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Research Article

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Microsatellite marker assisted molecular and morpho-physiological genetic diversity assessment in 38 genotypes of sesame (*Sesamum indicum* L.)

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ABSTRACT

Identification of genetic diversity and their relationships among breeding materials is crucial in crop improvement strategies. In this study, 38 sesame genotypes were characterized for their genetic diversity. The results revealed significant variations among various traits such as plant height, maturity, capsule plant^{-1} and seeds capsule^{-1}. The number of capsule plant^{-1} showed significant positive correlation with seeds capsule^{-1}. The highest heritability was found for the numbers of capsules plant^{-1} (98.67%). The 38 genotypes were separated into six distinct clusters. Comparison within the populations of the cluster IV and those of cluster VI had the highest capsules plant^{-1}, seeds capsule^{-1} with enormous genetic diversity. For molecular characterization, 7 microsatellite markers and 5 SSR primers with polymorphism were finally chosen for genetic diversity analysis. Altogether, 19 alleles were identified among the 38 genotypes, and the average number of alleles per locus was 3.80. The lowest and the highest numbers of alleles were 3 and 5, respectively. The polymorphism information content (PIC) ranged from 0.3201 to 0.5934 and SI-ssr30 showed to be highest at 0.5934. The UPGMA based clustering depicted a significant variation at molecular level among the sesame genotypes, having a coefficient of similarity between 0.29 and 1.00. The present study confirmed that extensive genetic diversity existed among the sesame genotypes and valuable agronomic traits may result in the development of high yielding genotypes.

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INTRODUCTION

Sesame (*Sesamum indicum* L.) belonging to the order tubiflorae, family Pedaliaceae, is an herbaceous annual plant cultivated for its edible seed, oil and flavorsome value (Wei *et al.*, 2022). It is the oldest known oilseed crop, domesticated nearly 5,000 years ago, and the first known oil consumed by human. Sesame is a short-duration (60-90 days), self-pollinated (although insect-driven cross pollination is common), temperature-loving, drought-tolerant crop, however, extremely intolerant of waterlogging conditions. Sesame is mostly produced in central Asia including Bangladesh and North Africa (Yadav *et al.*, 2022a) and *S. indicum* is the only cultivated species under the genus Sesamum that includes 38 species (McNeill *et al.*, 2006; Yadav *et al.*, 2022b). According to the Food and Agriculture Organization of the United Nations, the global production of sesame in 2017 was 5.899 million tons, of which 806,000 tons were produced in Tanzania and 733,000 tons in China (Wei *et al.*, 2022).

Chemical composition analysis showed that sesame seed is a primary source of oil (50-60%), protein (18-25%), carbohydrate (13.5%) and ash (5%) (Uzun *et al.*, 2003; Elleuch *et al.*, 2007). The bioactive components present in the seed include vital minerals, vitamins, phytosterols, polyunsaturated fatty acids, tocopherols and unique class of lignans (such as sesamin, sesamolin, sesamol, pinoresinol and lariciresinol), which play a principal role against oil oxidation and contribute to antioxidative activity (Senila *et al.*, 2020). The oxidative stability of sesame oil is eminent compared to other vegetable oils although it contains nearly 85% unsaturated fatty acids. Sesame seed oil shows an extraordinarily high oxidative stability compared to soybean, corn and most other popular vegetable oils due probably to the endogenous antioxidant contents in sesame

such as lignans and tocopherols (Minioti & Georgiou, 2010; Aglave, 2018). However, Sesame production and productivity are currently constrained severely by lack of high-yielding and locally adapted varieties; biotic and abiotic stresses; susceptibility to capsule shattering and low seed retention; and a lack of modern production and pre- and post-harvest technologies (Teklu *et al.*, 2022).

Sesame improvement has primarily focused on conventional breeding through germplasm characterization, selection, and variety recommendation. Determination of genetic diversity and their relationships among breeding materials is very crucial in crop improvement strategies and improving sesame yield and quality are possible by exploring and using its genetic diversity (Frison *et al.*, 2011; Teklu *et al.*, 2022). Plant genetic diversity offers opportunity for researchers selecting suitable parents in plant breeding and develop new improved varieties with desirable traits. Genetic diversity also facilitates crop cultivation at varied agroecological conditions and under varied degrees of biotic and abiotic stresses (Begna, 2021). Many wild sesame species are endowed with different desirable agronomic traits (such as plant height, branching pattern, leaf shape, number of capsules per axil, number of seeds per capsule, 1000 seed weight, oil content, seed color, etc.) and resistance to biotic/abiotic stresses which offer a broad foundation for breeding and develop modern sesame cultivars (Dossa *et al.*, 2017).

Genetic diversity among germplasms can be studied by physical observation of morphological characters as well as biochemical or molecular markers. So far, morphological characters have been mostly used as an easily usable tool to identify genetic diversity in sesame and have detected extensive diversity among the populations (Pham *et al.*, 2011; Pandey *et al.*, 2015; Stavridou *et al.*, 2021; Teklu *et al.*, 2021). However, many limitations are there to estimate genetic diversity through morphological characters due to strong influence from environmental factors. Microsatellites or Single Sequence Repeats (SSRs) have become very important tool in plant breeding and are extensively employed in plant genetics studies. Molecular marker techniques are based on the detection of sequence variation between varieties or accessions. However, for sesame, microsatellites have been used only in a couple of studies to evaluate genetic diversity (Wei *et al.*, 2015; Teklu *et al.*, 2021) to spot a limited number of morphological characters like growth habit and its capsule traits (Araújo *et al.*, 2019; Stavridou *et al.*, 2021). Genetic diversity evaluation in sesame germplasm could be the basis of its improvement.

