Original Research Article

Assessment of Genetic Diversity among 12 Ses*amum indicum* L. (Sesame) Genotypes from five states in Northern Nigeria using RAPD markers

ABSTRACT

Sesame (*Sesamum indicum*L.) is an important oil seed crop cultivated in many regions of the world. It is known as the king of oil seeds due to the high oil content (50-60%) in its seed. Nigeria being one of the top 10 largest producers of sesame has tremendous potentiality to increase production in order to promote exports. Vast genetic diversity is available in sesame which may facilitate the breeder to develop new varieties, provided that the genetic distance between accessions is properly understood. In this study, 12 sesame accessions collected from five states (Benue, Kaduna, Kogi, Nassarawa and Niger) in Northern Nigeria, were subjected to RAPD analysis to understand genetic differences among them. Among the 12 accessions, 10 RAPD primers efficiently amplified genomic DNA to produce a total of 63 RAPD fragments, of which 56 (88.89%) were polymorphic. Each primer generated 3 to 8 amplified fragments with an average of 6.3 bands/primer. A dendrogram constructed from pooled data revealed 11 clusters showing a high level of polymorphism. A single accession (BE-02) was relatively distinct from rest of the accessions at 4999.50 similarity coefficient exhibiting genetic divergent while two accessions (BE-01 and NA-02) were clustered together revealing genetic similarity which could be efficiently used in breeding programme.The results revealed that RAPD markers can efficiently evaluate genetic variation in the sesame germplasm.

Key words: genetic diversity,polymorphism, RAPD, sesame, *Sesamum indicum*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is a very ancient crop and one of the earliest domesticated oil crops in the world (Ashri, 1998). It is a very good source of cheap vegetable oil and protein in many parts of the world. The seed is an excellent source of high quality oil, which is very stable and free from undesirable nutrition or flavour components and has natural antioxidants that prevent aging and are vital for the production of liver cells (Falusi, 2007).These sterling attributes of sesame has stimulated the interest of farmers to produce and sell seed. Currently, about 25% of the world'ssesame seed is planted in Africa

(Ashri, 2007). In Nigeria, sesame seed is mostly produced in the Northern and North central states (Falusi and Salako, 2007). According to the federal ministry of agriculture and natural resources, Nigeria has a great market potential for sesame seed production for domestic and export markets noting that the production figures of the commodity has been on a steady increase since 1980, reaching 67000 MT by 1997 and was estimated to reach 139, 000 MT by the year 2010, (Joseph, 2009). . Generally, the cultivation of improved varieties has been limited due to insufficient information about varieties. Nigerian farmers continue to grow local varieties with low yields (RMRDC, 2004). Therefore, there is need for more genetic information in order to access useful traits present among adapted landraces in Nigeria. Characterization of accessions or species is an important step for germplasm conservation, maintenance and breeding studies. Molecular DNA marker analysis can be used to determine the genetic diversity among genotypes (Gilbert et al., 1999). Random Amplified Polymorphic DNA (RAPD) is one such method (Williams et al.,1990) of identifying polymorphism that can be used to elicit information on molecular differences among individuals of a population between lines or accessions or any breeding material. RAPD markers are simple, fast and cheap and analyses can be performed with small amounts of DNA. RAPD markers can provide robust classification criteria that could be useful in species separation and systematics. Therefore, the objective of the study was to assess the genetic diversity among 12 sesame genotypes collected from northern Nigeria and establish the genetic relationship between them using RAPD markers. This may be useful in improving the productivity and stability of the crop yield.

MATERIALS AND METHODS

Twelve accessions of sesame were procured from five states (Benue, Kaduna, Kogi, Nassarawa and Niger) in Northern Nigeria and were used as the source material for this study (Table 1). Seeds were sown and grown in the Department of Biological Sciences Experimental Garden, School of Natural and Applied Sciences, Federal University of Technology, Minna, Nigeria.

