



Assessment of Genetic diversity of Sesame accessions obtained from National Gene Bank using Simple Sequence Repeat

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Abstract

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops widely grown for its economic and nutritional importance. Knowledge of the genetic diversity of gene bank accessions is essential for crop improvement and a key factor for effective conservation and breeding strategies. The purpose of this study is to assess the genetic diversity among the sesame accessions in the gene bank to promote their utilization. Seventy-six accessions of sesame collected from the National gene bank were characterised using eight simple sequence repeat markers and data was analysed using Power marker and DARwin version 6. The Polymorphic Information content (PIC) ranged from 0.0.12 to 0.37 with an average of 0.30 while the Major allele frequency (MAF) ranged from 0.55-0.93 and the average was 0.71. The genetic diversity ranged from 0.12- 0.49 with an average value of 0.37 whereas the unweighted pair group method with arithmetic mean (UPGMA) revealed three main clusters with the largest cluster having five sub-clusters. The SSR markers used in this study were able to show the genetic variation that existed among the sesame accessions in the gene bank and this will promote their utilization for crop improvement.

Keywords: Accession, Gene bank, Genetic diversity, Sesame, Simple sequence repeat,

Introduction

Sesamum indicum L. (Sesame) belongs to the family Pedaliaceae and is one of the oldest and most important oil-seed crops valued for its high quality. The genus *Sesamum* has many species but *S. indicum* is the most widely grown species across the tropical and subtropical areas of Asia, Africa and South America –. The origin of cultivated sesame and the process underlying its domestication has been controversial. The African subcontinent is believed to be the primary centre of origin while the Asia subcontinent is thought to be the secondary centre of origin. Sesame, a diploid species with $2n = 26$ belongs to the Eudicotyledon clade and Lamiales order. Sesame seed has the highest oil contents (~55%) compared to other main oil crop like soya bean (~18%), oilseed rape (~41%), sunflower (~40–44%) and peanut (~51%). Sesame is a good source of protein (~ 20-30%) which is high in tryptophan and methionine, carbohydrates (~13.5%), unsaturated fatty acids (mainly Oleic acid and Linoleic acid) and minerals¹. Sesame annual production is reported to be around 3.84 million tons with Myanmar (890,000 tons), India (636,000 tons) and China (588,000 tons) being the world's leading producers followed by Tanzania, Ethiopia, Uganda and Nigeria respectively –

The occurrence of genetic diversity in crops is the core to the survival of crops in nature and plays an integral part in food security and sustainable development; as it reveals the relatedness and genetic differences that exist among crops. These intra and inter-specific differences are vital

information employed in the selection of breeding lines in crop improvement programs. The adverse climatic conditions are a threat to genetic diversity existing in crops, hence the need for the establishment of National gene banks for conservation of the genetic resources for future utilization. Conservation of genetic resources is the bedrock for research on crop improvement and breeding strategies and the various national gene banks are entrusted with the responsibility of accessing in detail the genetic diversity of the accessions of cultivated sesame by collection of germplasm for proper documentation and efficient utilization in strategy breeding, for present and future. The diversity existing among the genotypes of sesame in Africa remained unexplored.

Molecular markers are crucial to plant breeding in many aspects as the identification of genes that confer desirable traits and quantifying variation within arrays of germplasm. Different molecular markers have been used to understand the genetic diversity of sesame accessions. Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-simple Sequence Repeats (ISSR), Sequence-Related Amplified Polymorphism (SRAP), Expressed Sequence Tags-SSR (EST-SSR), Simple Sequence Repeats (SSR) or a blend of molecular markers for a better understanding. SSR markers have the advantages of high reproducibility and abundance, widespread genome coverage and co-dominance, therefore are broadly applied in genetic diversity studies.

