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Yield Evaluation and Genetic Variability Assessment in Sesame (*Sesamum Indicum L.*) Mutant Population Using Morphological Characters and Simple Sequence Repeat (SSR) Markers

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Abstract

The assessment of genetic variability is of utmost importance in crop improvement and the conservation of genetic resources. In the current study, two high-yielding sesame cultivars, namely SI 10 and SI 04, were subjected to treatment with ethyl methane sulphonate (EMS) mutagens. Four different concentrations of EMS (0.5%, 1.0%, 1.5%, and 2.0%) were applied to both cultivars. In this study we aimed to evaluate the genetic variability in a mutant population of sesame (*Sesamum indicum L.*) by employing morphological characters and Simple Sequence Repeat (SSR) markers. The morphological data collected were analyzed using R 4.2.2 software. Analysis of variance revealed significant differences (P=0.05) among most of the morphological traits. Notably, the mutant lines C1P18 SI 10, C3P06 SI 10, C4P10 SI 04, C4P13 SI 04, C1P10 SI 04, C1P18 SI 10, and C2P02 SI 10 exhibited the highest production of capsules per plant and seeds per capsule, indirectly indicating their potential as superior yielders. Furthermore, molecular genetic variation was assessed using twenty-eight SSR markers that were widely distributed across the sesame genome to characterize the mutants. Seventeen out of the 28 primers exhibited polymorphism. Cluster analyses, employing the Euclidean similarity test and a complete link clustering method, were performed to construct a dendrogram based on the morphological data. The mutants were clustered into two major groups and two minor groups. In contrast, the SSR marker-based dendrogram clustering resulted in the discovery of two major clusters, A and B, with a similarity index of 79%. The mutants from both genotypes displayed a diversity range of 10-20% based on the SSR markers, whereas morphological characterization revealed a diversity range of 10 to 51.2%. This study concluded that SSR markers provided a more accurate representation of the true variability in the mutants compared to morphological characterization. Moreover, the use of a lower concentration of EMS (0.5%), which does not cause chromosomal damage, appeared to be more effective in increasing variability in sesame. In summary, this study highlighted the importance of assessing genetic variability in sesame mutants using both morphological and molecular approaches. The findings shed light on the potential for improving sesame crops through the selection of promising mutants and the utilization of SSR markers for accurate characterization of genetic diversity.

Keywords: Mutant line; Morphological; Ethyl methane sulphonate; SSR Markers; Variability; Yield

Introduction

Sesame, scientifically known as Sesamum indicum L. and commonly referred to as simsim, belongs to the order Tub florae and the family Pedaliaceae (Pandey et al., 2015). It is a self-pollinated diploid species with 26 chromosomes (2n = 26). Sesame seeds have gained significant importance in the oilseeds sector in recent years and have become a highly sought-after product (Rutes et al., 2015). The global demand for sesame is substantial, indicating that increasing sesame yields can significantly contribute to the economic development of any country. Africa, known as the center of origin for sesame, possesses high genetic variability, which serves as a valuable resource for further crop improvement (Sarwar and Hussain, 2010) [1].

On a global scale, sesame cultivation covers approximately 9.98 million hectares, with an annual production of about 5.33 million tons and an average yield of 554.1 kg ha-1. However, in Africa, the figures stand at 5.76 million hectares, 3.15 million tons of annual production, and a mean yield of 546.4 kg ha-1 (FAOSTAT, 2017). In Benin, sesame production is relatively underdeveloped, and the reasons behind this lag remain unknown. In Ethiopia, sesame is cultivated over 0.29 million hectares, with an annual production of 0.23 million tons and an average productivity of 787.3 kg ha-1 (FAOSTAT, 2017) [2].

Sesame production varies depending on cultural practices, growing environments, and the choice of varieties. The major constraints in sesame production globally include the lack of adaptable cultivars, capsule shattering at maturity, asynchronous maturation, poor establishment of stands, un responsiveness to fertilizers, excessive branching, and low harvest index (Baraki and Berhe, 2019). Additionally, inadequate storage facilities and mechanical mixtures of different variety seeds have been reported as issues. Moreover, the progress in sesame improvement has been relatively slow due to insufficient research and effective breeding programs (Ashri, 1998). The attention given to improving this crop does not match its potential contribution.

Sesame plays a significant role in the food supply chain. Most sesame seeds are used for oil extraction, while the remainder is utilized

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for various food purposes (Goshme, 2019). Traditionally, sesame seeds were primarily valued for their oil extraction and their ability to add a nutty flavor or serve as a garnish for foods (Ghandhi, 2009). The seeds are rich in fat, protein, carbohydrates, fiber, and several minerals. Sesame oil is renowned for its stability, as it exhibits strong resistance to oxidative rancidity even after prolonged exposure to air (Kumari et al., 2016) [3].

Mutation breeding offers a highly effective alternative to conventional breeding methods for enhancing crops. By subjecting plant genetic material to chemical or physical mutagens, the chances of isolating exceptional genetic traits are significantly increased. Over the years, induced mutations have played a crucial role in developing new and desirable alterations in plant characteristics, resulting in improved yield potential. This technique enables the rapid creation of variability in both qualitative and quantitative traits inherited by plants (Maluszynski et al., 1995; Muduli and Mishra, 2007) [4]. Moreover, mutation breeding techniques not only introduce variation within crop species but also expedite the development of new cultivars compared to traditional hybridization methods. The successful application of mutagenesis has led to the generation of genetic diversity in numerous crops, allowing for the selection of mutants with desirable traits such as increased seed yield, early maturation (Wongyai et al., 2001), modified plant architecture, resistance to diseases (Cagirgan, 2001; Ashri, 1998), improved seed retention, larger seed size, attractive seed color, and higher oil content (Hoballah, 2001). As a result, induced mutations have played a significant role in the global release of many newly developed cultivars.

