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Assessment of Genetic Diversity in Indigenous Sesame Genotypes

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ABSTRACT

Thirty-three indigenous genotypes of sesame (*Sesamum indicum* L.) were evaluated to assess the extent of genetic diversity based on phenotypic and marker-based genotypic data. Multivariate analysis revealed considerable genetic diversity in the material and led to their grouping into 8 clusters. The yield per plant contributed most to genetic divergence followed by days to 50% flowering and 1000 grain weight, respectively. Altogether, 78 alleles were detected with 27 polymorphic SSR markers, of which 49 were polymorphic (62.82%) with an average of 2.89 alleles per locus. The polymorphism information content (PIC) value varied from 0.99 to 0.01 with a mean of 0.43. Dendrogram analysis grouped the 33 genotypes into three separate clusters exhibiting a genetic similarity coefficient from 0.931 to 0.591 with an average of 0.754. No relationship between geographic origin and genetic diversity was observed. Few genotypes were identified to be important for varietal improvement programme on the basis of their genetic distances and desirable cluster means. The result confirmed the presence of high genetic diversity among the local germplasm.

Keywords

D2 statistic, Genetic diversity, Polymorphism, Sesame, Simple sequence repeats (SSR) markers

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Introduction

Sesame (*Sesamum indicum* L.) is a diploid ($2n=2x=26$) oilseed crops belong to family *Pedaliaceae*. Sesame is basically a crop of the tropics and subtropics highly tolerate to drought because of its deep taproot system. Sesame has remarkable antioxidant function, rich in quality proteins and essential amino acids. The oil with 85% unsaturated fatty acids, is highly stable and has reducing effect on cholesterol and prevent coronary heart diseases. In spite of being a good source of

healthy oil, it is not grown on a large extent due to very poor yield. India, Myanmar and China are the top three sesame producing countries in the world. India is the second largest producer with the productivity of 413 kg/ha after China (1234 kg/ha) as compared to world's average of 535 kg/ha (Anon. 2017). In Assam, during 2013-14 to 2015-16 area, production and productivity was 0.12 lakh ha, 0.90 lakh tones and 746 kg/ha respectively (Anon., 2017). The low seed yield of sesame is a result of a lack in breeding attention (Ashri *et al.*, 1989), and a lack of improved

varieties for use by the farmers. Therefore serious efforts are necessary for developing high yielding varieties of good quality and high adaptive potential to the diverse climatic situations (Farooq *et al.*, 2016).

India in general, and North Eastern India in specific is a rich source of biodiversity in Sesame (Bisht *et al.*, 1998; Sharma *et al.*, 2014). Traditional sesame landraces are an important source of genetic diversity for breeders and form the backbone of sesame breeding programme, which remains largely unexplored and uncharacterized. Morphological characteristics, being the important determinants of the agronomic value and taxonomic classification are widely used in assessing the genetic diversity. However, morphological markers have limitations in their ability to estimate genetic diversity because of strong influence from environmental factors, which make them highly dependent on the cultivation conditions. Molecular marker mediated diversity analysis is considered ideal due to its abundance, hyper-variability, multi-allelic and codominant nature (Sharma *et al.*, 2009; Yepuri *et al.*, 2013; Wu *et al.*, 2014; Sehr *et al.*, 2016). Since the studies on genetic diversity analysis combining morphological and molecular markers are limited in Indian sesame germplasm, the present study was carried out to compare the genetic diversity in sesame genotypes using morphological traits and SSR markers.

Materials and Methods

Experimental materials and phenotyping

A set of 33 genotypes (Table 1) collected from different parts of the North Eastern India were grown during Kharif, 2016 in a randomized block design with 3 replications. Out of these 33 genotypes, 22 genotypes were collected from different agro-climatic zone of Assam.

Seven genotypes were collected from Calcutta University, one from Arunachal Pradesh, one from Umium (Meghalaya) and rest two were HYV. Each genotype was sown in 3 rows of 4 m length with spacing of 30 X 10 cm following recommended agronomic practices. Observations were recorded on height of first capsule bearing node (cm), plant height (cm), number of capsules per plant, number of branches per plant, capsule length (cm), capsule width (cm), number of seed per capsule, 1000 grain weight (g), days to 50% flowering, days to maturity and yield per plant (g). Multivariate analysis was done as per Mahalanobis's D^2 statistics described by Rao (1952) and the genotypes were grouped into different clusters following Tocher's method.

