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Research Note

# Analysis of genetic diversity in sesame (*Sesamum indicum* L.) germplasm lines based on agro-morphological traits and SSR markers

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**Abstract**
An investigation was carried out with a set of fifty sesame genotypes to study the degree of genetic diversity based on morphological and marker based genotypic data. The genomic isolation was carried out using CTAB method and the molecular diversity was estimated with the help of fifty SSR markers using per cent polymorphism, PIC content and represented in dendogram. Morphological diversity was estimated by using $D^2$ analysis. Quantitative traits, namely plant height (67.8-147.2 cm), number of capsules per plant (36-89), number of seeds per capsule (46-70), seed yield per plant (3.20-10.89 g), oil content (40.64-50.51 %), protein content (20.5-32.42 %), oleic acid content (31.25-42.85 %) and linoleic acid content (40.39-54.11 %) showed a significant amount of variation. Fifty genotypes were grouped into fourteen clusters based on data of 15 agro-morphological traits by following Tocher's method. SSR markers showed 36% polymorphism with an average of 2.39 alleles per locus and 0.34 PIC value indicated a trend of moderate level of genetic diversity at molecular level in the collection of genotypes. In order to assess genetic diversity and to select various lines for breeding programs, the results highlighted the importance of utilizing both agro-morphological and molecular data.

**Keywords:** Sesame, genetic diversity, molecular markers, $D^2$ analysis

Sesame (*Sesamum indicum* L.) is an important oilseed crop cultivated globally, renowned for its nutritional value, culinary adaptability, and economic significance. The sesame seed, comprising 44% to 58% oil, 18% to 25% protein, 13.5% carbohydrates, and 5% ash, plays a pivotal role in baking, confectionery, and in other food industries (Uzun and Hersek, 2002; Were *et al*., 2006; Elleuch *et al*., 2007). Sesame oil, characterized by its balanced fatty acid composition, contains almost equal amounts of oleic acid (35% to 54%) and linoleic acid (39% to 59%), accompanied by roughly 10% palmitic acid and around 5% stearic acid (Wacal *et al*., 2019). This intricate fatty acid composition not only enhances its culinary value but also holds potential health benefits.

The rich morphological and genetic diversity harboured within sesame populations offer a valuable resource for crop improvement. However, considering the changing

environmental conditions and evolving agricultural requirements, understanding the genetic diversity of sesame takes on heightened importance. Morphological and quality traits have long been indicators of genetic variability and adaptation, while molecular markers provide insights into the genetic underpinnings of these traits. The combined analysis of morphological attributes and molecular markers may unravel the complex relationships between genotype and phenotype, facilitating targeted breeding efforts and conservation strategies.

Knowledge about the nature and magnitude of genetic divergence is a prerequisite for an effective hybridization programme (Mukhthambica *et al*., 2023). According to Rao *et al.* (1981) and Jatasra and Paroda (1983), genetic divergence analysis helps in the classification of genotypes into discrete genotypic classes and the identification of parents for hybridization. The crosses between divergent parents usually produced greater heterotic effect than between closely related ones (Rodge *et al*., 2003). The $D^2$ statistic developed by Mahalanobis (Mahalanobis, 1936) is an effective method for estimating the level of genetic diversity. By using cluster analysis, genotypes are divided into several groups. In order to identify genetically varied high-yielding genotypes that could aid in crossing programs to produce more transgressive segregants, $D^2$ analysis would be more helpful.

According to Jones *et al.* (2009), molecular markers are highly reliable genetic tools that can be used along with phenotypic characterization in breeding. The SSR markers have been widely used in analysing the sesame genetic diversity, population structure and heterotic groups. According to Wei *et al.* (2014), Dossa *et al.* (2016), Asekova *et al.* (2018), and De Sousa *et al.* (2019), these markers are highly preferred for detecting higher degrees of polymorphism, reproducibility, allelic variation, and their abundance in the genomes.

The present investigation aimed to study the extent of genetic diversity present within sesame populations by considering both yield components and quality traits. Furthermore, the utilization of SSR molecular markers will provide a deeper understanding of the genetic basis underlying these variations. This study not only contributes to the fundamental understanding of sesame genetics but also holds practical implications for crop improvement strategies and germplasm conservation.

