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Genetic diversity of Bambara groundnut genotypes (*Vigna subterranea* [L.] Verdc.) revealed by SSR markers

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Abstract

Bambara groundnut is an under-utilized legume of African origin with the potential to alleviating food security issues in Africa. There is limited research output on genetic diversity, selection and breeding of the crop, especially using genomic tools. Landraces were mostly being characterized using morphological markers whose expression is heavily influenced by environmental factors. Molecular markers provide a better choice for genetic diversity studies, because crop species are not affected by environmental factors. SSR markers have been found to be most convenient for genetic analysis, especially that they are multiallelic, co-dominant and evenly dispersed throughout the genome. The objective of the study was to genotype 50 Bambara groundnut lines from single plant selection that were obtained from seven geographical regions across Africa using five polymorphic SSR markers. The analyses detected a total of 53 alleles, with a mean of 10.6 alleles per locus, while genetic distance measured by polymorphic information content ranged from 0.0 to 3.8, with a mean of 0.76. The neighbor-joining analysis generated seven major genetic groups, clustered irrespective of geographic origin.

Keywords: Bambara groundnut, Cluster, Genetic distance, Microsatellite markers, Neighbor-joining analysis

Introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc. 2*n*=2*x*=22) is an African legume which bears its origin at from West Africa (Hepper, 1963). The crop is primarily grown by resource-limited farmers as a source of cheap protein (Massawe *et al*., 2005). Seeds of Bambara groundnut are consumed in fresh form as a vegetable, while in dry form the seeds are processed into flour to prepare other kind of foods as snacks. This makes Bambara groundnut a complement to cereal-based diet (Olukolu *et al*., 2012), hence this crop has the potential of reducing food insecurity in Africa (Shegro *et al*., 2013).

Bambara groundnut is one of the most popular, but under-utilized grain legumes, with limited research interest by the scientific community (Amadou *et al*., 2001). Most frequently landraces have been developed by farmers through selection and maintenance as local varieties for production, which may be distinguishable by their names, seed coat colour, growing locations, or markets where found (Massawe *et al*., 2002). Therefore, one

landrace may bear several names due to the movement of seeds from one region to another. Presently, more than 2000 accessions have been collected and preserved by International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria (Olukolu *et al*., 2012), with little or no attention to genetic improvement activities. A major limitation to large scale production of Bambara groundnut in Africa is its low yield which is estimated to be as low as 68.5-159.9 kg ha^{-1} (Collinson *et al*., 2000). This has been attributed to lack of improved varieties (Mayes *et al*., 2008) and poor production technologies. Genetic enhancement of this valuable crop is essential to its productivity in the region. Genetic variation is the basis for breeding of this important crop.

For effective breeding in Bambara groundnut, characterization of any available germplasm/landraces becomes imperative. Both morphological and molecular diversity analysis can be employed for genetic diversity studies for subsequent breeding and release of varieties with desirable qualities including increased yield, resistance to pests and diseases, abiotic stress tolerance and seed quality. Molecular markers offer greater power for detecting diversity that exceeds that of traditional methods (Gupta and Varshney, 2000), because they are not environmentally dependent. DNA markers including SSRs that are linked to agronomic traits could increase the efficiency of classical breeding by significantly reducing the number of backcross generations required and by reducing expensive, tedious, phenotypic selection as well as germplasm conservation. They also have the benefit of being efficient, regardless of the developmental stage of the plant under investigation (Mondini *et al*., 2009). It therefore clear that molecular markers can be used for tracing the origin of genotypes and as well be employed breeding program for genetic improvement (Mayes *et al*., 2015).

Biochemical and molecular analyses of genetic diversity between and within Bambara groundnut landraces were reported, including amplified fragment length polymorphism (AFLP) (Massawe *et al*., 2002; Ntundu *et al*., 2004), randomly amplified polymorphic DNA (RAPD) (Amadou *et al*., 2001, and SDS-polyacrylamide electrophoresis technique (Odeigah and Osanyinpeju, 1998). The RAPD and AFLP markers showed high levels of polymorphism among Bambara groundnut landraces (Massawe *et al*., 2002). RADPs identified significant polymorphism among Bambara groundnut varieties grown in Namibia (Mukakalisa *et al*., 2013) with a range from 63.2 to 88.2% and a mean of 73.1% at Nottingham, UK (Massawe *et al*., 2003). SSR markers also known as microsatellites have been found to be markers of choice for diversity studies. Being PCR-based, SSRs are technically simple to deploy and are amenable to high throughput assays (Mansfield *et al*., 1994), as well as being easy to score and requiring small amount of DNA for analysis (Somta *et al*., 2011). In recent years, the application of SSRs has been established in early generation selections among breeding populations (Gupta and Varshney, 2000). SSR markers displayed sufficient diversity among Bambara groundnut landraces (Basu *et al*., 2007b; Somta *et al*., 2011).

There is scant information on the use of SSRs in Bambara groundnut genetic diversity studies in literature. A recent study found SSRs to be the markers of choice for Bambara groundnut genetic diversity studies (Somta *et al*., 2011). Somta *et al*. (2011) employed SSRs markers tested on other legumes belonging to the Bambara groundnut genus', the '*Vigna* cultigens' including adzuki bean (*Vigna angularis* [Willd.]) and mungbean. These markers identified sufficient variability among the assessed Bambara groundnut landraces. Bambara groundnut is a prominent member of the genus *Vigna;* hence its genetics may be similar or closely related to members of the same genus. SSRs markers were also employed by Basu *et al*. (2007a) to assess the genetic diversity of Bambara groundnut genotypes.

