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RESEARCH ARTICLE

Studies on Genetic Divergence in Tulsi (*Ocimum basilicum*) under Semi-Arid Conditions for the Development of Novel Cultivars

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Abstract

In the present investigation, 40 Tulsi (*Ocimum basilicum*) genotypes were grown in RBD in the Research Area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The observations were recorded for 17 quantitative traits. All the genotypes were grouped into 10 different clusters, demonstrating the presence of genetic divergence among the genotypes studied. The maximum intra-cluster distance was recorded for cluster VII and maximum inter-cluster distance was recorded between cluster II and cluster X. Relative contribution of diverse traits towards genetic diversity analysis revealed that 1000 seed weight contributed maximum (41.41%) towards genetic divergence followed by number of spikes per plant (17.31%), fresh herbage yield per plant (13.46%) and oil content (10.77%). Based on the maximum mean performance for seed yield, number of primary branches, fresh as well as dry herbage yield, oil content and seed vigour index-I & II, the genotypes EC 338772, EC 388890 and IC 326732 may be exploited in the breeding program for the development of novel improved cultivars of Tulsi for higher oil content, seed yield, and other economic traits.

Keywords: Genetic divergence, *Ocimum spp*., Semi-arid conditions, Tulsi

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Introduction

Tulsi is a medicinal and aromatic crop plant that belongs to the Lamiaceae family and the *Ocimum* genus. In India, nine 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 & Pushpangadan, 1988; Talawade *et al*., 2024). The *Ocimum basilicum* and *O. sanctum* species are most widely distributed in India (Arya *et al*., 2021). *Ocimum americanum* and *O. canum* are two species that are primarily found in north-western India, including Jammu and Kashmir, Punjab, Himachal Pradesh, Delhi and Uttar Pradesh. *O. gratissimum* is found across North India (Krishnamoorthy, 1989). 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,* which has a 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' (Talawade *et al*., 2024). 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).

Genetic diversity plays an important role in plant breeding, either to generate productive recombinants or to exploit heterosis (Chowdhury *et al*., 2017). The divergence analysis can be a valuable instrument in measuring the extent of divergence between distinct germplasm lines (Bompalli and Nallabilli, 2013). Thus, a detailed understanding of the type and extent of genetic divergence can aid plant breeders in identifying genetically diverse plants that can be used in crop improvement programmes for the development of new recombinants (Arunachalam, 1981).

Although *Ocimum* possesses medicinal and aromatic properties of high value, it is not commonly grown on a large scale. It may become a significant crop in the near future as a result of the increasing demand for aromatic compounds in the food, flavours and pharmaceutical industries. The low cultivation area and yield of *Ocimum* can be attributed to the unavailability of genotypes that are suitable for the specific regions. When selecting *Tulsi* for cultivation*,* farmers consider the yield and quality of the herb and oil, as well as other related characteristics that contribute to an increased economic yield and profit. It is essential to conduct studies of different genotypes across various agro-climatic regions to identify the best-yielding genotypes with unique attributes that influence the yield. This approach will allow us to recognize promising genotypes that can be used in breeding programmes to improve the yield of sacred basil. Therefore, keeping the above points in view, the present investigation has been planned to determine the extent of genetic diversity for different traits to classify the *Tulsi* genotypes into different clusters.

Materials and Methods

Experimental Material

The experimental material for the present investigation was comprised of 40 genotypes of Tulsi (*Ocimum basilicum*) (IC44681, EC388887, IC387837, EC388895, IC369247, EC388896, IC387838, EC388782, IC388785, EC388737, IC469938, EC388889, IC326735, EC338772, IC312264, EC388788, IC110207, EC388890, IC338794, IC436153, IC336833, IC381158, IC201223, IC326732, IC328582, RDV 45, IC338959, NSV38, IC333833, EC112548, IC281185, IC75730, IC381552, Local 1, EC469904, IC381185, EC326771, Local 2, EC388893, and DOS 1). 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, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar.