The experiment was carried out at Regional Agricultural Research Station, Polasa, Jagtial, during late *kharif*, 2022. The experimental material consisted of 50 genotypes collected from different sources like Tikamgarh, Madhya Pradesh (6), Project Coordinate unit, JNKVV, Jabalpur (15), ARS, Yelamanchili (1), Mauranipur, Uttar Pradesh (1), RARS, Polasa, Jagtial (26) and Agricultural Research Station (JAU) Amreli, Gujrat (1).

Morphological evaluation: The genotypes were sown in Randomized block design with two replications in which each genotype was sown in 2 rows of 3m length with 30 × 15 cm spacing. All recommended package and practices were followed. Data were recorded in five randomly selected plants in each genotype for six different traits namely plant height (cm), number of branches per plant, number of capsules per plant, number of seeds per capsule, 1000 seed weight (g) and seed yield per plant (g). The traits, days to 50% flowering and days to maturity were recorded on plot basis.vThe quality parameters of seed harvested from these entries were assessed at biochemistry laboratory of ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad. Oil content of sesame genotypes was estimated by using nuclear magnetic resonance (NMR)—Oxford- MQC-5 analyser (London, UK). Protein content was determined by micro-Kjeldahl method (Markham, 1942) and fatty acid composition *viz.* Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) were determined using an Agilent 7890B gas chromatograph (Santa Clara, California, USA) equipped with a flame ionization detector (FID) and an auto sampler.

Statistical methods: The Mahalnobis $D^2$ statistic (Rao, 1952) was used to calculate average intra and inter-cluster distances based on the data on quantitative traits. To evaluate the cluster formation, Mahalanobis distance matrix was subjected to Tocher's method (Rao, 1952). The cluster mean and the percentage contribution of the quantitative traits to genetic diversity were estimated.

Molecular characterization: The genetic diversity analysis based on molecular markers was carried out at biotechnology laboratory of the ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad. Genomic DNA was isolated using CTAB method (Doyle and Doyle, 1987). The quantification of extracted DNA was done by comparison with defined concentrations of λ DNA along with 100 bp DNA ladder in agarose gel. Genetic diversity at molecular level was assessed using 50 SSR markers. Data was scored and analysed using diversity analysis software DARwin 6.0 (Perrier and Flori, 2003). The number of alleles (n), major allele frequency (MAF), Polymorphism information content (PIC) and number of unique alleles (UA) were calculated to assess diversity of alleles of marker locus using POWERMARKER (Version 3.25).

In this study, the extent of genetic diversity among 50 genotypes of sesame was assessed based on 15 quantitative traits and genotypic data from 50 sesame SSR primers.

Diversity analysis based on agro-morphological traits: All the genotypes showed significant difference for all the

characters which indicated the existence of variability among the genotypes studied. The mean and range of 15 quantitative traits scored across 50 sesame genotypes are given in **Table 1**.

Cluster formation based on Tocher's analysis of collective field data on yield, yield components and quality traits generated 14 clusters (**Table 2**). Cluster II was the largest comprising of 18 genotypes followed by cluster I with 10 genotypes, cluster III with seven genotypes and cluster IX with three genotypes. Cluster XI and XIV each had two genotypes. Cluster IV, V, VI, VII, VIII, X, XII and XIII were solitary indicating that these clusters were represented by single genotype with high degree of heterogeneity among them. Formation of more number of clusters is an indication of higher divergence among the genotypes.

The inter cluster distance ranged from 127.28 (between cluster V and VI) to 21519.43 (between cluster XI and XIV). The crossing or hybridization made between the genotypes selected from the clusters (cluster XI and XIV) which showed the highest inter cluster distances may give high heterotic response and thus better segregants which were corroborated with the results of

