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Molecular Diversity Assessment in Selected Accessions of White Seeded Sesame (*Sesamum indicum* L.) using SSR Markers

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Fifty sesame accessions with 10 simple sequence repeat (SSR) markers were used for their molecular characterization and assessment of genetic diversity. It was observed through this study that the accessions have enough genetic variability at molecular levels. Thirty five alleles with mean polymorphism information content of 0.42 resulted from the molecular studies very explicitly indicate the superiority of SSR primers in assessment of genetic diversity. These primer bands size varied from 200 to 400 bp. The number of alleles per locus in selected accessions varied from 3 to 6 and heterozygosity per primer ranged from 0.00 to 0.40. The pair wise genetic similarity varied from 0.44 to 0.86. A closure view of dendrogram identified two major clusters, indicating high genetic resemblance among sesame accessions. Hence, under the study here, diversity assessment through SSR markers was proved to be stronger tools for discriminating *Sesamum indicum* accessions.

Keywords: Genetic diversity, Genetic similarity, Germplasm, Heterozygosity, Polymorphism

Introduction

The projected climate change scenarios indicates that the drier area and the number of hot days will be increasing which would put unprecedented abiotic stress for production of many crops in India in general and central India in particular. The rural livelihood of farmers in the area of Bundelkhand of central India is primarily dependent on crop production. This necessitates the farmers to select a crop suitable for production in drier and hotter environment for sustainable livelihood. Under this perspective, sesame (*Sesamum indicum* L.), a traditional (dates back to the 1500 B.C)[1] oilseed crop would be a suitable and sustainable choice for adoption. India leads in sesame production and holds third global rank after Tanzania and Myanmar.[2] The average sesame yield in India (3.30 q/ha) is far behind the global mean yield (5.11 q/ha).[2] It's a matter of concern that despite genetic and cultural advancement, the average yield of sesame has been stagnating during the past few years on global scale.

Sesame, being a self pollinated crop (although with limited out crossing from 5 to 50 per cent), prompts the breeding plan to be primarily focused on hybridization approach for its improvement. This approach has been using few selected genotypes as parental lines during the past few decades which narrowed down its genetic base. Although, a number of high yielding varieties have been released for cultivation in India, however, for having a sustainable yield under the projected climate scenarios, the untapped genetic diversity of sesame need to be utilized for broadening the genetic base. Estimates of genetic diversity and relationships among germplasm accessions are the very first step towards selection of elite germplasm and their management. Many advanced tools such as isozymes, and molecular markers are now available at ease for studying variability and relationships among accessions.[3,4]

Diversity analysis through molecular studies can have significant applications in discriminating genotypes within and between populations using informative markers or combinations of markers. Though the hybridization program alone develops many cultivars for maximizing production, but the program synergised with molecular characterization using DNA markers could be more stable. In view of above, the present study was carried out to assess the genetic diversity among the fifty elite accessions of white seeded sesame germplasm through SSR markers so as to have the information on the trait

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variability, which would be utilized in mitigating the climate change impact on sesame production.

Materials and Methods

Plant Materials

The sesame (*Sesamum indicum* L.) germplasm accessions were collected from the Research unit of College of Agriculture, Tikamgarh, under the All India Coordinated Research Project on Sesame and Niger (AICRP), JNKVV Campus, Jabalpur, Madhya Pradesh, India. The accessions consisted of collections of elite lines of white seeded type's sesame from the diverse regions of India.

Genomic DNA Extraction

Sowing of sesame accessions` seeds in sterilized soil in small plastic pots was accomplished after sterilizing the seeds with 0.1 per cent mercuric chloride. Pots were kept in growth chamber in light for 12 hours at 25 to 27°C. Plantlets were allowed to grow up to 2−3 leave stage (from 15 to 20 days old) in growth chambers. DNA of fresh leaves were isolated by following standard procedure. The procedure involved the treatment with the enzymes to denature the leaf sample at room temperature for 40 minutes. The fixing solutions comprised of alcohol (60 per cent, 70 per cent and 80 per cent), chloroform and alcohol: EDTA in varying ratios (60:40, 70:30 and 80:20). Other compounds were also used *e.g.* polyvinylpyrrolidone (PVP) (1 per cent, 2 and 4 per cent), β-mercaptoethanol (1 per cent and 2 per cent), CTAB (2 per cent and 5 per cent) and SDS (2 per cent and 20 per cent). Incubation time was also varied from 10 min to 60 min at 60ºC with centrifugation speed from 12000 rpm to 10000 rpm. Extraction was carried out with chloroform: isoamylalcohol (24:1) while supernatant was decanted in another tube. The precipitated DNA was washed thrice with 70 per cent ethanol. At last, pellet was air-dried and dissolved in TE buffer followed by RNase treatment at 37°C.