The present study engrossed to characterize the morpho-physiological and molecular based genetic diversity in 38 genotypes of sesame (collected from different agro-ecological regions of Bangladesh and some exotic); identifying populations with higher diversity for sesame improvement; and evaluating the genetic relationship among the accessions.

MATERIALS AND METHODS

This study was carried out in the research field and the molecular biology laboratory, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh during January 2019 to March 2020. Geographically the experimental site is located at 24°72′ N latitude and 90°48′ E longitudes at the elevation of 18m above the sea level. Thirty-eight (38) sesame genotypes were used in this study for phenotypic and genotypic characterization by SSR markers (Table 1), where 7 SSR Primer pairs (SI-ssr01, SI-ssr19, SI-ssr30, SI-ssr16, SI-ssr11, SI-ssr21, SI-ssr29) were evaluated initially for primer selection. Among the seven primers, five (SI-ssr01, SI-ssr19, SI-ssr30, SI-ssr16, SI-ssr29) were selected for final analysis based on their polymorphism and were used for diversity analysis. Microsatellite loci amplified were used for analysis to identify polymorphism using the images found in polyacrylamide gel electrophoresis (PAGE).

The experiment was conducted in trays. Each tray had a dimension of 1×1.5 m and 20 cm depth. All genotypes were tagged carefully in the trays. Fertilizers were applied into the soil of the tray as recommended doses @ Urea: 120-160 kg/ha, TSP: 140-150 kg/ha, MoP: 60-70 kg/ha, gypsum: 100-125 kg/ha, zinc sulfate: 4-6 kg/ha and boric acid: 8-10 kg/ha, for high yield in medium fertile soil. Seeds were sown on 07 January, 2019 with 1 g per 5 cm consecutively in two separate trays which were divided into four sections. Seeds were sown in 30 cm line

Table 1: Sesame genotypes collected and used in this study

Sl. No.	Genotypes	Source of collection
1	CW1	Exotic Material (China)
2	IB1	Exotic Material (Iran)
3	SMM-007	Binatil-1 (Mutant Line)
4	GM-11	Kushtia
5	GM-3	Ramnagar, Magura
6	IB2	Exotic Material (Iran)
7	GM-1	Ramnagar, Magura
8	Binatil-2	BINA, Mymensingh
9	GM-8	Natore
10	GM-5	Madarganj, Jamalpur
11	GM-7	Iswardi
12	SM-13	Binatil-2 (Mutant Line)
13	SMM-008	Binatil-1 (Mutant Line)
14	SM-17	Binatil-2 (Mutant Line)
15	GP-8	Mymensingh
16	SM-9	Binatil-4 (Mutant Line)
17	SM-5	Binatil-1 (Mutant Line)
18	SMM-001	Binatil-1 (Mutant Line)
19	GM-10	Kushtia
20	GM-2	Ramnagar, Magura
21	GM-9	Natore
22	BARI til-4	BARI, Joydebpur, Gazipur
23	Binatil-3	BINA, Mymensingh
24	Binatil-4	BINA, Mymensingh
25	IB-4	Exotic Material (Iran)
26	Binatil-1	BINA, Mymensingh
27	IB-3	Exotic Material (Iran)
28	GM-26	Paranganj, Mymensingh
29	GM-20	Faridpur
30	GM-6	Iswardi
31	SM-056	Mymensingh
32	CB-1	Exotic Material (China)
33	GM-25	Chapainawabganj
34	SM-10-4	Binatil-3 (Mutant Line)
35	GM-21	Faridpur
36	GM-4	Madarganj, Jamalpur
37	SM-067	Mymensingh
38	SM-15	Binatil-2 (Mutant Line)

spacing. Two trays are separated into four sections and each cultivar was sown with 1 g per 5 cm consecutively (Figure 1).

The data on yield and yield contributing characters were collected and analyzed. Data ware recorded from three randomly selected plants of each pot and the mean values were calculated. The analysis of variance (ANOVA) among the data was performed and the least significance differences (LSD) were calculated and presented at 5% and 1% probability in Duncan's Multiple Range Test using MSTAT-C software and following Gomez and Gomez (1984). Pearson correlation coefficient was performed using Minitab-18 to identify influential traits for selection. For molecular characterization, young, vigorously growing fresh leaves were collected for genomic DNA extraction. About 6-8 cm long apical part of leaf was cut apart and washed in sterile distilled water and ethyl alcohol and dried on fresh tissue paper to remove any sources of contamination of foreign DNA. Total DNA from leaf samples was extracted by the cetyltrimethyl ammonium bromide (CTAB) method (Zidani *et al.*, 2005) with some minor modifications. Seven primers of SSR developed by Yeh and Boyle (1997) were screened on randomly chosen two sesame genotypes. Out of seven primers, five were used for further analysis based on their clear polymorphism in banding. The details of the selected primers are given in Table 2. PCR was done by an initial denaturation for 3 min at 94°C, denaturation for 3 min at 94°C, annealing for 2 min at a temperature respective for individual primer for 1 min, polymerization at 72°C, cycle to step 2 for 32 min and incubation at 72°C for 5 min. The size (in bp) of the amplified bands was determined for each microsatellite marker based on its migration relative to a ladder with the help of Alpha Ease FC 5.0 software.

