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## Research Article

### Genetic variability and diversity analysis in sesame (*Sesamum indicum* L.) germplasm

K. Rahna<sup>1</sup>, R. Kalaiyarasi<sup>2\*</sup>, M. Umadevi<sup>2</sup>, A. Senthil<sup>3</sup> and M. Sudha<sup>4</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

<sup>2</sup>Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

<sup>3</sup>Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

<sup>4</sup>Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

\*E-Mail: kalaiyarasir@tnau.ac.in

#### Abstract

The evaluation encompassed 52 sesame germplasms to assess their variability and diversity. Number of primary branches, number of capsules per plant and single plant yield were the traits having high PCV and GCV along with high heritability and genetic advance as percent of mean. These findings suggest considerable variation for these traits among the germplasms and are strongly influenced by genetic factors, making them suitable candidates for effective selection strategies. The diversity among 52 sesame germplasm accessions were analysed using Mahalanobis D<sup>2</sup> analysis and Agglomerative hierarchical clustering. They were grouped into 11 clusters using Mahalanobis D<sup>2</sup> analysis. Cluster II was the largest, followed by cluster I. Highest intra-cluster distance was observed for cluster VIII, followed by cluster IV and cluster II. The greatest inter-cluster distance was observed among cluster VI and VII. Agglomerative hierarchical clustering (AHC) based on Ward's method was used to group the accessions, into clusters of increasing dissimilarity. The highest dissimilarity was observed between the genotypes CO1 and VRI-NIC-16381. Based on the AHC and D<sup>2</sup> analyses, genotypes with moderate to highest mean values from distant clusters are recommended as parental candidates in crop improvement programs to facilitate the creation of superior cross combinations.

**Keywords:** Sesame, variability, diversity, D<sup>2</sup> analysis, gingly, hierarchical clustering

#### INTRODUCTION

Sesame (*Sesamum indicum* L.), is one of the oldest crops and is grown widely in Asia and Africa (Kurdistani and Tohidinejad, 2011). The genus *Sesamum*, belongs to the family Pedaliaceae. It has more than 30 species, among which *Sesamum indicum* is the most cultivated species (Nayar and Mehra, 1970). Sesame primarily serves as an oilseed crop, valued for its oil extraction, while in certain regions, it also finds utilization in the preparation of sweets, snacks, chutneys, and spice blends. (Bedigian and Harlan, 1986). Sesame cultivation spans across more than 5 million acres (approximately 20,000 km<sup>2</sup>) globally. Among the 22 countries leading

in sesame production worldwide, Asia accounts for six, Africa for thirteen, and Latin America (Central and South America) for three. Among these nations, Myanmar, India, and China emerge as the top three producers (Dossa *et al.*, 2023). According to FAO statistics, the combined output from these prominent sesame-producing countries constitutes 92.6% of the global sesame production. Notably, India retains its position as the largest contributor, occupying over 40% of the global sesame cultivation area and contributing 27% to the total worldwide production. (UNSD, 2017; FAOSTAT, 2022). Despite having a large area under cultivation, India's overall production and

productivity (431 kg/ha) is low compared to the global average (512 kg/ha) (Myint *et al.*, 2020). This is because sesame is one of the most underutilised oilseed crops, usually grown on marginal lands with poor management and lack of improved varieties suited to various agro-climatic conditions.

At the national level, National Bureau of Plant Genetic Resources (NBPGR) is in charge of managing diverse crop plant germplasm safely and sustainably. A total of 10,507 sesame accessions have been conserved in NBPGR. One of the important concerns in the management and use of plant germplasm collections is, developing methods for shrinking collections to a manageable and easily accessible size (a core size) (Frankel, 1984; Brown, 1989; Marshall, 1990). Thus, it is crucial to understand the nature and structure of genetic diversity in order to conserve and utilise it effectively. Since a wide range of genetic diversity among parents is necessary for hybridization, information about the genetic diversity and relationships among these sesame populations is crucial for plant breeding programmes (Ganesh and Thangavelu, 1995). Utilising morphological and agronomic traits, it is possible to identify the genetic diversity of crop species (Liu, 1997). Hence, the present study was conducted to evaluate the genetic variability and diversity within a collection of 52 sesame genotypes, employing various methods, and subsequently selecting clusters that encompass superior germplasm exhibiting a range of agronomic performances and promising yield potential.