Among the influential mutagenic agents, Ethyl Methane Sulfonate (EMS) is a chemical compound known for its ability to induce random mutations in the genetic material of plants. It primarily causes nucleotide substitution, mainly through guanine alkylation, resulting in point mutations (Okagaki et al., 1991) [5]. Exposing plant material to chemical mutagens enhances the likelihood of generating unique genetic variations. Induced mutations have proven successful in creating valuable alterations in plant characteristics, contributing to increased yield potential. This technique allows for the rapid introduction of variability in both quantitative and qualitative traits inherited by the crops (Begum and Dasgupta, 2010; Gnanamurthy and Dhanavel, 2014). In comparison to hybridization, mutation breeding techniques not only foster genetic diversity within plants but also significantly reduce the time required for the development of new cultivars. It is essential to maintain genetic diversity within breeding programs, and the abundance of sesame genotypes in Ethiopia comes as no surprise, as Ethiopia, along with China, Central Asia, the Middle East, and India, is recognized as a center of sesame diversity (De Jesús Pérez-Bolaños & Salcedo-Mendoza, 2018; Pandey et al., 2015; Sarwar and Hussain, 2010) [6].

In conclusion, this study aimed to evaluate the genetic variability in a mutant population of sesame *(Sesamum indicum L.)* that was treated with ethyl methane sulphonate (EMS) mutagens. The effectiveness of morphological characters and Simple Sequence Repeat (SSR) markers in capturing genetic variability was assessed. Additionally, the impact of a lower concentration of EMS (0.5%) on increasing genetic variability without causing chromosomal damage was investigated.

The findings of this study contribute to the understanding of sesame crop improvement through the selection of promising mutants and the accurate characterization of genetic diversity. This research provides valuable insights into the potential of utilizing EMS mutagens to enhance genetic variability in sesame crops. By identifying and selecting promising mutants that exhibit desirable traits, the breeding programs for sesame crops can be improved. Furthermore, this study contributes to the overall knowledge of genetic diversity in sesame and can aid in the development of improved varieties, ultimately benefiting the sesame industry [7].

Materials and Methods

Description of the study area

The field and laboratory experiments for this study were conducted in the Republic of Benin, specifically at the University of Abomey-Calavi and the Laboratory of Genetics, Horticulture, and Seed Sciences (GBioS). These institutions are situated in West Africa, between the latitudes 6.4130° N and longitudes 2.3450° E, at an elevation of 54 meters above sea level. The study area is located eastward of the country's capital city, Porto-Novo, approximately 28 kilometers away.

The municipality of Abomey-Calavi is predominantly composed of tropical ferruginous and sandy soils. The region experiences an average annual temperature of 26.5°C and a rainfall of 1342 millimeters. Benin, situated in the savanna of Africa, exhibits a humid climate in the south and a semi-arid climate in the north. The study area falls under agro-ecological region II. It is bordered by Togo to the west, Burkina Faso and Niger to the north, Nigeria to the east, and the Bight of Benin to the south (Sedami et al., 2017) [8].

Collection of seed material

The seeds of two popular high yielding sesame cultivars, namely SI 10 and SI 04, were obtained from the laboratory of Genetics, Horticulture, and Seed Sciences (GBioS) at the University of Abomey-Calavi in Benin. The plant characteristics of these selected cultivars for induced mutation are provided in (Table 1).

Experimental procedure

In this methodically planned and executed research project, two locally high-yielding sesame cultivars were chosen as the initial materials for our study. These cultivars were subjected to four different doses (0.5%, 1%, 1.5%, and 2%) of Ethyl Methane Sulfonate (EMS) treatment, a mutagenic chemical. The experimental procedure kicked off by presoaking 400 sesame seeds in distilled water for a duration of 3 hours. Following the presoaking, the seeds were exposed to freshly prepared EMS solutions of varying concentrations (0.5, 1.0, 1.5, and 2.0 mM) for an additional 3 hours. This treatment aimed to induce genetic mutations in the sesame seeds. To eliminate any residual effects of the mutagenic chemicals, the treated seeds underwent thorough washing for 1 hour under running tap water. As a control group, untreated seeds were also presoaked in distilled water for 3 hours, ensuring a baseline for comparison [9].

Table 1: Characteristics of Sesame Cultivars Chosen for Mutation.

Cultivar	Branching	Carpel/Capsules	Seed Color	Capsule/Axil	Year of Registration as Variety	Source of Seed
SI 10	Multiple	2	Black	Single	1995	GBioS
SI 04	Multiple	2	White	Single	1999	GBioS

Moving forward, the treated seeds, along with the untreated control group, were carefully sown in separate rows to initiate the M1 generation. This generation served as the starting point for our evaluation and analysis. From the M1 generation, we obtained a total of 690 M2 generation seeds, which were subsequently sown during the dry season of Benin in November 2021. The sowing was conducted following a randomized block design with three replications, ensuring robustness and reliability in our experimental setup.

To ensure the quality of our samples, the M1 mutants were selected based on their normal appearance and agro-morphological characteristics. From the M2 generation, we handpicked twenty-three mutant lines of sesame, along with two control lines, for further evaluation and genetic variability analysis. These selected lines represented a diverse range of genetic variations within the sesame population [10].

Throughout the growth period, we diligently implemented all necessary agricultural practices such as weeding and irrigation to provide optimal conditions for the plants. Additionally, a comprehensive set of morphological and yield parameters were meticulously measured at different stages of growth, enabling us to capture valuable data on the performance and characteristics of the sesame mutants (MoARD, 2017).

Collection of data

Procedure for morphological data collection

- **Plant height (cm):** The height of the plants was measured at maturity using a measurement tape. Five random plants were selected from each plot, and the height was recorded from the bottom to the tip of each plant. The measurements from the five plants were averaged to obtain the plant height in centimeters.

- **Branches per plant:** The number of branches on each plant was counted. Five random plants were selected from each plot, and the branches on each plant were counted. The average number of branches per plant was calculated for each treatment.

- **Capsules per plant:** The number of capsules on each plant was counted. Five random plants were selected from each plot, and the number of capsules on each plant was recorded. The average number of capsules per plant was calculated for each treatment.

- **Seeds per capsule:** At maturity, the capsules from the same five random plants were threshed, and the number of seeds in each capsule was counted. The count of seeds from the five plants was averaged to determine the average number of seeds per capsule [11].

- **Seed index (1000 Seeds Weight, g):** One thousand seeds were collected from the seed lot in each plot. The seeds were threshed and weighed to obtain the seed index in grams.