Figure 1: Sesame plants grown in a tray

Table 2: List of selected primers used for diversity analysis among Sesame germplasm collections

Primers	Sequences	Annealing temperature (°C)	Expected band size (bp)
SI-ssr01			
F	AGCAAGAGACAAGATGACGA	60	161-170
R	TGGTGGATGAGCAGGTAATA		
SI-ssr19			
F	TCCATTGAGAACTACCAGCA	61	359-401
R	GCCACCTGAAAATCTGAAAA		
SI-ssr30			
F	GATTGCAGAAATTGACACCA	61	242-244
R	CACTAGGCGAAGAATTCAAGA		
SI-ssr16			
F	CGAAACTCTCATCTACCCAAG	60	388-422
R	CAGCTCGTACTTCCCATGTA		
SI-ssr29			
F	TACAGGCGGAGAGAGAGATT	60	402

A summary statistic was obtained comprised of the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values using a genetic analysis software; POWER MARKER version 3.23 (Liu & Muse, 2005). Molecular weights for microsatellite products were estimated using Alpha Ease 4C software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. The genetic distance determined by the allelic molecular weight data was used for phylogeny reconstruction based on the neighbor-joining method (Saitou & Nei, 1983). Expected heterozygosity for each SSR marker was calculated as the Polymorphism Information Content (PIC) using the formula $Hn = 1\text{-}Pi^2$ (Pi indicates the allele frequency for the i^{th} allele) (Nei *et al.*, 1983). Unweighted Pair Group Method with arithmetic averages (UPGMA) cluster analysis and dendrogram construction were performed with NTSYS-PC (version 2.1).

RESULTS

Morphological Study

Plant height, branches plant^{-1}, root length, shoot length, maturity, number of the capsule plant^{-1}, capsule length and seeds capsule^{-1} varied significantly among the varieties at the 1% level of probability (Table 3). The tallest plant heights (120 cm) were recorded for BARI til-4 and GM-11 followed by CW-1, GM-10, GM-9, SM-056 and the shortest (110 cm) was recorded for mutant line SM-10-4 followed by SM-5, Binatil-3, IB-4, IB-3, GM-4, SM-15, respectively (Table 3). The highest numbers of branches (4) per plant was found for GM-11, GM-8, GM-5, GP8, GM-2, BARI til-4, Binatil-4, IB-4, GM-26, GM-6, CB-1, SM-10-4, GM-4, SM-067 and the lowest numbers of branches (0) was counted for variety Binatil-1. The longest root (12 cm) was obtained for IB-1, GM-3, IB-2, GM-2, GM-9, GM-26, GM-21 and the shortest (7 cm) was recorded for genotypes GM-7 and SM-5 followed by GM-1, SM-13 and IB-4, respectively (Table 3).

The tallest plant (111 cm) was obtained for BARI til-4 which was followed by GM-11 and GM-10. The shortest (100 cm) was recorded from genotype SM-10-4 followed by IB-2, IB-3, GM-26, SM-15 and GM-4, respectively. The highest maturity period (88 days) was obtained for IB-4 followed by GM-8, GP-8, BARI-4, GM-6 and the shortest maturity period (55 days) was recorded for variety Binatil-1. The highest numbers of capsules (90) was harvested from BARI til-4 followed by GM-11, and GM-8, CB-1 while the lowest numbers of capsules (58) was collected from the variety Binatil-1 and GM-25, respectively (Table 3). The highest length of capsules (2.6 cm) was obtained for SMM-001 and the lowest length of capsules (1.6 cm) was recorded for genotypes GM-9, BARI til-4, GM-26 and GM-6 which was followed by Binatil-4. The highest numbers of seeds