Healthy leaves (4 g) were collected from six plants of each accession, chopped, surface sterilized and pulverized in liquid nitrogen. DNA extraction and RAPD were carried out at the International Institute for

Tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria.Genomic DNA was extracted according to a modified CTAB method described by Doyle and Doyle (1990). The concentration and quality of genomic DNA was determined using a spectrophotometer at 260 to 280 nm and the quality was confirmed by banding on a 0.8% agarose gel.

For each primer, the number of polymorphic and monomorphic bands was determined. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The similarity coefficient was used to construct a dendrogram to show the phylogenetic relationships among the accessions studied by the unweighted pair group method with arithmetic averages (UPGMA) using statistical software package NTSYS-pc, Version 2.1 package (Rohlf, 2000).

RESULTS AND DISCUSSION

This study aimed to identify, using RAPD analysis, genetic variation among 12 sesame accessions from the North of Nigeria. Out of the 24primers screened, only 10 could be successfully amplified resulting in 63 bands, 56 (88.89%) of which were polymorphic while 7(11.11%) were monomorphic(Table 2).An example of genetic polymorphism across the 12 sesame accessions using primer OPB10 and OPB04 are shown in Fig. 1A and 1B, respectively. Most of the accessions that clustered in the dendrogram were fromthe same origin, e.g., cluster with accessions NG01, NG02 and NG03 from Niger state or accessions KG01 and KG02 from Kogi state (Fig.2).On the other hand, the genotype of accession BE-02 was separated from all other genotypes at 4999.50 similarity coefficient.

This study has reflected the genetic diversity available among Sesame genotypes from Northern Nigeria.. The high level of polymorphism (88.89%) observed among the accessionsis analogous to the 86.75% polymorphism noticed in a study of genetic diversity in Indian and exotic sesame germplasm

(Bhat *etal*., 1999).The clustering together of accessions from the same origin within the same range of similarity are indications that genetic divergence followed geographical separation.Alege and Mustapha (2013) reported that sesame had a common evolutionary relationship but some accessions have adapted well to their local environments through gene rearrangement due to long periods of cultivation, making them to become ecotypes. In an earlier report Pham*et al*.(2010) stated that sesame from different areas clustered according to their geographic origins.

Accurate estimates of diversity are a pre-requisite for optimizing sampling strategies and for conserving genetic resources. According to Rodriguez et al.(1999) the potential responses from selection lie in genetic diversity, therefore, necessary precautions should be taken to increase genetic diversity in any breeding program.Genetic diversity can be enlarged by combining desired traits from different local and wild populations of different geographical origins into the breeding lines (Bisht*et al*., 1998). Since genetic differentiation was correlated with geographic isolation in this study, it may be appropriate to analyze accessions that represent a wide range of more geographical origins in order to maximize genetic diversity.Nevertheless, the rich genetic diversity available among the Sesame genotypes from Northern Nigeria can be utilized for current and future breeding programs in order to select genetically distinct parents.The use of RAPD for assessment of genetic diversity is preferred over the morphological parameters because it is completely devoid of the effects of environment and the stage of the experimental material, thus making them highly reliable. Salazar *et al*.(2006) reported that Random Amplified Polymorphic DNA (RAPD) is simple to use for the evaluation of genetic diversity in sesame. This has been confirmed by several authors (Bhat *et al*., 1999; Ercan*et al*., 2004).The present study also confirmed the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions available in the germplasm collection. The results from this study have revealed that RAPD markers can efficiently evaluate genetic variation in sesame germplasm.