## MATERIALS AND METHODS

A total of 52 genotypes of sesame collected from Department of Plant Genetic Resources, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu and Onattukara Regional Agricultural Research Station,

Kayamkulam, Kerala (**Table 1**) was grown in randomized block design with two replications in the research field of the Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during *rabi*/summer season in 2022-23. The crop was raised in rows that were 5 meters in length, with a spacing of 30 cm between rows and 15 cm between plants. Throughout the cropping period, recommended package of practices was followed for better crop growth.

Observations were recorded on 11 biometrical traits and the collected data were analysed for variability and genetic diversity using R software (R version 4.3.1). Analysis of Variance (ANOVA) was performed for the eleven quantitative traits (**Table 2**) by following the method suggested by Panse and Sukhatme (1967). The phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were calculated according to the method introduced by Burton and Devane (1953). PCV and GCV were classified as low (<10%), medium (10-20%) and high (>20%) as suggested by Sivasubramanian and Madhavamenon (1973). Furthermore, the heritability in the broad sense and the genetic advance were assessed using the procedure outlined by Johnson *et al.* (1955). Multivariate analysis was performed as per Mahalanobis'  $D^2$  statistics (1928), as stated by Rao (1952), and the genotypes were classified into separate clusters employing Tocher's method. In addition, the agglomerative hierarchical clustering (AHC) was done based on Ward's method.

## RESULTS AND DISCUSSION

The ANOVA (**Table 2**) indicated significant variation between genotypes for each biometrical character. It revealed the presence of sufficient variability among genotypes for the character under consideration. Apart from the traits number of primary branches and number

**Table 1. List of sesame germplasm used for diversity studies**

S. No.	Name of the germplasm	S. No.	Name of the germplasm	S. No.	Name of the germplasm	S. No.	Name of the germplasm
1	VRI-Si-3075	14	SI-R-22-07	27	VRI-NIC-2939	40	VRI-NIC-1633
2	VRI-KMS-370	15	SI-R-22-08	28	VRI-NIC-16208	41	VRI-Si-2630-B
3	VRI-3	16	SI-R-22-09	29	VRI-NIC-8202	42	Thilarani
4	VRI-Si-7217	17	SI-R-22-10	30	VRI-KMS-4-308	43	VRI-NIC-9853
5	SI-R-22-01	18	KAYAMKULAM-1	31	VRI-Si-3171	44	VRI-Si-1025-B
6	VRI-4	19	VRI-NIC-9858	32	VRI-Si-437	45	VRI-KMR-12
7	VRI-Si-1578	20	VRI-KMR-51	33	VRI-KM-10	46	VRI-NIC-17263
8	SI-R-22-02	21	VRI-NIC-8392	34	VRI-NIC-8315	47	VRI-NIC-8252
9	SI-R-22-03	22	VRI-KMS-4-300	35	VRI-NIC-9524	48	VRI-NIC-8045
10	SI-R-22-04	23	Co-1(check)	36	VRI-KMS-4-258	49	VRI-Si-3079-1
11	SI-R-22-05	24	VRI-KMS-4-360	37	VRI-Si-295	50	THILAK
12	SI-R-22-06	25	VRI-NIC-7817	38	VRI-KMR-81	51	THILATHARA
13	VRI-NIC-9852	26	VRI-NIC-7875	39	VRI-NIC-16381	52	AYALI

**Table 2. Analysis of variance of eleven biometrical trait**

	Mean Sum of Squares	Error sum of squares	SE	CD	CV (%)
Number of primary branches	2.86**	0.73	0.60	1.72	19.94
Number of secondary branches	4.47**	1.89	0.97	2.76	17.39
Days to flowering	19.24**	0.57	0.53	1.52	1.91
Days to 50% flowering	19.04**	0.90	0.67	1.91	2.28
Days to maturity	116.95**	5.98	1.72	4.90	2.74
Plant height (cm)	404.25**	5.83	1.70	4.85	2.68
Capsule length (cm)	0.22**	0.01	0.07	0.20	3.90
Number of capsules per plant	6326.85**	108.54	7.36	20.91	7.11
Number of seeds per capsule	143.26**	9.39	2.16	6.15	4.63
1000 seed weight (g)	0.24**	0.07	0.05	0.15	2.50
Single plant yield (g)	55.66**	1.77	0.94	2.67	9.95