Table 3: Varietal differences in morpho-physiological traits of sesame

Varieties	Plant height (cm)	Branches plant^{-1}	Root length (cm)	Shoot length (cm)	Days to maturity	Capsules plant^{-1}	Capsule length (cm)	Seeds capsule^{-1}
CW-1	118.0 ab	3.0 b	10.0 bc	108.0 a-d	82.0 c-e	78.0 e	2.20 cd	73.0 c-f
IB-1	116.0 b-d	2.0 c	12.0 a	104.0 d-h	75.0 fg	67.0 h	2.30 bc	71.0 e-g
SM-007	117.0 bc	2.0 c	10.0 bc	107.0 a-e	74.0 f-h	60.0 k	2.40 b	70.0 fg
GM-11	120.0 a	4.0a	10.0 bc	110.0 ab	86.0 ab	86.0 b	2.40 b	69.0 g
GM-3	115.0 c-e	3.0 b	12.0 a	103.0 e-h	81.0 de	76.0 f	2.30 bc	73.0 c-f
IB-2	114.0 d-f	2.0 c	12.0 a	102.0 f-h	72.0 g-j	65.0 i	2.20 cd	71.0 e-g
GM-1	116.0 b-d	3.0 b	8.0 d	108.0 a-d	81.0 de	75.0 f	2.40 b	74.0 b-e
BINA-2	115.0 c-e	3.0 b	10.0 bc	105.0 c-g	82.0 c-e	72.0 g	2.20 cd	74.0 b-e
GM-8	117.0 bc	4.0 a	11.0 ab	106.0 b-f	87.0 ab	87.0 b	2.40 b	75.0 a-d
GM-5	113.0 ef	4.0 a	10.0 bc	103.0 e-h	86.0 ab	85.0 bc	2.10 de	71.0 e-g
GM-7	114.0 d-f	2.0 c	7.0 e	107.0 a-e	71.0 h-j	60.0 k	2.10 de	74.0 b-e
SM-13	115.0 c-e	3.0 b	8.0d	107.0 a-e	82.0 c-e	76.0 f	2.00 ef	72.0 d-g
SMM-008	115.0 c-e	2.0 c	12.0 a	103.0 e-h	72.0 g-j	64.0 ij	2.40 b	72.0 d-g
SM-17	116.0 b-d	2.0 c	10.0 bc	106.0 b-f	74.00 f-h	63.0 j	2.30 bc	76.0 a-c
GP-8	113.0 ef	4.0a	10.0 bc	103.0 e-h	87.0 ab	84.0 c	2.20 cd	75.0 a-d
SM-9	116.0 b-d	3.033 b	11.0 ab	105.0 c-g	80.0 e	82.0 d	2.10 de	72.0 d-g
SM-5	112.0 fg	2.0 c	7.0 e	105.0 c-g	69.0 j	64.0 ij	2.20 cd	70.0 fg
SMM-001	113.0 ef	3.00b	10.0 bc	103.0 e-h	85.0 a-c	73.0 g	2.60 a	69.0 g
GM-10	118.0 ab	3.0b	9.0 c	109.0 ac	85.0 a-c	72.0 g	2.10 de	70.00 fg
GM-2	117.0 bc	4.0 a	12.0 a	105.0 c-g	86.0 ab	82.0 d	1.800 gh	71.0 e-g
GM-9	118.0 ab	2.0 c	12.0 a	106.0 b-f	73.0 g-i	59.0 k	1.60 i	74.0 b-e
BARI-4	120.0 a	4.0 a	9.0 c	111.0 a	87.0 ab	90.0 a	1.60 i	77.0 ab
BINA-3	112.0 fg	3.0 b	10.0 bc	102.0 f-h	85.0 a-c	86.0 b	2.10 de	75.0 a-d
BINA-4	114.0 d-f	4.0 a	10.0 bc	104.0 d-h	85.0 a-c	81.0 d	1.70 hi	74.0 b-e
IB-4	112.0 fg	4.0a	8.0 d	104.0 d-h	88.0 a	80.0 d	1.80 gh	76.0 a-c
BINA-1	116.0 b-d	0.0 d	9.0 c	107.0 a-e	55.0 k	58.0 k	2.10 de	75.0 a-d
IB-3	112.0 fg	3.0 b	10.0 bc	102.0 f-h	75.0 fg	76.0 f	2.00 ef	73.0 c-f
GM-26	114.0 d-f	4.0 a	12.0 a	102.0 f-h	84.0 b-d	81.0 d	1.60 i	75.0 a-d
GM-20	116.0 b-d	2.0 c	11.0 ab	105.0 c-g	70.0 ij	60.0 k	1.70 hi	71.0 e-g
GM-6	115.0 c-e	4.0 a	11.0 ab	104.0 d-h	87.0 ab	80.0 d	1.60 i	75.0 a-d
SM-056	118.0 ab	3.0 b	12.0 a	106.0 b-f	84.0 b-d	76.0 f	1.90 fg	72.0 d-g
CB-1	116.0 b-d	4.0 a	10.0 bc	106.0 b-f	85.0 a-c	87.0 b	1.80 gh	76.0 a-c
GM-25	117.0 bc	2.0 c	11.0 ab	106.0 b-f	77.0 f	58.0 k	2.00 ef	70.67 e-g
SM-10-4	110.0 g	4.0 a	10.0 bc	100.0 h	86.0 ab	84.0 c	1.90 fg	75.0 a-d
GM-21	117.0 bc	3.0 b	12.0 a	105.0 c-g	81.0 de	72.0 g	2.10 de	75.0 a-d
GM-4	112.0 fg	4.0 a	11.0 ab	101.0 gh	85.0 a-c	81.0 d	2.10 de	73.0 c-f
SM-067	113.0 ef	4.0 a	10.0 bc	103.0 e-h	86.0 ab	84.0 c	2.30 bc	76.0 a-c
SM-15	112.0 fg	3.0 b	10.0 bc	102.0 fh	80.0 e	77.0 ef	2.10 de	78.0 a
Level of sig.	**	**	**	**	**	**	**	**
$LSD_{(0.05)}$	2.197	0.345	0.879	3.856	2.791	1.825	0.136	3.077
CV (%)	1.17	7.02	5.28	2.26	2.14	1.5	4.03	2.58

In a column, mean values having similar letters are not significantly different at $p \leq 0.05$, whereas figures with dissimilar letters are significantly different as per DMRT; **= significant at 1% ($p \leq 0.01$) level of probability

capsule^{-1} (78) were obtained for SM-15 which was followed by BARI til-4 and the lowest number of seeds capsule^{-1} (69) was obtained for genotypes GM-11, SMM-001 which was followed by GM-10 and GM-25 (Table 3).

