Agricultural Science Research Journal Vol. 7(3): 96 – 102, March 2017
Available online at http://resjournals.com/journals/agricultural-science-research-journal.html
ISSN: 2026 –6073 ©2017 International Research Journals

Full Length Research Paper

Assessment of genetic diversity in Bambara groundnut (*Vigna subterranea (L.)* Verdcourt) landraces in Burkina Faso using microsatellite markers (SSR)

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Abstract

Bambara groundnut is an important source of protein to the rural majority in sub-Saharan Africa. However, small farmers grow locally adapted landraces which are generally low yielding. Adequate knowledge of variability within Bambara groundnut germplasm collections is crucial for crop improvement and has important implications for conservation, management and future usefulness of germplasm resources. The objective of this study was to assess the genetic diversity of Bambara groundnut cultivated in Burkina Faso. A total of 92 Bambara groundnut accessions mainly collected in the three agro-climatic zones of Burkina Faso were estimated using 10 SSR markers. For all the loci analyzed, results showed a low average value of gene diversity and polymorphic information content of 0.307 and 0.298 respectively and a low average number of 4.9 alleles per locus. A Neighbour-joining dendrogram based on the dissimilarity matrix clustered the 92 landraces into two major mixed groups supported by accessions from the three agro-ecological zones. Thus, the study gave the genetic diversity profile of the collection which can be used for selection of appropriate parental genotypes for breeding program.

Key words: *Vigna subterranea,* accessions, gene diversity, dissimilarity matrix analysis.

Introduction

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is originated from the african continent. The exact area of origin of the crop is in the region of northeastern Nigeria and northern Cameroon. This hypothesis is supported by the distribution of wild Bambara groundnut which is known to extend from Jos Plateau and Yola in Nigeria, to Garoua in Cameroon (Goli, 1997). Although occasionally grown in Asia and elsewhere, its cultivation is rare outside the African continent (Hillocks et al., 2012).

Its seed highly nutritious (63% of carbohydrate, 19% of protein and 6.5% of oil) making it a good supplement to diets of a large part of the population, particularly to poorer people who cannot afford expensive animal protein (Ouédraogo et al., 2008; Bamshaiye et al., 2011). The crop is widely adapted to all agro ecological areas of Burkina Faso including Sahelian, Sudan-sahelian and

Sudanian zones. It is the second most food legume after cowpea (*Vigna unguculata*) and the main food for some communities in western part of the country (Nadembèga, 2016). Burkina Faso is one of the main producers of the crop with Mali, Niger, Tchad, Ivory Coast, Ghana and Nigeria (CIRAD-GRET, 2002, Ouédraogo et al., 2012). In 2014, the crop production was estimated around 50 000 tonnes for Burkina Faso (www.fao.org/faostat/en/#data/QC).

In most producing countries including Burkina Faso, the great majority of the production is consumed locally. Despite the importance of the crop as a food legume in the country, Bambara groundnut production is limited on small scale (Ouoba et al., 2016).

The crop genetic diversity at global scale has been studied by using different markers including nuclear DNA markers (Amadou et al., 2001; Ntundu et al., 2004; Basu et al., 2007; Ouédraogo et al., 2008; Touré et al., 2013; Odongo et al., 2015). However, relatively very few studies have been conducted using specially SSR markers to assess Bambara groundnut genetic diversity. Nevertheless, these studies showed that SSR markers can be effectively employed to assess the genetic diversity of Bambara groundnut (Basu et al., 2007; Molosiwa, 2012; Odongo et al., 2015). Moreover, in Burkina Faso the work undertaken on Bambara groundnut landraces variability were focused only on agromorphological characterization (Ouédraogo et al., 2008; Déné, 2011; Ouoba, 2012). Also, no study on the genetic diversity of Bambara groundnut in Burkina Faso using SSR markers was reported.

The SSR markers tend to be largely used today in plants improvement strategies for their efficiency in diversity assessment. The objective of this study is to assess the genetic diversity in Bambara groundnut landraces in Burkina Faso using microsatellite markers (SSR).

Material and methods

Plant material

The plant material consisted of 92 Bambara groundnut accessions. The great part of them (86 accessions) were collected from farmers (March to April, 2014) in different sites situated in the three agro-ecological zones (sahelian, sudan-sahelian and sudanian zones) of Burkina Faso. Few of them (six accessions) were obtained from the gene bank of Environment and Agricultural Researches Institute of Burkina Faso (INERA). The study was carried out at the Environmental and Agricultural Researches and Training Centre in Burkina Faso.