DNA isolation

The DNA was extracted from newly emerged fresh leave by modified CTAB method (Murray and Thompson, 1980). The modifications were made to improve the quality and quantity of the DNA. Isolation of genomic DNA from sesame is strenuous due to the presence of mucilage (viscous glue) which make it difficult to pipette out aqueous layers interferes at nucleic acid purification step. In this method 1g of ground sesame leaves (using liquid nitrogen) were immediately transferred into extraction buffer solution consisting of 2.3g Sorbitol, 1g N-laurylsorcosine, 0.8g CTAB, 4.7g NaCl, 1g PVP-40, 20mM EDTA(pH8), 10mMTris-HCl(pH8), 20 μ l β -marcaptoethanol, 1 ml Triton X-100. Tubes containing the samples were then incubated for 10 min at 42°C, then 30 min 65°C in a water bath with gentle shaking and left to cool at room temperature for 5mins. Phenol: chloroform: Isoamyl alcohol mixture (25:24:1) was added to each tube and centrifuge for 20 minutes at 12000 rpm. After that final concentration of 6% PEG 600 was added to the aqueous phase and incubates for 30mins at room temperature.

Then centrifuged for 20 minutes at 12,000 rpm. The cell debris was washed with 70% ethanol. The last drop of ethanol was removed by placing the tube face down on tissue paper. Finally isolated DNA was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and stored at -20 °C. The isolated genomic DNA was quantified on Nanodrop N.D.1000 system (V.3.3.0, Thermo Scientific, USA). And quality and integrity was further analysed using 0.8 % agarose gel electrophoresis run with a power packset at 70 V for 1 h in 1X TAE buffer consisting of 0.04 M Tris base, 17.4 M Glacial acetic acid, 0.001 M EDTA. DNA was subsequently diluted to final concentration of 50 ng/µl for PCR based microsatellite analysis.

PCR Amplification and gel analysis

In total, 50 different SSR marker were used for PCR amplification (Table 2). The PCR was carried out in reaction volume 10 µl volume containing 50 ng template DNA, 1X PCR buffer, 0.2 mM dNTPs, 0.5 pM of each primer and 0.1 U Taq polymerase (Takara). The PCR reaction conditions were an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, melting temperature (primer specific) for 1 min, 72°C for 1 min and a final extension of 7 min at 72°C. The PCR products were analysed on 3% agarose gels electrophoresis (Lonza LE agarose) stained with ethidium bromide run with a power pack set at 100 V for 2.5 h and visualized under Bioered gel-doc system. The DNA polymorphism between the genotypes was observed based on length of amplified DNA fragment in terms of base pair compared with 100 bp DNA ladder.

Only clear and reproducible bands were selected for data analysis by scoring polymorphic bands qualitatively as present (1) or absent (0). The total numbers of bands, polymorphic bands, average number of bands

per primer, polymorphism percentage, and Polymorphism information content (PIC) (Anderson *et al.*, 1993) were calculated. These data were used to calculate the pair-wise genetic similarity index was calculated as per Jaccard's coefficient of similarity (Jaccard, 1908). This similarity matrix was subjected to the unweighted pair-group method for arithmetic averages analysis (UPGMA) to generate the dendrogram. These analyses were conducted using the software package, NTSYS-pc (Numerical Taxonomy System) Version 2.1 (Rohlf, 2000).

