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# Genetic diversity of Bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces in Kenya using microsatellite markers

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**The existence of genetic diversity in germplasm collections is crucial for cultivar development. Genetic relationships among 105 Bambara groundnuts (*Vigna subterranea* (L) Verdc.) accessions from Kenya were evaluated using 12 microsatellite markers. The Bambara landraces were collected from farmers in the western region and the National Genebank of Kenya. A total number of 24 alleles were revealed with a mean of 2 alleles per locus. The polymorphic information content and gene diversity values averaged 0.28 and 0.35, respectively indicating low genetic diversity among the evaluated Bambara groundnut germplasm. Genetic distance based on Jaccard's similarity coefficient from the simple sequence repeat (SSR) marker analysis ranged from 0.08 to 1.16 among the landraces. Cluster analysis distinctly grouped the 105 accessions into three major clusters. The analysis of molecular variance (AMOVA) revealed that 98% of the total genetic variation was within accessions whereas the genetic variation among accessions accounted for 2% of the total genetic variation. The genetic diversity observed in this study provides the basis for selection of appropriate parental genotypes for breeding programmes and mapping populations to further broaden the genetic base of Bambara groundnut germplasm in Kenya.**

**Key words:** *Vigna subterranean*, accessions, Kenya, microsatellite markers, gene diversity, cluster analysis.

## INTRODUCTION

Evaluation of available genetic diversity is a pre-requisite for genetic improvement in crop plants, especially in underutilized crops such as Bambara groundnut (Olukolu et al., 2012). Investigation of genetic diversity in both wild and domesticated species is equally important. Wild populations are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool (Cattan-Toupance et al., 1998). Wild populations in centers of diversity or domestication constitute the initial gene pool of crops species. Crop

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failures and dispersal of germplasm within the centre of origin or limited introduction or isolated locations could lead to reduced genetic diversity in particular breeding populations (Trethowan and Mujeeb-Kazi, 2008).

The genus *Vigna* (Family *Leguminosae*) is an important legume taxon. It comprises about 90 described species of which seven species are cultivated as economic crops in various regions. Several species are cultivated as minor crops and some wildly grown species are harvested for food and feed. Bambara groundnut is the third most important food legume of Africa after peanut and cowpea. The crop is a very important source of dietary protein for poor people in Africa who cannot afford expensive animal protein (Baryeh, 2001). Thus it has high potential for food security in unpredictable drought regions.

Average yield of Bambara groundnut is rather low compared with other cultivated *Vigna* crops; this, is mainly due to the fact that all of Bambara groundnut cultivars grown are landraces. No improved cultivars were developed by a selective breeding program because an efficient hybridization technique has just been developed (Suwanprasert et al., 2006). Before setting up a breeding program for Bambara groundnut, a thorough understanding on its genetic diversity is necessary. Like many other orphan crops, there are only a few studies on genetic diversity in a large set of Bambara groundnut germplasm. Goli et al. (1997) and Olukolu et al. (2012) studied diversity based on seed patterns in 1,384 and 1,973 accessions, respectively. Olukolu et al. (2012) found that Bambara groundnut from Cameroon/Nigeria region had a higher diversity than those from the other geographical regions. Diversity study in 124 accessions using 28 quantitative traits and in 40 accessions using 554 Diversity Arrays Technique (DArT) markers by the same authors revealed the highest diversity in Cameroon/Nigeria region. The results support the view of Hepper (1963) that center of origin/domestication of Bambara groundnut is in the Cameroon/Nigeria region. In contrast, Somta et al. (2011) studied diversity in a collection of 240 Bambara groundnut accessions using 22 simple sequence repeat (SSR) markers and found highest diversity in West African (excluding Cameroon and Nigeria). This was also reported by Rungnoi et al. (2012). These studies suggest that the center of diversity and origin of Bambara groundnuts is still inconclusive and more evidence is needed to elucidate them. The available literature reveals a number of studies of genetic diversity in Bambara groundnut in the wild and domestication material. They offer a reasonable start to understanding the genetic basis of the domestication event(s) in this crop, potentially enabling parents with a wide genetic base to be identified for developing mapping populations and subsequent QTL analysis.

In this study we evaluated diversity in a collection of 105 Bambara groundnut accessions from several geographical origins in Kenya using simple sequence repeat (SSR) markers. The objective was to provide more evidences on genetic diversity and genetic relationships among different Bambara groundnut accessions in Kenya.