Field layout of the Experiment

To conduct the field experiment, all 40 genotypes of Tulsi (*Ocimum basilicum*) were planted in the Research Field of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, during the 2022 and 2023 *kharif* seasons. Hisar is located in a semi-arid sub-tropical region at 29.10°N latitude and 75.46°E longitude with an elevation of 215.52 m above mean sea level.

Nursery Preparation and Transplanting

The nursery was raised by sowing the seeds of all 40 genotypes on separate raised beds. Light irrigation was given from sowing to the development of seedlings with good growth. Twenty-day-old young healthy seedlings of all the genotypes were transplanted in the Randomized Block Design with three replications. One seedling per hill was transplanted at 10 cm spacing between the plants in two rows, each of 3m 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 a good *Tulsi* crop.

Observations and Analysis

The observations for following 17 quantitative traits i.e. plant height (cm), number of primary branches/plant, days to 50% flowering, spike length (cm), number of spikes/plant, number of flowers' whorls/spike, fresh herbage yield/plant (g), oil content (%), dry herbage yield/plant (g), days to maturity, seed yield/plant (g), 1000 seed weight (g), standard germination (%), seedling dry weight (mg), seedling length (mm), seed vigour index – I, and seed vigour index – II were recorded under natural field conditions and in laboratory. The data for quantitative traits from 1 to 10 were recorded on five randomly selected competitive plants from each genotype in each replication of the

Tulsi crop grown under natural field conditions. Mean of the five plants was calculated to record the data on per-plant basis. The observations for the quantitative traits from 11 to 17 were recorded under laboratory conditions. The observations for 1000 seed weight (g), standard germination (%), seedling length (mm), seedling dry weight (mg), seed vigour index-I and seed vigour index-II were taken in Seed Testing Laboratory, Department of Seed Science and Technology, and for oil content (%) in the Quality Analysis Laboratory, Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Analysis of variance (ANOVA) for the observations recorded on different characteristics was carried out as per the standard procedure suggested by Panse and Sukhatme (1967).

Results

Analysis of Variance (ANOVA)

The mean sum of squares due to genotypes presented in ANOVA Table 1, were highly significant for all the 17 quantitative traits *viz*., days to 50% flowering, days to maturity, number of primary branches per plant, plant height (cm), number of spikes per plant, spike length (cm), number of flowers' whorls per spike, fresh herbage yield per plant (g), dry herbage yield per plant (g), seed yield per plant (g), 1000-seed weight (g), oil content (%), standard germination (%), seedling length (cm), seedling dry weight (mg), seed vigour index-I and seed vigour index-II. It showed that an adequate amount of genetic variability was present for all the traits among the 40 genotypes studied, which can be utilized for genetic enhancement of Tulsi genotypes.

Genetic Divergence Analysis

In the present investigation, the D^2 distance matrix given by Mahalanobis (1936) and further elaborated by Murthy and Arunachalam (1966) was used for the genetic divergence study among the 40 *Tulsi* genotypes. The 40 *Tulsi* genotypes were grouped into 10 clusters by following Tocher's method (Rao 1952). The D^2 statistical analysis revealed that a substantial amount of genetic diversity is available among the 40 *Tulsi* genotypes. The genotypes that belong to the same cluster were more closely related to each other. A maximum of 29 genotypes were grouped in cluster I, followed by cluster VII (3 genotypes), and only one genotype was included under the cluster II, cluster III, cluster IV, cluster V, cluster VI, cluster VIII, cluster IX and cluster X as revealed in Table 2 and Fig. 1. Intra and inter-cluster distances are presented in Table 3. The maximum intra-cluster distance (35.57) was noticed for

Table 1: Analysis of variance (ANOVA) for seed yield and its component traits in 40 Tulsi genotypes