Results and Discussion

The analysis of variance revealed significant differences between the 33 genotypes for all the 11 characters studied except the number of branches per plants. The aggregate effects of all the 11 characters were tested by the Wilk's criterion, indicating significant differences between the genotypes. Hence, the analysis of genetic divergence based on D^2 values was considered relevant. Based on D^2 values maximum diversity was observed between SaliTil_Darrang, collected from Darrang and NempoSoksu, collected from RARS (Diphu). Similarly, SaliTil_Koliabor and KolaTil_Majuli were shown to be close relative due to the minimum D^2 value between them. The constituents of different clusters with their source are presented in presented in Table 3. Multivariate analysis revealed that 33 genotypes were grouped into eight clusters with a single largest cluster of 23 genotypes (Cluster 1). Cluster 2 comprised of four genotypes and the rest six were solitary cluster indicating the impact of selection pressure in increasing the genetic diversity. Four lines collected from RARS, Diphu were placed in Cluster 2 because of high morphological similarity among the lines. Sesame lines from other regions were scattered across different clusters indicating substantial genetic variability among the other lines. Clustering of

germplasm was not associated with the geographical distribution and mainly grouped due to their morphological differences and breeding history. Similar results have been reported by Solanki and Gupta (2001), Parameshwarappa *et al.*, (2010), Abate and Mekbib (2015).

The intra- and inter-cluster D^2 values among 8 clusters are presented in Table 4. The maximum inter cluster distance was observed between the cluster 6 (TKG 308) and cluster 8 (Punjab Til 1), indicating a wide divergence among the clusters. A high inter-cluster distance was also observed between cluster 6(TKG 308) and cluster 7 (Nga Na) followed by cluster 5 (AhuTil_Koliabor) and cluster 8 (Punjab Til 1).

The magnitude of heterosis largely depends on the degree of genetic diversity in the parental lines. Therefore, the genotypes from these diverse clusters could be used in a hybridization programme to obtain a broad spectrum of genetic variability in the segregating generations. The minimum inter-cluster distance was observed between clusters 3 (KolaTil_Tezpur) and 5 (AhuTil_Koliabor), suggesting that the genetic constitution of the genotypes in one cluster was in close proximity with the genotypes in the other cluster.

Hence, the genotypes from these clusters may not be useful in the hybridization programme. Combining inter-cluster distance and cluster mean value (Table 5), the genotypes Rama, Punjab Til 1, TKG 308 and Nga Na could be useful for exploitation of hybrid vigour and for getting good recombinant. The relative contribution of different characters towards genetic divergence (Table 6) revealed that yield per plant had the highest contribution followed by days to 50% flowering and 1000 grain weight. Therefore, these characters should be given importance for the selection

of parents for further breeding programmes. These results are in agreement with that given by Velusami *et al.*, (2008) for seed yield and 1000 seed weight; Parameshwarappa *et al.*, (2010) for 1000 seed weight, number of capsules per plant and seed yield per plant, Bandila *et al.*, (2011) for seeds per capsule and number of capsules per plant and Gangadhara *et al.*, (2012) for yield per plant and 100 grain weight.

Out of 50 simple sequence repeat (SSR) primers tested initially, 27 polymorphic primers that gave clear banding pattern were used in the present study (Table 7). These 27 SSR primers produced 78 bands of which 49 were polymorphic (62.82%). The level of polymorphism obtained in this study (62.82%) was low compared with the previous study in which a high level of polymorphism was detected among Indian sesame genotypes were more than 73% (Kumar and Sharma, 2011). Each marker amplified 1 to 5 alleles with an average of 2.89 alleles per locus. The SSR markers in the present study showed a lower number of alleles than previous analysis of the diversity of sesame accessions conducted with SSR markers (Dixit *et al.*, 2005; Gebremichael and Parzies, 2011; Kumar and Sharma, 2011; Nweke *et al.*, 2015; Pandey *et al.*, 2015).

The polymorphism information content (PIC) value varied from 0.99 (SSR 46) to 0.01 (SSR 14) with a mean of 0.43. The highest PIC value of 0.99 was observed for marker SSR46. It reflected a better discriminatory power of this primer to reveal a higher level of genetic diversity. In spite of having all the polymorphic loci PIC of SSR 14 was very low (0.01) indicating a less discriminatory power of this to distinguish genotypes under present study. Since PIC value closer to 1 is desirable four primers (SSR 46, SSR 10, SSR 33 and SSR 28) found to be suitable to discriminate the accessions.

