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Molecular Characterization Using Microsatellites of Bambara Nut (*Vigna subterranea* [L] Verdcourt) Landraces Cultivated in Burkina Faso

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Abstract

Voandzou is a seed legume cultivated in Burkina Faso with significant nutritional potential. The objective of this study was to assess the genetic diversity of Bambara nut cultivated in Burkina Faso using microsatellite markers. For the study, fifteen SSRs markers were used for molecular characterization of 90 Bambara nut landraces from three agro-climatic zones of Burkina Faso. All markers were 100% polymorphic with an average value of 4.81 for effective alleles. The polymorphism information content (PIC) ranged from 0.654 to 0.867 with a mean of 0.775. Dendrogram classified the accessions in four mixed groups independently of the three agro-climatic zones. This distribution is consistent with the results on the agro-morphological characterization of the same landraces. This information was served as a basic model for breeding and conservation programs of *V. subterranea* in Burkina Faso.

Keywords

Bambara Nut, Landraces, Genetics Diversity, SSR Markers

1. Introduction

Bambara nut (*Vigna subterranea* [L] Verdcourt) is one of the most important

legumes in Burkina Faso [1]. Local population uses it as food and medicine for diseases treatment [2]. Bambara nut seeds are rich in many components such as protein, carbohydrate [3] and have high antioxidant activity [4]. The plant has a strong ability to adapt to poorly watered and not very fertile soils thanks to drought tolerance and its capacity to fix atmospheric nitrogen [5]. In Burkina Faso, Bambara nut has an economical importance for the producers and the traders as cash crop and generate substantial incomes for households [6]. Despite its importance country wide, there is a lack of high yielding cultivars to increase Bambara nut production and productivity. Improvement of this species will be of great benefit for producers, traders and population.

Therefore, it is very important to improve the level of knowledge of the genetic diversity within Bambara nut accessions in Burkina Faso. Few works have been realized on genetic variability of Bambara nut cultivated in Burkina Faso. Their studies were based on morphological markers [1] [7] which reported a significant agro-morphological variability. However, morphological markers, although effective and easy to study, can give a biased estimate of genetic diversity, as they could be influenced by environmental factors [8]. Molecular markers have become powerful tools in the assessment of genetic diversity compared to the conventional approach using phenotypic descriptors because they are independent of environmental factors [9]. The studies using molecular markers such as RAPD and SSRs were carried out [10] [11]. The microsatellite molecular markers used by [11] on the same genetic material of *V. subterranea* were not very informative because they showed a low polymorphism and genetic diversity. This genetic material has also been studied using RAPD markers [10]. Their authors reported moderate genetic diversity despite a high rate of polymorphism. According to [12], the heterozygosity and the polymorphism information content (PIC) are two distinct values, which can be employed to determine the level of polymorphism in markers. RAPD molecular markers have the disadvantage of being dominant, difficult to reproduce and not transferable between species [13]. Thus, a better estimate of the genetic diversity of Bambara nut requires suitable markers, which can provide information on the genetic diversity within this species. This study aims to contribute to a better knowledge of the level of diversity within 90 accessions of Bambara nut from three agro-climatic zones of Burkina Faso using microsatellite markers for conservation and breeding purpose.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of 90 Bambara nut cultivars from Burkina Faso. Most of the accessions were collected from the producers in Sahelian zone (18), Sudanian zone (17), and Sudan-Sahelian zone (49). Six varieties were obtained from National Institute for the Environment and Agricultural Research gene bank (**Table 1**).

Table 1. Origin of landraces.