The objective of this study was to genotype 50 contrasting Bambara groundnut lines obtained from seven geographical regions across Africa using five selected polymorphic SSR markers developed for Bambara groundnut.

Materials and Methods

Plant materials

Fifty (50) Bambara groundnut genotypes from seven geographical locations were used in the study (Table 1). All genotypes were pure breeding lines of single plants selected from a morphological diversity study of within and

between Bambara groundnut landraces (Mohammed et al., 2016a). Selection of the accessions was based on distinct features of seed and plant morphological diversity (Mohammed et al., 2016b).

DNA extraction and genotyping

Seeds were used for genomic DNA extraction. All samples were used in bulked amplification using DNA extracted from 7 coleoptiles per sample following the CTAB extraction procedure (CIMMYT, 2005). PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). Five SSR markers (Table 2) specific for Bambara groundnut (Basu *et al*., 2007a; Somta *et al*., 2011) were used to perform the PCR reactions and analysis for genetic diversity among the Bambara groundnut genotypes. The SSR primers used in this study were selected based on their high PIC and amplified alleles, and that they were developed being specific for Bambara groundnut (Basu *et al*., 2007a; Somta *et al*., 2011). Somta *et al*. (2011) compared PIC estimates among derived SSRs markers from three legumes including cowpea, adzuki bean and Bambara groundnut that revealed mean PIC estimates of 0.43, 0.61 and 0.78 for cowpea, adzuki bean and Bambara groundnut accessions, respectively. Means for allelic richness were 2.80, 2.90 and 3.75, respectively, for the same species. Among the Bambara groundnut SSRs markers used in this study, mBam2Co80 and mBam2Co33 had higher alleles score (8 and 12) per locus and PIC estimates (0.8 and 0.88) than seven others (Basu *et al*., 2007a). Sequences of the SSRs are presented in Table 2. An automated genetic analysis was employed to screen the SSR markers, using an automated gene sequencer (an ABI 3130 from Applied Biosystems, Johannesburg, South Africa). The analysis comprises the use of electrophoresis for amplification, wherein SSR loci that comprise of more than two base pairs may not be determined on agarose gel electrophoresis and nucleotides composed of up to 200bp (Sibov *et al*., 2003).

Data analysis

Analysis was performed using GeneMapper 4.1. The program GGT 2.0 (van Berloo, 2008) was used to calculate the Euclidian and Jaccard distances between bulked samples, and the matrix of the genetic distances was used to create a UPGMA and Neighbour Joining (NJ) dendrogram of the results.

Results and Discussion

Marker characterization

The SSRs markers detected a total of 53 alleles with a mean of 10.6 (Table 3). A minimum number of six alleles were detected by the SSR marker, mBamC039, while mBam2C033 detected the most alleles which as 17. The mean alleles observed in this study was higher than 7.59 (Somta *et al*., 2011) and 5.20 (Basu *et al*., 2007) who also used the SSR markers used in this study.

The PIC observed in this study varied from 0.5576 to 0.8486, with a mean of 0.7554, as revealed by mBam3C039 and mBamC017 markers, respectively. A mean PIC of 0.58 was previously generated by 22 polymorphic SSRs markers in a diversity study among Bambara groundnut accessions from diverse origins (Somta *et al*., 2011) with range of 0.10 to 0.91 and a higher PIC of 0.70 which also revealed 166 alleles from the same materials. Use of SSRs used on some legumes (mungbean and blackgram) were reported in which PICs generated were 7.3 and 4.1, respectively (Danzmann *et al*., 2009). The polymorphic information content (PIC) describes the usefulness of SSR markers in identifying genetic similarities and differences among the pure lines, in this case, of the Bambara groundnut genotypes. It also, confirms the validity of using specific maker(s) in the construction of genetic linkage maps for the crop (Massawe *et al*., 2002). This maximizes selection of genetically distinct parents that can be used for the genetic enhancement of the crop (Amadou *et al*., 2001; Massawe *et al.*, 2002).

The allelic diversity, as explained by heterozygosity (*He*), varied between 0.6261 and 0.8634 for mBam3C039 and mBamC017 markers, respectively. This range is higher than the scores of 0.54 and 0.77 reported for the same markers by Basu *et al*. (2007a). Somta *et al*. (2011) reported the highest mean PIC and *He* of 0.70 and 0.552, respectively. Bambara groundnut being self-fertilizing, the findings in this study compared favourably with

previous reports, because, the genotypes used were from single plant selection which were pure lines. As such it is probable that the selected plants used for the analysis in the previous study were from heterogeneous mixtures of landrace seeds. Somta *et al*. (2013) employed a cross-species amplification of SSRs on 34 Bambara groundnut accessions which detected between 2 and 8 alleles per marker, and a PIC estimate of 0.16 to 0.73, while none of the markers revealed any heterozygosity among the accessions. This underlines the detection power of the markers that were used in this study for effective genetic grouping of the 50 Bambara groundnut genotypes. The SSR markers which were developed for Bambara groundnut (Basu *et al*., 2007a), have generally revealed high correlations between the PIC and *He* estimates. They also match with the allelic detection by the corresponding markers, with mBamC017 and mBamCo33 markers presenting higher correlation between PIC values of 0.8486 and 0.8118, and *He* values 0.8634 and 0.8322, respectively. These means, they were higher than those reported by Basu *et al*. (2007a) and Somta *et al*. (2011) using SSRs including those used in this study. High PIC estimates describe the strength of the molecular markers, especially SSRs that have the advantage of being co-dominant and multiallelic (Gupta *et al*., 2003), to distinguish any variability among species, which is resolved by the number and frequency of alleles discovered (Somta *et al*., 2011). The results explained the homogeneity status of the genotypes used in this study as sourced from single plant selection, i.e. pure lines. Findings in this study suggest that these SSR markers could be used in any Bambara groundnut genetic diversity study and genetic map construction.