Variability, genetic advance (GA), percent (%) GA and heritability (h^2b) of the mean for eight growth and growth related characters of 38 genotypes of sesame are shown in Table 4. The traits evaluated in this study showed high heritability that ranged between 54.17% and 98.67%. Among the studied traits, the numbers of capsules plant^{-1} had the highest (98.67%) heritability and the numbers of seeds capsule^{-1} exhibited the lowest heritability (54.17%). The average plant height (115.11 cm), branch plant^{-1} (3.03), root length (10.24 cm), length of shoots (104.87 cm), days to maturity (80.26 days), capsules plant^{-1} (74.76), length of the capsule (2.07 cm), seeds capsule^{-1} (73.32) were recorded in the present study. The highest genetic advance was recorded for the number of branch^{-1} (62.13) followed by the number of capsule^{-1} (26.40) and the lowest genetic advance was recorded for shoot length (2.71) among the growth characters. The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) for all the characters studied in the present research. The branches plant^{-1} showed high PCV and GCV values preceding the length of root and number of capsule plant^{-1} (Table 4). Days to maturity exhibited moderately higher PCV and GCV than the remaining traits.

Correlation coefficients between various morphometric characters in sesame genotypes are shown in Table 5. Height of sesame plant exhibited a significantly positive correlation with the length of root and shoot, capsule plant^{-1} and seeds capsule^{-1}. Days to maturity showed a significantly positive correlation with the number of capsules plant^{-1}. A negative correlation

Table 4: Variability, heritability (h^2b) and genetic advance (GA) for eight growth characters of sesame

SL. No.	Characters	Mean	Phenotypic variance (δ^2p)	Genotypic variance (δ^2g)	PCV (%)	GCV (%)	Heritability (%)	GA	GA (%)
1	Plant height (cm)	115.11	6.93	5.11	2.29	1.96	73.70	4.00	3.47
2	Number of branches plant^{-1}	3.03	0.921	0.876	31.71	30.93	95.11	1.88	62.13
3	Length of root (cm)	10.24	2.16	1.87	14.37	13.37	86.51	2.62	25.61
4	Length of shoot (cm)	104.87	9.97	4.352	3.01	1.99	43.65	2.84	2.71
5	Days to maturity	80.26	52.48	49.54	9.03	8.77	94.39	14.09	17.55
6	Number of capsules plant^{-1}	74.76	94.32	93.06	12.99	12.90	98.67	19.74	26.40
7	Length of capsule	2.07	0.074	0.067	13.16	12.53	90.58	0.51	24.56
8	Number of seeds capsule^{-1}	73.32	7.802	4.226	3.81	2.80	54.17	3.12	4.25

PCV=phenotypic coefficient of variation, GCV=genotypic coefficient of variation, GA=genetic advance

Table 5: Correlation coefficients among the morphological characters in sesame

Characters	Genotypes	Shoot length (cm)	Capsule/plant	Maturity (days)	Branch/plant	Capsule length (cm)	Seed/capsule
Height (cm)	0.218*	0.836 ***	0.123**	-0.048	0.145	-0.051	0.184*
Root length (cm)		-0.354*	0.01	0.083	0.056	-0.105	-0.083
Shoot length (cm			0.123	-0.092	0.171	0.01	0.13
Capsules/plant				0.843**	0.898***	-0.153	0.267**
Maturity (days)					0.937	-0.136	0.154
Branch/plant						-0.246**	0.208***
Capsule length (cm)							0.360**

*Pearson correlation Here, *** indicates 0.1% and * indicates 5% level of significance respectively.*

of root length was found with shoot length. A significantly positive correlation of the number of capsules per plant was also recorded in this study with the number of days to maturity, and the number of branches plant^{-1} and number of seeds capsule^{-1}. Capsule length showed a positive significant correlation with seeds capsule^{-1}. Branches plant^{-1} on the contrary showed a negative correlation with capsule length. Shoot length showed a positive correlation with branches plant^{-1}.

Thirty-eight genotypes of sesame were separated in six clusters (Table 6). Cluster II (23.68%) and IV (23.68%) had nearly 50% of the genotypes in it containing nine genotypes each. Cluster I and VI contained five genotypes each (Table 6). On the contrary, clusters III contained seven genotypes (18.42%) and cluster V contained three genotypes (7.89%). The thirty-eight sesame genotypes were grouped by the Pearson method using quantitative and qualitative traits as shown in Figure 2. Five genotypes, CW-1, GM-11, GM-1, SM-13, GM-10 was grouped in Cluster I. Cluster II comprised of nine genotypes, IB-1, SMM-007, IB-2, SMM-008, SM-17, SMM-001, GM-9, GM-20, GM-25. Cluster III was formed by seven genotypes, GM-3, BINA-2, GM-8, SM-9, GM-2, SM-056, GM-21. Cluster IV consisted of nine genotypes, GM-5, GP-8, BINA-3, IB-4, IB-3, SM-10-4, GM-4, SM-067, SM-15. In cluster V, there were three genotypes GM-7, SM-5, BINA-1. Cluster VI consisted of five genotypes, BARI4, BINA-4, GM-26, GM-6, CB-1.

