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RESEARCH ARTICLE

GENETIC DIVERSITY OF BAMBARA GROUNDNUT ACCESSIONS FROM BURKINA FASO USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

[1,2]N'Golo Moussa KONATE, [2]Hervé NANDKANGRE, [2]Adjima OUOBA, [2]Serge Félicien ZIDA, [2]Mahama OUEDRAOGO, [1]Nerbewende SAWADOGO, [2]Sabine NADEMBEGA, [2]Abdou Kader CONGO and [1]Mahamadou SAWADOGO

[1]Université Ouaga I Professeur Joseph KI-Zerbo, Ecole Doctorale Sciences et Technologies, Laboratoire Biosciences, Equipe Génétique et Amélioration des Plantes, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]Institut de l'Environnement et de Recherches Agricoles (INERA), Département de Productions Végétales, Laboratoire de Génétique et de Biotechnologies Végétales, 04 BP 8645 Ouagadougou, Burkina Faso

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) originated from Africa where it is an important grain legume. The knowledge of genetic variability level within a crop species is an important step towards improving it. The objective of this study was to determine the level and structure of genetic diversity of bambara groundnut from Burkina Faso. In this study, 92 bambara groundnut accessions from three climatic zones of Burkina Faso were characterized with 17 random amplified polymorphic DNA markers. The results revealed a high level of polymorphism (100%) for all loci tested and a total of 161 alleles counted with an average allelic richness of 9.471 alleles per locus. The average expected heterozygosity (0.270) reflected a moderate genetic diversity within the collection. A dendrogram established by the "neighbour joining" method classified the 92 accessions into three mixed clusters. A weak genetic diversity between the clusters was also observed. These results could lead to the development of conservation strategies and the implementation of varietal breeding in Burkina Faso.

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INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a grain legume belonging to the *Leguminosae* family and *Papilionoideae* subfamily (Baudouin and Mergeai, 2001). It is generally cultivated on small land sizes and mainly by women in West Africa, particularly in Burkina Faso (Ouédraogo *et al.*, 2012; Ouoba *et al*., 2016). The grain is highly valued by the local populations and can thrive very well on poor soils compared to other crops such as common beans, cowpea (*Vigna unguiculata* (L.) Walp.) and groundnuts (*Arachis hypogaea*L.). Its seeds are an important source of dietary protein especially for rural populations (Baryeh, 2001; Bamshaiye *et al*., 2011). Bambara groundnut is ranked third among the grain legumes in sub-Saharan Africa, after groundnut and cowpea in terms of production (Siise *et al*., 2012; Touré *et al*., 2013) and second after cowpea at production and consumption levels in Burkina Faso (Ouédraogo *et al*., 2008). Despite the potential of the crop, it has long been on the fringe of the scientific and technical boom that other seed legumes, such as cowpea and groundnut, have benefited (Basu *et al*., 2007). Most part of studies conducted on bambara groundnut were limited to a mass selection of some local accessions based on physiological and agronomic traits (Baudoin and Mergeai, 2001). An important agro-morphological variability within bambara groundnut landraces has been reported by Ntundu *et al*. (2006), Ouédraogo *et al*. (2008) and by Touré *et al*. (2013). However, it does not provide a reliable measure of genetic diversity due to the strong influence of the environment on the various parameters considered. In order to achieve a real improvement of a given crop species, it is fundamental to improve our knowledge on the genetic diversity within this crop (Ndiang *et al*., 2012; Olukolu *et al*., 2012). To this end, molecular markers provide a more powerful tool to accomplish this objective. Molecular markers such as Simple Sequence Repeat (SSR) (Somta *et al*., 2011, Odongo *et al*., 2015, Ouoba *et al.,* 2017),

***Corresponding author:*[1,2]N'Golo Moussa KONATE,**
[1]Université Ouaga I Professeur Joseph KI-Zerbo, Ecole Doctorale Sciences et Technologies, Laboratoire Biosciences, Equipe Génétique et Amélioration des Plantes, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]Institut de l'Environnement et de Recherches Agricoles (INERA), Département de Productions Végétales, Laboratoire de Génétique et de Biotechnologies Végétales, 04 BP 8645 Ouagadougou, Burkina Faso