- **Seed yield (kg·ha^{-1}):** At maturity, the sesame crop in each plot was harvested and threshed. The seed yield per plot was recorded in kilograms. To calculate the seed yield per hectare, the following formula was used:

$$\text{Seed yield ha (kg)} = \frac{Seed\ yield\ plot\ (kg)}{\text{Plot size (m2)}} \times 10000$$

- **Chlorophyll content:** The chlorophyll content of the plants was measured during the flowering stage using a spectrophotometer.

- **Days to 50% flowering (DF):** The number of days from emergence to when 50% of the plants in each plot started flowering was recorded.

- **Days to 50% maturity (DM):** The number of days from emergence to when 50% of the plants in each plot reached maturity was recorded. These data collection procedures were adapted from the study conducted by Mank *et al.* (2018).

Genomic DNA extraction and PCR amplification

DNA extraction

At the Republic of Benin, University of Abomey-Calavi, and the laboratory of Genetics, Horticulture, and Seed Sciences (GBioS), molecular characterization was conducted. Twenty-five sesame accessions were cultivated in a screen-house, and young apical leaves weighing approximately 200 mg per sample were collected for genomic DNA extraction [12]. The extraction was performed using the CTAB Protocol. The samples were finely powdered with liquid nitrogen, and one ml of newly prepared CTAB buffer was added to all tubes. Nucleic acids were precipitated using Phenol Chloroform isoamyl alcohol, followed by washing with 70% ethanol alcohol. DNA precipitation was further carried out using low salt TE (1X) buffer. RNAase was added to eliminate RNA, and finally, DNA purification was conducted. The resulting DNA pellet was dissolved in TE (1X) buffer. Quality assessment was performed using a 0.8% agarose gel, and the quantification of genomic DNA was determined using a Nanaodrop 2000c Spectrophotometer.

Genotyping with simple sequence repeat (SSR) markers

To assess genetic variation, a total of 28 primers were employed to genotype 25 sesame lines. The objective was to identify polymorphic primers capable of producing scorable bands at the expected band size. The selection of these 28 primers was based on the need for comprehensive genome coverage, the detection of genetic variations in multiple regions, and the availability of previously validated primers [13]. The presence or absence of bands corresponding to each primer was recorded as '1' or '0,' respectively. Out of the 28 primers, 17 were found to be polymorphic and produced scorable bands. Hence, these 17 SSR primers were used to screen the twenty-five accessions using the SeeAMP™ PCR thermal cycler.

For the PCR reaction, a total volume of 20 µL was prepared. This contained 0.75 µL of freshly extracted DNA, 2.5 µL of 10x PCR buffer, 1.25 µL of MgCl2, 2.5 µL of each forward and reverse primers, 0.75 µL of dNTPs (dATP, dCTP, dGTP, dTTP), 9.55 µL of double-distilled water, and 0.2 µL of Taq polymerase [14].

Data analysis

The obtained morphological data for the quantitative traits were compiled and analyzed using the R 4.2.2 software. To assess the similarity between the accessions, a dendrogram was constructed. Additionally, yield parameters and growth data, including plant height at physiological maturity, capsules per plant, branches per plant, and seeds per capsule, were subjected to analysis of variance using R 4.2.2.

For molecular analysis, the genetic analysis package Power Marker version 3.25 (Liu and V. Muse, 2005) was utilized. Various parameters were generated, such as major allele frequency, number of alleles per locus, observed heterozygosity (HO), polymorphic information content (PIC), and expected heterozygosity (HE). The R function plot. hclust was employed to construct the dendrograms [15].

Results

Morphological Characterization

The morphological characterization of the mutants was conducted using analysis of variance (ANOVA) to comprehensively assess the traits and overall performance. The results, summarized in Table 2, revealed that the genotypes displayed significant variation ($p < 0.05$) for the majority of the agronomic traits studied, with the exception of TSW. These findings highlight the distinct phenotypic characteristics and potential impacts of the mutations on the observed traits (Table 2).

Among the different sources of variation, it is worth noting that the replication had a significant effect on yield (kg/ha) and days to 50% maturity (DM). The variation between accessions was also significant for most of the traits, including yield (kg/ha), plant height (PH), number of branches per plant (NBPP), number of capsules per plant (NCPP), days to 50% flowering (DF), number of seeds per capsule (NSPC), and thousand seed weight (TSW gm). The residual variation accounted for the remaining variation in the data [16].

Further analysis of the data revealed significant differences in the number of capsules formed per plant among the mutant lines. Mutants C1P18 SI10 and C1P02 SI10 exhibited the highest number of capsules per plant, with values of 130.6 and 90.3, respectively. Similarly, mutants C1P10 SI04 and C4P13 SI04 showed high capsule production, with values of 96.5 and 89.9, respectively. In contrast, the control genotypes SI10 and SI04 had lower capsule production compared to the mutants, with values of 54.3 and 54.6, respectively.

In terms of grain yield, the highest yield of 240.4 kg/ha was obtained from the C1P18 SI10 mutant, followed by C3P06 SI10 with a yield of 222.0 kg/ha, both of which were significantly different from the control genotypes. Conversely, the lowest grain yield of 84.5 kg/ha was recorded for the C1P02 SI10 mutant, which was significantly different from the other lines and controls. The control genotypes SI10 and SI04 yielded 145.5 kg/ha and 133.3 kg/ha, respectively [17].

The number of seeds per capsule exhibited significant variation among the mutant lines. Interestingly, the number of seeds per capsule followed a similar pattern as capsules per plant for the first four mutants, as shown. The four lines that produced the highest number of capsules also yielded the highest number of seeds per capsule. Notably, the two mutants derived from the SI 04 genotype, C4P13 SI04 and C1P10 SI04, not only had the highest number of capsules per plant but also produced the highest number of seeds per capsule.

In terms of thousand seed weight (TSW), most accessions did not show a significant difference at a p-value of 0.05. However, the mutant C1P18 SI10 stood out with the lowest TSW of 1.7 gm, which was significantly different from the other lines as well as the check genotype [18].

These findings highlight the complex relationship between the number of capsules per plant, number of seeds per capsule, and thousand seed weight. While some mutants exhibited consistent patterns across these traits, there were also notable exceptions that demonstrated unique characteristics. Further analysis of these traits will provide valuable insights into the genetic and physiological factors influencing seed production and quality in sesame (Table 3).