Table 6: Frequency distribution in different clusters of sesame genotypes

Cluster number	Number of genotypes	Percent (%)	Name of genotypes
I	5	13.16	CW1, GM-11, GM-1, SM-13 and GM-10
II	9	23.68	IB1, SMM-007, IB2, SMM-008, SM-17, SMM-001, GM-9, GM-20 and GM-25
III	7	18.42	GM-3, Binatil-2, GM-8, SM-9, GM-2, SM-056 and GM-21
IV	9	23.68	GM-5, GP-8, Binatil-3, IB-4, IB-3, SM-10-4, GM-4, SM-067 and SM-15
V	3	7.89	GM-7, SM-5 and Binatil-1
VI	5	13.16	BARI til-4, Binatil-4, GM-26, GM-6 and CB-1

Molecular Characterization

Among the 38 sesame genotypes, 19 alleles were detected using five SSR markers and the result are presented in Table 7. Average number of alleles per locus was calculated and it was 3.80. The highest allele number was 5.0 detected for SI-ssr29 and the lowest allele number was 3 in two markers, SI-ssr01 and SI-ssr19. The highest degree of gene diversity (0.6593) was recorded in locus SI-ssr30 and the lowest gene diversity was recorded (0.3726) in locus SI-ssr01 with a mean value 0.5640. The PIC values, that are considered to be the measure of the informative nature of microsatellites, ranged from the lowest 0.3201 to the highest 0.5934. The PIC value was highest for SI-ssr30 and the lowest value was found for SI-ssr01 (Table 7).

Pair-wise genetic distance (D) (Nei, 1973) between genotypes of sesame was calculated and the data ranged from 0.20 to 1.00. The highest value of genetic distance between genotypes indicated that the genotypes were genetically diverse compared to those having a lower value of genetic distance. A genetic distance of 0.20 was observed for genotype pairs CW-1 vs GM-5, GM-7 vs SMM-008, GM-7 vs GM-6, SM-13 vs SMM-008, SM-13 vs GM-26, SM-13 vsGM-25, SMM-008 vs SM-17, SMM-008 vs SM-15, SM-17 vs SMM-001, SM-17 vs GM-26,GP-8 vs GM-10, GP-8 vs IB-1, SMM-001 vs Binatil-1, SMM-001 vs SM-15, GM-10 vs IB-4, Binatil-3 vs Binatil-1, Binatil-3 vs GM-1, IB-4 vs SM-056, Binatil-1 vs GM-25, Binatil-1 vs SM-056, GM26 vs SM10-4, GM-26 vs GM-1, GM-20 vs GM-1, GM-6 vs SM-056, GM-25 vs SM-4, GM-25 vs GM-1, GM-25 vs SM-15, GM-21 vs SMM007, GM-21 vs SM-15, GM-4 vs GM-3, SM-15 vs GM-3, GM-1 vs Binatil-2 which had the lowest value. The highest value of genetic distance (1.00) was observed between a good numbers

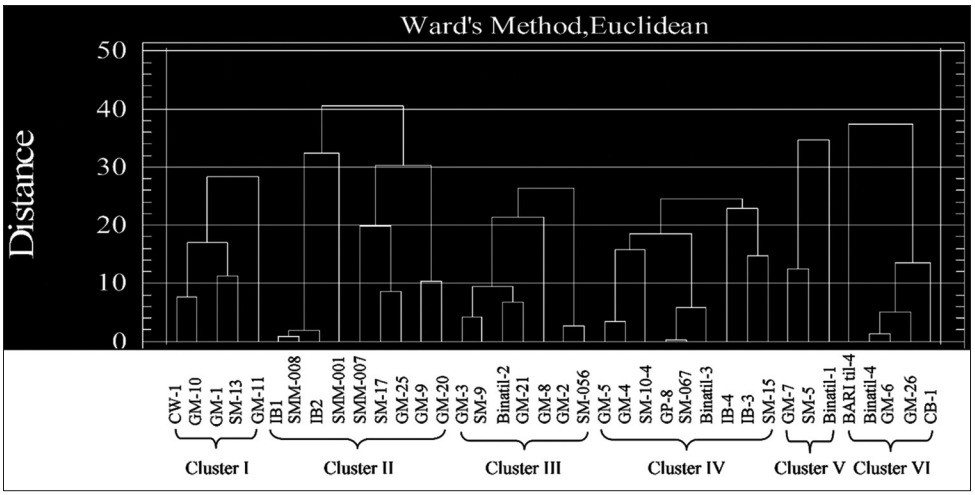

Figure 2: Dendrogram showing distribution of sesame genotypes into clusters using Ward's method

Table 7: Allele information, Gene Diversity and Polymorphism information content (PIC) Value for 5 SSR markers

Marker	Allele No	Major. Allele. Frequency	Null Allele	Gene Diversity	PIC
SI-ssr01	3.00	0.7632	1	0.3726	0.3201
SI-ssr19	3.00	0.6053	-	0.5554	0.4951
SI-ssr30	4.00	0.4474	-	0.6593	0.5934
SI-ssr16	4.00	0.5789	-	0.5997	0.5537
SI-ssr29	5.00	0.5526	-	0.6330	0.5929
Mean	3.80	0.5895	-	0.5640	0.5110

of genotype pairs. Those were CW-1 vs GM10, Binatil-4, IB-4 and GM-8; GM-5 vs GM-10, Binatil-4 and GM-8; GM-7 vs GM-9, SMM-007, IB-2; SM-13 vs GM-9, SMM-007 and IB-2; SM-17 vs GM-9, SMM-007 and IB-2; GP-8 vs SM-5, GM-2, IB-3, GM-21, IB-2; SM-9 VS Binatil-4 and GM-20; SM-5 VS GM20 and GM-8; GM-10 vs IB-2, IB-1 vs GM-9, IB-1 vs IB2; GM-9 vs CB1; BARI til-14 vs IB-4 AND SMM-007; BARI til-4 vs IB-2; Binatil-3 vs GM-8, Binatil-4 vs GN-11, IB2 and Binatil-2; IB-4 vs GM-11; IB4 vs IB-2 and Binatil-2; GM-20 vs CB-1, SMM-007 vs GM-26 and SM-056; GM-6 vs IB2 and GM-8; SM-056 vs IB-2; GM-4 vs GM-8; SM-067 VS GM-8, GM-15 vs GM-8.