Amplified Fragment Length Polymorphism (AFLP) (Massawe *et al*., 2002), Random Amplified Polymorphic DNA (RAPD) (Amadou *et al*., 2001; Massawe *et al*., 2003) were used to assess polymorphism within bambara groundnut. In order to estimate the level of genetic diversity in bambara groundnut produced in Burkina Faso our choice focused on the RAPD marker technique described by Williams *et al*. (1990). It is a PCR based method in which genomic DNA amplification is carried out from random primers sequences (10 pb approximately) generally dominant and used alone or by couple. The RAPD method has been used by many authors to assess the genetic diversity within crops such as bambara groundnut (Amadou *et al.,* 2001; Massawe *et al*., 2003), cowpea (Ba *et al*., 2004; Malviya *et al*., 2012, common bean (Tiwari *et al*., 2005) and soybean (Xu *etal*., 2003). The use of RAPD markers provides many advantages such as ease and speed of analysis, relatively low cost and very low DNA requirement for analysis (Weising *et al*., 1995). The objective of this study was to assess the level and structure of genetic variability among a collection of bambara groundnut accessions from Burkina Faso using RAPD markers.

MATERIAL AND METHODS

Plant material: A total of 92 bambara groundnut accessions were used in this study. Eighty-six accessions were collected from the three agro ecological zones of Burkina Faso (18 from the Sahelian zone, 50 from the Sudan-Sahelian zone and 18 from the Sudanian zone) and six were acquired from the Institute of Environment and Agricultural Research (INERA) germplasm (Ouoba *et al*., 2017).

Genomic DNA extraction: For DNA extraction, one seed of each accessions were sowed in seedling plates containing 20g of industrial compost in the laboratory culture room at INERA/Kamboinsé, Burkina Faso. Leaves of young plants (10 to 21 days) were sampled from one plant per accession for DNA isolation. Genomic DNA was extracted from young leaves of about two weeks of age using What man Flinders Technology Associates (FTA) cards. For each accession, a leaf was excised and then deposited on the FTA card at the corresponding number. Each leaf was pressed with a pestle through a parafilm. The fingerprint obtained on the FTA card was dried at room temperature and then placed in a desiccator. A puncher (Harrison) of 1mm diameter was used to sample discs on the FTA card. Each sampled disc was placed in separate Eppendorf tube and washed twice in 200 µL of 70% alcohol for 5 minutes and then rinsed twice with 200 µL of TE (Tris-EDTA) for 5 minutes as well. The tubes containing the disc were then dried for 24 hours and transferred into PCR tubes for amplification.

PCR amplification: The random primers used for DNA amplification were 10bp A, B, L, O, P and AI sequence kits (Operon Technologies Inc., Alameda, USA) (Table 1). The amplification of the genomic DNA was carried out in a final volume of 20 µl containing PreMix (Bioneer Corp., Republic of Korea) composed of Taq Polymerase (2.5 U), dNTPs (250 ML), Tris-HCl pH 9 (10 mM), KCl (30 mM), MgCl (1.5 mM), 2 µl primer (forward-reverse) and one disc of FTA card containing the genomic DNA. The amplification was performed as follow: 50 s at 90 °C followed by 40 cycles of 10 s at 93 °C, 10 s at 38 °C and 1 min at 72 °C and a final elongation at 72 °C for 2 min. Electrophoresis was been performed with the PCR products using 2% agarose gel and Ethidium Bromide staining on a tension of 100 V for 90 min in a buffer Tris Borate EDTA 0.5x (TBE).

Data analysis: The electrophoretics bands were identified on the basis of their position on the gel. The presence (1) and the absence (0) of the bands were recorded and used for statistical analysis. Data obtained were processed with Gen Alex software to determine the following genetic parameters for each tested loci: the total number of alleles (At), the effective number of alleles (Ae), the rate of polymorphism, the expected heterozygosity (He) and the polymorphic information content (PIC).

Structure of the genetic diversity was given by the dendrogram obtained with the "neighbour joining method" using DARwin 5.0.158 software version (Perrier and Jacquemoud-Collet, 2006)

RESULTS

Genetic diversity within accessions: All the RAPD markers used have all amplified DNA fragments (Figure 1). A total of 161 alleles were obtained with a 100% polymorphism rate for each marker (Table 1). The number of total alleles per marker (At) varied from seven (OPA 07; OPB 10; OPL 08) to 15 (OPL 12) with an average of 9.471 loci per marker. The number of effective alleles (Ae) varied from 4.533 (OPL 08) to 12.797 (OPL 12). The genetic diversity within the total population studied ranged from 0.129 to 0.400, respectively for OPB 08 and OPL 12, with an average of 0.27. The polymorphic information content (PIC) varied from 0.128 (OPB 08) to 0.396 (OPL 12) with an average of 0.267.