Genetic distance

The genetic distance (D_A) among the 50 Bambara groundnut genotypes from the seven geographical locations are presented in Table 4, with a minimum of 0.0 to a maximum D_A 3.8 among 11 pairs of genotypes. This difference in the D_A of (0.00 to 3.8) observed in this study is lower than the values 0.28 and 0.27 and 0.53 and 0.53 the minimum and the maximum distances among Bambara groundnut landraces from two extreme geographical locations of Togo (Africa) and Thailand (Asia) (Somta *et al*., 2011). The extent of variation among the landraces used in the previous study was higher than that observed in the current genetic analysis. The findings in the current study revealed that the Bambara groundnut genotype 211-68 from CAPS (South Africa) correlated at a D_A of 0.0 each with 211-83-2 also from CAPS, as well as N211K and M09-3, which originated from Kano in Nigeria and Zimbabwe, respectively. N211K had a close association with two genotypes, 211-51 and 211-83-2, which originated from CAPS. These correlations link genotypes from the two distinct geographical locations, Kano in Nigeria and CAPS in South Africa which suggested that the genotypes involved may have a common origin. Meaning that seeds may have been moved from one location to another across the African continent. In addition, the genotypes 101-2 and 101-2-1 from Zambia displayed similar relationship with D_A at 0.0; M12-1 from Zimbabwe is related to 211-91 from CAPS, and 211-57 and 211-55-1 suggests similar origin. TV-93 and TV-79-1 have a close association.

The distance of 0.30 on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram (Fig. 1) between M12-1 and 211-91, and that between TV-93 and TV-79-1, reflected the extreme similarity between the two pairs, suggesting that these two pairs may be the same genotypes, probably considering their common source (IITA). This D_A of 0.0 emphasizes the capacity of the SSR markers to discriminate among the Bambara groundnut genotypes, even between those that have close relationships. Similarly, it was observed that most of these genotypes, including M09-3, 211-68, 211-51 and 211-83-2, were grouped in the same cluster on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram (Fig.1). Furthermore, close and similar associations with a D_A of 3.6 were detected between KB 05 from ARC in South Africa and 211-551and 211-57 from CAPS and KB 08 from the ARC in South Africa and 211-55-1 and 211-57 from CAPS. These relationships may be explained by the fact that CAPS is a seed company that sells Bambara groundnut landraces composed of seed mixtures. It is based in South Africa where we have the ARC. We propose that the genotypes have common origins. Interestingly, KB 05 and KB 08 on one hand, and 211-55-1 and 211-57 were grouped on the same, but separate 'leaves' (simplicifolious) on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram in the II and III clusters, respectively. Hence, this result also showed the ability of the SSR markers to distinguish between genotypes that are distinct, similar or closely related. In their genetic diversity study using RAPD Massawe *et al*. (2003) found a similar trend of association, and proposed that such

close associations between Bambara groundnut landraces could mean that they were related or that they were the same genotypes. Similar suggestions were made by Ntundu *et al*. (2006) in a morphological diversity study among Bambara groundnut landraces in Tanzania. These authors proposed that unorganized collection and grouping of Bambara groundnut landraces would result in a single genotype bearing several names (Massawe *et al*., 2003).

The highest D_A of 3.8 was observed between two pairs of Bambara groundnut genotypes, M02-3 and 211-51-1, and M02-3 and 211-57 (Table 4). However, these two pairs were not grouped in the same cluster (Fig. 1). Amadou *et al*. (2001) used RAPD markers and found that Bambara groundnut accessions from Zambia and Zimbabwe were grouped in the same cluster, suggesting that the same seed material may have been taken from one of the location to the other. The D_A observed in this study revealed low minimum and maximum values, when compared with reports of other genetic studies based on SSRs (Somta *et al*., 2011), AFLP (Ntundu *et al*., 2004) and RAPD (Amadou *et al*., 2001; Massawe *et al*., 2003). These variations may be due to the of nature the germplasm used in this study, which consisted of pure lines from single plant selection, compared to the use of landraces composed of mixtures of a few to several seed morpho-types.

Genetic relationship

The levels of similarities and divergence among the 50 Bambara groundnut genotypes are presented in Fig. 1 and Table 4 using the Jaccard Neighbor-joining analysis. The analyses revealed the presence of significant genetic diversity among the tested genotypes. The genotypes were conveniently grouped into seven definite clusters, independent of geographical origin (Table 4). Conversely, Amadou *et al*. (2001) and Ntundu *et al*. (2004) collectively reported genomic grouping of Bambara groundnut landraces that were related to geographical origin using RAPDs and AFLP, respectively. The findings in this study demonstrated the ability of SSR markers to portion the genotypes into closer genetic groupings than other marker systems. The pattern was similar to that obtained in a morphological diversity study presented in the previous chapter.