Origin	*Accessions*
INERA	KVS 235, KVS 235 100 GY, KVS 246-1, KVS 246-2, KVS 246-3, Life 16-141
Sahelian zone	E 105b, E 108, E 110a, E 110b, E 111a, E 114, E 117, E 118, E 119, E 12, E 124a, E 124b, E 124c, E 125, E 126, E 13, E 59, E 61a
Sudan-Sahelian zone	E 01, E 03, E 04, E 09, E 103a, E 103b, E 105a, E 107, E 111b, E 131, E 132, E 16a, E 48, E 49, E 51, E 53, E 56a, E 56b, E 56c, E 58, E 61b, E 62a, E 62b, E 62c, E 65, E 70b, E 71, E 72, E 75, Nob-Loc, E 76a, E 76b, E 78a, E 83a, E 83b, E 88b, E 89a, E 89b, E90, E 92, E 93b, E 94, E 95a, E 95b, E 97, E 98, ED8, KAYA 2014
Sudanian zone	E 101b, E 123, E 130, E 16b, E 20, E 22, E 23, E 25, E 26, E 27, E 28, E 44, E 76c, E 78b, E 83c, E 86, E 88a

2.2. Microsatellite Markers

Fifteen SSR markers were used for molecular characterization due to their high polymorphism revealed in previous studies on 35 genotypes including 21 accessions from IITA (Nigeria), while 12 were from the University of Nottingham stocks and 2 were sourced from Botswana using 75 SSR markers [14]. The characteristics of these markers are mentioned in **Table 2**.

2.3. DNA Extraction

The accessions were sowed on seedling plates. Fourteen days after sowing, a piece of young leaf was removed with a pair of scissors and placed on FTA card with the accession number. The sheets were covered with plastic film and crushed using a laboratory porcelain pestle in order to obtain an imprint on the FTA cards. The cards bearing the paper were air-dried for 24 h at room temperature and kept in a desiccator away from any element liable to degrade DNA. At the end, disk of 2 mm in diameter was taken for each sample from the cards using a paintbrush. They have been placed in "Eppendorf" tubes at the rate of five disks per tube. Five disks of each tube are washed twice in 1000 µl alcohol 70˚ for five minutes and then rinsed twice also with the same amount of TE buffer (Tris-EDTA) during this same time. The discs containing the DNA were then dried at room temperature and kept at −20˚C for gradual use in PCR.

2.4. PCR Amplification

PCR reactions were carried out in a final volume of 20 µl of PreMix (Bioneer corp, Republic of Korea), 2 µl of each primer (1 µM) (Integrated DNA Technologies,) (foward-reverse) and one disk (2 mm) of FTA card containing genomic DNA in BIO-RAD thermocycler (BioRad MyCycler PCR System, Texas, United States). A program of initial denaturation phase at 94˚C (5 min) followed by 35 consecutive cycles of a denaturation at 94˚C (30 s), a hybridization of Primers at 44˚C - 51˚C (1 min), stretch at 72˚C (1 min) has been launched. A final elongation at 72˚C (10 min) followed by cooling to 10˚C. The amplification products were subjected to electrophoresis at 150 V using 2% agarose gel in which 7.5 µl

Table 2. SSR markers used and their characteristics.

Loci	Sequence (5'-3')	T (˚C)	Size (bp)
Primer 15	F: AGGAGCAGAAGCTGAAGCAG	46.9	20
	R: CCAATGCTTTTGAACCAACA		20
Primer 16	F: CCGGAACAGAAAACAACAAC	47.4	20
	R: CGTCGATGACAAAGAGCTTG		20
Primer 19	F: AGGCAAAAACGTTTCAGTTC	46.8	20
	R: TTCATGAAGGTTGAGTTTGTCA		22
Primer 26	F: CGCTCATTTTAACCAGACCTC	46.1	21
	R: CAAACAAACCAACGGAATGA		20
Primer 30	F: AATGCAAGATTTTGGCTTGG	47.1	20
	R: CCCACTCAAACCATACACCA		20
Primer 32	F: TTTACCTGAACCCCTTAACC	49.5	20
	R: AGGCTTCACTCACGGGTATG		20
Primer 33	F: ACGCTTCTTCCCTCATCAGA	48.9	20
	R: TATGAATCCAGTGCGTGTGA		20
Primer 37	F: CCGATGGACGGGTAGATATG	49.4	20
	R: GCAACCCTCTTTTTCTGCAC		20
Primer 48	F: TACCTGCATTCGGGACAGTT	48.4	20
	R: TTCACTCTTTCTTGATCACATGC		23
G33AB4-D1	F: TGCTTCTTCAAGGAGGAAGTAAGT	50.2	24
	R: ACAAACATACGCACAACAGAGAAT		24
G196AB4-D5	F: CCACGTTCTGGTTGTGAGTAGATA	50.9	24
	R: GTGCTTTCAGACCATTACTTGCTT		24
G180B2-D11	F: GAGGAAATAACCAAACAAACC	44.8	21
	R: CTTACGCTCAATTTTAACCAGACCT		24
G240-7-B2-D12	F: TTTTGTTGTTGTATGAATCCAGTG	47.1	24
	R: CCTCATCAGACGCTCATCATT		21
G240-9-B2-D14	F: GAACGAAGCCAGGATAATGATAGT	49.5	24
	R: CGAAAGCGACAACTCACTACTAAA		24
G358B2-D15	F: TGACGGAGGCTTAATAGATTTTTC	48	24
	R: GACTAGACACTTCAACAGCCAATG		24