Genetic diversity according to collection areas: Genetic diversity parameters of bambara groundnut accessions according to their collection zones revealed that accessions from Sudan-Sahelian zone showed the greatest value of genetic diversity parameters (Table 2). They totalled 156 alleles with an average of 6.803 effective alleles per locus. The polymorphism rate and expected heterozygosity were 96.89% and 0.271, respectively. The lowest values were observed for accessions from the INERA germplasm. They gave a total of 78 alleles with an average of 6.079 effective alleles and a low polymorphism rate of 48.45%.

Organization of the diversity within 92 bambara groundnut accessions: The construction of the dendrogram by the "Neighbour-Joining" method distributed the 92 accessions of bambara groundnut into three genetic groups (Figure 2). These genetics parameters results were recorded in Table 3. Cluster I is composed by 43 accessions coming from all the three climatic zones. Accessions of this group have a total of 152 alleles and a polymorphism rate of 94.41% with an expected heterozygosity of 0.275 and an effective number of alleles of 6.865 per locus.

The cluster II comprised 44 accessions and as cluster I it is composed by accession from all the climatic zones of Burkina Faso. This cluster contained 148 alleles for 6.650 effective alleles per locus and a polymorphism rate of 91.93% for an expected heterozygosity of 0.255. Cluster III had the smallest number of accessions (5) coming from the Sahelian zone (2) and the Sudan-Sahelian zone (3) with a total of 89 alleles with 6.339 effective alleles per locus. The rate of polymorphism and the expected heterozygosity are respectively 54.66% and 0.255.

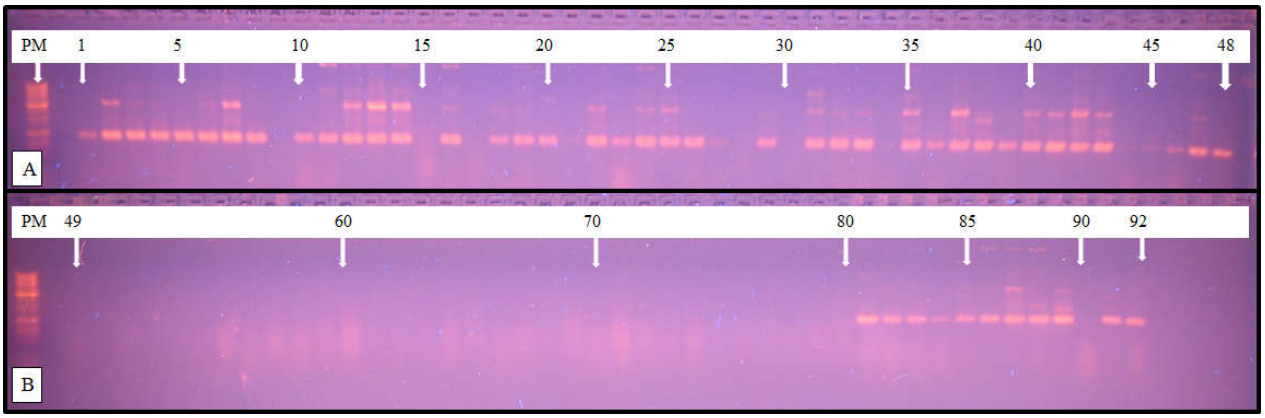

Figure 1: Electrophoresis profile of 92 bambara groundnut accessions obtained with RAPD marker OPA 07

A: Electrophoresis profile obtained for sample 1 to 48 with marker OPA 07;
B: Electrophoresis profile obtained for sample 49 to 92 with marker OPA 07.

Table 1. Genetic diversity parameters of 92 accessions revealed by 17 RAPD markers

Primers	Sequences 5'3'	At	Ae	He	PIC
OPA 07	GAAACGGGTG	7	4.691	0.221	0.218
OPA 08	GTGACGTAGG	10	6.672	0.229	0.226
OPB 08	GTCCACACGG	9	5.224	0.129	0.128
OPB 10	CTGCTGGGAC	7	4.845	0.223	0.221
OPL 08	AGCAGGTGGA	7	4.533	0.210	0.208
OPL 12	GGGCGGTACT	15	12.797	0.400	0.396
OPO 04	AAGTCCGCTC	9	6.719	0.320	0.316
OPO 11	GACAGGAGGT	9	7.393	0.377	0.373
OPO 12	CAGTGCTGTG	10	7.962	0.342	0.338
OPP 04	GTGTCTCAGG	9	6.206	0.228	0.226
OPP 08	ACATCGCCCA	9	6.266	0.252	0.250
OPP 11	AACGCGTCGG	13	7.742	0.158	0.156
OPP 13	GGAGTGCCTC	8	6.466	0.345	0.342
OPP 15	GGAAGCCAAC	10	6.873	0.261	0.258
OPP 19	GGGAAGGACA	10	5.899	0.147	0.145
OPAI 11	ACGGCGATGA	11	8.829	0.357	0.353
OPAI 15	GACACAGCCC	8	6.586	0.383	0.378
Total		161	-	-	-
Mean		9.471	6.806	0.270	0.267

At = Number of total alleles, Ae = Number of effective alleles, He = expected Heterozygosity or genetic diversity, PIC = Polymorphism Information Content.