Methods

DNA isolation

One seed of each accession was sown in an hole (4 cm diameter) of plastic planting plate of 72 holes containing industrial substrate NFU 44-551 with pH 6.5 (Jardinova Company, France) as a planting medium. Leaves were harvested from 10 to 21 days old plants and used for DNA isolation. Genomic DNA was extracted following the Flinders Technology Associates (FTA) plant cards method as described by Borman et al. (2008). Plant DNA was collected on the FTA card by placing the leaf over the card, overlaying it with plastic film and crushing the tissue with a hard object and the paper was air-dried for 24 h at room temperature. Using a Harris Micro Punch instrument, disks of 2-mm in diameter were punched out of FTA matrix impregnated with plant material. The sample disks were placed in separate Eppendorf tubes and were washed with FTA purification reagent. The washed disks were air-dried for 1 h at room temperature and stored at –20°C.

Molecular analysis

Ten microsatellite primers selected for their high polymorphism (Molosiwa, 2012; Odongo et al., 2015) (Table 1) were used to assess the genetic diversity of 92 Bambara groundnut accessions. The PCR amplification was performed in 20 µl of PreMix (Bioneer corp, Republic of Korea), 2 µl of each primer (1 µM) (Integrated DNA Technologies,) (foward-reverse) and one disk (2mm) of FTA card containing genomic DNA in BIO-RAD thermocycler (BioRad MyCycler PCR System, Texas, United States). The PCR conditions included an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54-59.7°C (depending on the primer) for 1 min, extension at 72°C for 1 min followed by one cycle of final extension at 72°C for 10 min, cooling at 10 °C. The PCR product were separated on agarose gel (2%) (Norgen Biotek Corp, Canada) stained with ethidium bromide (Norgen Biotek Corp, Canada) under a tension of 100 V during 1h 30 min in a 0.5x TBE (Tris-Borate-EDTA) buffer. The separated amplicons were visualized on a gel visualization system. The alleles were scored as absent (0) or present (1) based on the size of the amplified product using a 25 bp ready to use DNA Ladder (Norgen Biotek Corp, Canada).

Table 1: List of 92 Bambara groundnut accessions used in the study and their origin

Accessions N°	Number	Origin
In-1; In-2; In-3; In-4; In-5; In-6	06	INERA Genebank(In)
Zs-7; Zs-8; Zs-9; Zs-10; Zs-11; Zs-12; Zs-13; Zs-14; Zs-15; Zs-16 ;Zs-17; Zs-18; Zs-19; Zs-20; Zs-21; Zs-22; Zs-23; Zs-24	18	Sahelian zone (Zs)
Zsd-25; Zsd-26; Zsd-27; Zsd-28; Zsd-29; Zsd-30; Zsd-31; Zsd-32; Zsd-33; Zsd-34; Zsd-35; Zsd-36; Zsd-37; Zsd-38; Zsd-39; Zsd-40; Zsd-41; Zsd-42	18	Sudan zone (Zsd)
Zss-55; Zss-56; Zss-57; Zss-58; Zss-59; Zss-60; Zss-61; Zss-62; ;Zss-63;Zss-64; Zss-65; Zss-66; Zss-67;Zss-68; Zss-69; Zss-70; Zss-71; ,Zss-72; Zss-73; Zss-74; Zss-75; Zss-76; Zss-77; Zss-78; Zss-79; Zss-80; Zss-81; Zss-82; Zss-83; Zss-84;Zss-85; Zss-86; Zss-87; Zss-88; Zss-89; Zss-90; Zss-91; Zss-92	50	Sudan Sahelian zone (Zss)

Table 2: Primer information of ten SSR markers used for amplification of DNA isolated from 92 accessions of Bambara groundnut germplasm

Markers	Forward (F) and reverse (R) primer sequences (5'-3')	Annealing temperature (°C)	Alleles size (bp)
Primer 3	F : TTCACCTGAACCCCTTAACC R : AGGCTTCACTCACGGGTATG	57.6	247
Primer 4	F : ACGCTTCTTCCCTCATCAGA R : TATGAATCCAGTGCGTGTGA	57.6	197
Primer 5	F : TCAGTGCTTCAACCATCAGC R : GACCAAACCATTGCCAAACT	55.3	260
Primer 6	F : CCGGAACAGAAAACAACAAC R : CGTCGATGACAAAGAGCTTG	57.6	189
Primer 7	F : TGTGGGCGAAAATACACAAA R : TCGTCGAATACCTGACTCATTG	59.7	198
Primer 8	F : CAAACTCCACTCCACAAGCA R : CCAACGACTTGTAAGCCTCA	57.6	250
G358B2-D15	F : TGACGGAGGCTTAATAGATTTTTC R :GACTAGACACTTCAACAGCCAATG	59.0	193
mBam2co80F	F : GAGTCCAATAACTGCTCCCGTTTG R : ACGGCAAGCCCTAACTCTTCATTT	59.0	220
G180B2-D11	F : GAGGAAATAACCAAACAAACC R : CTTACGCTCATTTTAACCAGACCT	59.0	198
G358B3-D15	F : TGACGGAGGCTTAATAGATTTTTC R : GACTAGACACTTCAACAGCCAATG	59.0	196