A dendrogram, supported by genetic distance, was constructed (Nei, 1973). All the genotypes used in the present study were remarkably separated from each other. The cluster analysis by UPGMA showed a considerable genetic variation among the 38 genotypes under the study, characterized with the variable similarity coefficients ranging from 0.29 to 1.00. The cluster analysis discriminated the genotypes into three distinguished clusters formed at 0.35 cut off similarity coefficient (Figure 3). The similarity values narrowed outstandingly below this similarity coefficient. Based on UPGMA analysis the entire dendrogram was divided into 3 sub-clusters. Cluster 1 consisted with genotypes CW-1, GM-5, GM-11, SM-9, SM-007 and IB-2. Cluster 2 was composed of two sub-clusters (Sub-cluster 2.1 & sub-cluster 2.2). The sub-cluster 2.1 consisted with the genotypes named as GM-7, SM-13, SM-17, SMM-008, SMM-001, GM-26, GM-25, SM-10-4, GM-21, SM-067, GM-4, SM-15, GM-3, SM-5, GM-6, GM-2, IB-3, Binatil-1, Binatil-3, IB-4, SM-056 and CB-1, whereas the sub-cluster 2.2 was comprised with GM-9, BARI til-4, GM-1, Binatil-2, GM-20. The last Cluster 3 consisted of GP-8, GM-10, IB-1, GM-8 and Binatil-4, respectively (Figure 3).

DISCUSSION

Molecular and morpho-physiological genetic diversity of 38 sesame genotypes were assessed in this study. The results revealed that the morpho-physiological genetic characters such as plant height, branches plant^{-1}, shoot length, maturity duration, number of the capsule plant^{-1} and seeds capsule^{-1} significantly varied among the genotypes. The broad-sense heritability was estimated high (98.67% for number of capsules plant^{-1}, branches plant^{-1} (95.11%), maturity duration (94.39%), and capsule length (90.58%). Also, relatively high coefficient of variation was observed for branches plant^{-1}, capsule length and capsules plant^{-1} which indicated that genetic improvement is possible for these traits by visual selection. Very little or no variations were observed for root length and capsule length among the genotypes.

Heritability is a deciding factor in the suitability and strategy for the selection of a particular character. Rosmaina *et al.* (2016) proposed that the selection for morpho-physiological characters could be fairly easy, if their heritability is 80% or above. At high heritability, a close correspondence is established between the genotype and the phenotype due to the relatively small contributory effect of the environment to the phenotype. On the contrary, characters with low heritability (below 40%), selection could be relatively complicated or impossible due to the strong masking effect of the environment. Roy and Shil (2020) stressed that heritability and genetic advance are very important and should always be considered jointly while selecting an appropriate line or progeny. Existence of significantly high positive correlation coefficients as observed between capsule plant^{-1} and plant height with the number of seed capsule^{-1}, as seen in our study, leads to an increased yield. Our findings suggest that there is prospect of using those phenotypic characters as selection criteria in breeding programs

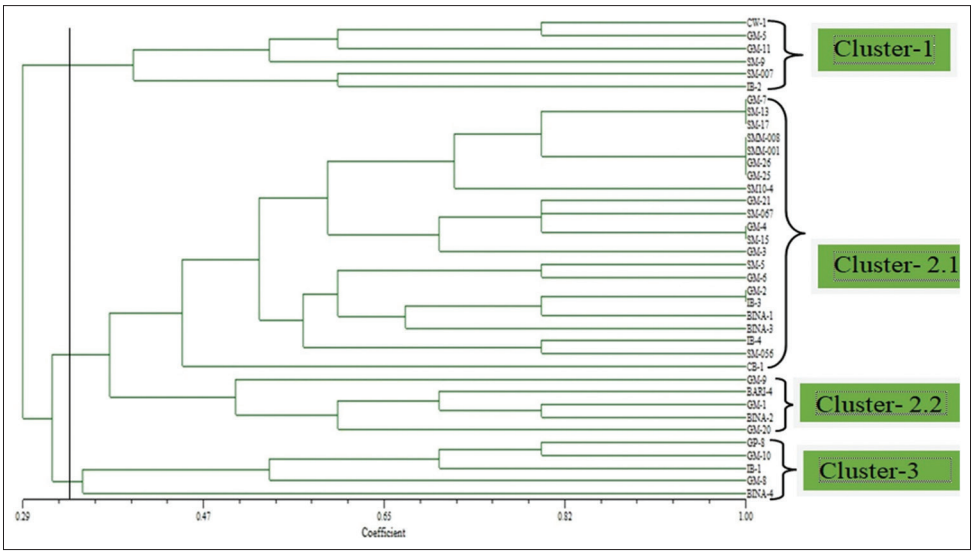

Figure 3: UPGMA dendrogram for 38 sesame genotypes showing the genetic similarity

for enhanced sesame yield. Ali *et al*. (2020) also reported that taller plants with the higher number of capsules are favorable for seed yield improvement in sesame. In our study, genotypes BARI til-4, GM-11, CW-1, GM-10, GM-9 and GM-8 had the tallest plants accompanied with higher capsules plant^{-1}.