of secondary branches, the remaining traits exhibited a slight variation between PCV and GCV values (**Table 3**). This implies that the impact of the environment on these traits was relatively low. The value of PCV was ranging from 7.57 to 40.04 and that of GCV was 7.22 to 38.79. Number of primary branches, number of capsules per plant and single plant yield were the traits having high PCV and GCV, suggesting that these traits possess substantial variation among individuals in the population, and a significant proportion of this variation can be attributed to genetic variability. Comparable findings were reported in the study conducted by Mohan (2014). The low values for both PCV and GCV have been observed in days to flowering, days to 50% flowering and days to maturity implies that these traits exhibit minimal variability among individuals. These observations are in accordance with Mohanty *et al.* (2020) and Kumar *et al.* (2022). Although these measures of variability elucidate the existing variation within the population, they fall short of accounting for the hereditary aspect of this variation. Estimation of

broad-sense heritability and genetic advance can offer insights into the heritable portion of this variation.

High heritability was observed across all assessed traits, with plant height (97.21) displaying the highest heritability value (**Table 3**). This similarity in results is also reflected in the studies conducted by Kiruthika *et al.* (2018) and Patidar *et al.* (2020), implying a consistent trend of genetic predominance over environmental factors in explaining the variations in these traits. Furthermore, in line with findings of Sasipriya *et al.* (2022), high genetic advance as percentage of mean, were observed for various traits including the number of primary branches, plant height, capsule length, number of capsules per plant, number of seeds per capsule, 1000 seed weight, and single plant yield. It suggests that, along with high heritability, selection on these traits will be favourable. Conversely, traits like number of secondary branches, days to flowering, days to 50% flowering, and days to maturity demonstrated a moderate level of genetic advance. In

**Table 3. Estimates of genetic components of variance, heritability and genetic advance for eleven traits**

Characters	Mean	Range		PCV (%)	GCV (%)	Heritability (%)	Genetic advance	GAM (%)
		Min	Max					
Number of primary branches	4.28	2.0	7.0	31.22	24.02	59.23	1.63	38.08
Number of secondary branches	7.24	2.0	13.0	22.56	14.37	40.62	1.37	18.86
Days to flowering	39.56	33.0	46.0	7.95	7.72	94.26	6.11	15.44
Days to 50% flowering	41.69	35.0	48.0	7.57	7.22	90.94	5.91	14.18
Days to maturity	89.17	69.2	107.8	8.79	8.35	90.33	14.58	16.35
Plant height (cm)	89.85	54.7	126.1	15.93	15.70	97.21	28.65	31.89
Capsule length (cm)	2.68	2.0	3.4	12.79	12.18	90.76	0.64	23.89
Number of capsules per plant	146.91	48.0	282.0	38.71	38.05	96.64	113.19	77.05
Number of seeds per capsule	66.09	48.0	85.0	13.22	12.38	87.72	15.78	23.88
1000 seed weight (g)	3.10	2.2	4.1	11.37	11.09	95.21	0.69	22.29
Single plant yield (g)	12.6	4.8	20.4	40.04	38.79	93.83	9.75	77.39

addition to gaining perspectives from variability analysis, conducting correlation studies is essential as they provide a deeper understanding of how different traits interact and influence each other in the given population.

Analysis of correlation among the 11 quantitative traits, shed light on their interrelationships and potential patterns (Table 4). The strong positive correlation observed between traits, days to first flowering and days to 50% flowering, suggests a close association in their developmental line. The single plant yield displayed a pronounced positive correlation with the number of capsules per plant (0.802), succeeded by plant height (0.534), days to maturity (0.49), and capsule length (0.465). These results align with Haibru *et al.* (2018) and Navaneetha *et al.* (2019), reinforcing the significance of these traits in contributing to overall plant yield. The correlation analysis not only

provides an understanding of the relationships between the quantitative traits, but it also offers the basis for further investigation into how these interrelated traits may impact the diversity dynamics within the population.