## MATERIALS AND METHODS

### Plant materials and DNA isolation

A total of 105 Bambara groundnuts accessions (Table 1) from Busia (39), the National Genebank of Kenya (37), Kakamega (6), Bungoma (6) and Vihiga (2) were planted in pots containing 0.00141 $m^3$ of soil in the greenhouse at the Kenya Agricultural Research Institute (KARI) Njoro, Kenya. To ensure sufficient tissue, young leaf samples (two weeks old) from four plants per accession were collected for DNA isolation and analysis using a modified protocol of the Doyle and Doyle (1990) CTAB protocol. The modifications involved omission of Ammonium acetate stage and longer (12 h) DNA precipitation. DNA Quantification was carried out by 0.8% agarose gel and Nanodrop 200c spectrophotometer (Thermo scientific corp.) and was diluted to 10 ng $\mu l^{-1}$ for PCR.

### Microsatellite marker analysis

Twelve (12) microsatellite primers (Molosiwa, 2012) (Table 2) were used to assess the genetic diversity of the 105 Bambara groundnuts accessions. The PCR amplification was performed in a 10 µl volume mix consisting of 5 U Dreamtaq polymerase enzyme (Thermo scientific corp, Lithuania), x 6 Dreamtaq buffer (Thermo scientific corp, Lithuania), 2.5 mM of each dNTPS (Bioneer corp, Republic of Korea), $MgCl_2$, 5 µM of each primer (Inqaba biotec, S.A) and 30 ng DNA template in an Applied Biosystems 2720 thermocycler (Life Technologies Holdings Pte Ltd,Singapore). The PCR cycles consisted 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), extension at 72°C for 1 min followed by one cycle of final extension at 72°C for 10 min. The amplicons were mixed with 6x Orange DNA loading dye (Thermo scientific corp, Lithuania) and separated on a 2% agarose gels (Duchefa, Netherlands) stained with Invitrogen life technologies ethidium bromide (Invitrogen corp, U.S.A) in a 0.5x TBE buffer. The separated amplicons were visualized on an Ebox-VX5 gel visualization system (Vilber Lourmat inc, France). The alleles were scored as absent or present based on the size of the amplified product using a 100 bp O'geneRuler ready to use DNA Ladder (Thermo Scientific Corp, Lithuania).

### Data analysis

Molecular data was recorded in binary fashion for SSR marker loci analysed and scoring was based on presence and absence of band for each primer set with one (1) and zero (0) being the respective scores. The summary statistics on major allele frequency, allele number, gene diversity, and PIC values (Botstein et al., 1980) were calculated using Power Marker version 3.25 (Liu and Muse, 2006) while Shannon's information index (*I;* Lewontin, 1972) of each locus was calculated using software popGene32 version 1.32 (Yeh et al., 2000). Analysis of molecular variance (AMOVA) was performed using Arlequin v.3.1 (Excoffier et al., 2005). Genetic dissimilarities between all the accessions was calculated using DARwin version 5.0 (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006) using simple matching coefficient. The dissimilarity coefficients were then used to generate an unweighted neighbour-joining tree