For the morphological traits based genetic diversity quantification among the sesame genotypes, clustering was done following Ward's linkage method using the Euclidean distance matrix. The 38 genotypes used in this study were separated into six distinct clusters. A greater inter- and intra-cluster distances proves existence of a higher diversity among the genotypes both within and between the clusters (Sen *et al*., 2021). The genotypes belonging to the cluster II and cluster IV seemed to have the highest inter-cluster distance. These two clusters contained the maximum number of genotypes. The differential mean values of the characters also reflected a distinct scenario of genetic diversity. Considerable variation in various traits was found between clusters II and IV which were considered outstanding considering the highest and lowest values of cluster means for yield and yield contributing characters. In this study, the clustering pattern of the genotypes was consistent with those of Khatun *et al*. (2010) and Rabbani *et al*. (2012) who also discriminated the genotypes into four and five clusters, respectively. Majority of the genotypes collected from the same or nearby areas in our study fell in the same clusters. However, there was a paradox showing that a few genotypes from the same region were distributed into different clusters, indicating that geographical proximity of the genotypes does not always result in genetic similarity. There might be other factors than geographical diversity that contributes to the genetic diversity, and it is not unlikely that genotypes collected from the same place or close areas may have different genetic make-up.

High genetic variation is considered important for breeding crops, however, crossing on the basis of the highest and lowest values of traits may not always result in better performance with higher yield, especially in short-term breeding programs. Consequently, Pavan *et al*. (2020) recommended to choose genotypes that have considerable genetic distance and valuable phenotypic characters for the development of high yielding good quality varieties through hybridization. Our research findings revealed that the genotypes belonging to clusters IV and that of cluster VI which had the highest number of capsules plant^{-1} and number of seeds capsule^{-1} with a sufficient genetic variations and desired agronomic traits required for higher yield. Therefore, hybridization between the genotypes belonging to clusters IV and VI may result desired sesame genotypes for greater yield potentials.

Other purpose our study was to detect the genetic diversity among the 38 sesame genotypes through molecular characterization. Markers SI-ssr29 yielded maximum number (5) of alleles while SI-ssr01 and SI-ssr19 yielded the least number (3) of alleles. Several factors including structure of primers and the availability of annealing sites within the genome cause observed variation in alleles amplified by different primers (Kadri, 2019). Admas *et al*. (2021) suggested that polymorphic bands showing the variability in the genotypes as suitable for examining and establishing a systematic relationship among the genotypes.

The polymorphism information content (PIC) values in this study ranged from 0.3201 in marker SI-ssr01 to 0.5934 in marker SI-ssr30 that averaged 0.5110. The PIC values similar to this study were also reported by Wu *et al*. (2014) for Chinese sesame. Markers that have PIC values of 0.5 or above are highly informative, indicative of high polymorphism and are used in genetic studies (Li *et al*., 2021). A pair of genotypes that have a higher PIC value is more dissimilar than a pair with a lower PIC. Therefore, the average PIC values observed in the SSR markers used in this study confirmed that these SSR markers can be considered sufficiently informative, the genotypes were genetically diverse from each other, and indicative of high polymorphism. To evaluate the genetic diversity of different sesame genotypes the means of genetic distances between genotypes were used and it also revealed wide variability among

the tested genotypes. Using SSR marker, Ahmed *et al.* (2019) recorded genetic distance between 48 genotypes of brinjal genotypes ranging from 0.250 to 1.00 indicating good capacity of SSR markers to clearly discriminate among plants of near or distant genetic backgrounds. The information on microsatellite markers collected in our research was also capable to analyze diversity among all the genotypes through cluster analysis. The genotypes of genetically similar type formed clusters together in the dendrogram. The genetic similarity result supports the preceding findings in sesame (Bhattacharjee *et al.*, 2019; Yadav *et al.*, 2022b).

CONCLUSIONS

The primary breeding objectives for sesame closely linked to current needs are increasing the seed yield, improving the canopy architecture, tolerance to biotic and abiotic stresses, indehiscent capsules, and improving oil quality. Some recent achievements have seen development of new genotypes with increased adaptability and resistance to biotic and abiotic stresses. However, significant obstacle to increasing sesame yield still continues. Identification of genetic diversity and their relationships among breeding materials is a pre-requisite to screen out the desired genetic materials for the genetic improvement. The polymorphism that was found among the genotypes in our study could be used in the breeding program to develop new sesame varieties with greater yields and other desired traits. The 38 genotypes used in this study were separated into six distinct clusters. A greater inter- and intra-cluster distances proved existence of a higher diversity among the genotypes both within and between the clusters. The information on microsatellite markers also proved diversity among the genotypes. The genotypes belonging to the cluster II and cluster IV seemed to have the highest inter-cluster distance. Also, these two clusters contained the maximum number of genotypes. The broad-sense heritability was estimated higher (>90%) for traits like capsules plant^{-1}, branches plant^{-1}, maturity duration, and capsule length. The genotypes which showed higher genetic distance in this study are very important to establish genetic bases for improved varieties through further breeding programs.

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Conflict of Interest

The authors report no conflicts of interest.

Authors Contributions

RME, MNS and MKK conducted the experiments, analyzed the data, wrote the manuscript; MAA reviewed and polished the manuscript; MAM and MSH read and approved the final version.

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