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RESEARCH ARTICLE

DETERMINATION OF GENETIC DIVERSITY OF NIGERIEN SESAME (*SESAMUM INDICUM* L.) USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS MARKERS

ZANGUI hamissou[1,*], AMOUKOU ADAMOU Ibrahim[2], BOUREIMA Seyni[3], THIERRY Robert[4] and TAKVORIAN Najat[5]

[1,2]Abdou Moumouni University, Faculty of Agronomy, Department of Plant Production, BP 10960 Niamey, Niger
[3]Dan Dicko Dankoulodo University, Faculty of Agronomy and Environmental Sciences, Department of Plant Production, BP 465 Maradi, Niger
[4,5]Ecology, Systematics and Evolution Laboratory, Building 360, Paris-Sud University, Orsay Cedex 91405, France

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ABSTRACT

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops cultivated by man. The improvement of this crop requires a large exploitation of the available genetic variability on which its success depends. The present study aims to analyze the genetic diversity within 127 accessions of the sesame collection carried out in 6 regions of Niger (Tillabéri, Dosso, Maradi, Zinder, Diffa, and Tahoua). The Amplified Fragment Length Polymorphism (AFLP) marker was used to assess the level and structure of genetic diversity among sesame accessions collected in Niger. DNA was extracted using the CTAB extraction method. A total of 179 bands were amplified with the EcoRI-ACT/MseI-CTA primer of which 83.80% were polymorphic. The population structure indicated that the material was divided into three populations. Sesame genetic diversity is not geographically structured in Niger. Principal Component Analysis (PCA) in Sesamum indicum accessions with AFLP markers showed that the variation explained by the first two axes was 10.46%. Analysis of Molecular Variance Analysis (AMOVA), based on the three populations, showed very high intra-population diversity. The hypothesis that selecting genotypes of different geographical origin will maximize the diversity available for a breeding project is not relevant for sesame in Niger.

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INTRODUCTION

Sesame, a diploid species (2n = 26), of the family Pedaliaceae is one of the oldest domesticated oil seed crops (Bedigian and Harlan, 1986). Compared to cereal crops, genetic research on oil seed crops is limited (Wang *et al*., 2011; Wei *et al*., 2013; Li *et al*., 2014; Hwang *et al*., 2014; Jiang *et al*., 2014). As for sesame, it lags behind other major oilseed crops in terms of genetic improvement. However, in recent years, extensive molecular analyses have been carried out to estimate genetic variation in sesame using RAPD (Bhat *et al*., 1999; Ercan *et al*., 2004; Zhang *et al*., 2004; Abdellatef *et al*., 2008; Pham *et al*., 2011) and SSR (Zhang *et al*., 2010, 2011, 2012a, b; Gebremichael *et al*., 2011; Yue *et al*., 2012; Pandey *et al*., 2015). Among oilseed crops, sesame maybe an interesting species for genetic studies because it has a small diploid genome (~ 350 Mb) (Wang *et al*., 2014). The use of AFLPs in genetic marker technologies has become the main tool because of its ability to disclose a high number of polymorphic markers by a single reaction (Vos *et al*., 1995). Amplified fragment length polymorphism (AFLP) has been used in binding analysis (Uzun *et al*., 2003).

Corresponding author: **ZANGUI hamissou**
Abdou Moumouni University, Faculty of Agronomy, Department of

AFLP is a promising technique for the characterization of sesame genetic diversity. AFLP markers have been successfully used to analyze the genetic diversity of some other plant species such as peanut (Herselman, 2003), soybean (Ude *et al*., 2003) and maize (Lübberstedt *et al*., 2000). These studies have indicated that the AFLP technique is highly applicable to molecular discrimination at the species level. It is a reliable genotyping method with a high degree of reproducibility and discriminating power with typically 10 to 20 or more fragments analyzed simultaneously (Savelkoul *et al*., 1999; Vos *et al*., 1995). The diversity of the Nigerien sesame collection, representing all agro-ecological regions, for a range of morphological and agronomic characteristics, has been studied in previous studies (Zangui *et al*., 2020). However, diversity analyses based on morphological characteristics are subject to environmental biases. The present study aims to analyze genetic diversity within 127 accessions of the sesame collection from 6 regions of Niger (Tillabéri, Dosso, Maradi, Zinder, Diffa, and Tahoua). This diversity analysis at the molecular level will be based on the use of amplified fragment length polymorphism (AFLP).

MATERIALS AND METHODS

Plant material: The study was conducted with 127 sesame accessions, 123 of which were from the collection made in 62

villages belonging to 6 agro-ecological zones of Niger, namely Tillabéri (29), Dosso (25), Maradi (28), Tahoua (02), Diffa (04) and Zinder (32) (Table 1). These are the main sesame-growing areas in Niger. The other four are accessions from Chad. All color categories of the seedcoat are represented.

Methods

Conduct of the trial: The seeds were sown in plastic pots of 12 cm diameter, filled with potting soil and earth (1/1) at a rate of fifteen seeds per pot. The crop was grown in a greenhouse under controlled conditions with a temperature maintained at 27C° and a relative air humidity of 40%. After 28 days of cultivation, one hundred milligrams of fresh young leaves were harvested and placed in a 2mL tube in the presence of a 3mm diameter ball. The whole was frozen in liquid nitrogen and crushed 2 x 30 seconds at 24 Hz using the Tissue Lyser II (QIAGEN) crusher. The ball is removed and the grinded material is stored at -80°C until use.