Table.1 Genotypes included in the present study

Sl. No.	Genotype Name	Source
1.	Nga Na	Arunachal Pradesh, local
2.	KolaTil_Tezpur	Tezpur,local
3.	AhuTil_Kalibor	Kalibor,local
4.	NeiIong_Umium	Umium, local
5.	AhuTil_Lakhimpur	Lakhimpur, local
6.	SaliTil_Biswanath	Biswanath,local
7.	SaliTil_Kaliabor	Kaliabor,local
8.	PahariaTilNagaon	Nagaon, local
9.	AhuTil_Sibsagar	Sibsagar,local
10.	CUHY 57	CalcuttaUniversity,mutant
11.	TKG 308	HYV, Madhya Pradesh
12.	CUMS 17	Calcutta University,mutant
13.	CUMS 04	Calcutta University,mutant
14.	Uma	Calcutta University,mutant
15.	AhuTil_Puranigudam	Puranigudam, local
16.	V 12	Calcutta University,local
17.	Rama	Calcutta University, selection from local 'Khosla'
18.	V 15	Calcutta University, local
19.	SaliTil_Nagaon	Nagaon,local
20.	KolaTil_Nalbari	Nalbari,local
21.	SaliTil_Ghilamara	Ghilamara, local
22.	KolaTil_Majuli	Majuli, local
23.	SaliTil_Darrang	Darrang, local
24.	AST 1	RARSDiphu, HYV local X ST1683
25.	NempoThepo	RARSDiphu, local
26.	Punjab Til 1	HYV
27.	NempoLongnicklu	RARSDiphu, local
28.	Nagaon Local	Nagaon, local
29.	NempoCharap	RARSDiphu, local
30.	NempoSoksu	RARSDiphu, local
31.	Kaliabor Local	Kaliabor, local
32.	Bahua Bheti	RARSDiphu, local
33.	NempoChindon	RARSDiphu, local