**Table 1. Mean and range of quantitative traits studied across the genotypes**

| S. No. | Character | Mean | Range | CV (%) | CD (at 5 %) |
|---|---|---|---|---|---|
| 1. | Days to 50% flowering | 40.82 | 35.00-48.00 | 3.10 | 2.55 |
| 2. | Days to maturity | 93.57 | 82.00 - 99.00 | 1.79 | 3.38 |
| 3. | Plant height (cm) | 116.60 | 67.8-147.2 | 8.74 | 20.53 |
| 4. | Number of branches per plant | 4.63 | 2.00-7.00 | 7.83 | 30.73 |
| 5. | Number of capsules per plant | 53.85 | 36.00-89.00 | 10.20 | 11.14 |
| 6 | 1000 seed weight (g) | 3.22 | 2.75-3.62 | 4.26 | 4.92 |
| 7 | Number of seeds per capsule | 57.00 | 46.00-70.00 | 2.64 | 0.17 |
| 8 | Seed yield per plant (g) | 5.71 | 3.20-10.89 | 12.17 | 1.38 |
| 9 | Oil content (%) | 46.85 | 40.64-50.51 | 0.49 | 0.46 |
| 10 | Protein content (%) | 25.75 | 20.5-32.42 | 0.74 | 0.38 |
| 11 | Palmitic acid (%) | 9.09 | 7.88-9.91 | 1.24 | 0.22 |
| 12 | Stearic acid (%) | 5.78 | 4.98-6.55 | 1.79 | 0.20 |
| 13 | Oleic acid (%) | 38.02 | 31.25-42.85 | 0.23 | 0.17 |
| 14 | Linoleic acid (%) | 46.73 | 40.39-54.11 | 0.19 | 0.18 |
| 15 | Linolenic acid (%) | 0.39 | 0.15-0.96 | 8.80 | 0.06 |

**Table 2. Clustering pattern of sesame genotypes based on $D^2$ values**

| Clusters | Number of genotypes | Name of genotypes |
|---|---|---|
| Cluster 1 | 10 | TKG 55, TKG 308, JTS 8, EC 3349998, FFAT 04,EC 182833, EC 3349997, ES 3196, ES 81 and JCS 4020. |
| Cluster 2 | 18 | JCS 4862, Telangana Til-1 (JCS 3202), JCS 3287, JCS 3758, JCS 3880, NIC 16095-A, JCS 4026, JCS 4047, Pragathi, JCS 3605, JCS 2698, JCS 4018, JCS 3889, Jagtiala Til-2 (JCS 2454), JCS 4904, JCS 4911, Jagtiala Til-1 (JCS 1020) and GT 10. |
| Cluster 3 | 07 | FFAT 16, Swetha, JCS 4022, JCS DT 26, JCS 3890, JCS RF4 and JCS 3888. |
| Cluster 4 | 01 | ES 28 |
| Cluster 5 | 01 | IS 35-1-A |
| Cluster 6 | 01 | EC 182835 |
| Cluster 7 | 01 | JCS 4894 |
| Cluster 8 | 01 | NIC 9843 |
| Cluster 9 | 03 | Madhavi, FFAT 13 and FFAT 17 |
| Cluster 10 | 01 | JCS 3604 |
| Cluster 11 | 02 | TKG 21 and TKG 306 |
| Cluster 12 | 01 | TKG 22 |
| Cluster 13 | 01 | EC 330005 |
| Cluster 14 | 02 | JCS RF2 and JCS 4917 |

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**Table 3. Average intra (diagonal) and inter cluster distances ($D^2$) of sesame genotypes**