DNA extraction: All accessions were grown under greenhouse conditions and the broth is contained in 900 µL of extraction buffer containing 2% CTAB, 2% polyvinylpyrrolidone (PVP), 1.4M NaCl, 20 mM EDTA and 100Mm Tris HCL, pH=8, 2 µL β-mercaptoethanol, 500µg/ml Proteinase K (Macherey-Nagel) and 10µg/ml RNase. The buffer ispreheated 15 min at 60°C before addition of Proteinase K, RNase and β-mercaptoethanol (98%). The homogenateisincubated at 60°C for 45 min. Duringthis incubation, the homogenates are mixed by inverting the tubes every 5 min. 900 µl chloroform-isoamylalcohol (24:1) isadded to eachhomogenate. The phases are separated by centrifugation for 10 min at 16 200 g (13 000 rpm). The aqueous phase obtained is re-extracted with 900 µl of chloroform-isoamylalcohol (24:1) followedd by centrifugation again for 10 min at 16,200 g (13,000 rpm). These two steps are important because they remove plant debris, proteins and polysaccharides. The supernatant containing the nucleic acids is transferred to a 1.5 ml tube. The same volume of cold is propanol (stored at -20°C) is mixed with the supernatant by inverting the tubes gently. A DNA precipitate is formed. The DNA pellet is obtained after centrifugation for 30 min at 6 200 g (8000 rpm). It is washed once with 600 µl of cold 75% ethanol followed by centrifugation at 6 200 g (8000 rpm) for 10 min and a second time with 600 µl of cold absolute (100%) ethanol and centrifugation at 6 200 g (8000 rpm) for 10 min. After removal of the ethanol, the pellet is dried at room temperature for 30 min. The DNA is resuspendedin 100 µl of TE-RNase (Tris HCl 10 mM, pH 8.0, EDTA 1 mM, Ph 8.0 and 2mg/ml RNase). The DNA is incubated at 37°C for 30 min before being assayed and stored at -20°C.

AFLP methodology: The AFLP analysis was performed according to the protocol of Vos *et al* (1995) with some modifications. Approximately 200 ng of DNA was digested at 37°C for 3 hours in 25 µL of Ligase Buffer 1x (Invitrogen) containing 5 U of the restriction enzymes EcoR1 and Tru1l (MseI). The restriction enzymes were denatured at 70°C for 20 min. All digestion products were used for ligation of adapters at 20°C for 2 hours 30 min in 50 µL of Ligase Buffer 1x (Invitrogen) containing 1 U of T4 DNA Ligase (Invitrogen), 0.1µM of the EcoRI adapters and 1µM of the Tru1l adapters (MseI). 5µL of the ligation were used for preamplification (PA) using primers containing a selective nucleotide at their 3' termini: adenine and cytosine for the Eco RI and MseI primers respectively (Table 2). The preamplification is per formed in 50µL of Dream Taq 1x buffer containing 0.4mM Mg Cl2 (Thermo Fisher Scientific), an additional 0.5mM MgCl2, 200µM dNTP (Quiagen), and 1 U Taq polymerase (Thermo Fisher Scientific, Lithuania). The preamplification program used includes: 1 minute of initial denaturation at 94°C, 20 cycles comprising 30sec at 94°C denaturation, 1mn hybridization at 56°C and 1mn elongation at 72°C followed by 5mn final elongation at 72°C. 4 µL of the 30x diluted preamplification were used for selective amplification with the combination of EcoRI primers labelled E-ACT_FAM and MseI primers labelled M-CTA. The direct primer used for the selective amplification step was marked with a 6-FAM modification at the 5' end to allow maximum detection on a capillary sequencer (Applied Bio system® 3730XL). EcoRI and MseI 10mM primers, dNTP Mix PCR Grade 10mM, (Quiagen kit, Germany), Dream Taq buffer (10 X) (with 20mM MgCl2) (Lithuania), 25mM MgCl2 and 5U/µl Taq polymerase (Thermo Fisher Scientific, Lithuania) in a total volume of 25µL were used. The selective amplification program starts with an initial denaturation step of 1mn at 94°C, a first segment of 10 cycles (denaturation of 1mn at 94°C, hybridization of 1mn with a temperature decreasing from 65ºC to 56ºC with each cycle, elongation of 1mn 30sec at 72°C), a second segment of 20 cycles (denaturation of 30sec at 94°C, hybridization of 30sec at 56ºC, elongation of 1mn at 72ºC) then a final elongation step of 3 min at 72°C. Two negative controls were included in the PCR and genotyping steps. In addition, six randomly selected individuals were repeated three times: two repeats were performed at the extraction stage and kept throughout the process, and the third was included after the digestion ligation step in the PCR and genotyping steps. These controls were designed to test for maximum reproducibility.