The largest among the seven clusters was Cluster III which consisted of 12 genotypes emanating from four geographical sources (Fig. 1). Five of these genotypes originate from CAPS, three from Zambia, two from Kano and one from IITA (Table 5). Two genotypes, 101-2 and 101-2-1, were positioned closely in this cluster, with the latter being a selection from the former, suggesting that they possess similar genes. Cluster I followed with ten genotypes, of which six originated from CAPS, while three were sourced from Zimbabwe, and one genotype was obtained from a farmers' collection in Pietermaritzburg that appeared as an outlier.

Capstone Seed Company is a seed company in South Africa that buys and sells Bambara groundnut seeds composed of mixtures of different morpho-types. The seed lots vary in seed coat colour and eye pattern. Hence there is the possibility that CAPS may have secured Bambara groundnut seed landraces from Zimbabwe and other neighboring countries hence the grouping pattern.

Cluster II comprised of nine genotypes collectively originating from CAPS, Zambia and ARC in South Africa. In this cluster, two pairs of genotypes KB 05 and KB 08 from ARC in South Africa, and 42-1 and 42-2 from Zambia, had strong similarities. However, the two pairs varied in seed coat colour: while 42-1 was light brown, 42-2-2 was cream. The smallest cluster was Cluster IV which had only three genotypes, M01-8, which originated from Zimbabwe, while N211K and TV-14 originated from Kano and Ghana, respectively, reflecting a close genetic relationship, despite their distant origins.

Pasquet *et al*. (1999) compared the genetic diversity between wild and domesticated Bambara groundnut accessions using isozyme markers and reported a close relationship between the two species suggesting that the former is the progenitor of the latter. However, Ntundu *et al*. (2004) discussed isozymes as having limited use for genetic analysis due to their low levels of polymorphism.

The findings in this study confirmed the detection power of the SSRs to resolve the genetic diversity of the Bambara groundnut genotypes into their similarity and divergent groups with great precision, while each genotype was derived from single plant selection that was presumed to be genetically uniform.

Conclusion

The genetic analysis using the SSR makers revealed the extent of similarity and differences among the 50 Bambara groundnut genotypes used in this study, which compared favourably with the results obtained in similar studies using SSRs which include those adopted in this study. In this study, PIC estimates varied from 0.5576 to 0.8486 with a mean of 0.7554, while heterozygosity (*He*) varied between 0.6261 and 0.8634 with a mean of 0.7865. These measurements were higher than the ranges of 0.70 and 0.552, and 0.54 and 0.77 of PIC and *He* found by Basu *et al*. (2007b) and Somta *et al*. (2011), respectively. There were also fewer alleles in other studies than those revealed in the present study: 6 to 17 per locus with a mean of 10.6. Also, the SSR analysis exhibited a comparable pattern between morphological diversity of the same genotypes (*Manuscript In Press: Legume Research Journal, No. LR-475*) and the result displayed in the Jaccard Neighbor-joining analysis. For genetic distance analysis, the Bambara groundnut genotypes were grouped into seven clusters, consisting of combination of genotypes from different geographical origin. Therefore, this study confirmed that the homogeneity of the genotypes used in this study was because they were sourced from single plant selections, i.e. pure lines; and that SSR markers were highly effective at discriminating between the Bambara groundnut genotypes.

Conflict of Interests

None.

Tables, Figures and Charts

Table 1 List of the Bambara groundnut genotypes used in the study and their origins

S/No.	Genotype	Origin	Seed coat colour	S/No.	Genotype	Origin	Seed coat colour
1	211-77	CAPS	Cream	26	211-75	CAPS	Cream
2	211-87	CAPS	Black	27	211-46-3	CAPS	Red
3	211-55	CAPS	Red	28	211-83-2	CAPS	Cream
4	32-1-1	ZM	Light brown	29	712-4	ZM	Tan
5	45-2	ZM	Tan	30	N211-1	KNG	Cream
6	211-55-1	CAPS	Red	31	KB 05	ARC	Cream
7	TV-79-1	IITA (Kenya)*	Cream	32	211-68	CAPS	Cream
8	211-90	CAPS	Black	33	101-2	ZM	Cream stripe
9	211-51	CAPS	Red	34	KB 08	ARC	Cream RBF**
10	211-91	CAPS	Light brown	35	M12-1	ZIM	Cream
11	42-2-3	ZM	Light brown	36	712-7	ZM	Tan
12	84-2	ZM	Red	37	211-45	CAPS	Red
13	N211K	KNG	Cream	38	101-2-1	ZM	Cream stripe
14	73-3	ZM	Red	39	42-2	ZM	Light brown
15	211-76	CAPS	Cream	40	M01-8	ZIM	Cream RBF
16	25-Jan	ZM	Light brown	41	TV-93	IITA (Kenya)	Cream
17	B71-2	ARC	Cream	42	M02-3	ZIM	Cream RBF
18	M09-4	ZIM	Cream	43	B71-1	ARC	Cream
19	N212-5	KNG	Brown	44	73-2	ZM	Red
20	TV-27	IITA (Nigeria)	Dark brown speckle	45	211-88	CAPS	Black
21	M09-3-1	ZIM	Cream	46	N212-4	KNG	Brown Dark brown
22	011-7	PMB	Cream stripe	47	TV-39	IITA Sudan)	speckle
23	N212-8	KNG	Brown	48	211-69	CAPS	Cream
24	211-57	CAPS	Red	49	M09-3	ZIM	Cream
25	42-1	ZM	Light brown	50	TV-14	IITA Ghana)	Cream