To gain insights into the genetic divergence patterns within the given population, an analysis utilizing the Mahalanobis D<sup>2</sup> method was performed. This analysis, employing Tocher's method (Rao 1952), resulted in the classification of 52 sesame genotypes into 11 distinct clusters based on their D<sup>2</sup> values (as shown in Table 5), with genotypes belonging to the same cluster having an average lower D<sup>2</sup> value than those belonging to different clusters. The pattern of clustering was arbitrary and independent. The present study showing cluster II as largest cluster comprised of 18 germplasm, followed by cluster I, having 10 sesame germplasm. The percentage

**Table 4. Correlation analysis of 11 studied quantitative traits**

	NPB	NSB	DF	DFF	DM	PH (cm)	CL (cm)	NCP	NSC	TSW (g)	SPY (g)
NPB	1	0.565***	0.011	-0.01	0.263**	0.159	0.103	0.216*	0.092	0.031	0.214*
NSB	0.565***	1	0.13	0.11	0.276**	0.04	0.265**	0.19	0.193*	0.11	0.222*
DF	0.011	0.127	1	0.967***	0.372***	0.082	0.008	-0.03	0.052	-0.09	-0.03
DFF	-0.01	0.113	0.967***	1	0.331***	0.053	-0.04	-0.05	0.001	-0.05	-0.07
DM	0.263**	0.276**	0.372***	0.331***	1	0.348***	0.375***	0.579***	0.29**	-0.01	0.49***
PH (cm)	0.16	0.04	0.08	0.05	0.348***	1	0.241*	0.488***	0.197*	0.03	0.534***
CL (cm)	0.103	0.265**	0.008	-0.04	0.375***	0.241*	1	0.434***	0.84***	0.126	0.465***
NCP	0.216*	0.186	-0.03	-0.05	0.579***	0.488***	0.434***	1	0.337***	0.147	0.802***
NSC	0.09	0.193*	0.05	0	0.29**	0.197*	0.84***	0.337***	1	0.01	0.386***
TSW (g)	0.031	0.108	-0.09	-0.05	-0.01	0.025	0.126	0.147	0.006	1	0.138
SPY (g)	0.214*	0.222*	-0.03	-0.07	0.49***	0.534***	0.465***	0.802***	0.386***	0.138	1

(NPB-Number of primary branches, NSB- Number of secondary branches, DF- Days to flowering, DFF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, CL- Capsule length, NCP- Number of capsules per plant, NSC- Number of seeds per capsule, TSW- Thousand seeds weight, SPY- Single plant yield)

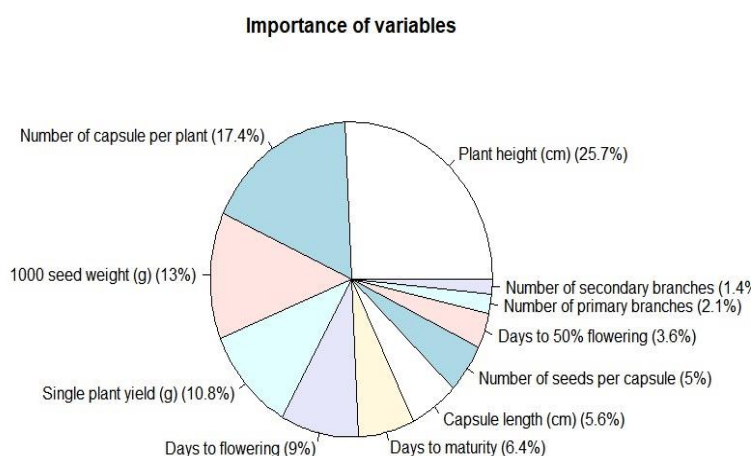
**Table 5. Distribution of 52 genotypes in to 11 clusters**

Cluster Number	Number of genotypes	Genotypes
I	10	SI-R-22-01, SI-R-22-03, SI-R-22-09, SI-R-22-02, SI-R-22-05, SI-R-22-08, SI-R-22-07, SI-R-22-04, VRI-NIC-9852, VRI-Si-2630-B
II	18	VRI-KMR-12, AYALI, VRI-NIC-8315, VRI-KMS-4-308, VRI-NIC-2939, VRI-NIC-9524, VRI-NIC-16381, VRI-NIC-8045, KAYAMKULAM-1, THILATHARA, THILAK, VRI-Si-1025-B, VRI-Si-3079-1, VRI-NIC-8252, VRI-NIC-16208, VRI-4, VRI-Si-437, VRI-NIC-17263
III	7	VRI-KMS-370, VRI-Si-7217, VRI-KM-10, VRI-NIC-9853, VRI-KMS-4-300, VRI-Si-3075, VRI-KMR-51
IV	7	VRI-NIC-9858, VRI-NIC-7875, VRI-NIC-7817, VRI-Si-3171, VRI-Si-295, VRI-KMR-81, VRI-NIC-8202
V	2	Thilarani, VRI-3
VI	2	SI-R-22-10, SI-R-22-06
VII	1	VRI-NIC-1633
VIII	2	VRI-Si-1578, VRI-NIC-8392
IX	1	VRI-KMS-4-360
X	1	Co-1(check)
XI	1	VRI-KMS-4-258