Table.2 SSR markers used in the present study

	Primer sequence	Annealing temp. °C	Source
SSR 1	F: 5'-TCATATATAAAAGGAGCCCAAC-3' R: 5-GTCATCGCTTCTCTCTTCTTC-3	55	Nweke, 2015
SSR 2	F: 5-GGAGAAATTTTCAGAGAGAAAAA-3 R: 5-ATTGCTCTGCCTACAAATAAAA-3	58	Nweke, 2015
SSR 3	F: 5-CCCAACTCTTCGTCTATCTC-3 R: 5-TAGAGGTAATTGTGGGGGA-3	58	Nweke, 2015
SSR 4	F: 5-TTTTCTGAATGGCATAGTT-3 R: 5-GCCCAATTTGTCTATCTCT-3	54	Nweke, 2015
SSR 5	F: 5-GCAGCAGTCCGTTCTTG-3 R: 5-AGTGCTGAATTTAGTCTGCATAG-3	61	Nweke, 2015
SSR 6	F: 5-CCACTCAAATTTTCACTAAGAA-3 R: 5-TCGTCTTCTCTCTCCCC-3	61	Nweke, 2015
SSR 7	F: 5-GCAAACACATGCATCCCT-3 R: 5-GCCCTGATGATAAAGCCA-3	61	Nweke, 2015
SSR 8	F: 5-TTTCTTCTCGTTGCTCG-3 R: 5-CCTAACCAACCACCCTCC-3	55	Nweke, 2015
SSR 9	F: 5-CCATTGAAAACCTGCACACAA-3 R: 5-TCCACACACAGAGAGCCC-3	55	Nweke, 2015
SSR 10	F: 5-TCTTGCAATGGGGATCAG-3 R: 5-CGAACTATAGATAATCACTTGGAA-3	55	Nweke, 2015
SSR 11	F:5-AGAACAGGATCTTTCCTCCT-3 R:5-CTTAAGGTCGGAGAAGTCAAT-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 12	F:5-CCACTTACATTGCACATACCT-3 R:5-CGTATTGCTGTTGGTCATATT -3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 13	F:5-GAATCGAACCTGATTGAACTA-3 R:5-GCCAGTGATAACCCATAAAGT-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 14	F:5-GTAGACGAGCGAGAAAGAAA-3 R:5-CTTATCGAGCATCCAATGTT -3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 15	F:5-GGCATCAGTCCGTCTTTC -3 R:5- AAAACTAGAGACGAACAGC-3	56	Bhattacharyya <i>et al.</i> , 2014
SSR 16	F:5-TTTTTACTTCCCGTTTCTTTC -3 R:5-GGTACTTGGTCTGGAATTCTT -3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 17	F:5-CATTGATTTTTATTTCGACCTC -3 R:5-CCAATCTGCGATAGTTGC -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 18	F:5-TTTCGTTTACACATTTGACCT -3 R:5-GATAGGCCTTCTTGATTTTCAT -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 19	F:5-CTCATCTACCCACACCATCTA -3 R:5-CACCAATTCTTTTGTGTCTT-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 20	F:5-TTCTGCCATGTTCACTATCTT -3 R:5-GACAAATATTGACGGTAGACG -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 21	F:5-CTGAACAAGACAAGGGAATC-3 R:5-GACAGGGATTCAAGAGACAG-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 22	F:5-ATTTGATACACCAACAGCAAC -3 R:5-TCCTTGCATCATTAGTGTGA -3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 23	F:5-ATCTACAAAAACATCCCCTTC-3 R:5- ACATGAAACCTTTGAGTGATG-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 24	F:5-CACAACAACCATCATCATGTA -3R:5-AGTGAAGTCGGAAGAGAAAAGT	55	Bhattacharyya <i>et al.</i> , 2014
SSR 25	F:5-GCGTTTTTGTATTGTTGAA -3 R:5-GTTTGCCTCTGAACAGTTTT-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 26	F:5- GAGTAGTGCTCATCCTCACAG -3 R:5-GGACGGACAGTAACAGTAAGA-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 27	F:5-CCGCTCTTACTGTTACTGTCC-3 R:5-TGACAAGAGAAGGAGAAGTGA	56	Bhattacharyya <i>et al.</i> , 2014
SSR 28	F:5-CTCCCTCTTCTTCTTCTT-3 R:5- CGAGCCATTCATAGATACAAC-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 29	F:5-TTTTTGTACACTCCCTTTC -3 R:5-GACATAGAGAAAGGTCTCTG-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 30	F:5-AATCAGTGAATTAGCAGCATC-3 R:5-GTTGAGAGTGTGCGAAGAAT -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 31	F:5-CAGATCTGTTTCTATGGGTTT-3 R:5- ATCTCCTTAATTAACCCAGCA-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 32	F:5-TTTTCTCTCTCTCTCTCTC -3 R:5- CCCTCTGTATGTCTTCAGTTG -3	53	Bhattacharyya <i>et al.</i> , 2014
SSR 33	F:5-ACAATCGTAGTCTTCTTCTGA-3 R:5- GCAAAGGTTGTTGTTGTCTC-3	54	Bhattacharyya <i>et al.</i> , 2014