Sr. No.	Characters	Mean sum of squares		
		Replication	Genotype	Error
	Degree of Freedom	2	39	78
1.	Days to 50% flowering	4.01	504.77**	4.85
2.	Days to maturity	5.73	371.32**	4.96
3.	Number of primary branches/plants	1.70	19.35**	1.10
4.	Plant height (cm)	25.80	275.33**	11.85
5.	Number of spikes/plant	17.79	876.71**	21.03
6.	Spike length (cm)	0.31	37.54**	0.86
7.	Number of flowers' whorls/spike	0.06	11.58**	0.83
8.	Fresh herbage yield/plant (g)	486.91	163239.79**	804.67
9.	Dry herbage yield/plant (g)	385.20	21752.76**	130.25
10.	Seed yield/plant (g)	5.59	561.88**	7.58
11.	1000 seed weight (g)	0.002	0.482**	0.001
12.	Oil content (%)	0.0003	0.0222**	0.0001
13.	Standard germination (%)	12.10	453.80**	6.19
14.	Seedling length (mm)	1.58	692.94**	37.05
15.	Seedling dry weight (mg)	0.29	16.36**	0.11
16.	Seed vigour index-I	79664.43	8615014.11**	224980.60
17.	Seed vigour index-II	2124.72	119627.79**	1203.10

** Significant at 1% level of significance, *Significant at 5 % level of significance, DF: Degree of freedom

Table 2: Clustering of 40 Tulsi genotypes based on the D^2 statistic

Cluster	No. of genotypes	Name of genotypes
1	29	EC 388887, NSV 38, IC 281185, EC 388895, IC 333833, IC 336833, EC 388737, IC 436153, IC 326735, EC 388782, EC 388896, EC 112548, IC 326732, IC 338794, EC 388788, EC 388889, IC 312264, EC 326771, IC 381552, EC 469904, EC 388893, IC 381158, IC 338959, IC 387838, Local 1, IC 369247, IC 110207, EC 338772, IC 387837
2	1	IC 44681
3	1	IC 201223
4	1	IC 328582
5	1	RDV 45
6	1	IC 469938
7	3	IC 75730, Local 2, IC 381185
8	1	IC 388785
9	1	EC 388890
10	1	DOS 1