| Clusters | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 | Cluster 6 | Cluster 7 | Cluster 8 | Cluster 9 | Cluster 10 | Cluster 11 | Cluster 12 | Cluster 13 | Cluster 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cluster 1 | **537.39** | 2132.69 | 6140.98 | 735.88 | 3472.54 | 2842.43 | 752.67 | 901.22 | 1624.93 | 2813.56 | 1679.53 | 1496.06 | 5471.57 | 13215.17 |
| Cluster 2 | | **563.68** | 1897.08 | 1323.05 | 892.23 | 877.77 | 1656.03 | 2639.62 | 1761.31 | 1125.13 | 5977.42 | 1305.54 | 1437.05 | 5889.21 |
| Cluster 3 | | | **352.1** | 4229.09 | 2243.11 | 2800.22 | 5709.83 | 7547.25 | 5413.34 | 2649.21 | 12252.52 | 4037.14 | 1206.52 | 1937.77 |
| Cluster 4 | | | | **0** | 2645.63 | 2232.68 | 711.99 | 1326.56 | 1428.88 | 2684.05 | 2582.61 | 1597.22 | 4258.92 | 10081.89 |
| Cluster 5 | | | | | **0** | 127.28 | 2080.47 | 3169.45 | 1722.29 | 1249.16 | 7933.39 | 1866.61 | 654.78 | 5087.00 |
| Cluster 6 | | | | | | **0** | 1602.71 | 2633.47 | 1466.09 | 1107.18 | 6898.48 | 1386.44 | 1129.94 | 6257.39 |
| Cluster 7 | | | | | | | **0** | 252.86 | 500.65 | 2721.36 | 2193.24 | 1724.97 | 4354.6 | 11698.34 |
| Cluster 8 | | | | | | | | **0** | 717.93 | 3689.43 | 1572.84 | 2448.33 | 5712.92 | 14341.92 |
| Cluster 9 | | | | | | | | | **860.31** | 2939.29 | 3527.22 | 2289 | 3682.24 | 10607.78 |
| Cluster10 | | | | | | | | | | **0** | 7428 | 579.94 | 1351.81 | 7077.24 |
| Cluster11 | | | | | | | | | | | **553.58** | 4758.59 | 11417.42 | 21519.43 |
| Cluster12 | | | | | | | | | | | | **0** | 2765.29 | 9775.08 |
| Cluster 13 | | | | | | | | | | | | | **0** | 3016.54 |
| Cluster 14 | | | | | | | | | | | | | | **687.47** |

Krishna *et al*. (2018) and Mohanty *et al*. (2020). Intra cluster distance revealed that the genotypes in cluster IX (860.31) were most diverse (**Table 3**). Promising genotypes of Cluster IX, which showed maximum intra-cluster distances, were Madhavi, FFAT 13, and FFAT 17. These genotypes can be considered highly divergent among themselves. Due to their solitary nature, clusters IV, V, VI, VII, VIII, X, XII, and XIII recorded zero intra-cluster distances.

The problem of selecting diverse parents for hybridization programme can be narrowed, if one can identify the characters responsible for the discrimination between the populations (Ramprasad *et al*., 2019). The per cent contribution towards genetic divergence by all the yield, yield contributing traits and quality parameters is presented in **Table 4**. The characters such as oleic acid (43.51 %), linoleic acid (33.87%), protein content (10.53 %), oil content (5.63 %), linolenic acid (5.38) and stearic acid (0.40 %) contributed 99.32% of the overall divergence. These traits were identified as key factors influencing genetic diversity among these genotypes.

The cluster mean values for eight yield, yield components and seven quality traits across 14 clusters are furnished in **Table 5**. There was wide range of variation in the cluster mean values for most of the characters except for 1000 seed weight and days to 50% flowering. These results are akin to the reports of Mohan (2014) for days to 50% flowering. On considering cluster means and inter cluster distances, the importance of cluster II becomes obvious, since it had the highest seed yield per plant and number of capsules per plant. The clusters XII and XI showed high cluster mean value for protein content and oleic acid, palmitic acid and oil content indicating their importance in the breeding programmes.

Diversity analysis based on SSR markers: Out of the 50 SSR markers used, 36% (18 markers) exhibited polymorphism, revealing a total of 43 alleles across the genotypes (**Table 6**). The extent of polymorphism was higher in this study compared to previous reports of Yepuri *et al.* (2013) and Ramprasad *et al*. (2017), who found only 12% and 29.4% polymorphism, respectively.

The number of alleles detected for each of the 18 polymorphic SSR loci were slightly variable and ranged from 2 to 3 per locus with a mean of 2.39 alleles per locus. The SSR allelic diversity in sesame is relatively limited, yet it is on par with the diversity found in other oilseed crops such as sunflower, 4.95 alleles/locus (Filippi *et al*., 2015), safflower, 3.6 alleles/locus (Kiran *et al*., 2017) and groundnut, 3.14 alleles/locus (Jiang *et al*., 2007). The mean value for number of alleles (2.39) obtained in the present study was closer to the study reported by Ramprasad *et al.* (2017), who reported 2.8 mean value for number of alleles. It also closely resembled the mean number of alleles reported by Dixit *et al.* (2005) and Cho *et al.* (2011) in sesame. The mean gene diversity value of 0.37 reported in the present study is lower than the findings of Asekova *et al*. (2018), who reported mean value of 0.72 in sesame. Moderate level of genetic diversity was observed among the 50 genotypes which might be due to non- inclusion of wild genotypes and also may be due to genetic relatedness among the genotypes. In contrast, the present study focused only on domesticated genotypes, which might have narrowed the genetic diversity. The allelic variation among the sesame genotypes is presented in **Fig 1**. In this study, the polymorphic information content (PIC) of the microsatellite markers ranged from 0.14 (SIM44) to 0.61 (SIM39), with an average PIC value of 0.34 which was considerably lower than the PIC values reported by Cho *et al.* (2011)