AFLP analysis

Peak detection and selection procedure: AFLP migration profiles were analyzed using Gene Mapper v.5 (Thermo Fisher Scientific), using the GS500 (-250) LIZ control for the fragment size scale. Default options were used as a first step for peak selection and phenotype assignment (presence or absence of detected peaks). Only peaks with a size between 60 bp and 500 bp were kept for analysis. Peaks were also checked by eye for each individual in the analyses. Individuals with poor profiles (high background noise or weak signals) were removed from the analysis. In order to reduce potential phenol typing errors, controls were used to select peaks on the basis of repeatability and reliability, as follows: (i) all peaks detected in negative controls were eliminated, (ii) a second peak selection step was based on the calculation of Bonin serror rate (Bonin *et al*., 2004) using control sample replicates. All peaks with an error> 0% were eliminated (meaning that all peaks retained for subsequent ones were 100% reproducible on the basis of replicate controls) and (iii) loci displaying alternative phenotypes (presence or absence of the detected peak in a single individual in the sample (singletons) were also discarded from further analysis as this type of variation can be easily obtained due to genotyping error. For each locus, the presence and absence of the detected peak is referred to phenotype [1] and phenotype [0] in the analysis.

Annotation and analysis of AFLP data: The number of polymorphic bands was identified and counted using GenAlEx 6.503 software. Genotypes were annotated based on the presence (1) or absence (0) of polymorphic bands. The result of

the analysis is an amplified band count for the primer pair and the presence (1) or absence (0) genotype of the band for each individual. The same GenAlEx software allowed us to calculate the percentage of polymorphic bands. To study the genetic structure of Sesamum indicum L. species, accessions were grouped into five sets according to geographical distribution. Heterozygosity cannot be directly observed in the AFLP data because the AFLP markers are dominant. To calculate allele frequencies, the absence of a band was considered as a homozygous state of a recessive allele (q2) and the presence of a band as dominant homozygous (p2) or heterozygous (2 pq).

The frequencies p and q are obtained accordingly. Unbiased measures of genetic identity and genetic distance between groups have also been determined (Nei, 1978). To identify genetic relationships between individuals, a dissimilarity matrix was calculated using DAR win v6 software. The individual relationships were analyzed with a tree construction based on the Neighbors Joining (NJ) method (DARwin v6). Then, using polymorphic bands, the diversity index between the different populations formed was calculated. This diversity index is estimated using the "polymorphism information content (PIC)" defined by Botstein *et al*. (1980) through the following formula used in the case of AFLPs: $PIC = 1 - p^2 - q^2 - 2p^2q^2$

Where

p = genotype 0 frequency and q = genotype 1 frequency.

The analysis of genetic diversity and molecular variance (AMOVA) was performed with GenAlEx v6.503 software using a binary AFLP database for the three populations. The genetic distance and geographic distance matrices resulting from the GenAlEx analysis were used in the R software through the ADGENET package to generate a histogram of similarity between the genetic distance matrix and the geographic distance matrix. The GenAlEx program was used to obtain allele frequencies, "p" and "q" values to calculate the polymorphism information content (PIC) between the groups formed. To verify the dispersion of individuals, a principal component analysis (PCA) was performed using R.3.5.1 software.

Analysis of the population structure using the Bayesian approach

In order to determine the number of genetically homogeneous groups, a Bayesian approach to assignment was used. For this purpose, the STRUCTURE 2.3.4 software (Pritchard *et al*., 2000; Falush *et al*., 2003) was used. It allows, by successive iterations by fixing a priori the number of groups (K), to determine the optimum number of groups to characterize the genetic diversity observed, assuming that mixing between genetic groups exists (model including the possibility of admixture). For each value of K ranging from 1 to 10, 10 independent runs were performed with for each run, a "burn-in" period (iterations not conserved to explore the landscape of possibilities) of 500,000 iterations, followed by 1,000,000 iterations to calculate the assignments (exploration of the landscape by the Markov Monte Carlo Chains Method (MCMC)). For each K, the run with the highest likelihood (Ln P (D|K)) was retained both to determine the most likely K (Evanno's method (Evanno *et al*., 2005) using the Structure Harvester program (Earl and VonHolt, 2012) and the percentage of assignment to each group within the genomes.

RESULTS

General analysis of diversity: In the analysis of the AFLP profile, only high-resolution loci were considered. A total of 179 loci were generated by the primer combination used, of which 150 were polymorphic, with a polymorphism rate of 83.80% (table 3). A high rate of polymorphism was observed, indicating a considerable amount of genetic variability among the genotypes studied. Across all 179 loci, the S110 accession had more polymorphic bands (21 in total), followed by S83 (20 bands), S68 (18 bands), S48, S52, S60, S57 (15 bands each) and S116, S4, S75 with 14 bands each.

Genetic relationships between sesame accessions: Factor analysis based on the Nei diversity index showed that for the overall collection, the first axis is the most important in explaining the organization of genetic diversity (Figure 1). Principal Component Analysis (PCA) with all sesame accessions showed the formation of three main groups, suggesting a broad genetic basis from the genetic material (Figure 2). From this figure it can be seen that there is no obvious relationship between geographical origin and clustering based on genetic similarities. In particular, accessions from Dosso, Tillabery, Zinder, Maradi, Tahoua, Diffa and those from Chad are distributed across clusters in the UPGMA analysis. The dendrogram produced using the Neighbour Joining method shows a structuring of the individuals in the entire collection (Figure 3). The general structure of the tree, grouping all the individuals, makes it possible to divide the min to three (3) clusters. The accessions of each provenance are mixed in the different defined groups. Thus, Group 1 includes 13 accessions distributed as follows: 9 from Tillabéri, 3 from Dosso, and 1 accession from Chad. Group 2 includes 49 accessions where all origins are represented except Chad: 16 from Maradi, 13 from Zinder, 8 from Tillabéri, 7 from Dosso, 3 from Diffa, and one from Tahoua. Group 3 includes 65 accessions, 19 of which are from Zinder, 15 from Dosso, 12 from Tillabéri, 12 from Maradi, 4 from Diffa, and 3 from Chad.