Quantification of Genomic DNA

Spectrophotometer was used to quantify the DNA at λ260 nm and λ280 nm along with DNA purity by calculating the absorbance ratio (λ260/λ280). The gel electrophoresis was also carried out using 8 per cent agarose gel to quantify DNA. Tris base, boric acid, EDTA (TBE) buffer was made and stored as 5X stock solution (54 g Tris base, 27.5 g boric acid and 20 ml 0f 0.5 M EDTA dissolved in 1 liter distilled water)[5] followed by heating at 90 to 95°C in microwave oven. The gel was cooled down to 60°C followed by addition of ethidium bromide (final concentration of 0.5 µg/ml) for visualization of bands under UV light. The tray was mounted in electrophoresis buffer tank containing 0.5 X TBE buffer. After that 5 µl DNA was mixed with 2 µl of 6X loading buffer (0.25 per cent bromophenolblue, 0.25 per cent xylene cyanol and 30 per cent (w/v) glycerol in distilled water) and loaded into the well made by comb.[5] After running gel to a sufficient distance as indicated by dye, λ DNA EcoRI/Hind III double digested was loaded for comparison.

SSR (Microsatellite) Markers and PCR Amplification

Polymorphism in the sesame genotypes were noticed using SSR and shown in Table1. These selected SSR primers used for detection are highly polymorphic and thoroughly distributed in the sesame genome.[6] PCR reactions were carried out in a Thermal cycler in a 10 µl reaction mixture in 96-well plates. PCR kits was used for the amplification. The kits composed of 2X PCR master mix containing DNA Polymerase (0.2 U per 10 µl reaction), PCR buffer, dNTPs (0.2 mM each at 1X), $MgCl_2$ (1.5 mM at 1X), stabilizers and loading dye. 1 µl genomic DNA and 0.5 µl each of forward and reverse primers were added to the PCR kits for DNA amplification. PCR was run as per standard protocol.

Gel Electrophoresis

All SSR primers used here in the study were tested for amplification using check entries of sesame. The PCR products were separated on 1.5 per cent agarose gel and denaturing polyacrylamide gel. The 10 labelled primers with 4 different fluorescent dyes (6-FAM, NED, VIC, PET) were used to measure the size of amplified fragments. In order to determine the peaks` size, the Peak scanner software v1.0 was used.

Statistical Analysis

Jaccord's coefficient of pair wise comparison of genotypes (based on the presence (1) or absence (0) of unique and shared polymorphic products) was estimated using NT-SYS-pc version 2.1 software.[7] Loci with more than one allele were considered polymorphic. The unweighted pair group method along with arithmetic mean (UPGMA)[8] was estimated and used for construction of a dendrogram. The PIC value for self-pollinated species suggested by

Botstein *et al.*[9] and modified by Anderson *et al.*[10] was estimated by the formula as given below:

$$PIC_i = 1 - \sum_{j=i}^{n} p_{ij}^2$$

Table 1 —Details of 50 sesame accessions used for molecular characterization and genetic diversity analysis