**Table 4. Relative contribution (%) of yield components and quality traits towards genetic diversity**

| S.No. | Character | Contribution % |
|---|---|---|
| 1 | Days to 50% flowering | 0.08 |
| 2 | Days to maturity | 0.08 |
| 3 | Plant height (cm) | 0.00 |
| 4 | Number of branches per plant | 0.16 |
| 5 | Number of capsules per plant | 0.00 |
| 6 | Number of seeds per capsule | 0.08 |
| 7 | 1000 seed weight (g) | 0.00 |
| 8 | Seed yield per plant (g) | 0.08 |
| 9 | Oil content (%) | 5.63 |
| 10 | Protein content (%) | 10.53 |
| 11 | Palmitic acid (%) | 0.16 |
| 12 | Stearic acid (%) | 0.40 |
| 13 | Oleic acid (%) | 43.51 |
| 14 | Linoleic acid (%) | 33.87 |
| 15 | Linolenic acid (%) | 5.38 |

**Table 5. Cluster means for yield, yield components and quality traits using Tocher's method**

| Clusters | DFF | DM | PH | NBP | NCP | NSC | TSW | SYP | Oil | PRO | PAL | STE | OLE | LIN | LNL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cluster 1 | 38.60 | 91.65 | 105.54 | 4.72 | 54.81 | 56.63 | 3.24 | 5.57 | 49.13 | 27.07 | 9.45 | 5.8 | 40.54 | 43.75 | 0.47 |
| Cluster 2 | 41.25 | 93.53 | 123.88 | 4.76 | 57.95 | 57.91 | 3.22 | 6.41 | 46.54 | 25.9 | 9.02 | 5.75 | 37.6 | 47.27 | 0.38 |
| Cluster 3 | 43.29 | 95.64 | 126.43 | 5.06 | 56.42 | 60.54 | 3.17 | 6.06 | 48.19 | 25.23 | 9.05 | 5.69 | 34.4 | 50.66 | 0.27 |
| Cluster 4 | 40.00 | 93.50 | 133.75 | 3.90 | 53.80 | 61.3 | 3.57 | 6.12 | 48.62 | 24.66 | 9.09 | 5.86 | 39.25 | 44.8 | 0.96 |
| Cluster 5 | 43.00 | 97.50 | 113.25 | 4.04 | 37.55 | 59.85 | 3.29 | 3.48 | 40.88 | 25.06 | 9.15 | 6.12 | 37.08 | 47.6 | 0.18 |
| Cluster 6 | 41.50 | 93.50 | 118.5 | 4.20 | 46.70 | 56.05 | 3.00 | 4.57 | 40.64 | 26.45 | 9.5 | 6.56 | 37.47 | 46.44 | 0.3 |
| Cluster 7 | 43.00 | 99.00 | 109.85 | 5.00 | 51.45 | 64.55 | 3.23 | 5.49 | 45.26 | 24.2 | 8.74 | 6.3 | 40.42 | 43.87 | 0.48 |
| Cluster 8 | 39.50 | 97.00 | 124.55 | 4.21 | 54.95 | 49.8 | 3.24 | 5.76 | 46.36 | 23.31 | 8.79 | 5.54 | 41.87 | 43.48 | 0.34 |
| Cluster 9 | 40.67 | 97.50 | 125.3 | 4.77 | 48.2 | 53.27 | 2.98 | 5.02 | 43.96 | 22.44 | 8.87 | 5.57 | 40.21 | 45.04 | 0.42 |
| Cluster 10 | 42.5 | 97.50 | 118.15 | 4.10 | 48.75 | 48.7 | 3.38 | 5.32 | 46.3 | 32.15 | 8.47 | 5.94 | 38.16 | 47.47 | 0.16 |
| Cluster 11 | 37.25 | 82.00 | 71.40 | 3.56 | 36.2 | 52.18 | 3.41 | 3.13 | 49.81 | 24.56 | 9.8 | 5.91 | 42.79 | 40.86 | 0.55 |
| Cluster 12 | 35.50 | 84.00 | 79.65 | 4.45 | 36.8 | 58.4 | 3.07 | 3.44 | 45.08 | 32.43 | 9.33 | 5.62 | 38.76 | 45.8 | 0.62 |
| Cluster 13 | 40.00 | 97.50 | 131.50 | 3.23 | 55.55 | 50.4 | 3.16 | 5.92 | 43.36 | 26.21 | 8.57 | 4.99 | 36.18 | 50.3 | 0.2 |
| Cluster 14 | 44.00 | 96.50 | 112.70 | 4.55 | 52.15 | 63.03 | 3.38 | 5.45 | 44.09 | 21.67 | 8.38 | 6.27 | 31.46 | 53.63 | 0.28 |