Analysis of diversity within groups: The diversity within the groups formed shows that the highest percentage of polymorphism (80%) and the highest ICP value (0.053) are obtained in the accessions composing group 3 (Table 4). However, overall, the information content of the polymorphism and the Shannon index are very low for the groups. This indicates a low diversity between the three groups. This diversity should be greater within the groups, especially group 1, which has the highest Shannon index (0.105). Indeed, the Shannon index is an index of abundance based on specific diversity; the larger it is, the less homogeneous the populations are. Therefore, Group 1 appears to be the most diverse. The least genetically diverse group is group 3 with a Shannon's diversity index of 0.082. When analyzing the total band profiles by group, Group 3 accessions had the highest number of bands (150), followed by Group 2 accessions (136), and Group 1 accessions (92). On the other hand, the accessions in this group have the highest number of bands with a frequency greater than 5%. In addition, the accessions in group 3 have the largest number of private bands (22), followed by group 2 (15) and group 1 with a total of 6 private bands (Figure 4). The analysis of molecular variance (AMOVA) allows groups to be compared and evaluated. The analysis of molecular variance

Table 1. Accessions used, their origin and the color of the seedcoat of their seeds

CA	Region	Village	CTeg	CA	Region	Village	CTeg
S1	Tillabéri	Koulbaga	Brown	S33	Maradi	Korgom	Beige
S100	Tahoua	Tsarnaoua	Brown	S34	Maradi	HawanDawaki	White
S101	Tahoua	Tsarnaoua	Beige	S35	Maradi	HawanDawaki	White
S102	Dosso	Gaya	Brown	S36	Maradi	Korgom	White
S103	Dosso	Tara	Brown	S37	Maradi	Korgom	Beige
S104	Dosso	Tara	Beige	S38	Maradi	Korgom	White
S105	Dosso	Tara	Brunettette	S39	Maradi	Aharon	Beige
S106	Dosso	SabonBirni	Brunettette	S4	Tillabéri	Koulbaga	Beige
S107	Dosso	Bengou	Brown	S40	Maradi	Maijirgui	White
S108	Dosso	Bengou	White	S42	Maradi	Tchadoua	White
S110	Tillabéri	Garbey kourou	Brunettette	S43	Maradi	Aguié	White
S111	Dosso	Gaya	White	S44	Maradi	Dan Jikaou	Beige
S112	Dosso	Guéza	White	S45	Maradi	Aguié	White
S113	Dosso	SantchéFarou	Brown	S46	Maradi	Tessaoua	White
S114	Dosso	SantchéFarou	Beige	S47	Maradi	Tessaoua	White
S115	Dosso	SantchéFarou	White	S48	Maradi	Dan Jikaou	White
S116	Dosso	Lisso Matché	White	S49	Maradi	Aguié	Brunette
S117	Dosso	Kodo	White	S50	Maradi	Kibya Ga Kougou	Beige
S119	Dosso	Kodo	White	S51	Maradi	Kibya Ga Kougou	White
S12	Dosso	Kodo	Beige	S52	Maradi	Kibya Ga Kougou	Beige
S120	Tillabéri	Garbey kourou	Brown	S54	Maradi	SabonMachi	Beige
S121	Dosso	Kodo	Brunettette	S55	Maradi	Gangara Dan Ido	White
S122	Dosso	Kodo	Brunettette	S56	Maradi	Gangara Dan Ido	White
S124	Dosso	Kodo	Beige	S57	Maradi	Jaja	White
S125	Dosso	Madina	Beige	S58	Maradi	Kiré	Beige
S126	Dosso	Madina	Brown	S59	Maradi	Tibiri	Beige
S127	Dosso	Tanda	Black	S6	Tillabéri	Guérokiraï	White
S128	Dosso	kouka mai lamba	Black	S60	Maradi	Tibiri	Beige
S129	Dosso	Tanda	Brown	S61	Maradi	Tibiri	Brown
S130	Tillabéri	Garbey kourou	White	S62	Maradi	Tibiri	Brunette
S131	Diffa	Diffa	Brown	S63	Zinder	Matameye	Beige
S132	Diffa	Diffa	Brown	S64	Zinder	Takiéta	Beige
S133	Diffa	Diffa	Brown	S65	Zinder	Takiéta	White
S134	Diffa	Diffa	Grey	S66	Zinder	Kantché	White
S135	Diffa	Diffa	Beige	S67	Zinder	Dan Bouba	Beige
S136	Diffa	Gandaré	Beige	S68	Zinder	Dan Bouba	Brunette
S137	Diffa	Gandaré	White	S7	Tillabéri	Guérokiraï	Beige
S138	Tchad	Geida Sila	Beige	S71	Zinder	Ga Allah	Grey
S139	Tchad	Geida Sila	Beige	S73	Zinder	Malawa	Grey
S140	Tchad	Geida Sila	Beige	S74	Zinder	Malawa	Brunette
S14b	Tchad	Geida Sila	Beige	S75	Zinder	Malawa	Beige
S16	Tillabéri	Djabou	Beige	S76	Zinder	Malawa	Beige
S17	Tillabéri	Djabou	White	S79	Zinder	Hayaniya	Brunette
S18	Tillabéri	Say	White	S80	Zinder	Hayaniya	Beige
S19	Tillabéri	Diguimassa	Beige	S81	Zinder	Malawa	Brunette
S2	Tillabéri	AnamaTondi	Beige	S82	Zinder	Katta Kara	Brunette
S20	Tillabéri	Koulbaga	Beige	S83	Zinder	Malawa	Beige
S21	Tillabéri	GouTiyéna	Brown	S84	Zinder	Dan Marké	Beige
S22a	Tillabéri	Boura	Brown	S85	Zinder	Hayaniya	Beige
S22b	Tillabéri	Kossa	Brown	S86	Zinder	Hayaniya	White
S23a	Tillabéri	Kossa	Beige	S87	Zinder	Malawa	Beige
S23b	Tillabéri	Namari peulh	Brown	S88	Zinder	Dogo	Beige
S24	Tillabéri	Namari peulh	Beige	S89	Zinder	Dogo	Beige
S25	Tillabéri	Sona	Brown	S9	Tillabéri	Tchoumbo	Beige
S26	Tillabéri	Sona	Beige	S90	Zinder	Dogo	Beige
S27	Tillabéri	Lossa	Beige	S91	Zinder	Dogo	Brown
S28	Tillabéri	Lossa	Brown	S92	Zinder	Dogo	Beige
S29	Tillabéri	N'Dounga	Brown	S93	Zinder	Kaba	White
S3	Tillabéri	Kollo	Brown	S94	Zinder	Bandé	Beige
S30	Tillabéri	Koulbaga	Beige	S95	Zinder	Kaba	Brunette
S31	Tillabéri	Kollo	Brown	S96	Zinder	Bandé	Brunette
S32	Tillabéri	N'Dounga	Brown	S97	Zinder	Bandé	Beige
S33	Tillabéri	Liboré	Brown	S98	Zinder	Gada	Beige
				S99	Zinder	Dogo	Beige