S.#	Accessions	Name under study
1	KMR-60	TKGSE-11-1
2	KMR-31	TKGSE-11-2
3	SI-233	TKGSE-11-3
4	KMR-51	TKGSE-11-4
5	SI-3263-1	TKGSE-11-5
6	KMR-61	TKGSE-11-6
7	KMR-394	TKGSE-11-7
8	SI-107-B	TKGSE-11-8
9	IS-405	TKGSE-11-9
10	IS-8	TKGSE-11-10
11	NIC-10622	TKGSE-11-11
12	IS-35-1-A	TKGSE-11-12
13	EC-303311-1	TKGSE-11-13
14	SI-2039-A	TKGSE-11-14
15	SI-8459	TKGSE-11-15
16	SI-1782A	TKGSE-11-16
17	EC-52000145	TKGSE-11-17
18	S-01159-B	TKGSE-11-18
19	KMR-39	TKGSE-11-19
20	KIS-300-A	TKGSE-11-20
21	IS-390	TKGSE-11-21
22	SI-3257	TKGSE-11-22
23	KMR-89	TKGSE-11-23
24	NIC-17912-B	TKGSE-11-24
25	NIC-8588	TKGSE-11-25
26	NIC-8584	TKGSE-11-26
27	NIC-10630	TKGSE-11-27
28	KMS-4-258	TKGSE-11-28
29	(JTS-8 x SI-225-3)-2-2-1	TKGSE-11-29
30	(VKS-272 x SI-250)-2-2-1	TKGSE-11-30
31	(TC-25 x KMR-115)-6	TKGSE-11-31
32	VKS-272 x SI-1446)-5-1-1	TKGSE-11-32
33	(GT-1 x TKG-22)-1-2-2	TKGSE-11-33
34	(TKG-307 x N-32)-1	TKGSE-11-34
35	(GT-1 x TKG-22)-1-4-2	TKGSE-11-35
36	(GT-1 x TKG-22)-4-1-2	TKGSE-11-36
37	(JTS-8 x SI-225-3)-2-1-2	TKGSE-11-37
38	(RT-46 x N-32)-1-2	TKGSE-11-38
39	(SI-911 x N-32)1-1	TKGSE-11-39
40	(SI-1556 x SI-250)-6-2	TKGSE-11-40
41	(SI-928 x N-32)-3-1-1	TKGSE-11-41
42	(SI-928 x N-32)-3-1-2	TKGSE-11-42
43	(SI-1556 x TKG-308)-1	TKGSE-11-43
44	(RT-46 x TKG-306)-1-1	TKGSE-11-44
45	(RT-46 x TKG-306)-2-2	TKGSE-11-45
46	(AT-66 x TC-25)-1-2	TKGSE-11-46
47	GT-2	TKGSE-11-47
48	JTS-8	TKGSE-11-48
49	Phule Til-1	TKGSE-11-49
50	TKG-55	TKGSE-11-50

where, p_i equals the frequency of the i^{th} allele and p_j the frequency of the allele. Only data from polymorphic loci were used for this analysis. The above mentioned methods are used for estimating the results. Primers, where no result was obtained was shown as (-) symbol.

Results and Discussion

Molecular Characterization of Sesame Accessions

These 10 SSR primers used for this study are shown in Table 2. From the table values, it is inferred that they are highly polymorphic and thoroughly distributed and also in conformity with the findings of other reported sesame genomes.[6] Eight SSR primers produced clear bands for determination of genetic variability while the other two primers were monomorphic in nature. The level of polymorphism among accessions was evaluated by calculating allelic number, and PIC values for each of eight loci evaluated. The results clearly expressed that the primers showed distinct polymorphism among the accessions and thus indicating the robust nature of microsatellite in expressing polymorphism. The banding patterns of PCR products for GBssr-sa-173 and GBssr-sa-33 are shown in Figs 1 and 2. The figures show that these primers producing bands varied considerably in size from 200 to 400 bp and the observed number of alleles per locus ranged from 3 to 6. It was also found that there is a high variability between observed and expected heterozygosity per primer and ranged from 0.00 to 0.40 0.437 to 0.858 respectively, thus indicating a high degree of variation.

The molecular information obtained from PCR amplification of genomic DNA along with allele frequency and gene diversity, heterozygosity and PIC values are presented in Table3. Primers Sesame 09 and GBssr-sa-108PCR products were not used in the analysis as they were monomorphic. Primers mean value of alleles per locus was found to be 4.38, while the number of alleles ranged from 3 to 6 per locus. There was a high frequency of allele observed which ranged from 0.30 to 0.88 with a mean of 0.68. The locus GBssr-sa-05 revealed the highest PIC value of 0.74 and gene diversity value of 0.77. GBssr-sa-33 had the highest heterozygosity of 0.45 followed by GBssr-sa-182 (0.41) with the least heterozygosity of 0.07 in GBssr-sa-123.

The results obtained in this study indicate high genetic diversity (80 per cent) among the accessions.

