contributing to the total variability were seed length and seed width.The third PC was mostly

Table 2 Eigen values and the proportion of total variability among cultivars of *O. tenuiflorum,* as explained by the first 6 Principal Components for quantitative characters

Component	Total variance explained			Traits with greater weightings
	Initial eigen values			
	Total	% of variance	Cumulative % of the variance	
1	3.101	22.147	22.147	LL, LW, LPL, Pht
2	1.710	12.212	34.359	SL, SW
3	1.532	10.946	45.305	DFI, EO
4	1.453	10.382	55.686	SLWR
5	1.195	8.536	64.223	LWR
6	1.135	8.107	72.330	NBP, YFH

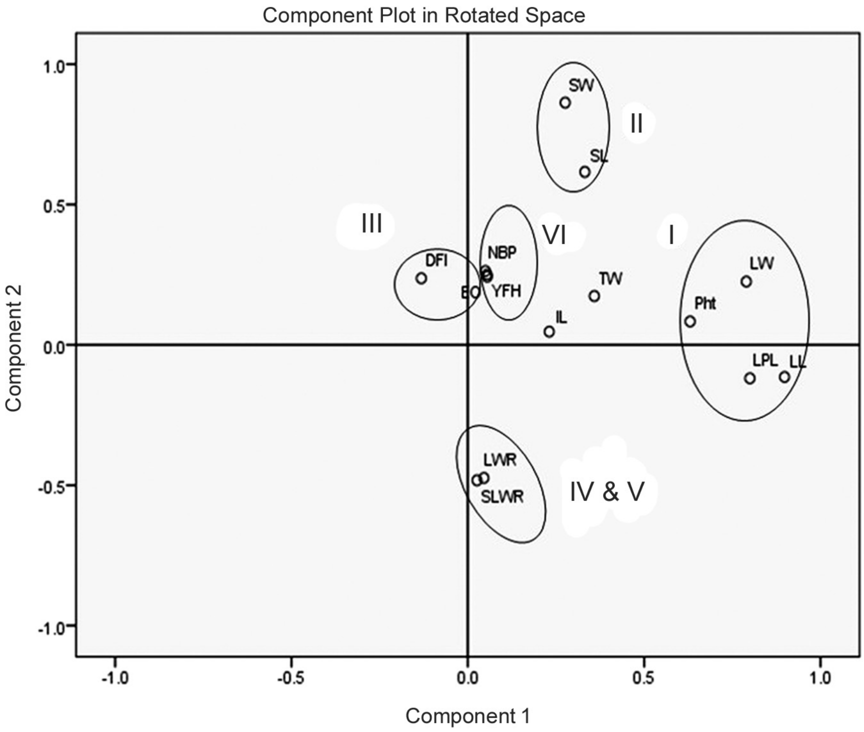

Fig 5 2-D Scatter Plot derived from the PCA analysis of 14 quantitative traits of *O. tenuiflorum*.

influenced by days to flower initiation and essential oil percentage. The fourth PC was mostly influenced by seed length/width ratio, the fifth PC influenced by leaf length-width ratio and the sixth PC was influenced by the number of primary branches and fresh herbage yield. Percent variations are higher in case of fresh herbage yield, seed length and minimum in case of seed length width ratio and essential oil% and moderate in other characters.

The 2D scatter plot of PC scores of the first two PCs is depicted in Fig. 5,confirmed by the clustering pattern traits relatedness. The Component Plot in rotated space gives a visual representation of the loadings plotted in a 2-dimensional space. The plot showed how closely related traits are to each other and the two components. Two components were rotated, based on the eigen values over 1 criterion and the scree plot.After rotation, the first component accounted for 22.14% of the variance and the second component accounted for 12.21% of the variance. 2D scatter plot help to determine potential relationships among scale variables for plotting multi-variate data.

In north Indian condition, *O. tenuiflorum* is grown as an annual due to extreme cold and severe frost condition in December, though it persists as a perennial.The data on all accessions of *O. tenuiflorum* were recorded for two consecutive years 2018 and 2019 are discussed here.