CA: Accession Code; CTeg: Colour of Integument.

Table 2. Oligonucleotideadapters and combinations of primers used

Name		Sequences	
EcoRI adapter MSEI adapter			5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATCGTTAA-5' 5'-CAGGATGAGTCCTGA G-3' 3'-TACTCAGGACTCAT-5'
Primers used in preamplification			
EcoRI + 1-A		E-PA-A	5'- ACTGCGTACCAATTCA -3'
MseI +1-C		M-PA-C	5'- GATGAGTCCTGAGTAAC -3'
Primer combinations used in selective AFLP amplification			
Combination	EcoRI	E-ACT (FAM)	5'- ACTGCGTACCAATTCACT -3'
	MseI	M-CTA	5'- GATGAGTCCTGAGTAACTA -3'

Table 3. Polymorphism generated by the EcoRI-ACT/MseI-CTA primer couple

Pair of primers	Species	NTB	NBP	% P	I
EcoRI-ACT/MseI-CTA	*Sesamum indicum*	179	150	83,80	0,093

NTB: Total number of bands; NBP: Number of polymorphic bands; %P: % polymorphism; I: Shannon Diversity Index

Table 4. polymorphism and value of the PIC generated by the pair of primerswithin the groups

Pair of primers	Groups	NTB	NBP	% P	I	PIC
EcoRI-ACT/MseI-CTA	Group1	92	47	51,09	0,105	0,035
	Group2	136	103	75,74	0,093	0,042
	Group3	150	126	84,00	0,082	0,053

NTB: Total number of bands; NBP: Number of polymorphic bands; %P: % polymorphism;
I: Shannon Diversity Index; PIC: Polymorphism Information Content

Table 5. Molecular analysis of variance (AMOVA)

Source	ddl	SCS	Variance	Pourcentage of variation
Between groups	2	14,312	0,012	0%
Intra groups	124	833,106	6,719	100%
Total	126	847,417	6,730	100%

ddl: degree of freedom. SCS: Sum of the squares of the deviations.