Table 2. Genetic diversity parameters within collection areas

Climatic zones	N	At	Ae	P (%)	He	PIC
INERA	6	78	6.079	48.45	0.206	0.169
Sahelian. zone	18	137	6.744	85.09	0.272	0.257
Sudan-Sahelian zone	50	156	6.803	96.89	0.271	0.265
Sudanian zone	18	140	6.751	86.96	0.274	0.259

N = Number of accessions, P = Polymorphism rate, At = Number of total alleles, Ae = Number of effective alleles, He = expected Heterozygosity or genetic diversity, PIC = Polymorphism Information Content

Table 3. Genetic diversity parameters of the three groups

Clusters	N	At	Ae	P (%)	He	PIC
Cluster I	43	152	6.865	94.41	0.275	0.269
Cluster II	44	148	6.650	91.93	0.255	0.249
Cluster III	5	89	6.339	54.66	0.255	0.204

N = Number of accessions, P = Polymorphism rate, At = Number of total alleles, Ae = Number of effective alleles, He = expected Heterozygosity or genetic diversity, PIC = Polymorphism Information Content

DISCUSSION

RAPD technic have been used by many authors in order to estimate the variability among many species of plants such as cowpea, common bean, groundnut, bambara groundnut, soybean, coffee.

Genetic diversity study using RAPD markers to assess the variability of bambara groundnut cultivated is the first attempt in Burkina Faso. All the 17 RAPD markers A, B, L, O, P and AI used in this study for the genotyping of 92 bambara groundnut accessions amplified DNA fragments. This result confirms those obtained by Amadou *et al*. (2001), who found

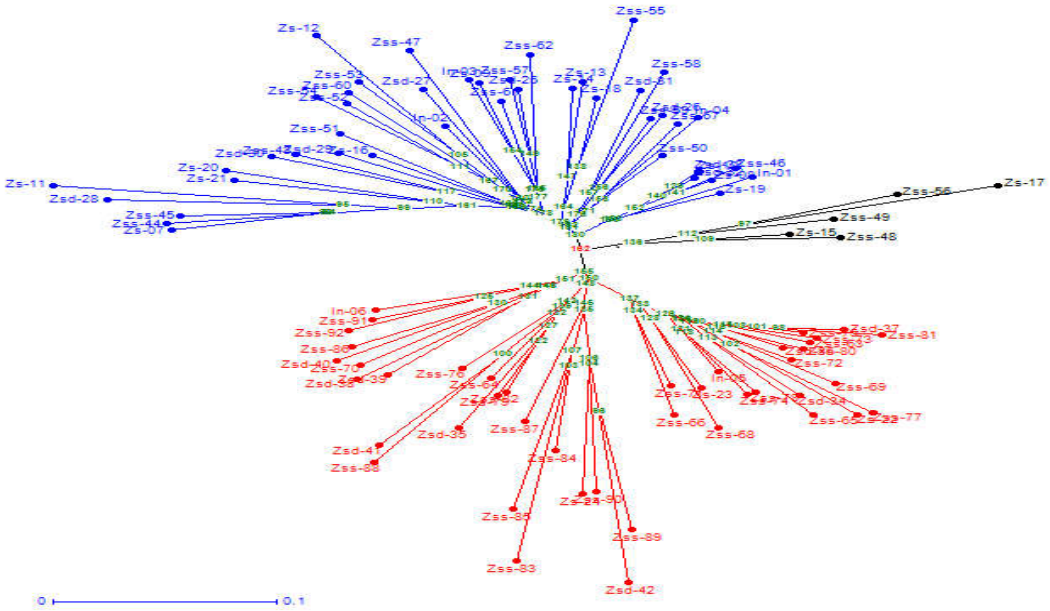

Figure 2: Neighbour-Joining tree based on allelic data from 17 RAPD loci among 92 bambara groundnut landraces from Burkina Faso.