of Ethidium Bromide (BET) during 1 h 30 min in 0.5x Tris Borate EDTA (TBE) buffer. The wells were loaded with 10 µl of PCR product in the presence of a marker of molecular weight varying from 25 bp to 600 bp. The migration gel was read in UV light and then was photographed for evaluation.

2.5. Data Analysis

The molecular data obtained from the binary coding made it possible to deter-

mine genetic diversity within accessions with genetic parameters such as number of alleles (Na), number of effective alleles (Ne), polymorphism (P), expected heterozygosity (He). They were determined with the GenALEx 6.501 [15]. The polymorphism information content (PIC) was estimated using Genetix 4.04 software [16]. Aptitude of the different loci to structure diversity between the accessions was carried out using the DARwin v 5.0.158 [17] through the construction of a dendrogram from dissimilarity matrix by the "Neighbour Joinning" method.

3. Result

The molecular data obtained from the binary coding made it possible to determine genetic diversity within accessions with genetic parameters such as number of alleles (Na), number of effective alleles (Ne), polymorphism (P), expected heterozygosity (He). They were determined with the GenALEx 6.501 [15]. The polymorphism information content (PIC) was estimated using Genetix 4.04 software [16]. Aptitude of the different loci to structure diversity between the accessions was carried out using the DARwin v 5.0.158 [17] through the construction of a dendrogram from dissimilarity matrix by the "Neighbour Joinning" method.

3.1. Genetic Diversity of *Vigna subterranea*

The results of the analysis of genetic diversity using 15 SSR markers are in Table 3. One hundred and one alleles in total were detected. Each primer revealed a

Table 3. Estimation of genetic diversity of 90 Bambara nut landraces using 15 SSR markers.

Loci	Na	Ne	P	He	PIC
Primer 15	6	4.720	6 (100)	0.788	0.788
Primer 16	5	4.336	5 (100)	0.769	0.769
Primer 19	8	5.777	8 (100)	0.827	0.827
Primer 26	6	5.246	6 (100)	0.809	0.809
Primer 30	8	5.322	8 (100)	0.812	0.812
Primer 32	5	2.977	5 (100)	0.664	0.664
Primer 33	4	2.892	4 (100)	0.654	0.654
Primer 37	5	3.629	5 (100)	0.724	0.724
Primer 48	10	7.562	10 (100)	0.868	0.867
D1	6	3.321	6 (100)	0.699	0.698
D5	6	4.931	6 (100)	0.797	0.797
D11	6	3.895	6 (100)	0.743	0.743
D12	5	4.188	5 (100)	0.761	0.761
D14	12	7.002	12 (100)	0.857	0.857
D15	9	6.443	9 (100)	0.845	0.844
Total	**101**	72	101	12	12
Average	6.73	4.816	6.73 (100)	0.775	0.775

Na: number of allele, Ne: effective allele, P: polymorphism, He: expected heterozygosity, PIC: polymorphic information content.

polymorphism (P) of 100%. The number of alleles (Na) ranged from 4 (Primer 33) to 12 (D14) with an average of 6.73 alleles per locus. The total number of effective alleles was 72 with an average of 4.816. Expected heterozygosity (He) ranged from 0.654 for Primer 33 to 0.868 for primer 48 with an average of 0.775 alleles per locus. The least discriminating marker was primer 33 with a Polymorphism Information Content (PIC) value of 0.654 and the most discriminating marker was primer 48 with a PIC value of 0.867.