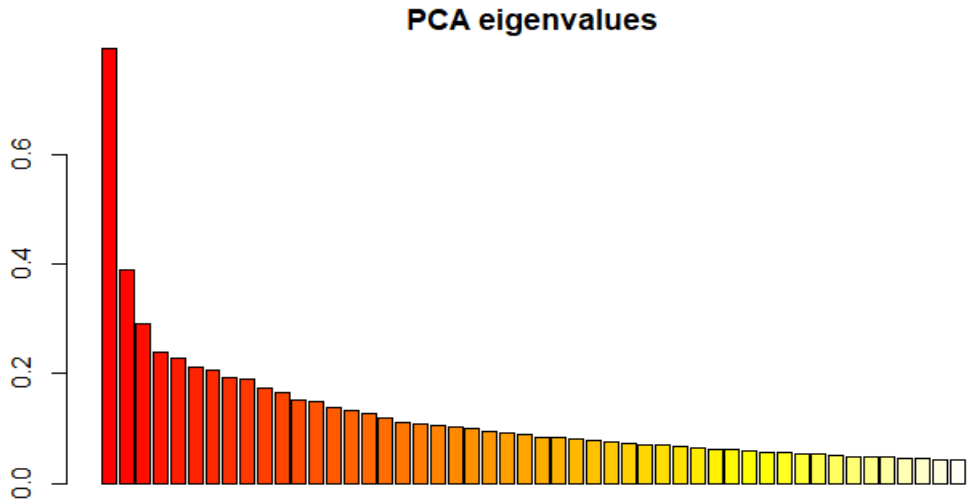

Figure 1 : Partition to total inertia at each axis

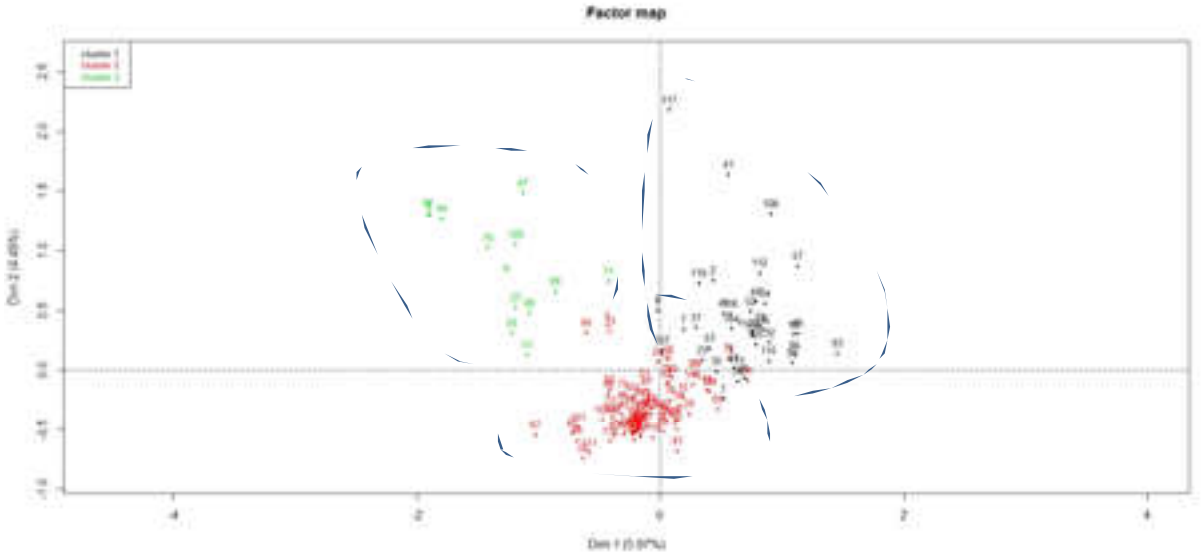

Figure 2. Genetic Relationship of 127 sesame (Sesamum indicum) accessions based on principal component analysis (PCA).

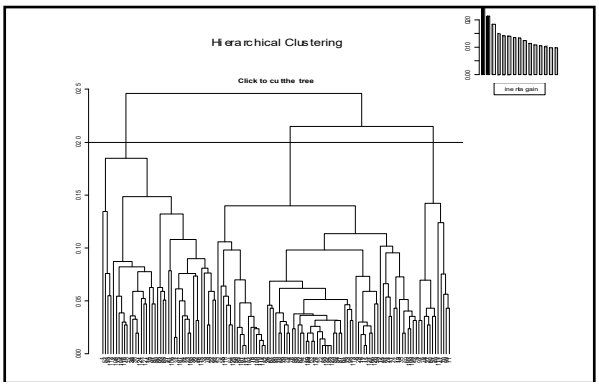

Figure 3 : Dendrogram showing phylogenetic relationships between Sesamum indicum accessions using AFLP markers.

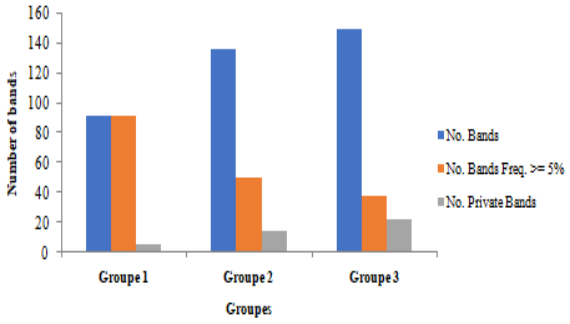

Figure 4 : Schematic of bands between different groups of sesame accessions.