**Table 1.** Names and sources of one hundred and five Bambara groundnut landraces used in the study.

| Accession | County | Characteristic features | Accession | County | Characteristic features |
|---|---|---|---|---|---|
| KE/BN/1/1 | Busia | Black | KE/BN/23/2 | Kakamega | Dark Red |
| KE/BN/1/2 | Busia | Dark Red | KE/BN/23/3 | Kakamega | Light Red |
| KE/BN/2/1 | Kakamega | Cream entire | KE/BN/24 | Vihiga | Dark Red |
| KE/BN/2/2 | Kakamega | Cream spotted | KE/BN/25/1 | Kakamega | Black |
| KE/BN/3/1 | Bungoma | Red | KE/BN/25/2 | Kakamega | Light Red |
| KE/BN/4/1 | Kakamega | Black | KE/BN/26/1 | Busia | Light Red |
| KE/BN/4/2 | Kakamega | Brown | KE/BN/26/2 | Busia | Dark Red |
| KE/BN/4/3 | Kakamega | Light Red | KE/BN/27 | Busia | Dark Red |
| KE/BN/5/1 | Busia | Cream entire | KE/BN/28 | Busia | Brown red black spotted |
| KE/BN/5/2 | Busia | Cream one side spotting | KE/BN/29 | Busia | Light Red |
| KE/BN/8/1 | Busia | Lght Red | KE/BN/30/1 | Busia | Brown |
| KE/BN/8/2 | Bungoma | Dark Red | KE/BN/30/2 | Busia | Red |
| KE/BN/9 | Kakamega | Dark Red | KE/BN/31/1 | Busia | Black |
| KE/BN/10 | Vihiga | Brown | KE/BN/31/2 | Busia | Dark Red |
| KE/BN/12/1 | Kakamega | Black | KE/BN/32/1 | Busia | Black |
| KE/BN/12/2 | Kakamega | Light Red | KE/BN/32/2 | Busia | Light Red |
| KE/BN/12/3 | Kakamega | Brown spotted | KE/BN/34/1 | Busia | Brown |
| KE/BN/13/1 | Busia | Black | KE/BN/35/1 | Busia | Black |
| KE/BN/13/2 | Busia | Light Red | KE/BN/35/2 | Busia | Dark Red |
| KE/BN/13/3 | Busia | Dark Red | KE/BN/36 | Busia | Cream Red spotted |
| KE/BN/13/4 | Busia | Brown | KE/BN/37/1 | Bungoma | Light Red |
| KE/BN/13/5 | Busia | Brown Black spotted | KE/BN/37/2 | Bungoma | Dark Red |
| KE/BN/14/1 | Kakamega | Brown entire | KE/BN/38/2 | Busia | Light Red |
| KE/BN/14/2 | Kakamega | Brown spotted | KE/BN/39/1 | Kakamega | Cream |
| KE/BN/15/1 | Kakamega | Black | GBK/050490 | Genebank | Cream entire white eye |
| KE/BN/16/1 | Busia | Dark Red | GBK/050491 | Genebank | Cream entire white eye |
| KE/BN/16/2 | Busia | Light Red | GBK/050492 | Genebank | Cream spotted white eye |
| KE/BN/16/3 | Busia | Black | GBK/050493 | Genebank | Red brown white eye |
| KE/BN/17/1 | Kakamega | Dark Red | GBK/050494 | Genebank | Red Brown spotted white eye |
| KE/BN/17/2 | Kakamega | Light Red | GBK/050495 | Genebank | Orange brown white eye |
| KE/BN/18/1 | Kakamega | Black | GBK/050496 | Genebank | Orange brown white eye |
| KE/BN/19/1 | Busia | Cream entire | GBK/050499 | Genebank | Brown white eye |
| KE/BN/19/2 | Busia | Cream spotted | GBK/050501 | Genebank | Cream white white eye |
| KE/BN/20/2 | Busia | Light Red | GBK/050502 | Genebank | Cream white white eye |
| KE/BN/21/1 | Bungoma | Black | GBK/050649 | Genebank | Black white eye |
| KE/BN/21/2 | Bungoma | Light Red | GBK/050650 | Genebank | Black white eye/red brown white eyes |
| KE/BN/22/2 | Busia | Dark Red spotted | GBK/050653 | Genebank | Dark brown white eye |
| KE/BN/22/3 | Busia | Light Red | GBK/050654 | Genebank | Red brown white eye |
| KE/BN/23/1 | Kakamega | Brown | GBK/050655 | Genebank | Black white eye |
| GBK/050656 | Genebank | Black white eye | GBK/050671 | Genebank | Black white eye |
| GBK/050657 | Genebank | Light red white eye | GBK/050672 | Genebank | Black white eye |
| GBK/050658 | Genebank | Black white eye | GBK/050673 | Genebank | Black white eye |
| GBK/050659 | Genebank | Black brown white eye | KE/BN/40 | Busia | Black white eye |
| GBK/050660 | Genebank | Cream spotted white eye | KE/BN/41 | Busia | Black white eye |
| GBK/050661 | Genebank | Black white eye/ Brown spotted white eye | KE/BN/42 | Busia | Black white eye |
| GBK/050663 | Genebank | Light red white eye | KE/BN/43 | Busia | Black white eye |
| GBK/050664 | Genebank | Black white eye | KE/BN/44 | Busia | Black white eye |
| GBK/050665 | Genebank | Black white eye/light red white eye | KE/BN/45 | Busia | Black white eye |
| GBK/050666 | Genebank | Black white eye/light red white eye | KE/BN/46 | Busia | Black white eye |
| GBK/050667 | Genebank | Light red white eye | KE/BN/47 | Busia | Black white eye |
| GBK/050668 | Genebank | Brown white eye/black white eye | KE/BN/48 | Busia | Black white eye |
| GBK/050669 | Genebank | Light red white eye | KE/BN/49 | Busia | Black white eye |
| GBK/050670 | Genebank | Black white eye | | | |

**Table 2.** Primer information of twelve SSR markers used for amplification of DNA isolated from 105 accessions of Bambara groundnut germplasm.