Where, DFF-Days to 50% flowering, DM-Days to maturity, PH-Plant height (cm), NBP-Number of branches per plant, NCP-Number of capsules per plant, NSC-Number of seeds per capsule, TSW-1000 seed weight (g), SYP-Seed yield per plant (g), Oil-Oil content (%), PRO-Protein (%), PAL-Palmitic acid (%), STE-Stearic acid (%), OLE-Oleic acid (%), LIN-Linoleic acid (%), LNL- Linolenic acid (%).

**Table 6. Genetic diversity measures in the sesame genotypes using Simple Sequence Repeat (SSR) loci**

| S.No. | Marker | Allele No. | Gene diversity | PIC |
|---|---|---|---|---|
| 1 | SIM5 | 2 | 0.26 | 0.29 |
| 2 | SIM7 | 3 | 0.61 | 0.55 |
| 3 | SIM12 | 2 | 0.35 | 0.31 |
| 4 | SIM16 | 2 | 0.51 | 0.42 |
| 5 | SIM39 | 3 | 0.66 | 0.61 |
| 6 | SIM44 | 3 | 0.15 | 0.14 |
| 7 | SIM54 | 2 | 0.44 | 0.40 |
| 8 | SIM57 | 2 | 0.17 | 0.19 |
| 9 | SIM59 | 3 | 0.21 | 0.20 |
| 10 | SIM63 | 3 | 0.48 | 0.56 |
| 11 | SIM76 | 2 | 0.24 | 0.21 |
| 12 | SIM79 | 2 | 0.29 | 0.32 |
| 13 | SIM86 | 2 | 0.52 | 0.43 |
| 14 | SIM89 | 3 | 0.33 | 0.28 |
| 15 | SIM91 | 2 | 0.25 | 0.22 |
| 16 | SIM108 | 2 | 0.38 | 0.32 |
| 17 | SIM114 | 3 | 0.36 | 0.37 |
| 18 | SIM116 | 2 | 0.36 | 0.28 |
| | **Mean** | **2.39** | **0.37** | **0.34** |

and Yepuri *et al*. (2013). The high PIC value of primers indicated the highly informative nature of these SSRs in revealing genetic diversity and allelic variation in sesame genotypes. The genetic diversity observed in the present investigation can provide information in selecting of some parental lines along with phenotypic data for selecting few of the parental lines for crossing programme. Studies on sesame microsatellite markers have revealed variable allelic diversity, as reported by multiple research groups (Anandan *et al*., 2018; Iqbal *et al*., 2018;