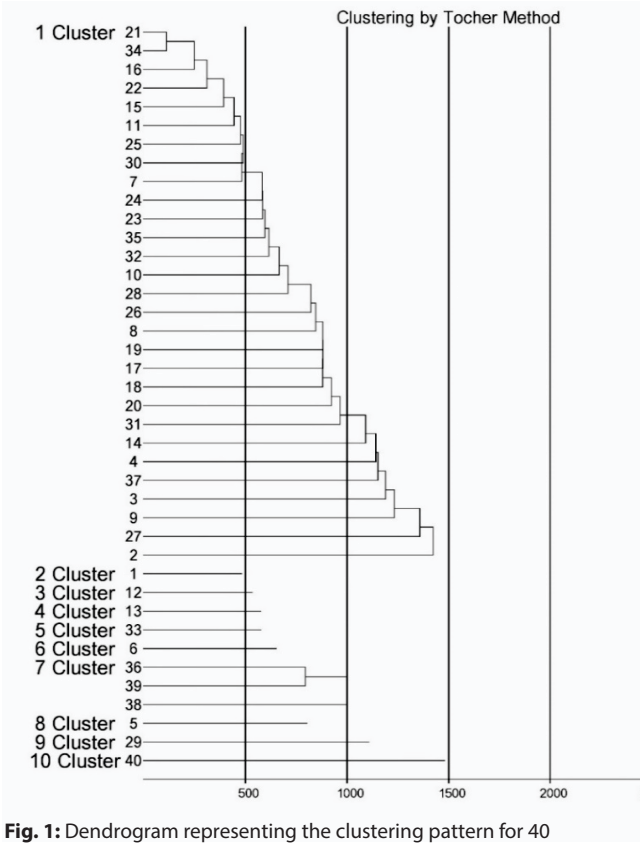

Fig. 1: Dendrogram representing the clustering pattern for 40 genotypes of Tulsi

cluster VII, followed by cluster I (31.73). For the rest of the clusters (cluster II, cluster III, cluster IV, cluster V, cluster VI, cluster VIII, cluster IX and cluster X) intra-cluster distance was zero. The maximum inter-cluster distance (105.80) was observed between cluster II and cluster X followed by between cluster IV and cluster X (99.80), cluster III and cluster X (98.65), cluster I and cluster X (83.26), cluster VIII and cluster X (83.08), cluster III and cluster VII (77.76), cluster II and cluster VII (77.11), cluster IV and cluster VII (76.30), cluster VI and cluster X (76.18), cluster V and cluster X (75.90), cluster V and cluster VII (68.68), cluster IX and cluster X (62.76) and cluster IV and cluster IX (59.63).

Cluster Means for Different Quantitative Traits of Tulsi Genotypes

The genetic divergence among 40 genotypes of *Tulsi* was also supported by cluster means computed for 17

Table 3: Intra and Inter Cluster distances (D^2) among ten clusters of Tulsi genotypes

Clusters	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10
C 1	31.73	38.53	41.39	43.81	45.80	40.57	57.51	40.25	48.21	83.26
C 2		0.00	23.47	26.99	49.15	51.68	77.11	33.57	59.16	105.80
C 3			0.00	33.16	36.29	60.03	77.76	32.34	60.18	98.65
C 4				0.00	51.17	54.59	76.30	32.08	59.63	99.80
C 5					0.00	62.53	68.68	39.94	50.34	75.90
C 6						0.00	45.85	41.28	33.71	76.18
C 7							35.57	65.27	52.13	58.04
C 8								0.00	36.69	83.08
C 9									0.00	62.76
C 10										0.00

C 1: Cluster 1, C 2: Cluster 2, C 3: Cluster 3, C 4: Cluster 4, C 5: Cluster 5, C 6: Cluster 6, C 7: Cluster 7,C 8: Cluster 8, C 9: Cluster 9, C 10: Cluster 10

Table 4: Cluster means for 17 different quantitative traits of 40 Tulsi genotypes

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
DF	58.02	73.33	80.33	78.33	49.33	57.33	50.33	79.33	46.00	41.00
DM	165.67	168.00	166.67	160.00	160.33	159.33	153.89	156.67	168.13	140.00
PB	15.96	17.67	14.60	15.60	14.87	14.27	17.83	10.67	19.13	17.13
PH	83.96	88.70	104.60	104.03	85.97	84.90	93.77	103.13	87.13	81.00N
SP	101.92	66.00	120.00	89.80	232.03	81.00	121.12	146.60	195.23	326.40
SL	16.36	23.30	16.87	17.23	17.10	17.77	12.12	22.47	16.73	10.43
NFW	12.99	15.13	16.40	16.07	16.60	14.60	13.71	17.80	12.73	14.27
FHY	520.72	713.27	458.87	1003.43	382.50	1087.77	683.29	1026.33	1249.97	825.23
DHY	154.02	215.77	123.77	319.30	117.67	382.10	182.71	382.87	414.87	206.77
SY	27.76	33.73	20.80	48.70	60.90	16.13	13.52	38.17	57.07	30.47
SW	1.02	1.59	1.86	1.54	1.61	0.63	0.36	1.38	0.81	0.24
OC	0.21	0.26	0.21	0.15	0.22	0.40	0.31	0.33	0.49	0.24
SG	67.22	66.00	70.67	68.00	52.67	44.67	43.78	54.00	70.87	62.67
SDL	100.75	104.00	94.00	93.67	86.00	81.33	61.22	110.00	97.33	63.33S
DW	10.04	11.53	10.23	11.63	7.90	9.43	3.49	9.47	10.03	3.77
SVI-I	6781.65	6861.33	6644.00	6365.33	4537.33	3628.00	2694.22	5941.33	6878.00	3969.33
SVI-II	673.24	761.07	723.33	790.93	416.87	421.73	154.22	511.67	709.20	236.47