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa; ***RBF***=Red butterfly eye

Table 2 Description of the SSRs markers used in this study

Marker name	Forward primer	Reverse primer	Source
mBamCo17	AACCTGAGAGAAGCGCGTAGAGAA	GGCTCCCTTCTAAGCAGCAGAACT	(Somta *et al.*, 2011)
mBam3Co39	CAGTAGCCATAATTTGCTATGAACA	CACATCAATCAAAAATCTCGGTAG	(Basu *et al.*, 2007b)
mBam2Co33	ATGTTCCTTCGTCCTTTTCTCAGC	AAAACAATCTCTGCCCCAAAAAGA	(Somta *et al.*, 2011)
mBam3Co07	GGGTTAGTGATAATAAATGGGTGTG	GTCATAGGAAAGGACCAGTTTCTC	(Somta *et al.*, 2011)
mBam2Co80	GAGTCCAATAACTGCTCCCGTTTG	ACGGCAAGCCCTAACTCTTCATTT	(Basu *et al.*, 2007b)

Table 3 Information of the SSR loci repeat type, bin location, number of alleles, PIC values and heterozygosity (He) for five SSR markers that were applied on fifty Bambara groundnut genotypes

SSR locus	Repeat type	No. of alleles	PIC value	He
mBam3C07	(CT)22	9	0.7641	0.7940
mBamC017	(GA)12	11	0.8486	0.8634
mBam2C033	(CT)12N47(CT)16(CA)9	17	0.8118	0.8322
mBam3C039	(GT)9(GA)4	6	0.5576	0.6261
mBam2C080	(TG)17(GA)13	10	0.7948	0.8170
Total		53	3.7769	3.9327
Mean		10.6	0.7554	0.7865

Table 4 Similarity matrix based on Euclidean NJ coefficient for the 50 Bambara groundnut genotypes used in the study

Genotypes	011-7	25-1	32-1-1	42-1	42-2	42-2-3	45-2	73-2	73-3	84-2	101-2-1	101-2	211-45	211-46-3	211-51	211-55-1
25-Jan	3.6															
32-1-1	2.5	2.2														
42-1	2	2.4	2.4													
42-2	1.9	2.1	2.3	0.5												
42-2-3	2.3	2.1	1	1.9	1.8											
45-2	1.6	3.5	2.1	2	2.2	1.9										
73-2	2.5	2.7	2.9	1	1.3	2.1	2.2									
73-3	1.5	3.6	2.4	1.9	2.1	1.8	1.1	2.1								
84-2	1.6	2.5	1.2	1.9	1.7	1.2	1.9	2.5	1.8							
101-2-1	2.3	2.7	1.2	2.1	2.2	0.7	1.6	2.3	1.5	1.4						
101-2	2.3	2.7	1.2	2.1	2.2	0.7	1.6	2.3	1.5	1.4	0					
211-45	1.5	3.7	2.6	1.9	2.1	2.5	1.1	2.5	1.7	2.1	2.3	2.3				
211-46-3	1.5	3.6	2.6	1.7	1.9	2.3	1.5	2.3	1.4	1.8	2.1	2.1	1			
211-51	2.1	2.8	1.9	1.9	1.9	1.5	2.3	2.5	1.7	1.1	1.5	1.5	2.4	1.7		
211-55-1	3.1	2.3	1.6	2.8	2.6	1.6	3.2	3.3	2.9	1.6	1.9	1.9	3.5	3	1.5	
211-55	2.3	2.8	2.2	2.4	2.1	2.5	2.9	3.4	3	1.5	2.7	2.7	2.4	2.2	2	2.3
211-57	3.1	2.3	1.6	2.8	2.6	1.6	3.2	3.3	2.9	1.6	1.9	1.9	3.5	3	1.5	0
211-68	1.5	2.6	1.3	1.7	1.6	1.1	1.5	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1	1.8
211-69	1.4	3.2	2.3	1.7	1.7	2.1	2.1	2.5	1.8	1.2	2.1	2.1	1.8	1.1	1.1	2.3
211-75	1.4	3.2	2.3	1.7	1.7	2.1	2.1	2.5	1.8	1.2	2.1	2.1	1.8	1.1	1.1	2.3
211-76	1.6	2.9	1.6	1.6	1.7	1.2	1.2	2.1	1.1	1	1	1	1.5	1.1	1.1	2.1
211-77	3	2.3	1.2	2.4	2.4	1.2	2.5	2.9	2.5	1.6	1.2	1.2	2.9	2.5	1.5	1.2
211-83-2	1.5	2.6	1.3	1.7	1.6	1.1	1.5	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1	1.8
211-87	2.3	2.8	1.7	2.8	2.5	1.5	2.5	3	2.2	1.5	1.8	1.8	3.2	3	2	1.8