contribution of each character towards genetic divergence was represented in **Fig. 1**, revealing that, plant height (25.7%) has the greatest impact on genetic divergence, followed by the number of capsules per plant (17.4%). Single plant yield, 1000 seed weight, days to flowering, number of secondary branches per plant, capsule length, number of primary branches per plant, days to 50% flowering and days to maturity revealed moderate to low contributions. The inter and intra cluster  $D^2$  mean value have been presented in table 3. Cluster VIII reported the maximum intra-cluster distance (177.41), followed by cluster IV (156.96) and cluster II (155.50) (**Table 6**). Highest inter cluster distance between the clusters VI and VII suggesting crosses involving genotypes from these two clusters would offer sufficient amount of heterosis for selection. Cluster mean of each character have been shown in **table 7**, in which most characters recorded

highest mean value for cluster X as reported in the study by Mukhthambica *et al.* 2020, in which cluster VII showed highest mean value for most of the characters. Hence, utilising either of the parent for breeding programme from cluster X, will provide enhancement of particular traits. Following the Mahalanobis  $D^2$  method, to reveal genetic divergence patterns, a complementary approach has been used through Agglomerative Hierarchical Clustering (AHC), which further organized the genotypes into cohesive clusters based on their dissimilarity.

Agglomerative hierarchical clustering (AHC) based on Ward's method was used to sort the data provided, into clusters of increasing dissimilarity. AHC have grouped fifty-two genotypes in to three main clusters and to further subclusters (**Fig. 2**). The highest dissimilarity was observed between the genotype "Co-1" and "VRI-



**Fig 1.** Pie diagram depicting the importance of each variable in distinguishing and characterizing different clusters in Mahalanobis  $D^2$  Method

**Table 6.** Intra and inter cluster distance of 11 clusters obtained through Mahalanobis  $D^2$  analysis. (intra-cluster distance in bold)

CLUSTER	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	<b>108.41</b>	283.80	269.85	541.34	279.98	222.99	767.78	216.95	372.86	309.98	535.12
II		<b>155.50</b>	247.95	369.91	204.31	566.18	514.72	323.62	331.24	610.22	371.13
III			<b>136.61</b>	370.61	264.51	612.96	381.44	239.27	478.38	817.59	615.56
IV				<b>156.96</b>	462.45	1075.2	212.36	480.49	833.57	985.26	414.59
V					<b>97.19</b>	535.68	599.30	367.06	496.63	517.41	402.34
VI						<b>82.20</b>	1466.7	541.14	332.48	273.59	730.31
VII							<b>0.00</b>	490.60	1116.1	1439.8	705.22
VIII								<b>177.41</b>	629.71	536.69	755.70
IX									<b>0.00</b>	738.83	473.71
X										<b>0.00</b>	675.57
XI											<b>0.00</b>

Table 7. Cluster mean of 11 studied quantitative traits.

Clusters	NPB	NSB	DF	DFF	DM	PH (cm)	CL (cm)	NCP	NSC	TSW (g)	SPY (g)
I	4.05	7.55	38.50	40.25	92.55	98.74	2.97	209.90	70.25	3.20	18.34
II	4.39	7.19	42.06	44.06	89.33	90.51	2.62	119.08	65.86	2.92	10.70
III	4.00	6.64	35.57	37.86	84.14	87.83	2.46	135.71	62.21	2.81	11.84
IV	3.29	5.93	38.57	41.29	79.43	70.78	2.55	105.07	62.21	3.49	8.62
V	4.50	8.00	42.00	44.25	99.50	83.48	2.20	157.25	52.25	2.89	14.43
VI	<b>6.00</b>	8.25	38.75	41.50	98.75	118.38	3.03	252.25	73.50	3.37	19.67
VII	5.00	6.50	33.50	35.50	86.00	61.80	2.50	50.00	60.50	3.27	6.14
VIII	5.75	9.75	36.25	38.25	94.75	83.30	3.25	178.00	79.50	2.94	16.92
IX	5.00	7.00	40.50	42.50	87.00	<b>125.25</b>	2.75	106.50	66.50	3.05	11.29
X	<b>6.00</b>	<b>11.00</b>	<b>45.50</b>	<b>47.50</b>	<b>105.50</b>	93.90	<b>3.25</b>	<b>279.00</b>	<b>83.00</b>	3.56	<b>20.39</b>
XI	4.50	9.00	42.50	45.00	93.00	94.45	2.25	81.00	57.00	<b>3.7</b>	13.80