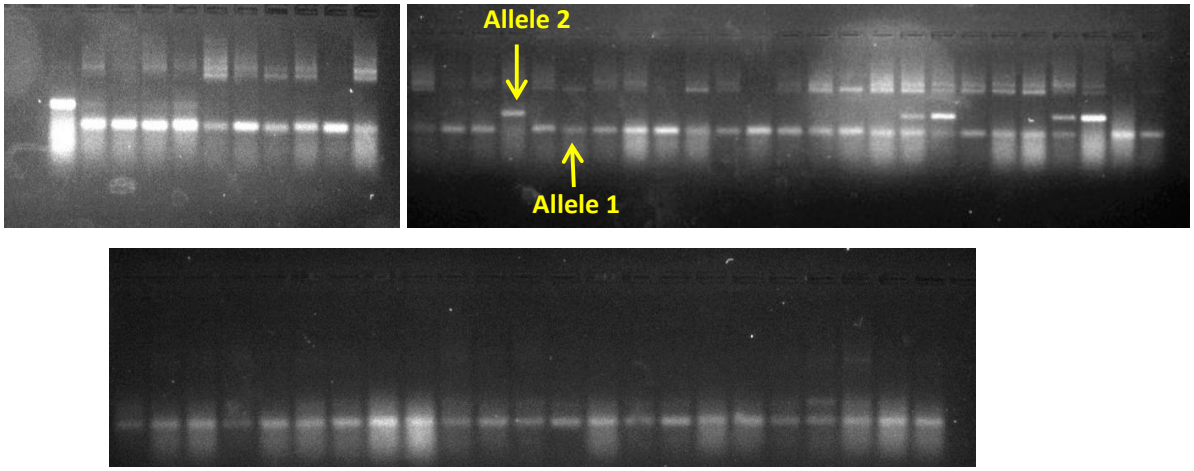


**Fig. 1. Allelic variation observed with primer SIM7 in sesame genotypes**

Anggraeni, 2022).This diversity is attributed to their multi-allelism, meaning that microsatellite markers often exhibit a high number of alleles per locus, resulting in significant genetic variation.

Genetic diversity in sesame germplasm accessions: The dissimilarity matrix was used to construct a dendrogram using the unweighted Neighbor-Joining (NJ) analysis method implemented in DARwin. The resulting dendrograms are displayed in **Fig 2**. The 50 genotypes were categorized into three main groups, with clusters II and III each subdividing into two subgroups. Cluster I consisted of only one genotype. Cluster II emerged as the largest cluster, containing 32 genotypes, covering 64% of the total genotypes in the study. The NJ analysis demonstrated that geographic origin did not play a significant role in the clustering, indicating a more complex genetic structure among the sesame accessions. This information is critical for guiding future breeding programs, germplasm conservation and the selection of diverse parental lines to develop improved sesame varieties with desirable traits. Most of the genotypes did not show much genetic variation with the SSR markers and this was reflected in the clustering pattern. This finding aligns with the conclusions of Wu *et al.* (2014), who suggested that domestication and advanced plant breeding techniques have likely contributed to narrowing the genetic basis of cultivated sesame. While molecular measures of genetic diversity are valuable, Zhang *et al.* (2012) emphasized that they may not fully explain quantitative genetic variability. Therefore, a comprehensive approach that combines both phenotypic and molecular-based analyses in the assessment of genetic diversity in sesame is crucial for the development of effective breeding programs. This study demonstrated significant genetic diversity at the phenotypic level and observed no similarity between the clustering patterns derived from $D^2$ analysis and those based on SSRs. These results were corroborated with the findings of Gogoi *et al.* (2018). Parental lines with complementary traits selected from different clusters could be utilized to develop new breeding populations possessing desirable yield components and quality traits.

Molecular and agro-morphological studies revealed significant genetic diversity. Cluster analysis showed extensive diversity, with 14 clusters formed. The highest inter-cluster distance was observed between clusters XI and XIV, indicating most divergent genotypes were grouped here. Hybridizing genotypes from clusters XI (TKG 21, TKG 306) and XIV (JCS RF2, JCS 4917) may yield promising segregants for yield, quality, and other traits. These genotypes can be used as parents in breeding programs to produce diverse breeding material. Eight SSR primers (SIM3, SIM22, SIM27, SIM32, SIM39, SIM41, SIM42, SIM47) emerged as valuable markers, exhibiting high polymorphism in distinguishing sesame genotypes. Gene diversity and polymorphic information content (PIC) values ranged from 0.15 to 0.66 and 0.14 to 0.61, respectively. The average PIC value of 0.34 underscored the markers' informativeness in uncovering genetic diversity and allelic variation. This study's findings can inform the selection of parental lines, and when combined with phenotypic data, facilitate the identification of optimal parental lines for breeding programs.