Table 4, cont'd

Genotypes	211-55	211-57	211-68	211-69	211-75	211-76	211-77	211-83-2	Genotypes	011-7	25-.1	32-1-1	42-1	42-2	42-2-3	45-2
011-7									211-88	2.5	1.7	2.4	0.9	0.7	1.8	2.5
25-Jan									211-90	2.1	2.5	1	2.5	2.3	1.6	2.1
32-1-1									211-91	1.1	3.2	2.2	1.7	1.6	2.1	1.8
42-1									712-4	2.3	1.9	2.2	0.9	0.7	1.5	2.4
42-2									712-7	2.1	2.5	2.8	0.9	0.7	2.3	2.6
42-2-3									B71-1	2.1	2.1	1.4	1.6	1.5	1.2	1.6
45-2									B71-2	1.9	2.7	1	2.1	2.1	1.2	1.2
73-2									KB05	3	2.5	3	1.3	1.6	2.6	2.6
73-3									KB08	2.5	2.7	3	0.9	1.2	2.4	2.2
84-2									N211-1	2.5	3.2	2.2	2.6	2.5	1.5	2.5
101-2-1									N211K	1.4	2.6	1.2	1.7	1.6	1.1	1.1
101-2									N212-4	2.3	3.6	2.6	2.3	2.5	1.9	2.1
211-45									N212-5	3.1	1.8	1.9	2	1.9	1.2	2.8
211-46-3									N212-8	2.1	3.7	2.8	2.2	2.3	2.1	2.1
211-51									M01-8	1.6	2.7	1.7	2.1	1.8	1.9	2.3
211-55-1									M02-3	1.9	3	3	1.2	1.5	2.6	1.8
211-55									M09-3-1	1.1	3.3	1.9	2.2	2.1	1.8	1.1
211-57	2.3								M09-3	1.5	2.6	1.3	1.7	1.6	1.1	1.5
211-68	1.7	1.8							M09-4	2.5	3	3.3	0.9	1.2	2.7	2.5
211-69	1.5	2.3	1.1						M12-1	1.1	3.2	2.2	1.7	1.6	2.1	1.8
211-75	1.5	2.3	1.1	0					TV-14	1.9	2.5	1	1.9	1.8	0.7	1.6
211-76	2.1	2.1	0.5	1.2	1.2				TV-27	2.1	2.7	1.2	2.2	2.2	0.7	1.7
211-77	2.3	1.2	1.5	2.2	2.2	1.6			TV-39	1.2	2.9	1.6	1.7	1.7	1.2	1.4
211-83-2	1.7	1.8	0	1.1	1.1	0.5	1.5		TV-79-1	1.2	3	1.9	2.1	1.9	1.6	1.9
211-87	2.8	1.8	1.7	2.5	2.5	2.1	2.3	1.7	TV-93	1.2	3	1.9	2.1	1.9	1.6	1.9

Table 4, cont'd

Genotypes	73-2	73-3	84-2	101-2-1	101-2	211-45	211-46-3	211-51	211-55-1	211-55	211-57	211-68	211-69	211-75	211-76	211-77
211-88	1.1	2.4	2.1	2.3	2.3	2.6	2.4	2.2	2.7	2.6	2.7	2	2.3	2.3	2.1	2.5
211-90	3.1	2.5	1.2	1.9	1.9	2.5	2.7	2.3	2.1	2.1	2.1	1.5	2.3	2.3	1.9	2.1
211-91	2.5	1.7	1.1	2.1	2.1	1.4	1	1.4	2.5	1.4	2.5	1	0.5	0.5	1.1	2.3
712-4	1.3	2.1	1.7	1.9	1.9	2.5	2.1	1.6	2.2	2.3	2.2	1.6	1.8	1.8	1.7	2.1
712-7	1.7	2.3	1.9	2.6	2.6	2.3	1.9	1.9	2.8	2.1	2.8	1.9	1.5	1.5	1.9	2.7
B71-1	1.9	2.1	1.6	1.6	1.6	2.1	2.3	2.3	2.5	2.5	2.5	1.5	2.3	2.3	1.6	2.1
B71-2	2.5	1.8	1.2	1.2	1.2	1.8	2.1	2.1	2.3	2.3	2.3	1.1	2.1	2.1	1.2	1.9
KB05	1.7	2.9	2.8	2.8	2.8	2.3	2.3	2.9	3.6	2.9	3.6	2.5	2.7	2.7	2.4	2.9
KB08	0.9	2.3	2.6	2.6	2.6	2.1	2.1	2.7	3.6	3.1	3.6	2.3	2.5	2.5	2.2	3
N211-1	2.7	1.7	1.8	1.5	1.5	3.2	2.6	1.4	1.8	3.2	1.8	1.7	2.3	2.3	1.8	2.1
N211K	2.3	1.4	0.5	1.1	1.1	1.4	1.4	0	1.5	1.7	1.5	0	1	1	0.5	1.4
N212-4	2.3	1.1	2.1	1.6	1.6	2.7	2.1	1.5	2.5	3.4	2.5	1.8	2.1	2.1	1.6	2.3
N212-5	2.2	2.5	1.9	1.6	1.6	3.2	2.7	1.5	1.4	2.7	1.4	1.8	2.3	2.3	1.9	1.2
N212-8	2.3	1	2.1	1.8	1.8	2.4	1.7	1.4	2.7	3.2	2.7	1.7	1.8	1.8	1.5	2.5
M01-8	2.9	2.3	0.7	2.1	2.1	2.3	2.1	1.5	1.9	1.1	1.9	1.1	1.2	1.2	1.6	2.1
M02-3	1.5	2.1	2.5	2.7	2.7	1.5	1.8	2.9	3.8	2.7	3.8	2.3	2.3	2.3	2.1	3.1
M09-3-1	2.5	1.4	1.5	1.8	1.8	1.7	2	2.2	2.9	2.6	2.9	1.4	2.1	2.1	1.5	2.7
M09-3	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1	1.8	1.7	1.8	0	1.1	1.1	0.5	1.5
M09-4	1.1	2.4	2.7	2.9	2.9	2.2	2	2.6	3.6	3	3.6	2.4	2.3	2.3	2.3	3.2
M12-1	2.5	1.7	1.1	2.1	2.1	1.4	1	1.4	2.5	1.4	2.5	1	0.5	0.5	1.1	2.3
TV-14	2.3	1.5	0.7	0.7	0.7	2.1	1.8	1.1	1.6	2.1	1.6	0.5	1.6	1.6	0.7	1.2
TV-27	2.4	1.5	1.2	0.7	0.7	2.5	2.3	1.5	1.7	2.7	1.7	1.1	2.1	2.1	1.2	1.6
TV-39	2.2	1.1	0.7	1.2	1.2	1.8	1.5	1.1	2	2.1	2	0.5	1.2	1.2	0.7	1.9
TV-79-1	2.5	1.5	1	1.7	1.7	2.3	2.1	1.5	2.1	2.3	2.1	1.1	1.6	1.6	1.4	2.3
TV-93	2.5	1.5	1	1.7	1.7	2.3	2.1	1.5	2.1	2.3	2.1	1.1	1.6	1.6	1.4	2.3