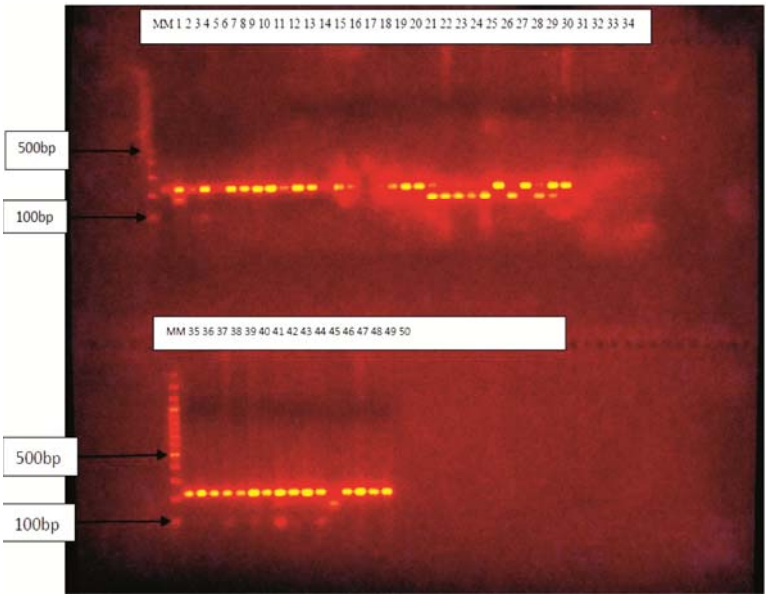

Fig. 1 — Banding pattern of PCR amplified products of GBssr-sa-173 among the 50 sesame accessions

Lane MM 100 bp ladder, 1: TKGSE-11-1, 2:TKGSE-11-2, 3:TKGSE-11-3, 4:TKGSE-11-4, 5:TKGSE-11-5, 6:TKGSE-11-6, 7:TKGSE-11-9, 8:TKGSE-11-10, 9:TKGSE-11-11, 10:TKGSE-11-12, 11:TKGSE-11-13, 12:TKGSE-11-14, 13:TKGSE-11-15, 14:TKGSE-11-16, 15:TKGSE-11-17, 16:TKGSE-11-18, 17:TKGSE-11-19, 18:TKGSE-11-20, 19:TKGSE-11-21, 20:TKGSE-11-22, 21:TKGSE-11-23, 22:TKGSE-11-24, 23:TKGSE-11-25, 24:TKGSE-11-26, 25:TKGSE-11-27, 26:TKGSE-11-30, 27:TKGSE-11-31, 28:TKGSE-11-32, 29:TKGSE-11-34, 30:TKGSE-11-35, 31:TKGSE-11-36, 32:TKGSE-11-40, 33:TKGSE-11-41, 34:TKGSE-11-42, 35:TKGSE-11-43, 36:TKGSE-11-44, 37:TKGSE-11-46, 38:TKGSE-11-47, 39:TKGSE-11-48, 40:TKGSE-11-49, 41:TKGSE-11-50, 42:TKGSE-11-51, 43:TKGSE-11-52, 44:TKGSE-11-54, 45:TKGSE-11-55, 46:TKGSE-11-56,47:GT-2, 48:JTS-8, 49:Phule Til-1 and 50:TKG-55