Informative molecular markers, ultra-dense genetic maps,

transcriptome assemblies, and multi-omics online platforms are part of the landmark progress made in the development of large-scale genomic resources. The draft genome of sesame released in 2013 has led to functional analysis of genes of interest related to key agronomic traits. The success recorded in the sesame studies holds some important genomic resources and platforms for its improvement which for the time being are nonexistent in some important oilseed crops. The Illumina Hiseq2000 platform generated 54.5 Gb of high-quality data from the elite cultivar 'Zhongzhi No.13' of the sesame nuclear genome. This has been the major breakthrough in sesame research for decades. A genome size of 293.7 Mb out of the 354 Mb was assembled from the variety 'Yuzhi 11' and calculated the function of 23,713 genes. And, sesame research has also documented the release of two new genome sequences from sesame landraces ('Baizhima' and 'Mishuozhima') .

Sesame research has witnessed three different periods: the germplasm collection era, the classical breeding and genetics era and presently the Omics era. Advancement in the Omic era has projected sesame research to a higher level of seed improvement . The completion and availability of the full nuclear genome sequence gives unlimited access to reference genome information for the study of genetic traits and comparative genomics . The whole sesame genome was sequenced with the help of high-throughput next-generation sequencing and informative SSRs were identified to be distributed throughout the whole genome sequence . An important sesame trait is the seed coat colour which varies from white to black with black dominant over white. Information on the genetic diversity of sesame using molecular markers is limited compared to other crop species. Three sites were identified to be pleiotropic for sesame seed coat and these are pseudo-chromosome 4, 8 and 11. The QTLs of the colour spaces L^* , a^* and b^* contributed 3-46% phenotypic variation in sesame . Thus, in this study, SSR markers were designed to target these regions on the chromosome. Therefore, we aimed to determine the genetic diversity among the accessions of sesame conserved in the National Centre for Genetic Resources and Biotechnology to provide genetic information that will promote their utilization in the breeding programme.

MATERIALS AND METHODS

A total of seventy-six (76) accessions of sesame (Table 1) conserved in the medium-term gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB) were collected for molecular characterisation using eight (8) simple sequence repeat (SSR) markers. DNA extraction was done using a modified CTAB protocol (Doyle and Doyle, 1990). 0.2 g of sesame seed was weighed and crushed in preheated mortar and 800 μ L of 10x CTAB extraction buffer (2 % CTAB, 100 Mm Tris-HCl, 20 Mm EDTA, 1.4M NaCl, 1 % w/v PVP and 0.1 % β mercaptoethanol) preheated at 65 °C was added. The

slurry was dispersed into 1.5 ml Eppendorf tube and incubated at 65 °C for 20 minutes. Afterwards, an equal volume of SEVAGE (Chloroform: iso-amylalcohol at a ratio of 24:1) was added and the tube was inverted gently. The mixture was homogenised on the orbital shaker for 1 hour at 450 rpm and then centrifuged at 10,000 rpm for 7 minutes. The supernatant was dispensed in a freshly labelled tube and ice-cold isopropanol and left overnight in a -40 °C freezer. The precipitated samples were washed thrice in 70 % ethanol and allowed to dry at room temperature. The DNA pellets were eluted using 100 μ L sterile water. The DNA was quantified using a Nanodrop Spectrophotometer and quality was checked using 1 % w/v agarose gel electrophoresis.

The amplification reaction was carried out in a 21 μ L total reaction mixture containing 1 μ L DNA template, 1 μ L each for both forward and reverse primers (Inqaba Biotec), 10 μ L mastermix (New England BioLabs® Inc.) and makeup with 8 μ L water. The amplification condition consisted of initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 20 seconds, annealing was for 20 seconds with temperature dependant on the particular primer pair where the least temperature was obtained for primer ODS1 (60 °C) and the highest primer ODS4 (64 °C), extension at 72 °C for 20 seconds repeated for 35 cycles and final extension at 72 °C for 7 minutes. The polymerase chain reaction was done using Eppendorf 96 well Thermal cycler nexus gradient. The amplicons were separated on 1 % w/v agarose gel using 1 x TBE (Tris-Borate- EDTA) and Safeview™ Classic stain (Canada) along with 1 Kb ladder (Thermo scientific).