3.2. Genetic Diversity Organization

The dendrogram base on the "Neighbour-Joining" method revealed four genetic groups (Figure 1). The distribution of accessions in groups took place independently of agro-climatic zones. Group I gathers 28 accessions including 17 from Sudan-Sahelian zone, 08 from Sudanian zone and 03 from Sahelian zone. Group II contains 22 accessions including 13 from Sudan-Sahelian zone, 04 from Sudanian zone, 03 from Sahelian zone and 02 from INERA gene bank. The group III comprised of 29 accessions including 15 from Sudan-Sahelian zone, 07 from Sahelian zone, 04 from INERA gene bank and 03 from Sudanian zone. Group IV gathers 11 accessions including 04 from Sudan-Sahelian zone, 02 from Sudanian zone and 05 from Sahelian zone.

The genetic parameters of the different genetic groups are scored in Table 4.

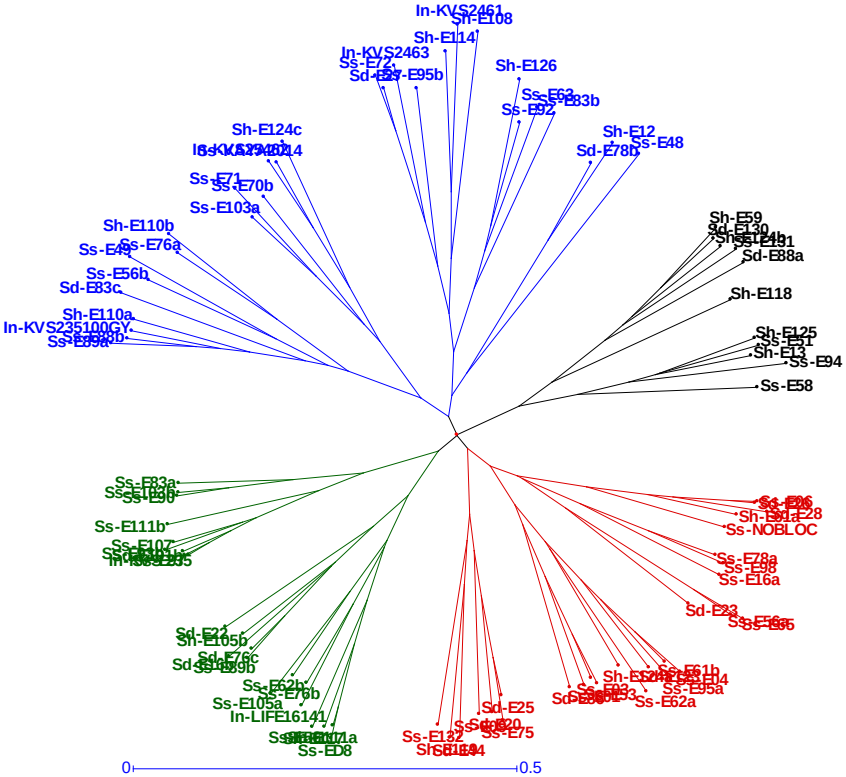

Figure 1. Dendrogram based on Neighbour-joining on SSR for 90 Bambara nut genotype from Burkina Faso. Group 1 in red, Group 2 in green, group III in blue and group IV in black.

Table 4. Diversity parameters of four genetic groups of Bambara nut from Burkina Faso.

Factor	N	Na	Ne	P (%)	He
Group I	28	4.067	2.798	82.52%	0.595
Group II	22	3.733	2.940	78.50%	0.566
Group III	29	5.133	4.055	92.96%	0.720
Group IV	11	2.400	2.082	65.78%	0.477

Na: number of allele, Ne: effective allele, P: polymorphism, He: expected heterozygosity, PIC: polymorphic information content.