The results of the study revealed significant differences (P=0.05) among the accessions in terms of plant height at physiological maturity, as presented in. The range of plant height varied from 75.2 cm to 132.3 cm. The tallest plant height was observed in the mutant line C4P13 SI04, while the shortest height was recorded in C1P14 SI10. Interestingly, it was noted that higher concentrations of EMS (0.2%) resulted in maximum plant height compared to the controls and other lines treated with a lower concentration [19].

Moving on to the number of branches per plant, the mutants C2P16 SI10 and C1P18 SI10 displayed the highest number of branches, with values of 9 and 8, respectively. These values were significantly different from the number of branches in other mutants. In contrast, the control genotypes had the least number of branches per plant, which was significantly different from the number of branches in some other lines. Notably, the mutant C2P18 SI10 exhibited the lowest number of branches per plant, with a value of 3, which was significantly different from the control and some other lines.

Regarding the days to 50% flowering, the maximum duration was observed in the mutant C3P12 SI04, with a value of 48 days, which was almost similar to the control SI04 (47 days). On the other hand, the minimum duration was recorded in the mutant C2P15 SI10, with a value of 32 days, which was significantly different from some other lines and the control (SI04). Similarly, the maximum days to 50% maturity were observed in the mutant C1P18 SI10, while the minimum days were recorded in the mutant C3P04 SI10, both of which were significantly different from other mutants and controls [20].

In the M2 generation of sesame, most of the morphological characters exhibited a declining trend with increasing concentrations of EMS. However, in the case of the flowering date and plant height, there was an increasing trend in most of the treated progenies. This indicates the complex interaction between EMS concentration and the expression of these traits.

The Duncan multiple range test highlighted the variations between the parental lines (SI10 and SI04) and the mutants derived from them in terms of all agronomic traits. The parental lines exhibited certain trait values that were either higher or lower than the mutants, suggesting the potential impact of mutation on these traits. These findings provide valuable insights into the changes induced by mutation and the potential for trait improvement through mutation breeding (Table 4) [21].

Clustering by morphological traits

In this study, dendrogram clustering was performed using Principal Clustering Analysis in Studio. The analysis resulted in the formation

Table 2: Analysis of variance for different agronomic characters in M2 of sesame.

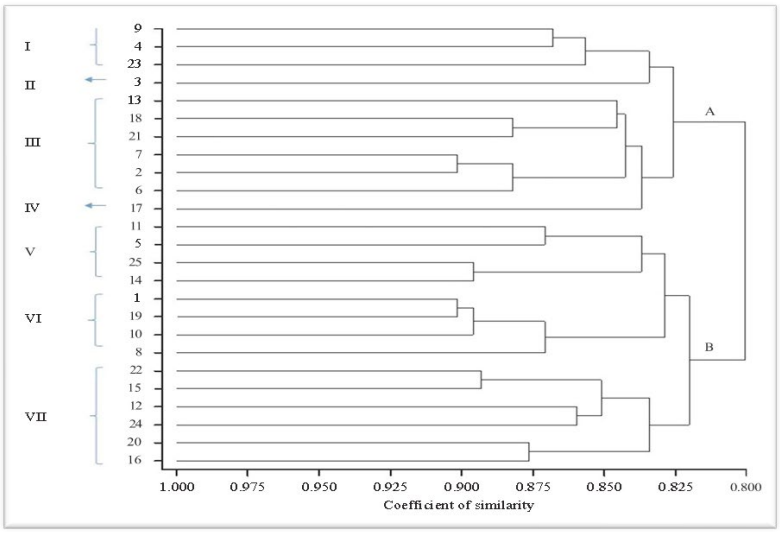
Source of variation	df	Y (kg/ha)	DF	PH	NBPP	NCPP	DM	NSPC	TSW (gm)	CC
Replication	1	30867.1	7.692	1095.42	4.2885	6336.1	94.231	29.25	0	0
Accession	24	3179.9	35.338	483.55	5.0036	683.3	126.784	136.16	0.43460ns	10128
Residual	46	2279.2	14.912	361.04	2.7004	454.3	189.746	89.818	0.19275	4215.3

ns: nonsignificant, ∗=significant ($p < 0.05$), df.: degree of freedom, Y= yield, NCPP= number of capsules per plant, NSPC=number of seed per capsule, TSW=thousand seed weight, DF=days to 50% flowering, PH=plant height, NBPP= number of branches per plant, DM=days to 50% maturity, CC=chlorophyll contents

Table 3: Duncan test results for different yield and yield component traits.