Data analysis

Molecular data was recorded in binary fashion for SSR marker loci analyzed and scoring was based on presence and absence of band for each primer set with one (1) and zero (0) being the respective scores. Only reproducible bands were scored. The summary statistics on total allele's number (Na), number of effective alleles (Ne), expected heterozygosity or gene diversity (He) and polymorphic information content (PIC) were performed using GenALEX version 6.41.

Genetic dissimilarity between all the accessions was calculated with DARwin 6.0.4 (Perrier and Jacquemoud-Collet, 2006) using simple matching coefficient. The dissimilarity coefficients were then used to generate an unweighted neighbor-joining tree with Jaccard's Similarity Coefficient with a bootstrapping value of 1,000 using the same software (DARwin 6.0.4). Genetic differentiation based on Fst was calculated between the clusters using Genetix 4.04 (Weir et Cockerham, 1984).

Results

Polymorphism

The analysis of ten SSR loci revealed a total of 49 alleles across the 92 Bambara groundnut accessions and all of them were polymorphic (Table 3). The number of alleles (Na) per primer pair varied from 2 (primer 7FR) to 7 (primers 3FR and 5FR) alleles with an average value of 4.9. Expected heterozygosity (He) ranged from 0.176 (primer B2D15FR) to 0.440 (primer 4FR) with an average of 0.307 per primer. Polymorphic information content (PIC) ranged from 0.174 (primer B2D15FR) to 0.435 (primer 4FR) with an average of 0.298 per primer.

Table 3: Estimate of genetic diversity of Bambara groundnut germplasm using ten SSR markers

Markers	Na	Ne	He	PIC
Primer 3FR	7	5.335	0.331	0.328
Primer 4FR	4	3.622	0.440	0.435
Primer 5FR	7	4.986	0.276	0.273
Primer 6FR	6	4.175	0.231	0.229
Primer 7FR	2	1.698	0.411	0.406
Primer 8FR	4	2.982	0.337	0.278
G180B2-D11FR	4	2.977	0.265	0.262
B2D15FR	5	3.129	0.176	0.174
OAB3D15FR	4	3.006	0.303	0.299
mBam2co80FR	6	4.357	0.299	0.296
TOTAL	49	36.267	3.069	2.98
MEAN	4.9	3.627	0.307	0.298

Na = number of alleles, Ne= number of effective alleles, He = Expected heterozygosity or Nei's genetic diversity index, PIC = polymorphic information content.

Genetic diversity

The dendrogram based on Neighbour-joinning methods partitioned the 92 landraces into four clusters (Figure 1). The genetic diversity parameters of each cluster are shown in Table 4. Each cluster was composed of a mixture of accessions from different origins. The four groups define the highest level of genetic divergence of the set of accessions. Genetic differentiation indexes (Fst) between the groups showed significant difference between the four genetic groups (Table 5). All the clusters had sub-clusters.