DF: Days to 50% flowering, DM: Days to maturity, PB: Number of primary branches/plant, PH: Plant height (cm), NSP: Number of spikes/plant, SL: Spike length (cm), FW: Number of flowers' whorls/spike, FHY: Fresh herbage yield/plant (g). DHY: Dry herbage yield/plant (g), SY: Seed yield/plant (g), SW: 1000 seed weight (g), OC: Oil content (%), SG: Standard germination (%), SDL: Seedling length (mm), SDW: Seedling dry weight (mg), SVI-I: Seed vigour index-I, SVI-II: Seed vigour index-II.

different quantitative traits (Table 4). The results revealed that cluster II showed the maximum cluster mean for spike length (23.30), cluster III for days to 50% flowering (80.33), plant height (104.60) and 1000-seed weight (1.86), cluster IV for seedling dry weight (11.63) and seed vigour index-II (790.93), cluster V for seed yield (60.90), cluster VIII for number of flowers' whorls per spike (17.80) and seedling length (110.00), cluster IX for days to maturity (168.13), number of primary branches per plant (19.13), fresh herbage yield per plant (1249.97), dry herbage yield per plant (414.87), oil content (0.49), standard germination (70.87) and seed vigour index-I (6878.00) and cluster 10 for number of spikes per plant (326.40). From this, it is clear that cluster IX exhibited the highest cluster means for the maximum (i.e. 7) number of characters. Whereas, the minimum cluster mean was shown by cluster II for number of spikes per plant (66.00), cluster IV for oil content (0.15), cluster V for fresh herbage yield per plant (382.50) and dry herbage yield per plant (117.67), cluster VII for seed yield per plant (13.52), standard germination (43.78), seedling length (61.22), seedling dry weight (3.49), seed vigour index-I (2694.22) and seed vigour index-II (154.22), cluster VIII for number of primary branches per plant (10.67), cluster XI for number of flowers' whorls per spike (12.73) and cluster X for days to 50% flowering (41.00), days to maturity (140.00), plant height (81.00), spike length (10.43) and 1000 seed weight (0.24).

Relative Contribution of Different Traits Towards Genetic Diversity

Based on the study of 40 genotypes of *Tulsi* regarding the relative contribution of the 17 yield and its attributes towards genetic divergence is presented in Table 5. It was observed that 1000 seed weight (41.41%)

Table 5: Relative contribution towards the genetic diversity of yield and its attributes

Trait	Contribution (%)
1000 seed weight (g)	41.41
Number of spikes/plant	17.31
Fresh herbage yield/plant (g)	13.46
Oil content (%)	10.77
Seedling dry weight (mg)	5.00
Standard germination (%)	3.59
Days to 50% flowering	2.69
Days to maturity	2.05
Seed vigour index-II	1.54
Dry herbage yield/plant (g)	1.15
Seed yield/plant (g)	0.64
Spike length (cm)	0.38

Trait	Contribution (%)
Plant height (cm)	0.00
Number of primary branches/plants	0.00
Seedling length (mm)	0.00
Number of flowers' whorls/spike	0.00
Seed vigour index-I	0.00

contributed maximum towards genetic divergence followed by number of spikes per plant (17.31%), fresh herbage yield per plant (13.46%), oil content (10.77%), seedling dry weight (5.00%), standard germination (3.59%), days to 50% flowering (2.69%), days to maturity (2.05%), seed vigour index-II (1.54%), dry herbage yield per plant (1.15%), seed yield per plant (0.64) and spike length (0.38%).