Accession	Y (kg/ha)	Accession	NCPP	Accession	NSPC	Accession	TSW (gm)
C1P18 SI10	240.4^{a}	C1P18 SI 10	130.6^{a}	C4P13 SI 04	74.0^{a}	C1P18 SI 10	3.6^{a}
C3P06 SI10	222.0^{ab}	C1P10 SI 04	96.5^{ab}	C1P10 SI 04	73.3^{ab}	C2P14 SI 10	3.6^{a}
C4P10 SI04	201.9^{abc}	C1P02 SI 10	90.3^{ab}	C1P18 SI 10	72.2^{ab}	SI 10	3.5^{a}
C2P14 SI10	178.5^{abcd}	C4P13 SI 04	89.3^{ab}	C2P02 SI 10	71.3^{ab}	C2P18 SI 10	3.5^{a}
C1P12 SI04	176.1^{abcd}	C3P06 SI 10	85.8^{abc}	C4P36 SI 04	70.6^{ab}	C2P18 SI 04	3.4^{ab}
C3P04 SI10	175.9^{abcd}	C1P12 SI 04	84.3^{abc}	C1P22 SI 10	70.0^{abc}	C3P04 SI 10	3.4^{ab}
C4P13 SI04	166.7^{abcd}	C1P31 SI 04	82.7^{abc}	SI 10	68.6^{abcd}	C4P10 SI 04	3.4^{ab}
C3P12 SI04	163.0^{abcd}	C2P16 SI10	81.2^{bc}	C3P14 SI 10	68.0^{abcd}	C2P34 SI 04	3.3^{ab}
C2P34 SI04	158.8^{abcd}	C2P14 SI 10	75.6^{bc}	C2P04 SI 10	67.5^{abcd}	C1P14 SI 10	3.3^{ab}
C2P18 SI04	145.7^{abcd}	C3P11 SI 04	70.6^{bc}	C4P11 SI 04	67.5^{abcd}	C3P16 SI 10	3.2^{ab}
SI 10	145.3^{abcd}	C3P03 SI 04	70.4^{bc}	C3P12 SI 04	67.0^{abcd}	C2P15 SI 10	3.3^{ab}
C2P02 SI10	139.3^{abcd}	C3P04 SI 10	70.1^{bc}	C1P12 SI 04	66.6^{abcd}	C3P03 SI 04	3.2^{ab}
C3P03 SI04	134.0^{abcd}	C4P03 SI 04	63.6^{bc}	C3P11 SI 04	65.0^{abcd}	C2P04 SI 10	3.1^{ab}
SI 04	133.6^{abcd}	C3P16 SI 10	61.1^{bc}	C3P16 SI 10	64.0^{abcd}	C3P06 SI 04	3.1^{ab}
C3P16 SI10	128.6^{abcd}	C4P11 SI 04	59.8^{bc}	C2P18 SI 10	63.0^{abcd}	C2P09 SI 04	3.0^{ab}
C3P14 SI10	124.5^{bcd}	C2P04 SI 10	58.8^{bc}	C1P08 SI 04	62.6^{abcd}	C1P12 SI 04	2.9^{ab}
C3P11 SI04	122.4^{bcd}	C1P22 SI 10	58.3^{bc}	C2P34 SI 04	62.0^{abcd}	C3P12 SI 04	2.9^{ab}
C4P36 SI04	120.3^{bcd}	C2P18 SI 04	57.3^{bc}	C2P14 SI 10	59.0^{abcd}	SI 04	2.9^{abc}
C2P16 SI10	118.5^{bcd}	C2P02 SI 10	57.0^{bc}	C4P03 SI 04	59.0^{abcd}	C1P08 SI 04	2.8^{abc}
C2P18 SI10	116.7^{bcd}	SI 04	54.6^{bc}	C2P15 SI 10	56.6^{abcd}	C2P16 SI 10	2.8^{abc}
C4P03 SI04	112.1^{bcd}	SI 10	54.3^{bc}	C1P31 SI 04	55.6^{abcd}	C1P31 SI 04	2.7^{abc}
C4P11 SI04	111.4^{bcd}	C3P14 SI 10	51.7^{bc}	C3P03 SI 04	55.0^{abcd}	C4P03 SI 04	2.7^{abc}
C2P15 SI10	102.2^{cd}	C2P15 SI 10	50.5^{bc}	SI 04	55.0^{abcd}	C4P11 SI 04	2.7^{abc}
C1P14 SI10	86.1^{cd}	C4P36 SI 04	47.5^{bc}	C3P06 SI 10	47.0^{cd}	C1P22 SI 10	1.9^{cd}
C1P02 SI10	84.5^{d}	C1P14 SI 10	36.5^{c}	C1P18 SI 10	46.0^{d}	C1P18 10	1.7^{d}

Y= yield, NCPP= capsule per plant, NSPC=seed per capsule, TSW=thousand seed weight
The letters (a, b, c, d) in the table indicate the statistical significance levels of the corresponding values. The letter "a" represents the highest level of significance, while "d" represents the lowest.



1= C4P10 SI04, 2= SI 04, 3= C4P13 SI04, 4= C3P12 SI04, 5= C2P34 SI04, 6= C3P06 SI04,7= SI 10, 8= C3P11 SI04,9= C1P31 SI04,10= C1P08 SI04,11= C2P18 SI04, 12= C3P16 SI10, 13= C4P03 SI04, 14= C3P14 SI10, 15= C2P18 SI10, 16= C4P36 SI04, 17= C2P09 SI04, 18= C2P16 SI10, 19= C1P22 SI10, 20= C2P15 SI10, 21= C4P11 SI04, 22= C3P03 SI04, 23= C1P12 SI04, 24= C3P04 SI10, 25= C1P02 SI10

Figure 1: Dendrogram of 25 sesame accessions screened with 17 SSR markers using complete link Euclidean cluster method.

of four distinct clusters, as shown in Figure 1. The clustering analysis revealed that mutants within the same cluster exhibited a higher degree of relatedness compared to individuals in different clusters.

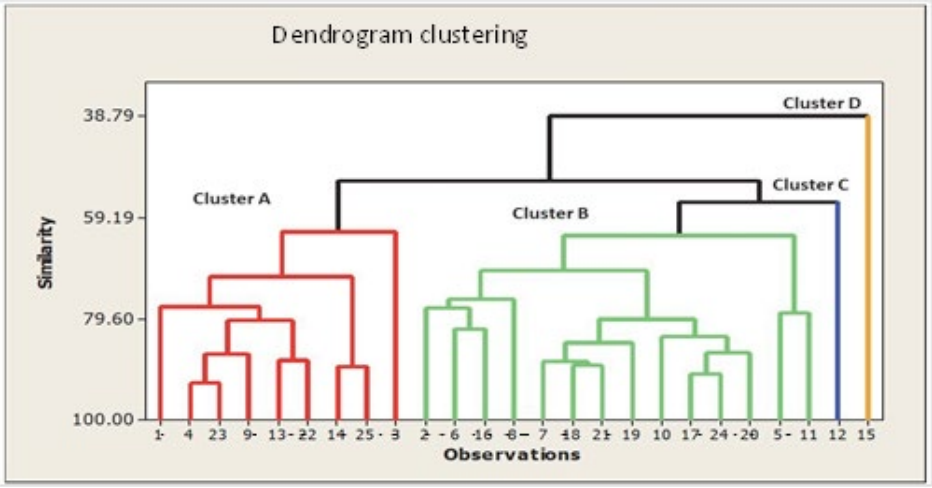
Notably, the dendrogram revealed some variation among the mutants, with similarity indices ranging from 38% to 90%. The mutants were categorized into two main clusters, namely Cluster A and Cluster B, as well as two minor clusters, Cluster D and Cluster C, which comprised a smaller number of lines [22].

Cluster B emerged as the major cluster, encompassing a total of 14 lines. It is interesting to note that Cluster B consisted of mutants derived from both genotypes, with 5 mutants originating from SI 04 and 9 mutants originating from SI 10. On the other hand, the largest

Table 4: The Duncan's new multiple range test result for morphological traits.