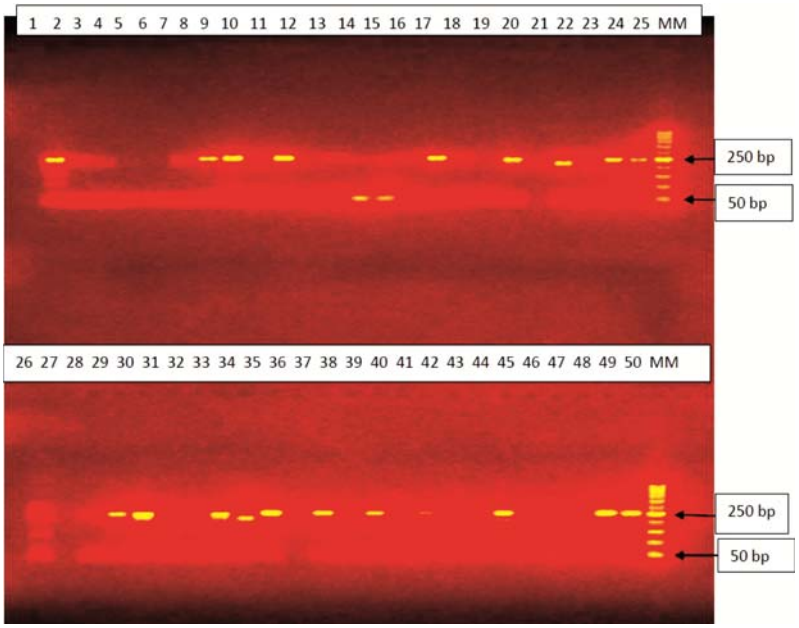

Fig. 2 — Banding pattern of PCR amplified products of GBssr-sa-33 among the 50 sesame accessions

Lane MM 100 bp ladder, 1: TKGSE-11-1, 2:TKGSE-11-2, 3:TKGSE-11-3, 4:TKGSE-11-4, 5:TKGSE-11-5, 6:TKGSE-11-6, 7:TKGSE-11-9, 8:TKGSE-11-10, 9:TKGSE-11-11, 10:TKGSE-11-12, 11:TKGSE-11-13, 12:TKGSE-11-14, 13:TKGSE-11-15, 14:TKGSE-11-16, 15:TKGSE-11-17, 16:TKGSE-11-18, 17:TKGSE-11-19, 18:TKGSE-11-20, 19:TKGSE-11-21, 20:TKGSE-11-22, 21:TKGSE-11-23, 22:TKGSE-11-24, 23:TKGSE-11-25, 24:TKGSE-11-26, 25:TKGSE-11-27, 26:TKGSE-11-30, 27:TKGSE-11-31, 28:TKGSE-11-32, 29:TKGSE-11-34, 30:TKGSE-11-35, 31:TKGSE-11-36, 32:TKGSE-11-40, 33:TKGSE-11-41, 34:TKGSE-11-42, 35:TKGSE-11-43, 36:TKGSE-11-44, 37:TKGSE-11-46, 38:TKGSE-11-47, 39:TKGSE-11-48, 40:TKGSE-11-49, 41:TKGSE-11-50, 42:TKGSE-11-51, 43:TKGSE-11-52, 44:TKGSE-11-54, 45:TKGSE-11-55, 46:TKGSE-11-56,47:GT-2, 48:JTS-8, 49:Phule Til-1 and 50:TKG-55

Table 2 — SSR primers and their sequences

Primer	Sequence (5'-3')	motifs	*Ta* (°*C*)	Expected Size bp	Observed Size bp
GBssr-sa-05	F-TGCCACATAGGTTGGCTTTC R-CAAAGCCAATGCACATAATCA	(gt)13	60	233	245
GBssr-sa-08	F-AAAAACATGCACCAGTCCTT R-CAACCGCCTGAATTTTCTCT	(ac)11	61	157	153
Sesame09	F-TTCCCTTTCCCAACATGGTA R-ACACCCGAAGATGGGTTAGA	(tg)10	61	154	150
GBssr-sa-33	F-GAGGGCTAAGCAAAGCAGAA R-TGCATGTTTCCCTTAGTTTCC	(ta)5(tg)13	60	241	239
GBssr-sa-72	F-GTCGGCCGTCATTCACTATT R-AGCTTGATGCACCTGGTCTT	(ag)12	61	240	244
GBssr-sa-108	F-TAAAAATCGCGGAAAGTTGC R-GTCGTTCTATGCGGCATTTT	(ga)8	60	184	182
GBssr-sa-123	F-TTTTCCCTTTCCAGTTGTTCA R-ATGGGTGAAGTGTGAAAAGAA	(tc)5	60	156	164
GBssr-sa-173	F-TCGATGACGAAAAAGTGTGAA R-AGGGCAAAAGACCCATTCTT	(ag)6	60	225	223
GBssr-sa-182	F-TCCTCCTCCTTCTCGTGTTC R-AAAGAAGCAGCAGGTGAAGA	(ctt)5	60	225	228
GBssr-sa-184	F-ACAAAGCATCGGCTGAAAAT R-GCGACCAAGTACCAACAGGT	(gaa)14	61	—	150