The data was scored, 1 for the presence and 0 for the absence of an amplified DNA band for each locus across all the samples for each of the primers used, thus generating the 0 and 1 matrix. For the SSR primers (see Table 2) used, each peak is considered as an allele at a co-dominant locus and the genotype of each sample is recorded and used for various genetic diversity analyses that include PIC, MAF and genetic diversity values by means of the computer software Power marker version 3.25. The dendrogram was constructed based on an unweighted pair group mean with an arithmetical average (UPGMA) clustering method via MEGA 7.

RESULTS AND DISCUSSION

Assessment of genetic diversity is very important among gene bank accessions, especially for application in crop improvement. Molecular markers have proven to be efficient in studying diversity among and within species for they are not influenced by the environment or by the stage of development of the crop.

The result of the DNA yield ranged from 93.1-709 ng/ μ L while the A260/A280 which gives the purity of the DNA is 1.36-2.60. The quality and quantity of the DNA indicated that the genomic DNA of sesame accessions could be used for downstream analysis.

Our analysis was based on the use of eight SSR primers

and the dendrogram obtained revealed the extent of genetic variation. The hierarchical cluster analysis was performed using UPGMA, and the graphical representation of the dendrogram was generated using Power marker software (V3.25). The dendrogram showed the phylogenetic relationship among the accessions of sesame in the gene bank. The Polymorphic Information content (PIC) ranged from 0.12- 0.37 with an average value of 0.30 (Table 2). The PIC value revealed in this study is lower than what Nwalo (2015) reported among 30 accessions of Nigeria sesame (0.34-0.80) but similar to that obtained by using thirty-two genic-SSR primers in 24 cultivated accessions. Major allele frequency (MAF) ranged from 0.55 - 0.93 and the average was 0.7, this is higher than that earlier reported.

The UPGMA - generated dendrogram grouped the accessions into three main clusters and five sub-clusters (Figure 1). The largest cluster has thirty-six sesame accessions while the smallest cluster has just two sesame accessions.) also reported three main clusters among common Chinese varieties using 218 SSRs. In contrast to this, Woldesenbet et al. (2015) reported that 120 accessions of Ethiopian sesame gave two major clusters and three outliers using ISSR. They however suggested that few polymorphic markers might not show distinction among samples. The average number of alleles produced by the SSR markers was 2.00. The genetic diversity ranged from 0.12- 0.49 with an average value of 0.37 (Table 2). This is similar to the findings of -) who obtained the range of 0.0-0.48 and an average value of 0.2 for 78 landraces and

15 cultivars of sesame. Genetic associations among individual genotypes were determined based on a dissimilarity matrix using DARwin version 6.0. A range (0.1 - 1) of dissimilarity value was observed among the accessions.

A detailed review of the various investigations on sesame genetic diversity revealed that different methods give different results regarding the level of genetic diversity, depending upon marker type, composition, and size of germplasm. The differences in genetic diversity reported by employing SSRs are not only due to the aforementioned factors but could also be due to the type of SSRs used. The SSR primers used in this study were able to show the genetic variation that existed among the sesame accessions in the gene bank and this will promote their utilization for crop improvement.