NPB-Number of primary branches, NSB- Number of secondary branches, DF- Days to flowering, DFF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, CL- Capsule length, NCP- Number of capsules per plant, NSC- Number of seeds per capsule, TSW- Thousand seeds weight, SPY- Single plant yield

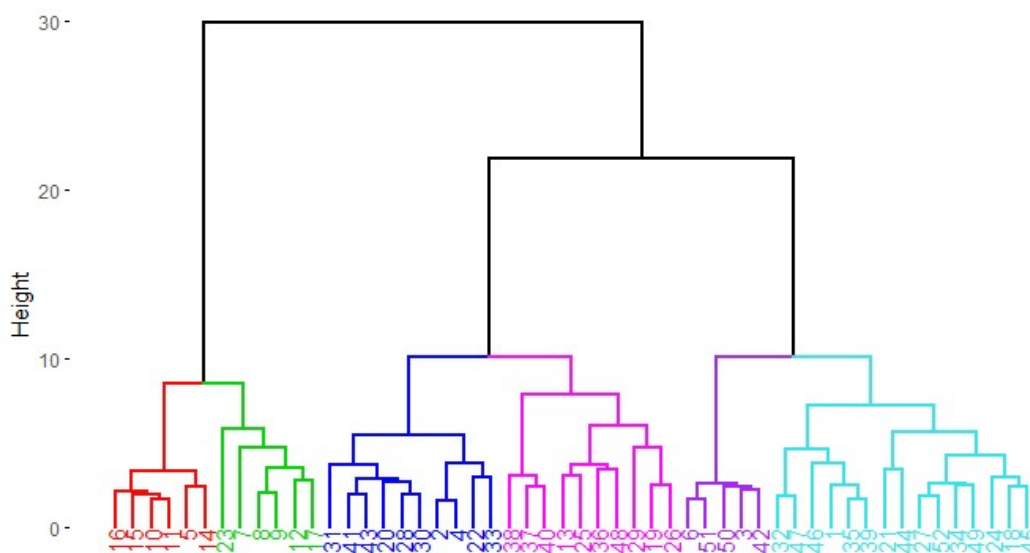


Fig. 2. Agglomerative Hierarchical Cluster Diagram illustrating the clustering of genotypes based on dissimilarity. (Genotypes are indicated by numbers given in the Table 1.)

NIC-16381". The genotypes "Kayamkulam-1", "Ayali", "Thilak", "Thilathara" and "Thilarani" from Kerala fell in to different sub clusters. "Kayamkulam-1" and "Ayali" were grouped into same subclusters while, the remaining Kerala genotypes were clustered to another. The findings showed that many genotypes that were geographically adjacent to one another fell into separate clusters and *vice versa*. Furthermore, according to Laurentin and Karlovsky (2006), geographical isolation was not the sole factor fostering variation in a natural population.

In summary, due to a significant inter cluster distance observed between cluster VI (high-yielding cluster) comprising genotypes "SI-R-22-10" and "SI-R-22-06", and cluster VII (low-yielding cluster) represented by "VRI-NIC-

1633", these clusters hold potential for parental selection in crossbreeding programs. Moreover, both  $D^2$  analysis and hierarchical clustering have given almost similar clustering pattern except for some genotype like, "VRI-Si-3075", "VRI-KMS-4-360", "VRI-Si-3171", "VRI-KMS-4-258" and "VRI-NIC-1633".

For the purpose of advancing research on breeding strategies and the choice of parental lines, it is crucial to characterise the genetic variability and diversity found in sesame germplasms. Overall, the results showed that existence of diversity within the collected sesame germplasms. Therefore, using the cultivars from such heterotic group could be effective for enhancing the variation in hybridization programme. The current

research found several factors that are highly variable in explaining the morpho-physiological diversity of the evaluated genotypes, notably plant height, days to flowering and capsule features. To break the stumbling block of local phenotypic variability and produce new cultivars with desirable qualities, selective breeding of these traits might be useful.

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