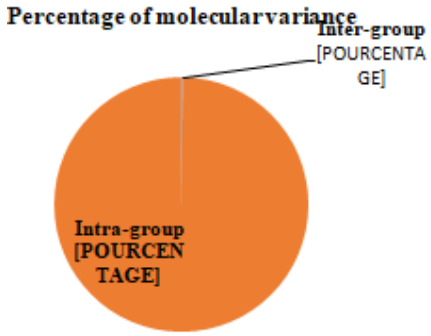

Figure 5 : AMOVA showing the variability between and within three groups

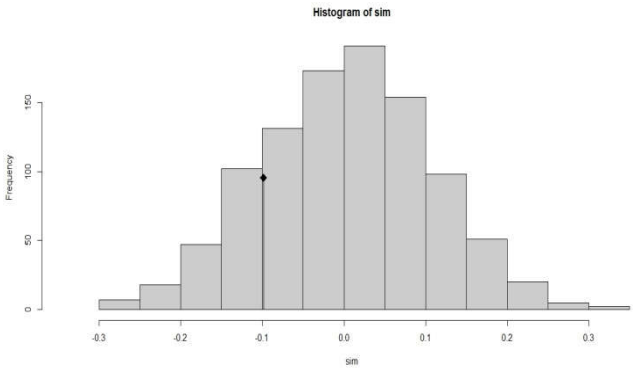

Figure 6. Similarity histogram between the genetic distance matrix and the geographic distance matrix

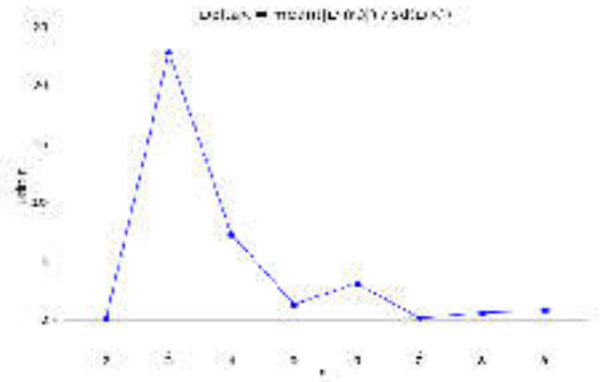

Figure 7. Representation according to the admixture model of the number of populations of sesame accessions (Sesamum indicum L.) as a function of Delta K (K groups k ranging from 1 to 3 (Evanno et al., 2005), Delta k = f (K)).

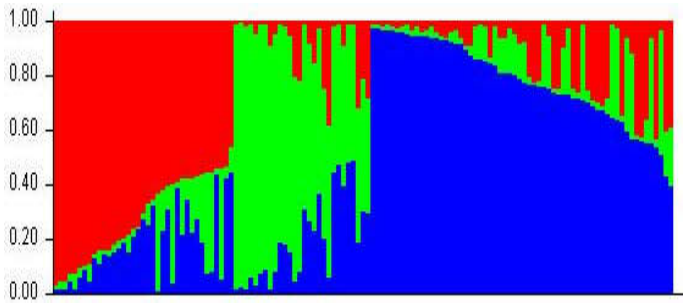

Figure 8 : Graphical representation of the estimation of membership coefficients for the three populations identified by the Bayesian method of Pritchard et al. (2000). Each accession is represented by a vertical line according to the membership coefficient for each population. Membership of the 3 populations is indicated by different colors: green for population1, red for population2 and blue for population 3

(AMOVA) of AFLP data performed on the different groups indicates that there is a significant genetic difference between the groups formed, but that the genetic diversity is high within the groups (100%) (Figure 5). This shows the existence of very strong intra-accession diversity (Table 5).

In order to study the geographical and genetic relationships of the accessions making up each constituted group, we tested the relationship between the matrix of geographical distances and the matrix of genetic distances using the Mantel test. A correlation was calculated between the two matrices to test the significance of the correlation; a simulation procedure was carried out. Under the hypothesis of interdependence between the two matrices, the rows and columns of a matrix are randomly interchanged and the correlation recalculated. The distribution for 10,000 simulations in the case of independence is given in grey (figure 6) and the initial value by a black square. The initial value is included in the distribution suggesting an absence of significant correlation between the geographic distance matrix and the genetic distance matrix. This test, which validates the information provided by figure 3, shows that there is no correlation between genetic distance and geographic distance. Therefore, there does not seem to be a relationship between genetic isolation and geographical isolation of the groups formed. Analysis of the structuring of accessions using the Bayesian approach. The pattern of genetic diversity and population structure of the sesame accessions studied was more characterized by a Bayesian approach.

Classification of sesame accessions according to the model of belonging to groups or populations characterized by their allelic frequencies on the basis of 179 AFLP loci, with admixture (Pritchard *et al*., 2000) and the search for the optimal number of groups according to the method of Evanno *et al*. (2005), showed the existence of three groups (populations) for K=3 (Figure 7).Thus, the STRUCTURE analysis divided all accessions into three groups (populations) (Figure 8), showing a high correspondence with the dendrogram and PCR results, but with some individuals being reassigned. Population 1, shown in red, includes 37 accessions, 13 of which are from Maradi, 10 from Tillabéri, 8 from Zinder, 4 from Dosso, 1 from Tahoua, and 1 from Diffa. The second population includes 28 accessions, 8 from Tillabéri, 7 from Dosso, 6 from Zinder, 3 from Maradi, 2 from Diffa, 1 from Tahoua, and 1 from Chad. The third population includes the maximum number of accessions, 62 in total, including 18 from Zinder, 14 from Dosso, 12 from Maradi, 11 from Tillabéri, 4 from Diffa, and 3 from Chad. In addition, of the 127 accessions with distinct genotypes, 78 had a coefficient of belonging to one of the three populations greater than or equal to 80% (pure population) and could therefore be assigned to one of the three populations. The remaining accessions that had membership coefficients in all three populations of less than 80% (mixed population) were considered "unclassified" or "admixed" and were not assigned to a population. Most of these accessions shared alleles from population 1, population 2, and population 3 in proportions ranging from 0.01% to 0.76%. Thus, in population 1, 21 pure and 16 mixtures, population 2, 14 pure and 14 mixtures, and population 3, 43 pure and 19 mixtures, for a total of 78 pure and 49 mixtures were identified.