Table 1: List of the 76 accessions

SN	ACCESSION NO	CODE	SN	ACCESSION NO	CODE
1	NGB00444	Sample1	39	NGB00387	Sample 43
2	NGB00421	Sample 2	40	NGB00404	Sample 44
3	NGB00400	Sample 3	41	NGB00389	Sample 45
4	NGB00402	Sample 4	42	NGB00398	Sample 46
5	NGB00368	Sample 5	43	NGB00418	Sample 47
6	NGB00406	Sample 6	44	NGB00399	Sample 48
7	NGB00420	Sample 7	45	NGB00410	Sample 49
8	NGB00382	Sample 8	46	NGB00403	Sample 50
9	NGB00370	Sample 9	47	NGB00377	Sample 51
10	NGB00379	Sample 10	48	NGB00412	Sample 52
11	NGB00378	Sample 11	49	NGB00429	Sample 53
12	NGB00371	Sample 12	50	NGB00445	Sample 54
13	NGB00381	Sample 16	51	NGB00417	Sample 55
14	NGB00422	Sample 17	52	NGB00411	Sample 56
15	NGB00405	Sample 18	53	NGB01285	Sample 57
16	NGB00425	Sample 19	54	NGB00298	Sample 58
17	NGB00428	Sample 20	55	NGB01273	Sample 59
18	NGB00433	Sample 21	56	NGB00345	Sample 60
19	NGB00419	Sample 23	57	NGB00409	Sample 61
20	NGB00449	Sample 24	58	NGB00386	Sample 62
21	NGB00446	Sample 25	59	NGB00453	Sample 63
22	NGB00437	Sample 26	60	NGB01408	Sample 64
23	NGB00441	Sample 27	61	NGB00396	Sample 65
24	NGB0042	Sample 28	62	NGB00385	Sample 66
25	NGB00438	Sample 29	63	NGB00423	Sample 67
26	NGB00440	Sample 30	64	NGB00391	Sample 68
27	NGB00450	Sample 31	65	NGB00408	Sample 69
28	NGB00413	Sample 32	66	NGB00369	Sample 70
29	NGB00415	Sample 33	67	NGB00376	Sample 71
30	NGB00390	Sample 34	68	NGB00427	Sample 72
31	NGB00407	Sample 35	69	NGB00394	Sample 73
32	NGB00456	Sample 36	70	NGB01336	Sample 74
33	NGB00367	Sample 37	71	NGB00426	Sample 75
34	NGB00401	Sample 38	72	NGB00393	Sample 76
35	NGB00454	Sample 39	73	NGB01335	Sample 77
36	NGB00374	Sample 40	74	NGB00424	Sample 78
37	NGB00627	Sample 41	75	NGB00312	Sample 79
38	NGB00452	Sample 42	76	NGB00392	Sample 80

Table 2: Characteristics of the SSR primers among 76 sesame accessions

Primer ID	Primer Sequence	Tm(°C)	Major Allele Frequency	Gene Diversity	PIC	Allele size(bp)
ODS1	F-ATGCTACCAATCCGGGCAA R-CTGAGTGGTTGATAGCCCCG	60	0.93	0.12	0.12	200
ODS2	F-GGATAGCCGAGCCATTCTCC R-TGGCTGAGTGGTTGATAGCC	64	0.68	0.43	0.39	200
ODS3	F-GGGATAGCCGAGCCA R-GGTTGTAGCCCCGGTCTTG	63	0.79	0.33	0.28	150
ODS4	F-GGTTTTCAAGACCGGGGCTA R-CACCCCCCTATGGCCATGTTC	63	0.86	0.25	0.22	100
ODS6	F-CAAAGAAACGGGCGGTAAACG R-CTGTATGTCCTCCGTCAGCC	64	0.57	0.49	0.37	100
ODS7	F-GCCGAGACGTCGGAATTTA R-TTCTGAAAAGGCGAGCTCAGG	62	0.55	0.49	0.37	150
ODS8	F-GAATCGACCTCTTGGAGCCC R-ACTGTTATGCGGTCCTCTGC	61.5	0.71	0.41	0.33	130
ODS10	F-TCCTCAATGGGAGCAACCAC R-GTAGACTGTGAAGACCCCGC	63	0.64	0.46	0.35	300
Mean			0.72	0.37	0.30	