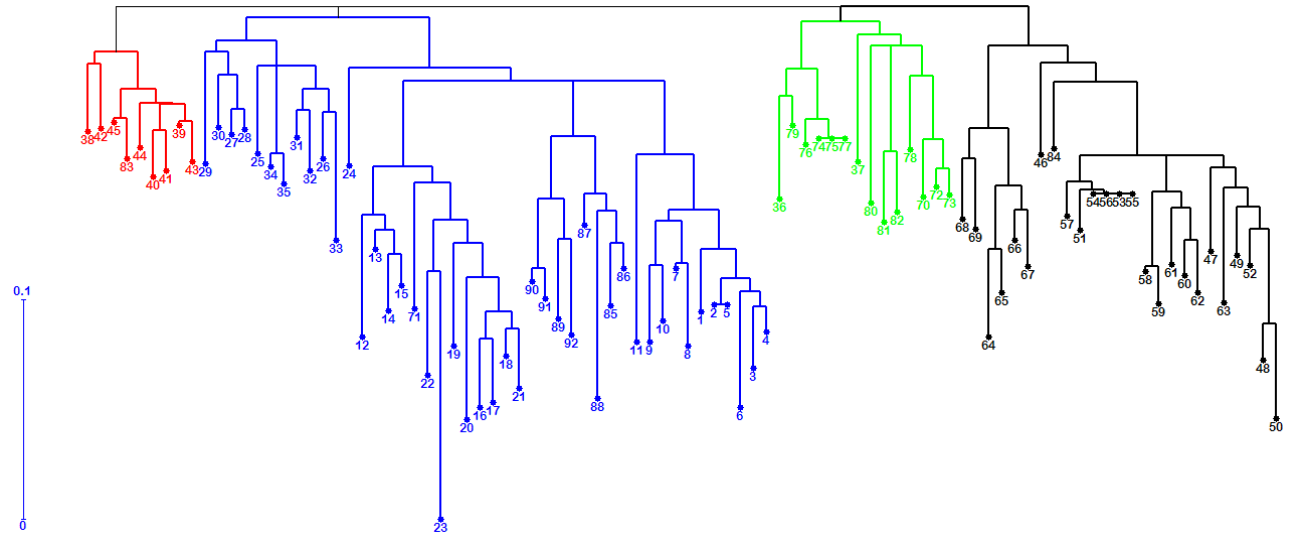

Figure 1: Neighbour-joinning (DARwin version 6.0.4) tree based on allelic data from ten SSR loci among 92 Bambara groundnut accessions from Burkina Faso.
Cluster given in red was cluster I, Blue cluster II , green cluster III and black cluster IV

Table 4: Diversity parameters of the four genetic groups estimated on GenALEX version 6.41

Clusters	N	Na	Ne	P (0,95)	He
	92	49	-	-	-
Cluster1	9	18	1.157	26.53%	0.114
Cluster2	44	35	1.337	71.43%	0.219
Cluster3	14	36	1.397	73.47%	0.247
Cluster4	25	42	1.425	85.71%	0.261

Table 5: Genetic differentiation (Fst) of four groups, estimated using Weir and Cockerham (1984) on Genetix version 4.04

Clusters	Differentiation index (Fst)		
	Cluster1	Cluster2	Cluster 3
Cluster2	0.25*	0	-
Cluster3	0.35**	0.16*	-
Cluster4	0.37**	0.20*	0.27**

*(high differentiation); ** (Very high differentiation)

The cluster I was the smallest in terms of number of accessions with a certain genetic proximity. It consisted of nine accessions including one from the sahelian zone, six from the Sudanian zone, and two from the Sudan-Sahelian zone. The parameters of genetic diversity within the accessions of this group yielded 18 alleles in total and 26.53% for polymorphism rate. Expected heterozygosity and effective alleles per locus were respectively 0.114 and 1.57. The parameters for this group were the lowest indicating a low variability of the accessions that composed it.

The cluster II had the highest number of accessions (44) with high diversity parameters. In fact, this group displayed 71.43% of polymorphism, a total allele's number of 35 and an effective allele number of 1.337 per locus for an expected heterozygosity of 0.219. It included five accessions from INERA, 12 from Sahelian zone, five from Sudanian zone and 22 from Sudan-Sahelian zone. It was subdivided into sub-groups reflecting a high level of variability within this group. This would explain the high values of the genetic parameters observed for this group.

The cluster III consisted of 14 accessions including two from Sahelian zone, three from Sudanian zone and nine from Sudan-Sahelian zone. It was organized into subgroups and had higher genetic parameters than group II, even though the number of its accessions was three less than that of cluster II. Despite its lower number of accessions, this group had a high potential genetic diversity.

The cluster IV was the most genetically diversified. Indeed, it gave the highest genetic parameters with a number of alleles of 42, a polymorphism rate of 85.71%, a number of effective alleles of 1.57 per locus and an expected heterozygosity of 0.114.

Discussion

Microsatellite markers were developed and used to evaluate genetic diversity of several leguminous plant species such as cowpea (*Vigna unguiculata*), common bean (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea* L.) and in particular Bambara groundnut (*Vigna subterranea*) (Diouf and Hilu, 2005; Masi et al., 2003; Basu et al., 2007; Molosiwa, 2012; Odongo et al., 2015, Gaikpa et al., 2015). In Burkina Faso, to our knowledge, it is the first time that SSR markers are used to evaluate the genetic variability of Bambara groundnut cultivated in this country.

The 10 microsatellite markers used here showed polymorphism among the set of 92 Bambara groundnut accessions. According to Shete et al. (2000), the heterozygosity and the polymorphism information content (PIC) are two distinct values which can be employed to determine the level of polymorphism in markers. According to Botstein et al. (1980), markers with polymorphic information content above 0.5 are considered highly informative. The average PIC of 0,298 found among the 10 markers in the present study proves that the majority of markers were slightly informative in this germplasm.