Accession	DF	Accession	PH	Accession	BPP	Accession	DM	Accession	CC
C3P12 SI 04	48.0^{a}	C4P13 SI04	132.3^{a}	C2P16 SI10	9.2^{a}	C1P18 SI10	110.0^{a}	C4P03 SI04	439 ^{a}
SI 04	46.6^{ab}	C3P06 SI04	128.3^{ab}	C1P18 SI 10	8.3^{ab}	C1P18 SI10	106.0^{ab}	C2P18 SI10	430^{ab}
C3P03 SI 04	46.0^{ab}	C1P18 SI10	126.6^{ab}	C3P06 SI 10	7.1^{abc}	C1P22 SI10	104.3^{ab}	C2P18 SI04	421^{abc}
C4P10 SI 04	45.6^{ab}	C3P06 SI10	125.8^{ab}	C1P18 10	7.0^{abcd}	C2P16 SI10	104.3^{ab}	C2P16 SI10	408.6^{abc}
C3P04 SI 10	44.3^{abc}	C2P34 SI04	121.5^{abc}	C4P13 SI 04	6.3^{abcd}	C3P14 SI10	101.7^{ab}	C2P34 SI04	396.3^{abc}
C4P03 SI 04	43.0^{abcd}	C2P18 SI04	118.5^{abcd}	C3P16 SI 10	6.0^{abcd}	C3P11 SI04	101.0^{ab}	C3P12 SI04	387.3^{abc}
C1P14 SI 10	42.6^{abcd}	C3P12 SI04	117.2^{abcd}	C3P04 SI 10	5.8^{abcd}	C4P13 SI04	101.0^{ab}	C1P14 SI10	385.6^{abc}
C2P34 SI 04	42.6^{abcd}	C2P09 SI04	116.7^{abcd}	C4P10 SI 04	5.6^{abcd}	C3P12 SI04	99.0^{ab}	C1P18 SI10	382^{abc}
C3P14 SI 10	42.2^{abcde}	C1P1810	113.8^{abcd}	SI 10	5.4^{abcd}	C3P03 SI04	96.0^{ab}	C3P16 SI10	369.6^{abcd}
C3P11 SI 04	42.0^{abcde}	C1P22 SI10	113.4^{abcd}	C4P03 SI 04	5.3^{abcd}	C2P18 SI10	95.0^{ab}	C1P31 SI04	362.6^{abcd}
C1P12 SI 04	41.3^{abcdef}	C1P31 SI04	113.4^{abcd}	SI 04	5.2^{bcd}	C4P10 SI04	95.0^{ab}	C1P12 SI04	361^{abcd}
C1P18 10	41.0^{abcdef}	C2P16 SI10	113.4^{abcd}	C1P08 SI 04	4.8^{bcd}	SI 10	94.6^{ab}	C4P10 SI04	354.6^{abcd}
C3P06 SI 10	40.7^{abcdef}	C3P16 SI10	112.7^{abcd}	C2P15 SI 10	4.7^{bcd}	C3P16 SI10	93.0^{ab}	SI 04	349.6^{abcd}
C1P22 SI 10	40.3^{abcdef}	C3P11 SI04	110.0^{abcd}	C1P31 SI 04	4.6^{bcd}	C2P34 SI04	92.6^{ab}	C1P18 SI10	340^{abcd}
C2P18 SI 10	40.0^{abcdef}	C4P36SI 04	108.5^{abcd}	C3P14 SI 10	4.6^{bcd}	C4P11 SI04	92.0^{ab}	C3P03 SI04	329^{abcd}
C1P31 SI 04	39.6^{abcdef}	SI 10	105.0^{abcd}	C2P04 SI 10	4.5^{bcd}	C1P14 SI10	90.6^{ab}	C3P06 SI04	318^{abcd}
C2P16 SI 10	39.3^{abcdef}	C2P15 SI10	104.6^{abcd}	C1P14 SI 10	4.3^{cd}	C4P36 SI04	90.6^{ab}	C1P08 SI04	317.3^{abcd}
C2P04 SI 10	39.0^{abcdef}	C4P11 SI04	103.3^{abcd}	C2P14 SI 10	4.3^{cd}	C3P06 SI10	90.2^{ab}	C2P09 SI04	313.3^{abcd}
C3P16 SI 10	38.3^{bcdef}	C1P08 SI04	101.1^{abcd}	C4P36 SI 04	4.2^{cd}	C2P14 SI10	90.0^{ab}	C3P04 SI10	312.6^{abcd}
C1P18 SI 10	38.0^{bcdef}	C3P04 SI10	100.5^{abcd}	C2P34 SI 04	3.8^{cd}	C3P06 SI04	90.0^{ab}	C3P06 SI10	302.7^{abcd}
C4P13 SI 04	38.0^{bcdef}	SI 04	95.3^{abcd}	C4P11 SI 04	3.6^{cd}	C1P12 SI04	89.0^{ab}	C2P04 SI10	277.5^{bcde}
C4P36 SI 04	38.0^{bcdef}	C2P18 SI10	93.3^{abcd}	C1P22 SI 10	3.3^{cd}	C1P31 SI04	87.3^{ab}	C2P15 SI10	271^{cde}
C2P18 SI 04	36.0^{cdef}	C2P04 SI10	86.1^{abcd}	C2P09 SI 04	3.3^{cd}	SI 04	87.3^{ab}	SI 10	223de
SI 10	34.3^{def}	C3P14 SI10	84.0^{bcd}	C3P06 SI 04	3.3^{cd}	C2P09 SI04	86.6^{ab}	C2P14 SI10	144^{e}
C2P15 SI 10	32.3^{f}	C1P14 SI10	75.2^{d}	C2P18 SI 10	3.0^{d}	C3P04 SI10	75.0^{b}	C4P13 SI04	135^{e}

DF=days to 50% flowering, PH=plant height, BPP=branch per plant, DM=days to 50% maturity, CC=chlorophyll contents. The letters (a, b, c, d) in the table indicate the statistical significance levels of the corresponding values. The letter "a" represents the highest level of significance, while "d" represents the lowest.