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Table 1 Description and sources of the 12 collected sesame accessions					
Accession number	Local name	Source	Seed colour	Colour of flowers	Seed length (mm)
KD	*Riddi*	Kafanchan, Kaduna	white	white	3-3.5
NG-01	*Anufi*	Paiko, Niger	light brown	white	2-2.5
NG-02	*Ishwa*	Saminaka, Niger	light brown	white	3
NG-03	*Esso*	Katcha, Niger	light brown	white	2-3
NG-04	*Anufi*	Mayaki, Niger	creamy white	white	2-3
NA-01	*Riddi*	Nassarawa	black	purple	2-3
NA-02	*Riddi*	Nassarawa	brown	white	2-3
NA-03	*Riddi*	Nassarawa	white	white	2-3
BE-01	*Ishwa*	Benue	white	white	2
BE-02	*Ishwa*	Benue	creamy white	white	3-3.5
KG-01	*Gogori*	Kogi	creamy white	purple	3
KG-02	*Gogorigo*	Kogi	light brown	white	2-3

Table 2. Successful Primers used, their sequence, Number of polymorphic products and percentage of polymorphic bands produced by each primer.

Primer	Sequence(5′-3′)	Totalnumberof bands	Numberofpolymorphicbands	Polymorphism(%)
OPT08	AACGGCGACA	8	7	87.5
OPB06	TGCTCTGCCC	7	6	85.71
OPB10	CTGCTGGGAC	6	6	100
OPH09	TGTAGCTGGG	3	2	66.7
OPB07	GGTGACGCAG	8	5	62.5
OPB01	GTTTCGCTCC	7	7	100
OPB03	CATCCCCCTG	8	8	100
OPB04	GGACTGGAGT	8	7	87.5
OPH03	AGACGTCCAC	4	4	100
OPH04	GGAAGTCGCC	4	4	100
Total		63	56	88.89