SSR 34	F:5-CTAAGAAAACCGTAGCTCCA-3 R:5-GTAGGTTTCAGATTTTGCCTCT -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 35	F:5-CCTCTCCACTGCTACCTACTA -3 R:5-TCAACAGGAAAAGAGAGAGAA-3	53	Bhattacharyya <i>et al.</i> , 2014
SSR 36	F:5-CTACTGTCAACAGCAACAA -3 R:5- AACCTCAACGGTAGGATTTTC	54	Bhattacharyya <i>et al.</i> , 2014
SSR 37	F:5-GAACCCAGTCATTCTTTATCC-3 R:5- TTTAAAGGGTGGTTATGTGTG-3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 38	F:5-AGTTCAAAGAATCCCTCGAC-3 R:5- GCGAGTCGTCCTCTCAT -3	55	Bhattacharyya <i>et al.</i> , 2014
SSR 39	F:5-TTCCTTCTCACACATTCACC -3 R:5- GATCTGATTCACCTCCTTCTG -3	56	Bhattacharyya <i>et al.</i> , 2014
SSR 40	F:5-CCAACTTAAGACCCAAGAAC-3 R:5- GTCATCAATGGAAGAACTCTG-3	53	Bhattacharyya <i>et al.</i> , 2014
SSR 41	F:5-AGAAGATCAAGGACAAGATCC-3 R:5- GATTCAAGCACACCTGAATTA -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 42	F:5-ATCCCACCATACTTTCTTCTC-3 R:5-CAATTGGGAGTAAATGACAAC-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 43	F:5-ACCTTTGGAATGAGAGGTAAC-3 R:5-GCAGCTGATTGTAAGAGAAGA -3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 44	F:5-GAAGAAGAGGAGGAAGAAGAA-3 R:5-CCTTGTCTAGTCTTGAGTTGG-3	54	Bhattacharyya <i>et al.</i> , 2014
SSR 45	F:5'-GAAGGTCTGCGAGTACTTTG-3' R:5;-CCTCCAAGGAGATACAAATTC -3'	55	Bhattacharyya <i>et al.</i> , 2014
SSR 46	F:5'-GCAAACACATGCATCCCT-3' R:5'-GCCCTGATGATAAAGCCA-3'	58	Pandey <i>et al.</i> , 2015
SSR 47	F:5'-GCAGCAGTTCCGTTCTTG-3' R:5'-AGTGCTGAATTTAGTCTGCATAG-3'	52	Pandey <i>et al.</i> , 2015
SSR 48	F:5'-GCTGAGGAGTCTTGAAGCAGA-3' R:5'-CAAAATCCCCCAACTCGATA-3'	60	Pandey <i>et al.</i> , 2015
SSR 49	F:5'-AAACCCGCTAAGGGACTCAT-3' R:5'-CATGGCTTCTGGCTTTCTTC-3'	60	Pandey <i>et al.</i> , 2015
SSR 50	F:5'-TGCAGGAATGAACTCAAGGA-3' R:5'-ACCTTATCCCAGCCCACTT-3'	60	Pandey <i>et al.</i> , 2015

Table.3 Clustering pattern among sesame genotypes in D² analysis using morphological traits

Cluster no.	Number of genotypes	Name of the genotypes
1	23	PahariaTil_Nagaon, CUHY 57, KolaTil_Majuli, NeiIong_Umium, Uma, AST 1, SaliTil_Kaliabor, AhuTil_Sivsagar, KolaTil_Nalbari, CUMS 17, V12, Bahuabheti, CUMS 04, Koliabor Local, SaliTil_Ghilamara, SaliTil_Nagaon, AhuTil_Lakhimpur, SaliTil_Darrang, SaliTil_Biswanath, Nagaon Local, NempoChindon, V 15, AhuTil_Puranigudam
2	4	NempoLongnicklu, NempoSoksu, NempoThepo, NempoCharap
3	1	KolaTil_Tezpur
4	1	Rama
5	1	AhuTil_Koliabor
6	1	TKG 308
7	1	Nga Na
8	1	Punjab Til 1

Table.4 Intra diagonal and inter cluster D² values for different characters in sesame genotypes

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Cluster 1	9.27	22.18	17.63	16.22	21.26	20.36	37.02	45.49
Cluster 2		3.62	27.24	26.40	38.86	59.11	34.54	20.59
Cluster 3			0.00	32.60	8.28	29.94	18.61	56.54
Cluster 4				0.00	27.89	36.36	34.99	47.51
Cluster 5					0.00	32.97	10.65	67.27
Cluster 6						0.00	69.92	79.59
Cluster 7							0.00	61.58
Cluster 8								0.00

Table.5 Cluster mean for different characters in sesame genotypes

	Height of 1 st capsule bearing node cm	Plant height cm	Capsules/ plant no.	Branches/ plant no.	Capsule length cm	Capsule width cm	Seeds/ capsule no.	1000 grain wt. g	Days to 50% flowering	Days to maturity	Yield/ plant g
Cluster 1	30.48	67.70	25.94	2.55	2.09	0.71	43.56	2.83	36.90	94.26	9.50
Cluster 2	39.10	71.01	26.77	2.95	2.22	0.74	37.48	2.09	44.33**	97.00	9.06
Cluster 3	37.73	85.87**	32.20	2.40	2.06*	0.75	53.83**	2.74	35.33	94.33	6.43
Cluster 4	39.27	70.00	72.40**	3.83 **	2.33	0.65*	46.80	2.92	40.33	93.33	9.63**
Cluster 5	23.07*	55.20*	18.27	2.27	2.17	0.68	51.73	2.83	35.67	99.33	6.07
Cluster 6	30.47	62.33	15.07*	1.63*	2.15	0.65*	46.93	3.40**	29.33*	83.67*	9.30
Cluster 7	32.80	65.63	39.33	3.75	2.13	0.69	36.90*	2.60	39.33	104.33**	5.10*
Cluster 8	54.24**	57.80	15.08	2.93	2.79**	0.87**	46.60	2.04*	42.00	102.00	9.53