Thus, the current study demonstrated moderate level of genetic diversity at the DNA level and found no similarity between the clustering patterns obtained from $D^2$ analysis and those based on DNA markers. The mismatch between the results obtained from both morphological and molecular diversity analysis may be due to the influence of environment on the expression of morphological characters, as phenotypes mostly reveal functional diversity confounded with environmental influence whereas, DNA markers reveal both neutral and functional diversity.

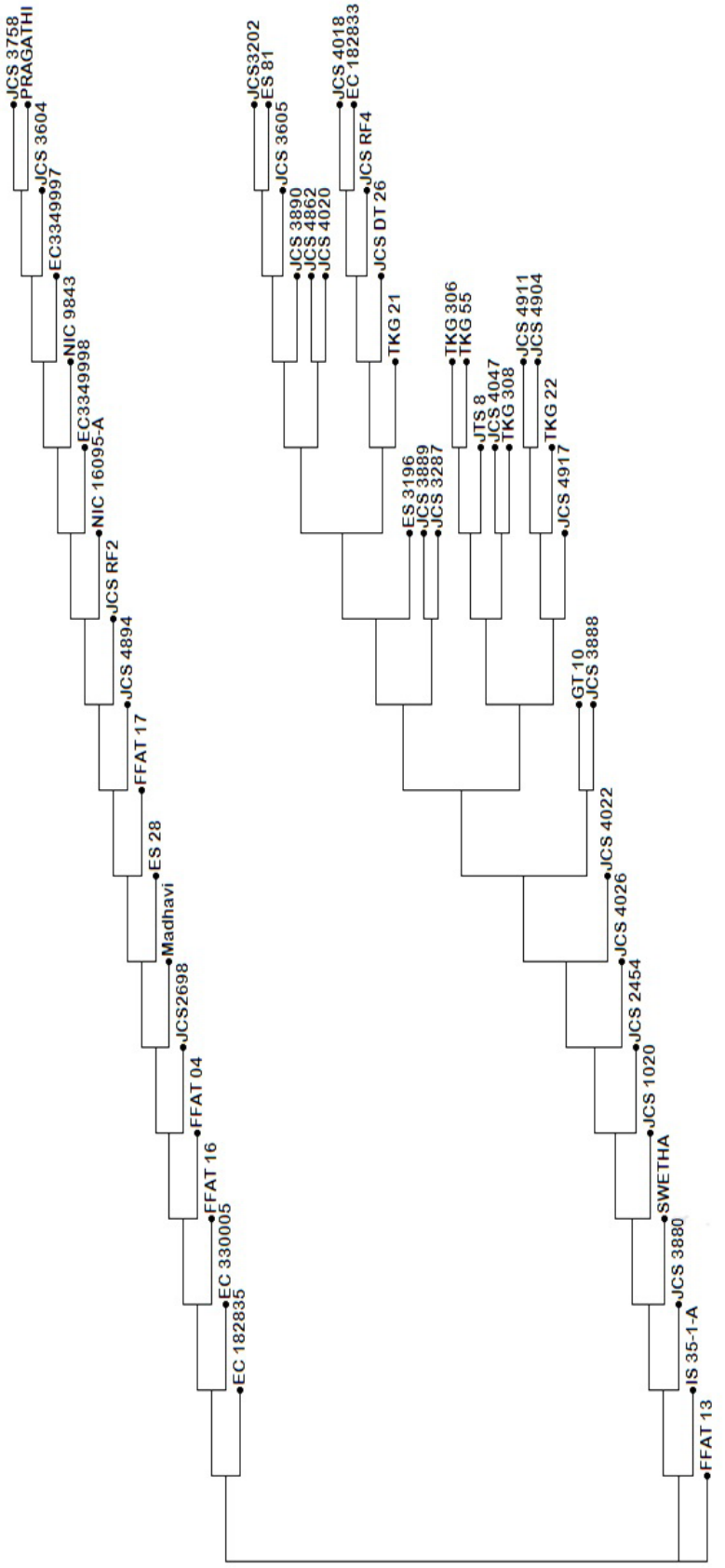


**Fig. 2. Dendrogram depicting the genetic diversity among sesame genotypes**

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