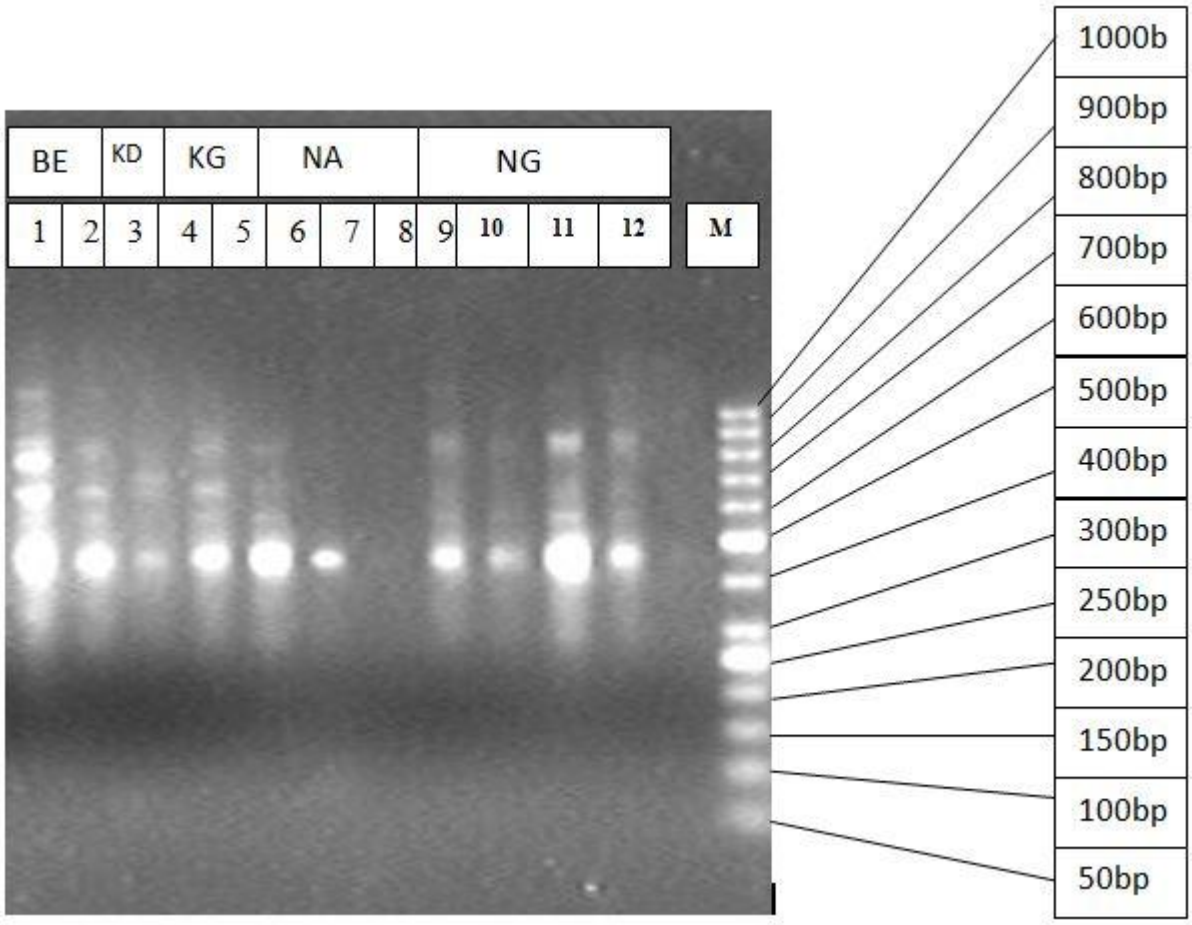

Figure 1A: RAPD pattern generated by the primer OPB10 with 12Sesame Accessions Studied. lanes1= BE01, 2= BE02, 3= KD, 4=KG01, 5= KG02, 6= NA01, 7=NA02, 8=NA03, 9=NG01, 10=NG02, 11= NG03,12 =NG04 and M=Ladder