** = highest mean, * = lowest mean

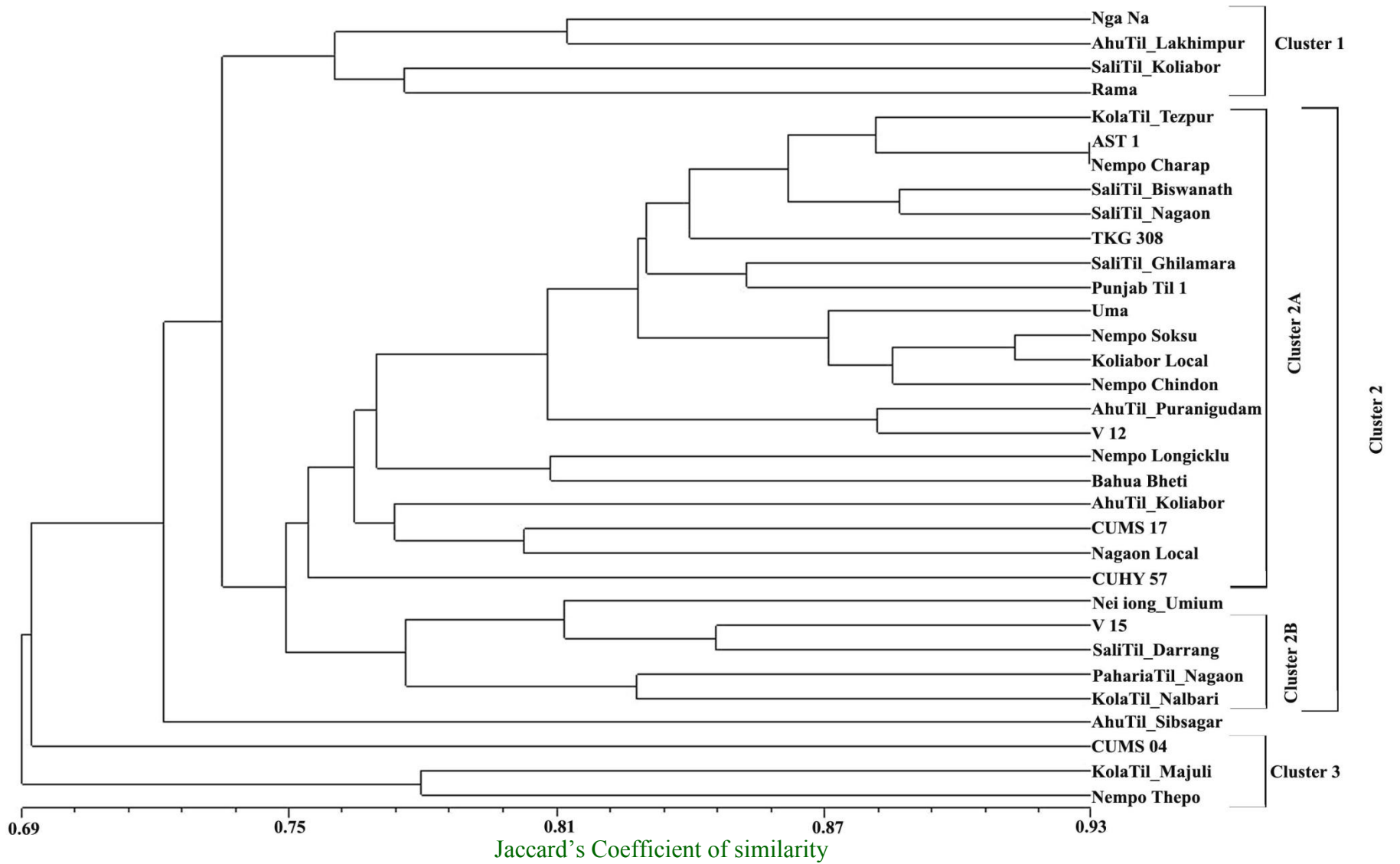
Table.6 Contributions of different characters towards clustering in sesame genotypes