Table 4, cont'd

Genotypes	211-83-2	211-87	211-88	211-90	211-91	712-4	712-7	B71-1	B71-2	KB05	KB08	N211-1	N211K	N212-4	N212-5	N212-8
211-88	2	2.6														
211-90	1.5	1.5	2.5													
211-91	1	2.4	2.2	2.1												
712-4	1.6	2.3	0.7	2.4	1.9											
712-7	1.9	2.9	1.2	2.8	1.6	1										
B71-1	1.5	2.1	1.5	1.4	2.1	1.7	2.2									
B71-2	1.1	1.8	2.3	1	1.8	2.2	2.6	1								
KB05	2.5	3.8	1.6	3.2	2.5	1.9	1.9	2.1	2.7							
KB08	2.3	3.4	1.2	3	2.3	1.6	1.6	1.8	2.5	1						
N211-1	1.7	1.4	2.6	2.5	2.4	2.1	2.7	2.5	2.3	3.7	3.2					
N211K	0	1.7	2	1.1	1	1.5	1.8	1.1	0.5	2.5	2.3	1.4				
N212-4	1.8	2.3	2.7	3	2.3	2.2	2.6	2.6	2.4	3.4	2.9	1.1	1.5			
N212-5	1.8	2.3	1.8	2.5	2.5	1.3	2.2	2.1	2.3	2.6	2.6	1.8	1.5	2.1		
N212-8	1.7	2.4	2.6	3	2	2.1	2.3	2.7	2.5	3.2	2.7	1.4	1.4	0.5	2.3	
M01-8	1.1	1.8	2.3	1.4	1.1	1.9	1.9	2	1.7	3	2.9	2.3	1.1	2.6	2.3	2.5
M02-3	2.3	3.4	1.8	2.9	2.1	2.1	1.8	1.9	2.3	0.9	0.9	3.4	2.1	2.9	3	2.7
M09-3-1	1.4	1.7	2.4	1.5	1.7	2.3	2.5	1.5	1.1	3.1	2.5	2.2	1	2.3	2.9	2.2
M09-3	0	1.7	2	1.5	1	1.6	1.9	1.5	1.1	2.5	2.3	1.7	0	1.8	1.8	1.7
M09-4	2.4	3.6	1.4	3.4	2.2	1.6	1.2	2.3	2.9	1.2	0.7	3.3	2.4	2.9	2.7	2.6
M12-1	1	2.4	2.2	2.1	0	1.9	1.6	2.1	1.8	2.5	2.3	2.4	1	2.3	2.5	2
TV-14	0.5	1.5	2.1	1.4	1.5	1.7	2.2	1.4	1	2.7	2.5	1.5	0.5	1.7	1.6	1.8
TV-27	1.1	1.1	2.3	1.6	2.1	1.9	2.6	1.6	1.2	3.1	2.8	1.1	1.1	1.6	1.7	1.8
TV-39	0.5	1.5	2.1	1.6	1.1	1.7	1.9	1.6	1.2	2.8	2.4	1.5	0.5	1.6	2	1.5
TV-79-1	1.1	1.1	2.3	1.6	1.5	1.9	2.2	1.9	1.6	3.3	2.8	1.5	1.1	1.9	2.3	1.8
TV-93	1.1	1.1	2.3	1.6	1.5	1.9	2.2	1.9	1.6	3.3	2.8	1.5	1.1	1.9	2.3	1.8

Table 4, cont'd

Genotypes	M01-8	M02-3	M09-3-1	M09-3	M09-4	M12-1	TV-14	TV-27	TV-39
M02-3	2.7								
M09-3-1	1.8	2.1							
M09-3	1.1	2.3	1.4						
M09-4	2.9	1.1	2.8	2.4					
M12-1	1.1	2.1	1.7	1.0	2.2				
TV-14	1.4	2.5	1.5	0.5	2.7	1.5			
TV-27	1.9	2.9	1.5	1.1	3.0	2.1	0.7		
TV-39	1.2	2.3	1.1	0.5	2.5	1.1	0.7	1.0	
TV-79-1	1.2	2.5	1.1	1.1	2.9	1.5	1.2	1.2	0.7