Discussion

Tulsi (*Ocimum spp.*) is one of the most important medicinal and aromatic crop plants that have been traditionally used in *Ayurvedic* medicine for its therapeutic properties (Saran *et al*., 2017). It is a versatile crop that can be grown by farmers with limited resources. The conservation and utilization of genetic resources by providing the genetically divergent germplasm lines to develop the improved genotypes (cultivars) for higher yield, disease resistance and high essential oil content, which are important for commercial cultivation (Patel *et al*., 2015; Koli *et al*., 2022). Yield is a complex trait that is influenced by various factors. In order to select the best genotypes for a particular environment, it is important to understand the relationship between yield and its attributing traits (Gowda *et al*., 2019). Wider adaptability and morphological stability an important criteria to improve the herbage yield, and quality of oil and economic products. Arya *et al*., (2024) reported a wide variation in adaptability and morphology in lemongrass. Although advances have been made in the breeding of some medicinal plants, several challenges remain, owing to the particularity and complexity in determining the breeding target. Additionally, in medicinal plants, there are limitations associated with research on traditional and modern breeding methods (Koli *et al*., 2021).

Genetic diversity analysis in plant breeding refers to the assessment of genetic variations within populations, which helps in selecting diverse parents for the improvement of traits and enhances the overall adaptability. Genetic diversity studies are also essential to ensure the resilience and adaptability of crop plants to changing environmental conditions, pests, and diseases. These studies help to identify and conserve diverse genetic resources, enabling breeders to develop new varieties with improved traits, higher productivity and enhanced resistance to biotic and abiotic stresses (Singh *et al*., 2010). Genetic divergence analysis (D^2 analysis) was done using the method suggested by Mahalanobis (1936) and elaborated by Murty and Arunachalam (1966). The level of genetic divergence between two genotypes increases with an increase in the range of D^2 values between them. The grouping of genotypes into different clusters was done following the Tochers' method as described by Rao (1952). A dendrogram was also plotted for genetic divergence studies of 40 *Tulsi* genotypes, which offered a visual representation that simplifies the understanding of genetic divergence. The D^2 analysis grouped the 40 *Tulsi* genotypes into 10 different clusters, which supports the existence of a substantial amount of genetic diversity among them.

After grouping the 40 genotypes of *Tulsi* into 10 different clusters based on 17 different quantitative traits, the mean values of the different clusters revealed the existence of adequate genetic variability among the 10 clusters in terms of the traits studied. In cluster IX, the highest cluster mean values were found for the large number of traits *viz*., seed vigour index-I (6878.00), fresh herbage yield per plant (1249.97), dry herbage yield per plant (414.87), days to maturity (168.13), standard germination (70.87), number of primary branches per plant (19.13) and oil content (0.49). Therefore, the genotype EC 388890 of cluster IX may be utilised comprehensively for further genetic improvement of the *Tulsi* crop. Relative contribution (%) of all 17 traits in the genetic diversity discovered that 1000 seed weight (41.41%) gave the maximum contribution in total genetic divergence expressed in the study. It was followed by the number of spikes per plant, with a 17.31% contribution, fresh herbage yield per plant, with a 13.46% contribution, and oil content, with a 10.77% contribution. The earlier research workers, Patel *et al*., (2018), Singh *et al.,* (2018), and Srivastava *et al.,* (2018) also reported similar findings regarding genetic diversity studies in *Ocimum spp*.

Conclusion

The present investigation on *Tulsi* genotypes revealed high genetic diversity among the genotypes. The maximum intra-cluster distance was observed for the cluster VII and cluster I whereas, the maximum inter-cluster distance was observed between cluster II and cluster X. The highest cluster means were observed in cluster IX for maximum number of characters *viz*. days

to maturity, number of primary branches per plant, fresh herbage yield per plant, dry herbage yield per plant, oil content, standard germination and seed vigour index-I. Relative contribution of different traits towards genetic diversity revealed that 1000 seed weight contributed the maximum towards genetic divergence, followed by number of spikes per plant, fresh herbage yield per plant and oil content. The maximum seed yield per plant and number of primary branches per plant were observed for the genotype EC 338772, maximum fresh as well as dry herbage yield per plant and maximum oil content were recorded for EC 388890, and seed vigour index I and II were found to be maximum for the genotype IC 326732. Hence, the genotypes EC 338772, EC 388890 and IC 326732 can further be utilised for the development of novel cultivars.

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