Cluster I in red*, *Cluster II in blue*, *Cluster III in black

that the same markers give a better amplification in comparison to 50 others tested. Moreover, the 161 alleles with an average value of 9.471 alleles per locus obtained in this study are higher than those obtained by Amadou *et al.* (2001), Massawe *et al*. (2002) and Ouoba *et al*. (2017). Indeed, Amadou *et al*. (2001) recorded on 25 accessions subjected to the same primers, 63 alleles for an average value of 3.706. Massawe *et al*. (2002), obtained 72 alleles in total with seven AFLP markers on a population of 16 individuals. As for Ouoba *et al*. (2017), they had found a total of 49 alleles with an average value of 4.9 alleles per locus with the same bambara groundnut accessions evaluated this time with 10 SSR markers. The low values obtained by these authors could result in the reduced size of their samples on one hand and on the other by the difference of markers used. However, the genotyping performed by Massawe *et al*. (2003) with 16 RAPD markers detected 222 alleles with an average of 13.9 alleles per locus with 12 bambara groundnut accessions. Difference of results obtained here could be explained by the difference between the RAPD markers used (sequence kits C, D, AA, Operon Technologies Inc., Alameda, USA) and also by the diversified origin and the great geographical distance between the samples tested. Similarly, Ntundu *et al*. (2004) with AFLP markers on a population of 100 individuals and Odongo *et al*. (2015) using SSR markers on a population of 105 individuals found an allele number greater than ours respectively, 364 total alleles for an average of 31.5 per locus and 958 total alleles for an average value of 79.83. The higher values obtained by these authors could be explained in the large sample size tested, the difference in molecular markers tested and the diversified origin of the accessions used. All the RAPD markers tested in this study were polymorphic and gave a 100% rate of polymorphism. This maximum value of polymorphism rate reflects the important level of polymorphism of the accessions studied and the effectiveness of the markers used. This fully justifies the choice of RAPD markers as a tool for evaluating genetic diversity within bambara groundnut accessions.

These values are similar to those obtained by Ouoba *et al*. (2017) with SSR markers. The values obtained in our study are higher than those obtained by Amadou *et al*. (2001) as well as Massawe *et al*. (2003), which obtained respectively an average value of 60% and 73.1%. The high level of polymorphism in our accession collection compared to those observed among these authors may be due to the geographical position of Burkina Faso, which is a crossroads between Mali, Niger, Ivory Coast and Ghana. This position would support exchanges of accessions with producers in the various neighbouring countries. The average value of the expected heterozygosity of 0.270 calculated here indicate a low genetic diversity among this collection. Indeed, bambara groundnut reproduces in the autogamous manner. Fecundation of the autogamous plants is done within the same flower including at the same time male and female's organs. The crossing between the varieties is thus limited and consequently the genetic diversity.

Moreover, the moderate genetic diversity of the bambara groundnut accessions from Burkina Faso could be the result of farmers' management practices of the seeds like exchange between farmers and selection of varieties for a particular agronomic trait. These results recorded here were closed to those obtained by Odongo *et al*. (2015), Ouoba *et al.* (2017) who founded respectively an average value of 0.345 and 0.307 for this parameter. The analysis of 92 accessions genetic diversity with 17 RAPD markers allowed distinguished three genetic clusters by the "neighbour joining" method. Bambara groundnut accessions which composed each genetic cluster formed a mixed group with accessions from different climatic zones. Similar observations were made by Ouoba *et al*. (2017). Accessions within the same cluster probably have the same origin. Accessions from INERA germplasm were found in cluster I (2) and II (4) which were mainly made up by accessions from Sudan-Sahelian zone. Sudan-Sahelian zone could be the first collected area of these accessions. The mixed distribution of accessions coming from different climatic zones

within each cluster suggest that the genetic diversity does not depend on the geographical location of the accessions. Mixing accessions from different collection areas within different clusters could be due to the movement of producers within different climatic zones, thus promoting seed exchange between producers. The moderate genetic diversity between the different groups obtained in this study could suggest that accessions from the same cluster may have the same origin. In conclusion the use of RAPD markers enabled the molecular scale to establish a genetic variability among the collection of bambara groundnut accessions from the three climatic zones of Burkina Faso. The 17 molecular markers showed an important rate of polymorphism and allowed to classify the accessions in three groups from the dissimilarity matrix according to the "neighbour joining" method. They also revealed a moderate genetic diversity between the different clusters formed by mixed accessions from different climatic zones. RAPD molecular markers are therefore an important tool for estimating the genetic diversity of bambara groundnut. These results could be exploited in order to set up an important database for the Bambara groundnut varietal breeding in Burkina Faso.

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