Ta: annealing temperature, *Na*: Number of alleles, *He*: expected heterozygosity, *Ho*: observed heterozygosity

Table 3 — Allele frequency, allele number, gene diversity, heterozygosity and Polymorphism Information Content (PIC) values generated from molecular data

Primer	Allele frequency	Allele number	Gene diversity	Heterozygosity	PIC
GBssr-sa-05	0.30	6	0.77	0.39	0.74
GBssr-sa-08	0.88	3	0.22	0.17	0.20
GBssr-sa-33	0.65	5	0.52	0.45	0.48
GBssr-sa-72	0.75	4	0.41	0.17	0.38
GBssr-sa-123	0.87	4	0.24	0.07	0.23
GBssr-sa-173	0.79	3	0.33	0.23	0.29
GBssr-sa-182	0.38	7	0.76	0.41	0.73
GBssr-sa-184	0.83	3	0.29	0.17	0.27
Mean	0.68	4.38	0.44	0.26	0.42

The number of alleles varied from 3 to7 with a mean value of 4.38 alleles per locus; which is also supported by other studies.[11] They observed 2 to 6 alleles with mean value of 2.7 alleles per locus. As an indication of the polymorphic nature of the primers, 35 alleles were observed. Expected heterozygosity ranged from 0.07 to 0.45 with an average of 0.26, which confirms the very instructive nature of the SSR markers. These markers will be markedly helpful in diversity assessment of a large germplasm collection of sesame present in our gene bank and also for the establishment of core collection of germplasm.

Above reported results are also supported by other studies[12] where fifty SSRs sequences were isolated from an enriched library of sesame. In this study, they reported that allele numbers ranged from 3 to 6 alleles per locus with a mean of 4.6 alleles. The variability in expected heterozygosites and PIC values were also reported by them; which varied from 0.437 to 0.858 and 0.34 to 0.80, respectively. The other investigators[13,14] used sixteen SSR markers, developed from genomic DNA library of sesame to assess genetic diversity and phylogenetic relationships of 150 sesame accessions from 22 countries and reported the allele number and PIC values. They reported the number of alleles varied from 2 to 18, with a mean value of 7.6 alleles per locus. The PIC values ranged from 0.03 to 0.79, with a mean of 0.42. They reported accession distribution pattern with mean genetic similarity coefficient of 0.45 by accession and 0.52 by population. These findings are in conformity with our reported results and thus all the sesame accessions represent a high genetic variability. In another study,[15] the genetic diversity of 41 sesame varieties was assessed with 68 SSR markers and reported PIC value 0.41; which is indicative of a moderate level of

genetic diversity. The high genetic variability in horticultural and pulse crops such as cassava,[16] sweetpotato,[17] apple[18] and *Cajanus cajan*[19] was also reported by other workers

Genetic dissimilarity evaluated by the generated dendrogram (Fig. 3) varied from 0.26 to 0.90 clearly indicates high variability and justifies the use of SSR in revealing genetic divergence as compared to phenotypic traits. SSR primers effectively discriminates the genotypic and phenotypic variability.[16] These reported findings are in similarity with that of our results. In addition to assessment of the genetic variability, the genetic similarity was also estimated through cluster analysis. The sesame