1= C4P10 SI04, 2= SI 04, 3= C4P13 SI04, 4= C3P12 SI04, 5= C2P34 SI04, 6= C3P06 SI04,7= SI 10, 8= C3P11 SI04,9= C1P31 SI04,10= C1P08 SI04,11= C2P18 SI04, 12= C3P16 SI10, 13= C4P03 SI04, 14= C3P14 SI10, 15= C2P18 SI10, 16= C4P36 SI04, 17= C2P09 SI04, 18= C2P16 SI10, 19= C1P22 SI10, 20= C2P15 SI10, 21= C4P11 SI04, 22= C3P03 SI04, 23= C1P12 SI04, 24= C3P04 SI10, 25= C1P02 SI10

Figure 2: Average linkage using dendrogram clustering for 25 sesame accessions.

cluster, Cluster A, demonstrated a high degree of association with Cluster B, having a similarity index of 58.0%. Cluster A consisted of nine mutants from both genotypes. Furthermore, it was observed that the mutants derived from SI 10 exhibited greater diversity, as they were found in all clusters. This highlights the genetic variability present within the SI 10 genotype (Figure 2) [23].

Molecular characterization

Genetic variability

Genetic variability plays a crucial role in sesame breeding programs. Evaluating the amount of genetic variation in sesame is therefore essential to develop effective breeding strategies. In a recent study, a comprehensive analysis was conducted to assess the genetic variability in sesame mutants.

A total of 325 alleles were detected in the mutants, with an average of 19.12 alleles per locus. The primer AC558318 exhibited the highest number of alleles, while also displaying the lowest major allele frequency. Interestingly, the primer AC557375 yielded the smallest number of alleles but indicated higher diversity in terms of genotype number, heterozygosity, gene diversity, and polymorphic information

content (PIC). The major allele frequency ranged from 0.07 to 0.21, with an average frequency of 0.16 across the population [24].

The mean gene diversity among the mutants was found to be 0.95, which was comparable to the PIC. The polymorphic information content ranged from 0.75 to 0.94, reflecting the diverse nature of the mutants. This indicates that the mutants possess a high degree of genetic diversity, providing a valuable resource for future breeding programs.

Heterozygosity is a measure of the proportion of individuals in a population that possess two different alleles at a particular locus. In the mutants, heterozygosity ranged from 0.04 to 1.00, with a mean value of 0.53. This indicates a wide range of heterozygous individuals within the mutant population, suggesting the presence of diverse genetic backgrounds (Table 5) [25].

Link euclidean clustering

The molecular study conducted on sesame mutants revealed a relatively low level of variability, ranging from 10% to 20%, when compared to the morphological results. However, through the application of dendrogram clustering, two major clusters, labeled as A and B, were identified with a similarity index of 79%.

Cluster A, the larger of the two clusters, exhibited further subdivision into four distinct subclusters (I, II, III, and IV). Subgroup I consisted of three accessions from SI 04 (C1P31SI04, C3P12 SI04, and C1P12 SI04), indicating a close genetic relationship among these mutants. Interestingly, another mutant from SI 04, C4P13 SI04, was found to be closely related to subgroup I mutants, with a similarity index of 80.5%.

Moving on to subcluster III, it included accessions from both SI 04 and SI 10, suggesting a potential genetic link between these two groups. Within subcluster III, the SI 04 mutants were further divided into three subclusters, highlighting additional genetic diversity within this subgroup. Notably, mutant C2P09 SI04 stood alone in subcluster IV, displaying a unique genetic profile. Furthermore, a distinct subcluster was formed by two accessions from SI 04 (C2P18 SI04 and C2P34 SI04) and two mutants from SI 10 (C1P02 SI10 and C3P14 SI10), indicating a close association between these accessions [26].

The Euclidean clustering analysis of the sesame mutants revealed relatively low variability compared to the morphological results. The clustering analysis identified two major clusters, A and B, with Cluster A further subdivided into several subclusters. These subclusters provided insights into the genetic relationships among the mutants, highlighting shared genetic characteristics within certain subgroups and potential connections between different accessions. The findings of this molecular study contribute to a better understanding of the genetic variability among sesame mutants and can aid in the development of targeted breeding strategies (Figure 1) [27].

Discussion

The results of our study revealed interesting findings. We observed variation among the different mutant lines examined, particularly in terms of capsule numbers. The number of capsules showed a wide range, from 36 to 130. This pattern is consistent with previous studies conducted by Caliskan et al. (2004), Morrell et al. (2012), and Frary et al. (2015), who also reported a similar variation in capsule numbers.

Remarkably, our research produced capsule numbers lower than those reported by Caliskan et al. (2004) but aligned with the findings of Morrell et al. (2012) and Frary et al. (2015). This suggests that there is variability in the mutant population, but it falls within the range observed in other studies. Additionally, the number of seeds and capsules per plant, as well as the 1000 seed weight, have been found to strongly correlate with yield, which is consistent with the findings of Morrell et al. (2012) and our study [28].

The seed count per capsule in our study was lower than that reported by Caliskan et al. (2004) but similar to the range observed by Frary et al. (2015). This indicates that there is variability in the seed production of the mutant population, but it falls within the range observed in other studies. It is worth mentioning that the correlation between seed number, capsule number, and yield has been established in previous research (Morrell et al., 2012), further supporting the importance of these traits in sesame breeding.

In terms of plant height, we observed significant variation among the sesame mutants. The plant heights reported by Morrell et al. (2012) and Caliskan et al. (2004) in different environments exceeded the

Table 5: Summary statistics of genetic variability among sesame mutant lines using SSR markers.

Marker	No. of Allele	Major Allele Freq.	Genotype No	Gene Diversity	Heterozygosity	PIC
AC557375	9	0.12	9	0.95	0.2	0.8
AC558318	33	0.08	24	0.95	1	0.95
AC558525	14	0.12	17	0.92	0.15	0.91
AC558951	25	0.12	23	0.94	0.8	0.94
AC559409	22	0.14	21	0.93	0.92	0.93
AC559452	14	0.2	18	0.9	0.32	0.89
UB35490	17	0.15	15	0.91	0.35	0.9
AC559908	21	0.24	22	0.93	0.88	0.92
AC570128	13	0.28	12	0.85	0.12	0.84
AC570254	21	0.15	20	0.93	0.32	0.93
AC570334	24	0.1	25	0.82	0.55	0.94
AC570515	18	0.2	18	0.91	0.4	0.9
AC570590	25	0.07	24	0.95	0.8	0.95
AC570450	7	0.28	8	0.82	0.04	0.8
AC559957	22	0.2	17	0.92	1	0.91
AC570003	20	0.14	19	0.93	0.54	0.92
AC559584	18	0.14	24	0.92	0.58	0.92
Mean	19.12	0.15	18.55	0.91	0.53	0.9

observations in our study. However, our findings aligned more closely with the plant height reported by Frary et al. (2015). This suggests that the sesame mutants in our study exhibit a range of plant heights, with some displaying shorter heights but still maintaining productive capsule and seed production [29]. These mutants hold great promise for future breeding programs, with one particular mutant, C2P14 SI10, emerging as one of the top 5 mutants in terms of capsule and seed production. It can be considered a strong candidate for parental line selection in future breeding activities.