Group I gave a number of alleles of 4.067, a polymorphism rate and expected heterozygosity of 82.52% and 0.595, respectively. For Group II, the number of alleles was 3.733, a polymorphism of 78.50% and expected heterozygosity of 0.566. Group III gave a number of allele of 5.133, the greatest effective allele and respectively a polymorphism and expected heterozygosity of 92.96% and 0.720. For Group IV, the number of allele was 2.400 with a lowest number of effective allele, a polymorphism and expected heterozygosity of 65.78% and 0.477.

4. Discussion

Analysis of Bambara nut accessions with 15 SSR DNA markers identified 101 alleles in this study with an average of 6.73 alleles per locus. This result showed an important level of diversity within the Bambara nut collection. These values are higher than the previous results reported by [18] on 18 Bambara nut landraces using 10 SSR marker, which had 52 alleles and an average of 5.20 alleles per locus. The values of PIC, which ranged from 0.654 to 0.867 show those SSR markers set used, were very discriminating. [19] reported that molecular markers are highly informative if the PIC value is greater than 0.5. The most polymorphic primers were D14 and Primer 48 with respective values 0.857 and 0.867. However, these results differ from those of [11] who showed 0.174 (primer B2D15FR) to 0.435 (primer 4FR) with an average of 0.298 per primer. The microsatellite markers used to characterize the same collection of *V. subterranea* were weakly are not very discriminating. According to [20], factors such as population size, origin of landraces, type of markers used and human community's impacts could influence the level of polymorphism within a population. In this study, results would be due to the use of a higher number of markers, which were in addition selected for their high rate of polymorphism according to a screening of 24 accessions of Bambara nut by 68 SSRs carried out by [14]. Therefore, there is genetic diversity within the Bambara nut cultivated in Burkina Faso. The SSR markers set used in this study could be used for efficient management, conservation, and utilization of Bambara nut germplasm. The allelic diversity explained by expected heterozygosity (He) varied from 0.654 to 0.868 respectively for Primer 33 and Primer 48 with an average of 0.775. These values are similar to those of [21] who found values ranged from 0.626 to 0.863 with and an average of 0.787. A moderate diversity for expected heterozygosity was reported by [10] on

92 landraces of *V. subterranea* screened with 17 RAPD markers. [22] obtained moderate diversity with an average of expected heterozygosity of 0.64 in 604 genotypes of common bean (*Phaseolus vulgaris*) using 36 SSR markers. High value of expected heterozygosity reflects a significant genetic diversity of Bambara nut landraces studied.

Bambara nut accessions were divided into four clusters. This gathering could reflect an existence of significant genetic diversity in the study population. The different genetic groups obtained are composed of individuals from all agro-climatic zones. The mixture of accessions from different agro-climatic zones shows that the gathering of accessions is not based on their origin. These results corroborate those of [11] which led to this same organisation. This weak genetic differentiation between accessions of different climatic zones could be explained by seed exchanges during migration of population and social cultural activities. [21] reported a distribution of Bambara nut accessions from seven geographic areas into seven groups independently of their geographic origins. On the other hand, the studies of [23] on Bambara nut with AFLP markers showed that accessions were regrouped according to their origin when they are collected in isolated localities or very distant from each other. These differences are due to the ability of SSR markers to divide genotypes into smaller genetic groups than other marker systems [21]. The gathering of accessions in four genetic groups independent of agro-climatic zones has been reported by previous studies on the same genetic material with phenotypic markers in an agro-morphological diversity study [7]. The selective pressure of genotypes and adaptations to the environment are sources of genetic modification.

5. Conclusion

Analysis of genetic diversity using SSRs markers revealed a significant genetic diversity of *Vigna subterranea* cultivated in Burkina Faso. The SSR markers used exhibited a high polymorphism and made it possible to structure the diversity into four genetic groups independent of their origin. This study allowed a better understanding of the structuring of genetic variability. The knowledge of the genetic diversity of *V. subterranea* available in Burkina Faso and the different profiles of this genetic diversity would serve in breeding and varietal improvement programs for this crop. Bambara nut is a growing crop and many studies remain to be done to enhance both production and economic profitability.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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