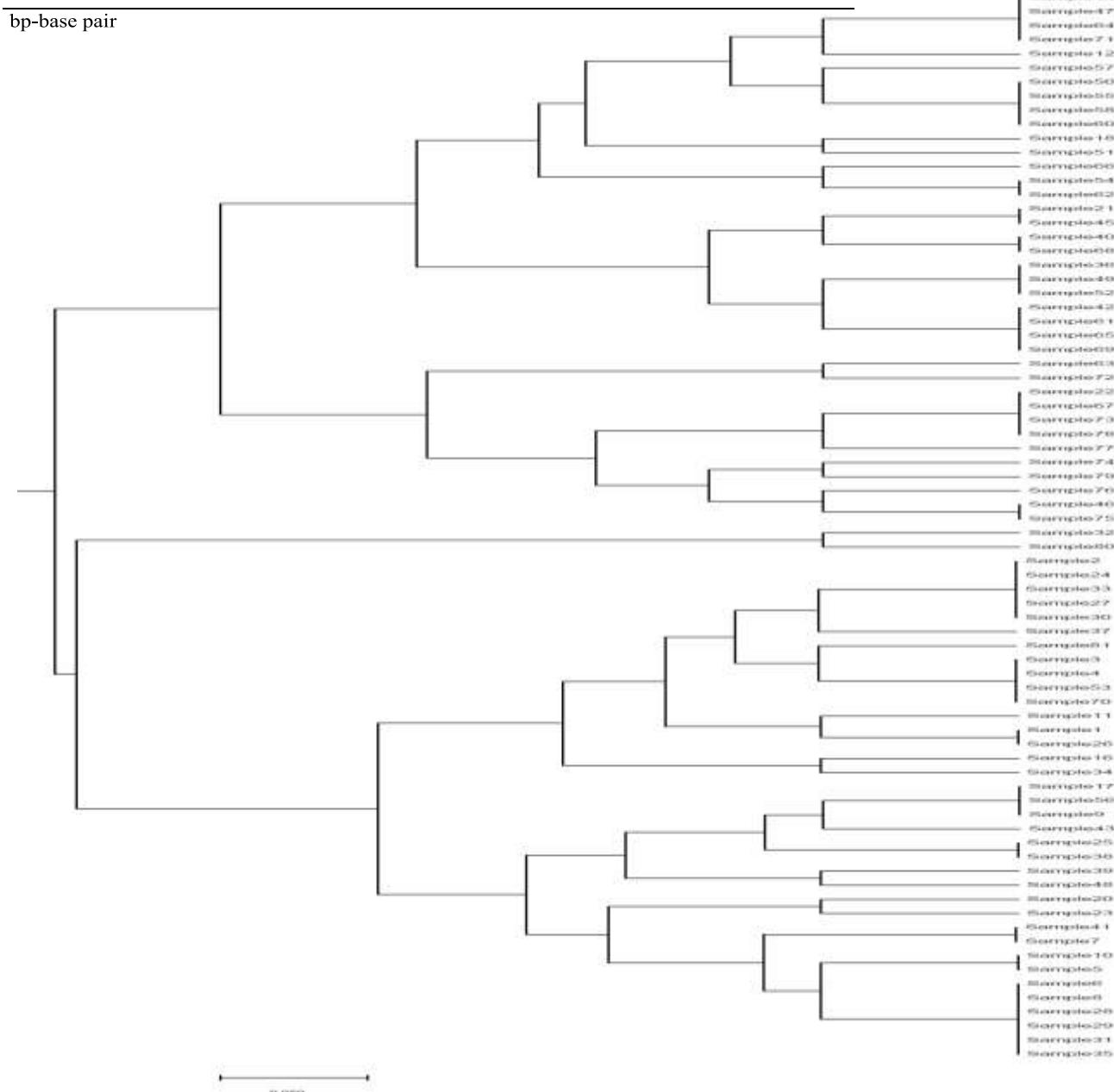


Figure 1: UPGMA tree illustrating relationships among 76 sesame genotypes based on genetic distances using 8- SSR primers

CONCLUSION AND RECOMMENDATION

In this study, we demonstrated that the SSR markers revealed genetic variation that existed among sesame accessions in the gene bank and this will promote the effective utilization. To have a comprehensive understanding of the patterns of diversity and population structure of the gene bank sesame accessions, further studies should be conducted using more SSRs.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

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