DISCUSSION

Nigerien sesame has a high genetic variability that must be taken in to account when planning conservation strategies or when its variability is used in breeding programs. This high level of polymorphism in sesame has already been reported for its morphology (Bedigian *et al*., 1986 and Bisht *et al*., 1998). Amplified Fragment Length Polymorphism (AFLP) has been shown to be a powerful process that uses molecular markers to efficiently detect polymorphisms in plant species (Bensch *et al*., 2005), including Sesamum indicum. As a result, this method allows a better estimation of genetic relationships between closely related individuals. In this study, AFLP analysis detected high levels of polymorphism (83.80%) among 127 sesame accessions collected in Niger. These results are consistent with those of Laurentin & Karlovsky, 2006, who worked on 32 sesame accessions from the Venezuelan germplasm collection, representing genotypes from five centers of diversity (India, Africa, China-Korea Japan, Central Asia and West Asia), where AFLP technique allowed to estimate genetic relationships of genetic material (Sesamum indicum) and to detect a high level of polymorphism, and with those of Pham *et al*. (2011) in their study of comparative analysis of sesame genetic diversity in Vietnam and Cambodia. The combination of primers named E-ACT (FAM)/M-CTA used in this study proved to be very effective in detecting a higher number of fragments. Therefore, this combination of primers is useful for the detection of informative fragments in sesame genetic material and can be recommended in future studies evaluating the diversity of Sesamum indicum. The low level of inter-access diversity observed can be explained at several levels: (i) the self-pollinating mode of reproduction of the species even if the rate of allogamy due to insects is not negligible; (ii) the cultural practice where all the varieties are mixed in the field; (iii) migration and genetic mixing through seed renewal which leads most farmers in the different regions of Niger to renew their seeds at the beginning of wintering on the markets or through trade; (iv) seed flow linked to late sowing carried out voluntarily by farmers in order to be able to take care of the main crops. In case of early cessation of rainfall, the cycle will not be completed, a situation that is quite frequent in most regions of Niger, therefore seeds migrate over long distances; (v) finally, the seeds used in this study come from a sample evaluated at the same site. The combination of all these factors can contribute to the homogenization of sesame in Niger, not only through seed exchange but also through cultural practices coupled with the mode of reproduction of the species. The distribution of genetic diversity of a plant species depends on its evolution and reproductive system, ecological and geographical factor sand often on human activities (Rao & Hodgkin, 2002). Cross-pollination may play a role, with up to 60% in sesame, depending on the presence of suitable insects at flowering time (Bhat *et al*., 1999).

The analysis therefore confirms that there is no relationship between genetic structure and geographical isolation, which reinforces the thesis of a significant migratory flow of seeds across the country. These results are corroborated by those of Laurentin & Karlovsky, (2017) in their study on the genetic diversity of German sesame. These authors found no association between geographical origin and AFLP profiles and report that most of the variation is explained by genetic diversity within regions of origin rather than between regions of origin. In determining the association between geographical origin and genetic diversity by amplified fragment length polymorphism (AFLP) between 32 sesame accessions from the Venezuelan germplasm collection, Laurentin & Karlovsky, (2006) reached the same conclusion. Bayesian statistics applied to the molecular data obtained with the primer pair of an AFLP analysis, structures our sesame accessions into three populations as revealed by the UPGMA analysis, but with a reassignment of individuals with admixture. This confirms Zhivotovsky (1999) and Krauss (2000), who mention that for essentially self-pollinated species, the Bayesian procedure allows a better estimation of the genetic diversity of dominant markers. These results are similar to those reported by Ercan *et al* (2004) on the analysis of genetic diversity in Turkish sesame populations. Several studies based on molecular markers on sesame have also revealed detailed information on its genetic diversity (Bhat *et al*., 1999; Kim *et al*., 2002; Ercan *et al*., 2004; Laurentin & Karlovsky 2006; Salazar *et al*., 2006), which is consistent with the present study.

Conclusion

The combination of EcoRI-ACT/MseI-CTA primers used was effective in differentiating sesame (Sesamum indicum) accessions because it yielded a large number of polymorphic markers. However, the use of more primer pairs (2-3 pairs) could have further improved the robustness of the analysis. AFLP molecular markers, combined with other analytical techniques such as the Bayesian approach, represent valuable and robust tools for identifying structure in populations of less polymorphic species such as sesame. We have shown that, up to a certain limit, the genetic diversity of sesame is not geographically structured in Niger. The different groups obtained are defined more on the basis of certain agro

morphological traits. This shows the reliability of the use of molecular markers and the limit of the use of morphological markers which are subject to the effect of the environment in a work of structuring a species or between species. The heterogeneity of the populations obtained could underline the flow of sesame seeds across the country. However, further studies are needed to assess the consequences of this seed flow and its importance for the adaptation and production of sesame in Niger.

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