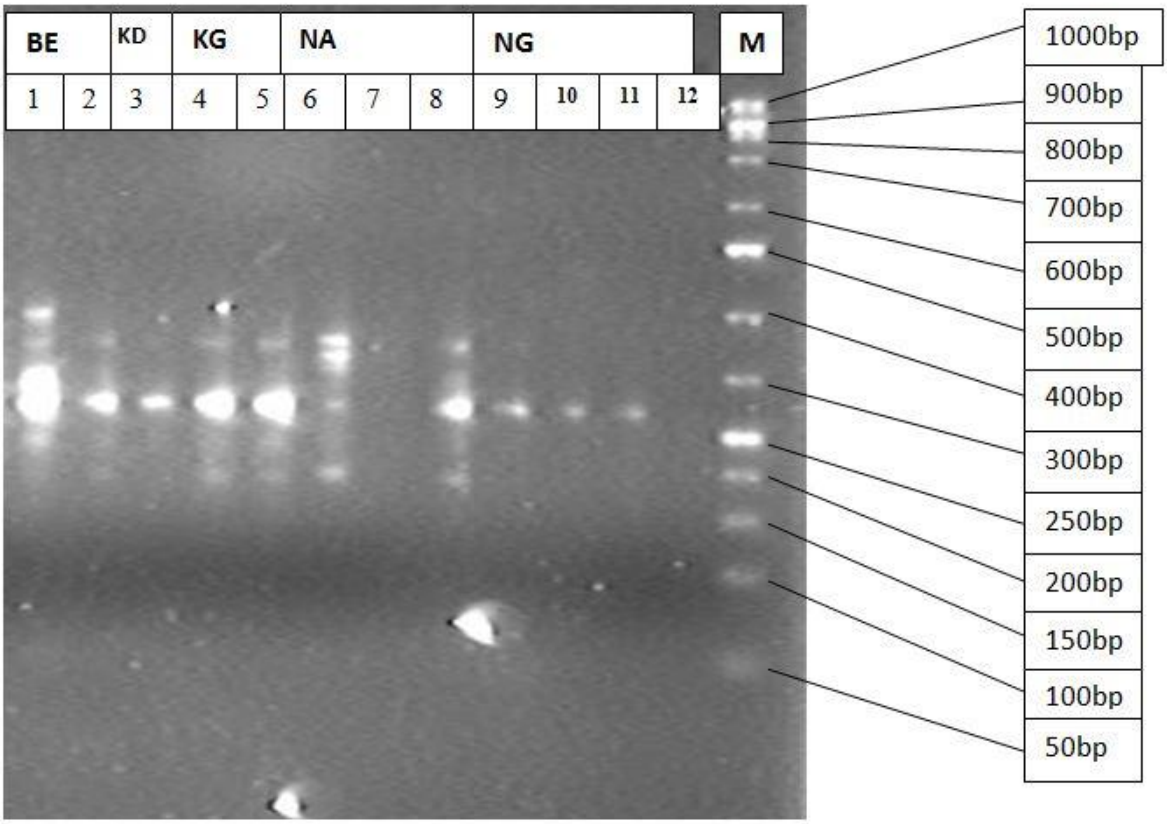

Figure 1B:RAPD pattern generated by the primer OPB04 with 12Sesame Accessions Studied. lanes1= BE01, 2= BE02, 3= KD, 4=KG01, 5= KG02, 6= NA01, 7=NA02, 8=NA03, 9=NG01, 10=NG02, 11= NG03,12 =NG04 and M=Ladder

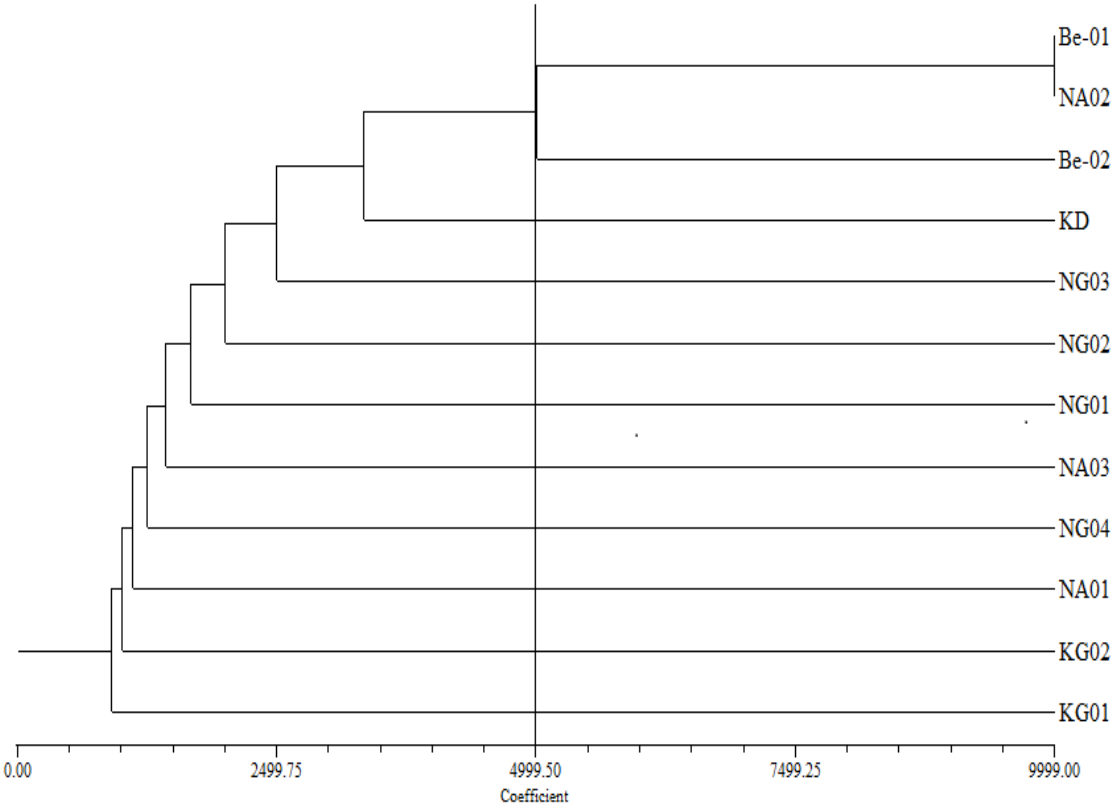

Figure 2: Dendrogram Showing cluster analysis and similaritycoefficient and diversity among 12 Nigerian sesame genotypes