Table 5 Cluster grouping of the fifty Bambara groundnut genotypes and their origin

Cluster	Genotype	Origin
Cluster I	211-46-3, 211-45, 211-91, 211-69, 211-75, 211-55	CAPS, South Africa
	M09-4, M02-3, M12-1	National Program, Zimbabwe
	011-7	PMB farmer collection
Cluster II	211-88	CAPS, South Africa
	25-1, 73-2, 42-1, 42-2, 712-1, 712-7	National Program, Zambia
	KB 05, KB 08	ARC, South Africa
Cluster III	211-51-1, 211-57, 21187, 211-77, 211-76	CAPS, South Africa
	32-1-1, 42-2-3, 101-2-1, 101-2	Zambia National Program
	TV-27	IITA, Ibadan Nigeria
	N212-5, N211-1	Kano farmers' collection
Cluster IV	M01-8	National Program, Zimbabwe
	TV-14	IITA
	N211K	Farmers' collection from Kano
Cluster V	211-68, 21151, 211-83-2	CAPS, South Africa
	M09-3	National Program, Zimbabwe
Cluster VI	211-90	CAPS, South Africa
	45-2	National Program, Zambia
	M09-3-1	National Program, Zimbabwe
	TV-39	IITA, Ibadan Nigeria
	B71-1, B71-2	ARC, South Africa
Cluster VII	84-2,73-3	National Program, Zambia
	TV-93, TV-79-1	IITA, Ibadan Nigeria
	N21-4, 212-8	Farmers' collection form Kano

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

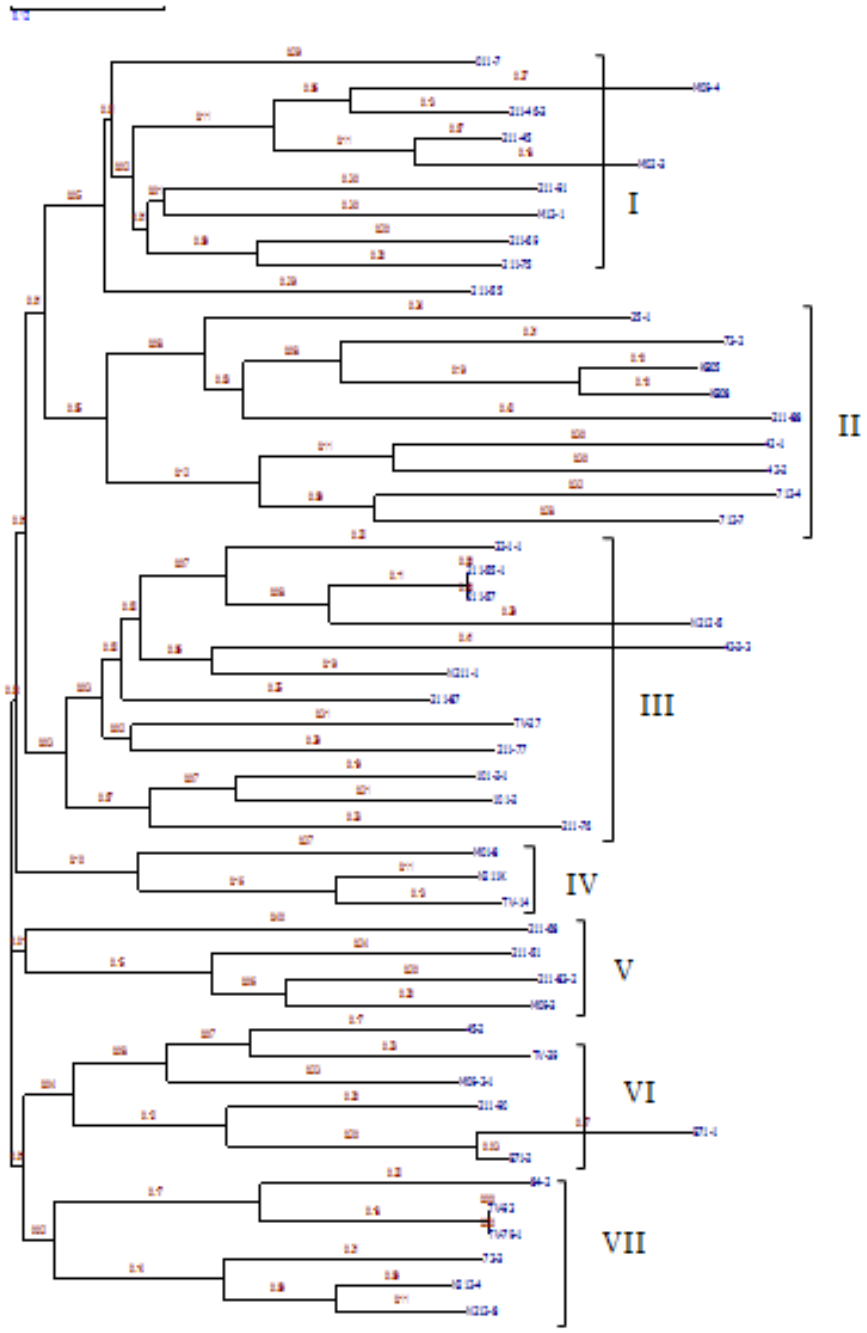

Fig. 1 The Jaccard Neighbor-joining dendrogram illustrating genetic diversity and relationships among 50 Bambara groundnut genotypes used in the study
50 Bambara groundnut genotypes used in the study

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