Variability recorded in characteristics of different accessions that showed the clustering pattern of the genotypes ata high degree of genetic diversity among different accession, as was reported by Ahmad *et al.* (2002). Genetic diversity is best estimated if agro-morphological studies are used together with other modern tools. Morphological trait measurements are commonly used parameters since they provide a simple technique of quantifying genetic variation while simultaneously assessing genotype performance under relevant growing environments (Fufa *et al.* 2005). The study carried out on the morphological variability of *Ocimum* spp. for phenotypic characters like leaf-ovate shape, flat surface and entire margin of leaf, green colour of stem, bract and leaf and flower had significant effect on all characters with much pronounced influence of genotype and environment (Tepi *et al.* 1991; Szabo *et al.* 1996; Agarwal *et al*. 2013). The phenotypic relationship among the basil genotype was assessed by an un-weighted pair group method with an arithmetic average (UPGMA) cluster analysis of the similarity matrix to inter genetic relationships and phylogeny among genotypes (Shazia *et al*. 2011). The study was undertaken on variation in leaf characters of basil to facilitate identification of types for herbage/essential oils yield and the best stage of harvesting to select superior accessions from western regions of India (Malav *et al.* 2015; Saran *et al*. 2017).Taxonomy of basil is complicated due to inter and intra-specific hybridization and the existence of numerous cultivars and chemotypes within species, which are significantly different in morphology (Tucker 1986;

Table 3 Descriptive statistic of quantitative variables

Descriptive statistics					
Trait	Minimum	Maximum	Mean	Std. error	Std. deviation
LL	5.04	11.83	9.32	0.11	1.14
LW	2.06	4.73	3.59	0.05	0.49
LWR	1.96	3.34	2.60	0.02	0.25
LPL	1.58	4.16	2.79	0.04	0.42
Pht	52.84	109.00	81.74	1.02	10.68
IL	8.85	16.55	13.03	0.15	1.57
NBP	9.34	21.50	13.39	0.24	2.47
DFI	43.00	72.00	60.03	0.48	5.04
YFH	360.00	1710.00	942.28	32.92	343.64
SL	1055.40	1406.50	1220.04	6.43	67.14
SW	752.06	1078.09	895.47	5.38	56.14
SLWR	1.25	1.50	1.365	0.01	0.06
TW	13.10	40.90	28.70	0.61	6.40
EO	.05	.37	0.17	0.01	0.06

Vieira 1999, Carovic *et al*. 2006).

In the present study, populations of *O. tenuiflorum* collected from different states of India were studied and compared for morphological characters (qualitative and quantitative). This study on morphological variation pattern in different accessions of *O. tenuiflorum*, on one hand, will support the crossability of discrete types and crop improvement programme and on the other hand, will support the crop gene pool study. The evidence from morphology, phenology and phyto-geograpgy could help in understanding the development and crop evolution.

Characterization of accessions provides information on the traits of the material essential for their identification, conservation, management and utilization in the crop improvement programme. The morphological variation studied among 109 germplasm accessions represented diverse agro-climatic regions of India, provided scope for screening and selection of morphotypes and identification of newer types. Based on the above distinct morpho-profiling of the "elite" traits, the accessions could be used for commercial exploitation. Further, information can be utilized for the identification of gaps in the conserved germplasm. Thus, from the present study, it is revealed that augmentation of germplasm accessions from Uttar Pradesh, Odisha, Rajasthan, Bihar, Chhattisgarh and adjoining areas contributed maximum herbage yield characters (as per cluster 3 and 4).

Wild germplasm from Southern part of India and cultivated types from unrepresented remote areas such as the North Eastern region, West Bengal, Andaman and Nicobar areas and diverse distribution range (including primitive types) from diversity rich pockets areas from Madhya Pradesh and adjoining areas have been identified.

Genetic enhancement in identifying potential value and documentation of data on the collection and conservation of populations can be achieved through the present investigation.

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