The average value of PIC (0.298) for this study was close to that found by Odongo et al. (2015) which was 0.280. On the contrary, this value is very lower than that of Molosiwa (2012) who found an average value of PIC of 0.670 using 20 markers SSR within 105 Bambara groundnut accessions. The very high value of the PIC obtained by this author could be explained by the large number of SSR primers selected for their high polymorphism after a screening of 75 SSRs

The number of alleles revealed by the 10 SSRs within the 92 accessions which was of 49 with an allelic richness of 4.9 was lower than that of Ntundu et al. (2004) and Amadou et al. (2001). Indeed Ntundu et al. (2004) had detected 346 alleles with an allelic richness of 31.5 within 100 Bambara groundnut accessions using 11 AFLP markers. Amadou et al. (2001) obtained a total of 63 alleles with an allelic richness of 3.7 with 25 accessions using 17 RAPD markers.

Several reasons could explain the discrepancies observed on the allele's number and allelic diversity as compared to the present study. First, the size of the population used could be one of the reasons. Indeed the number of accessions (92) used for the present study was higher than that of Amadou et al. (2001) (25 accessions) but lower than that of Ntundu et al. (2004) (100 accessions). According to Kalinowski (2004) large samples usually contain more alleles than small samples. In addition the number and the type of markers used could explain the difference observed in the allelic diversity. These authors used respectively FLP markers (17) and RAPD markers (11 markers). Microsatellite markers compared with others markers has many advantages: they are specific, highly polymorphic, co-dominant inherited, multi-allelic, reproducible and reliable (Missihoun et al., 2015; Li et al., 2015).

Expected heterozygosity which gave for all accessions an average of 0.307, was relatively low and indicates a moderate variability within accessions. This value was close to that of Odongo et al., 2015 who found for this parameter an average of 0.345. However this value was lower than that found in common bean (*Phaseolus vulgaris*) by Blair et al. (2009) who observed an expected heterozygosity of 0.64 among 604 genotypes analyzed using a set of 36 SSR markers. The size of the population, the number of markers and the type of species could explain differences observed. The value relatively low of expected heterozygosity calculated in our study, indicates a moderate variability within accessions.

The structuration of 92 accessions diversity into four genetically distinct groups, obtained from the dendrogram with Neighbor-Joining method gave the links existing between the accessions. The accessions have been grouped independently from their origins and formed composite groups. The mixture of accessions in all the clusters made them more heterogenic groups. The grouping of the genotypes of these accessions into sub-clusters in each cluster indicated substantial level of intra-landrace polymorphism.

Gathering of the accessions independently of their origins was also observed with several accessions (105) from different localities in Kenya (Odongo et al., 2015). According to these authors, this is explained by the existence of a very high flow of accessions following the exchange of seeds between farmers from different geographically close zones. The autogamous mode of reproduction of the plant could be one of the reasons that favored the seeds exchange between farmers.

All the accessions (excepted one) from INERA were gathered in cluster II where the majority of accessions from Sudan-sahelian zone are located. The Sudan-sahelian zone would be the origin of accessions from INERA. The works of Amadou et al. (2001) and of Ntundu et al. (2004), have shown that accessions collected in isolated localities or very distant from each other tend to group together according to their origins. This may be attributed to the fact that movement of plant germplasm between isolated localities may have been limited, which may have created isolation from the rest of Bambara groundnut accessions (Ntundu et al., 2004).

Conclusion

The extent of genetic diversity within and among 92 Bambara groundnut accessions mainly collected in the three agro-ecological zones of Burkina Faso has been investigated using 10 SSR markers. All markers showed relatively low polymorphism within the set of 92 Bambara groundnut accessions. The genetic diversity of Bambara groundnut evaluated in this study is relatively low among the accessions and moderate within them. These landraces constitute an important source of genetic variability to exploit. The application of cluster analysis revealed that Bambara groundnut accessions mainly grouped into four mixed and composites clusters. The genetic diversity profile of the collection given here can be

used for selection of appropriate parental genotypes for breeding program. The selection for genetic improvement in Bambara groundnut depends on the availability of genotypes having a favorable allele for good agronomic traits, which depends on the available genetic diversity. Thus, it is crucial to investigate about a possible genetic relationship between morphological and molecular traits.

Acknowledgements

The authors are grateful to Mcknight Foundation for funding this research work through the Mcknight 14-152 project. The farmers also are highly acknowledged for providing major part of accessions used in this study.

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