Source	Times ranked 1 st	Contribution %
Height of 1 st capsule bearing nodecm	3	0.57%
Plant height cm	4	0.76%
Capsules/ plant no.	34	6.44%
Branches/ plantno.	2	0.38%
Capsule length cm	34	6.44%
Capsule width cm	11	2.08%
Seeds/ capsuleno.	37	7.01%
1000 grain wt.g	104	19.70%
Days to 50% flowering	108	20.45%
Days to maturity	43	8.14%
Yield/ plantg	148	28.03%

Table.7 Marker polymorphism analysis in sesame genotypes using SSR marker

Marker name	Total band amplified	Number of polymorphic bands	% polymorphism	PIC
SSR 1	3	1	33.33	0.43
SSR 2	2	2	100.00	0.27
SSR 5	2	1	50.00	0.56
SSR 6	4	4	100.00	0.24
SSR 7	3	2	66.67	0.17
SSR 8	3	3	100.00	0.68
SSR 9	2	1	50.00	0.47
SSR 10	3	3	100.00	0.95
SSR 11	3	2	66.67	0.09
SSR 12	3	1	33.33	0.51
SSR 14	4	4	100.00	0.01
SSR 17	2	1	50.00	0.23
SSR 19	4	1	25.00	0.74
SSR 22	3	2	66.67	0.16
SSR 28	3	1	33.33	0.85
SSR 29	3	2	66.67	0.26
SSR 30	5	2	40.00	0.33
SSR 32	2	1	50.00	0.47
SSR 33	2	1	50.00	0.87
SSR 34	4	3	75.00	0.29
SSR 36	2	2	100.00	0.23
SSR 44	3	1	33.33	0.43
SSR 46	3	1	33.33	0.99
SSR 47	3	2	66.67	0.34
SSR 48	3	3	100.00	0.16
SSR 49	2	1	50.00	0.51
SSR 50	2	1	50.00	0.33
Average	2.89	1.81	62.82	0.43

Fig.1 Dendrogram Showing Genetic Relationship among the Sesame Genotypes



Diversity analysis with the use of molecular markers determines the degree of relatedness and help in an accurate grouping of the genotypes to identify parents for exploitation of heterosis and to detect the duplication. Jaccard's similarity coefficients ranged from 0.931 to 0.591 with an average of 0.754.

The maximum similarity between NempoCharap and AST1 (0.931) indicated close correspondence in their DNA based on present marker analysis. The minimum similarity was exhibited between AST 1 and KolaTil_Majuli (0.591) indicated diverse nature of those genotypes. Such diverse nature is obvious as AST 1 is an HYV developed by AAU, whereas KolaTil_Majuli is a local genotype collected from largest river island Majuli. Genetic relationships based on UPGMA cluster analysis identified three major clusters (Fig: 1). The cluster analysis revealed that KolaTil_Majuli and NempoThepo (Cluster 3) showed maximum divergence from the rest of the genotypes followed by CUMS 04 and AhuTil_Sibsagar. For the rest of the genotypes, two clusters were observed. Cluster 1 comprised of four genotypes and cluster 2 included 25 genotypes divided into two sub-clusters at a similarity coefficient of 0.75.

High polymorphic and genetic diversity will be useful for the breeder to incorporate them in a breeding programme. The present study indicated sufficient genetic diversity at DNA level and no congruence between clustering pattern done by D^2 and that based on DNA was recorded. Most of the materials use in the present study were from different location of North East India which form different groups at morphological and DNA level. Such clustering pattern indicated that there is greater diversity among the sesame genotype grown in the location and these germplasms will be the source of valuable gene system for the sesame breeder.

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