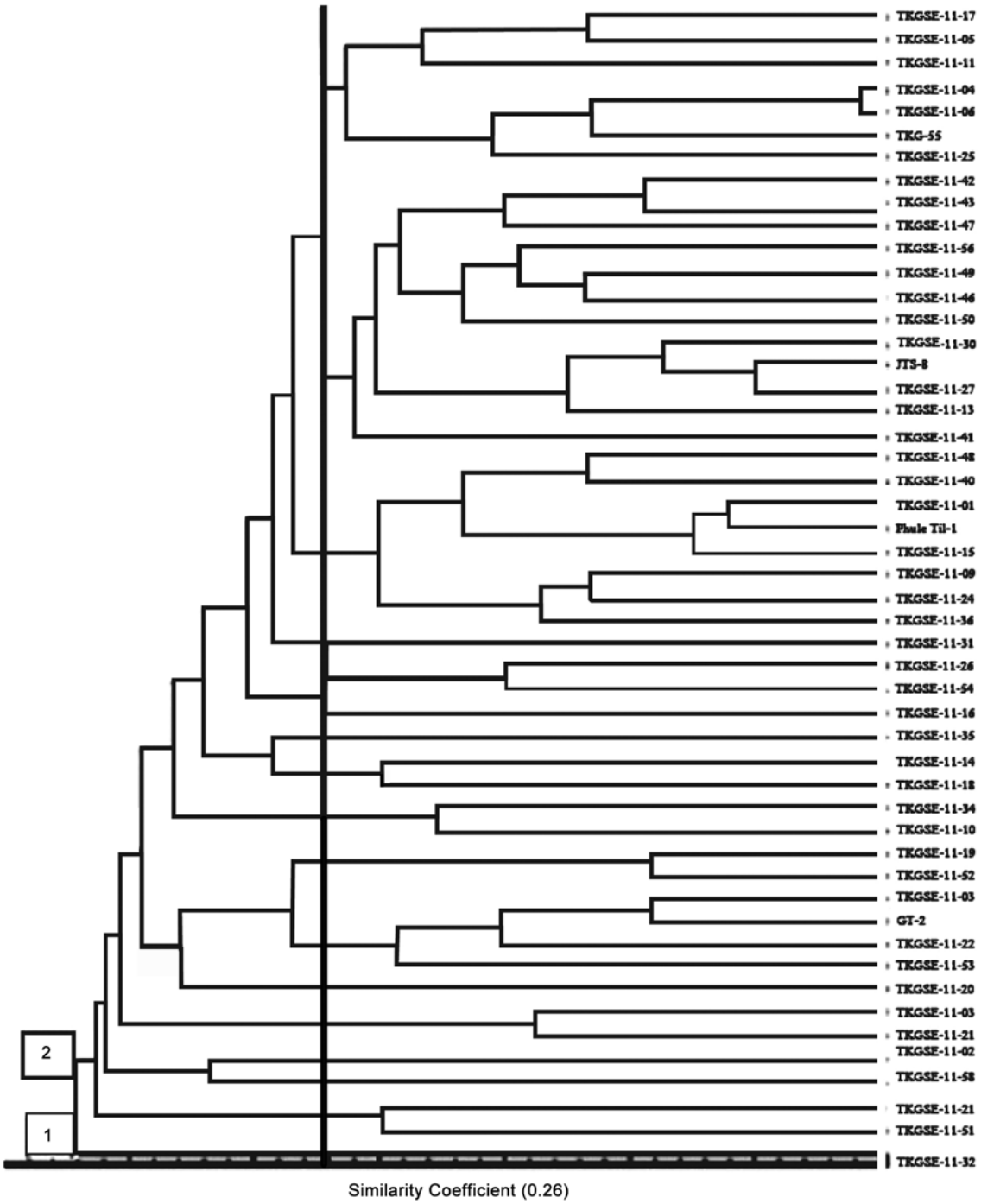

Fig. 3 — Genetic relationships among 50 sesame germplasm accessions based on similarity matrix using unweighted pair group method with arithmetic mean (UPGMA)

accessions were grouped mainly into two main clusters to evaluate the genetic similarity. The genetic similarities of the two clusters were found to be 0.26 and 0.46 respectively; which represents a wide genetic variability among the accessions.

Although, the dendrogram revealed a wide variations among the genotypes the same is not consistent with the groupings created by morphological descriptors based dendrogram. Several accessions, which were morphologically grouped as close relatives, were notably different accessions. For example TKGSE-11-21 and TKGSE-11-31 were grouped into different clusters morphologically were identified by the molecular markers as possible duplicates. Similarly, TKGSE-11-30 and TKGSE-11-27 were identical by the SSR primers but placed in different clusters based on morphological descriptors. On the other hand, genotypes TKGSE-11-46 and TKGSE-11-49 were morphologically similar but were regarded as genotypically distant by the molecular analysis.

Conclusions

The SSR primer based information indicates high genetic resemblance among several genotypes which differed morphologically. This indicates that SSR markers (which are free from environmental upshots), are the stronger and reliable tools for discriminating sesame accessions. SSR marker based information of genetic divergence may be preferred in selecting the genetic variation of diverse sesame accessions. Thus it is concluded that SSR based information may be used for future breeding strategies and germplasm conservation programs for identifying traits for abiotic tolerance to have a sustainable sesame production under climate change scenarios.

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