To assess genotype variability, we employed morphological characterization and molecular markers, specifically SSR markers. Morphological characterization revealed a higher degree of variation ranging from 10% to 51.2% among the mutants. The morphological dendrogram analysis resulted in two major clusters, consisting of mutants from both parental genotypes. This finding is consistent with the study conducted by Pandey et al. (2015), who also observed the grouping of sesame genotypes from different geographical locations [30].

The use of molecular markers, such as SSR markers, has proven to be a powerful tool for studying plant diversity in previous studies (Huang et al., 2002; Khlestkina et al., 2004; Singh et al., 2015; Pandey et al., 2015). These markers provide valuable information about the genetic variability within the mutant population and can aid in further understanding the relationships between different mutants and parental genotypes.

In addition to the morphological characterization, our study incorporated the use of SSR markers to assess genetic variability in the sesame mutant population. The SSR markers revealed a greater number of alleles compared to previous studies (Dixit et al., 2005; Singh et al., 2015), indicating a higher level of genetic diversity among the mutants. The polymorphic information content (PIC) values of the SSR markers demonstrated their informativeness, with a mean PIC value of 0.90 across all 17 markers. This value is higher than the value reported by Singh et al. (2015), suggesting a higher level of diversity within our mutant population [31].

Assessing heterozygosity, which serves as a reliable estimator of genetic variation, further supported the diversity level of the SSR markers. Our mutants displayed an average heterozygosity of 0.52, comparable to the value reported by Singh et al. (2015). This indicates that the mutants in our study exhibit a significant level of genetic variation, contributing to their potential for future breeding programs [32].

The clustering analysis using SSR markers and dendrograms proved to be more effective in revealing the true extent of variation among the mutants compared to the morphological dendrogram clustering. The molecular markers provided a narrower range of diversity among the mutants, ranging from 10% to 23%, compared to the wider range of 10% to 51.2% observed through morphological characterization. This difference can be attributed to the influence of environmental factors on the phenotype of morphological parameters, while molecular markers remain unaffected by these factors. Moreover, SSR markers are known to provide better distinction between closely related plant species, further highlighting their effectiveness in capturing genetic variation (Powell et al., 1996) [33].

To identify the highest-yielding mutants, we conducted Duncan's new multiple range test. Among the mutants, C1P18 SI10, C3P06 SI10, and C4P10 SI04 emerged as the top three mutants in terms of yield. When it came to seed production per capsule, C4P13 SI04, C1P10 SI04, C1P18 SI10, and C2P02 SI10 were the top five mutants. It is interesting to note that most mutants treated with lower concentrations of EMS displayed an increase in yield component characters, particularly seed yield and the number of capsules.

The molecular-based dendrogram clustering further emphasized the grouping of mutants with high capsule production, while the top three seed-producing mutants were also clustered together. Based on their exceptional seed production and number of capsules per plant, C4P13 SI04 stands out as a potential mutant line. Additionally, C1P18 SI10, being the highest yielder and capsule producer, as well as the third-highest seed producer, holds great promise. Similarly, C4P13 SI04, as the top seed producer and the third-highest capsule producer, is also a strong candidate. Lastly, C1P10 SI04, with its second-highest seed production and capsule numbers, deserves recognition as a potential line [34].

Overall, our study has provided valuable insights into the variability and characteristics of sesame mutants. By integrating morphological and molecular approaches, we have gained a comprehensive understanding of the genotype variations observed. These findings have significant implications for the development of improved breeding programs, as they identify potential mutant lines with high yield, seed production, and capsule numbers. Further research and evaluation of these mutants will be crucial to harness their potential and contribute to the advancement of sesame breeding programs [35].

Conclusions and Recommendations

In conclusion, the molecular study utilizing SSR markers demonstrated a more accurate assessment of genetic variability compared to morphological characterization. The SSR markers exhibited a diversity range of 10-20% among mutants, while the morphological characterization displayed a broader diversity range of 10% to 51.2%. This highlights the significance of incorporating molecular markers, specifically SSR markers, for a more precise understanding of genetic variability in sesame mutants.

Additionally, the study identified five promising mutant lines, namely C1P18 SI 10, C3P06 SI 10, C4P10 SI 04, C4P13 SI 04, C1P10 SI 04, and C1P18 SI 10, which exhibited high capsule per plant and seed per capsule production. These mutants have shown potential for future breeding programs aimed at enhancing sesame yield. It is recommended to further evaluate these mutants in other desirable traits such as seed quality, fatty acid composition, disease resistance, and drought tolerance to determine their overall suitability for practical implementation and commercial cultivation.

Based on the findings of this study, several recommendations were proposed for future research. Firstly, conducting field evaluations of agronomic traits across multiple test locations and seasons is recommended. This comprehensive approach will provide a more comprehensive understanding of the mutants' performance under varying environmental conditions, aiding in the identification of consistently high-yield potential mutants.

Secondly, further evaluation of selected mutants displaying promising yield components, such as high seed per capsule and capsule per plant production, is crucial. This evaluation will help confirm their potential in other important traits and identify mutants with multiple desirable characteristics suitable for practical implementation and commercial cultivation.

Thirdly, exploring the use of a lower concentration of EMS (0.5%)

that does not cause chromosomal damage to increase variability in sesame is recommended. Future research should investigate the effectiveness of EMS at this concentration or lower to induce desired variations and identify valuable genetic variants.

Lastly, investigating the effectiveness of EMS at lower concentrations to identify valuable genetic variants is essential for further enhancing the genetic diversity of sesame mutants.

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