| Marker | Sequence (5´-3´) | Product size (bp) | Ta (°c) |
|---|---|---|---|
| PRIMER 1F | AGGCAAAAACGTTTCAGTTC | | 55.3 |
| PRIMER 1R | TTCATGAAGGTTGAGTTTGTCA | 273 | 55.3 |
| PRIMER 2F | AGGAGCAGAAGCTGAAGCAG | | 55.3 |
| PRIMER 2R | CCAATGCTTTTGAACCAACA | 212 | 55.3 |
| PRIMER 3F | TTCACCTGAACCCCTTAACC | | 57.6 |
| PRIMER 3R | AGGCTTCACTCACGGGTATG | 247 | 57.6 |
| PRIMER 4F | ACGCTTCTTCCCTCATCAGA | | 57.6 |
| PRIMER 4R | TATGAATCCAGTGCGTGTGA | 197 | 57.6 |
| PRIMER 5F | TCAGTGCTTCAACCATCAGC | | 55.3 |
| PRIMER 5R | GACCAAACCATTGCCAAACT | 260 | 55.3 |
| PRIMER 6F | CCGGAACAGAAAACAACAAC | | 57.6 |
| PRIMER 6R | CGTCGATGACAAAGAGCTTG | 189 | 57.6 |
| PRIMER 7F | TGTGGGCGAAAATACACAAA | | 59.7 |
| PRIMER 7R | TCGTCGAATACCTGACTCATTG | 198 | 59.7 |
| PRIMER 8F | CAAACTCCACTCCACAAGCA | | 57.6 |
| PRIMER 8R | CCAACGACTTGTAAGCCTCA | 250 | 57.6 |
| G358B2-D15F | TGACGGAGGCTTAATAGATTTTTC | | 59.0 |
| G358B2-D15R | GACTAGACACTTCAACAGCCAATG | 193 | 59.0 |
| mBam2co80F | GAGTCCAATAACTGCTCCCGTTTG | | 59.0 |
| mBam2co80R | ACGGCAAGCCCTAACTCTTCATTT | 220 | 59.0 |
| G180B2-D11F | GAGGAAATAACCAAACAAACC | | 59.0 |
| G180B2-D11R | CTTACGCTCATTTTAACCAGACCT | 198 | 59.0 |
| G358B3-D15F | TGACGGAGGCTTAATAGATTTTTC | | 59.0 |
| G358B3-D15R | GACTAGACACTTCAACAGCCAATG | 196 | 59.0 |

(Saitou and Nei, 1987) with Jaccard's Similarity Coefficient with a bootstrapping value of 1,000 using the same software (DARwin 5.0).

## RESULTS

### Marker polymorphism, diversity within accessions and genetic distance among accessions

SSR analysis in the 105 Bambara groundnut accessions (Table 3) revealed that the number of reproducible DNA bands per primer ranged from 70 (Primer 1) to 97 (mBam2co80) totalling to 958 with an average of 79.83 bands.

Polymorphic information content (PIC) ranged from 0.13 to 0.35, (marker 10 and 5, respectively) with an average of 0.28 (Table 3). Accessions from Kakamega, Bungoma and Vihiga counties had the greatest gene diversity ($H_{E\ =}$ 0.5) and Shannon's diversity index ($I$ =0.6931), followed by those from the National Genebank of Kenya ($H_{E\ =}$ 0.49, $I$ =0.6928) and Busia ($H_{E\ =}$ 0.47, $I$ =0.6663). The National Genebank of Kenya accessions had the lowest $H_E$ and $I$ with 0.1023 and 0.2103, respectively followed by accessions from Busia county with 0.1420 and 0.2712. Kakamega and Bungoma counties accessions both had genetic diversity of 0.2778

**Table 3.** Estimate of genetic diversity of Bambara groundnut germplasm collections using 12 SSR markers.

| Locus | na* | ne* | h* | I* | Major allele frequency | PIC | No. of amplified bands |
|---|---|---|---|---|---|---|---|
| Primer 1 | 2.0000 | 1.8000 | 0.4444 | 0.6365 | 0.67 | 0.35 | 70 |
| Primer 2 | 2.0000 | 1.2771 | 0.2170 | 0.3744 | 0.88 | 0.19 | 92 |
| Primer 3 | 2.0000 | 1.8202 | 0.4506 | 0.6429 | 0.66 | 0.35 | 69 |
| Primer 4 | 2.0000 | 1.4953 | 0.3312 | 0.5133 | 0.79 | 0.28 | 83 |
| Primer 5 | 2.0000 | 1.8396 | 0.4564 | 0.6489 | 0.65 | 0.35 | 68 |
| Primer 6 | 2.0000 | 1.5448 | 0.3527 | 0.5375 | 0.77 | 0.29 | 81 |
| Primer 7 | 2.0000 | 1.9489 | 0.4869 | 0.6800 | 0.58 | 0.37 | 61 |
| Primer 8 | 2.0000 | 1.5448 | 0.3527 | 0.5375 | 0.77 | 0.29 | 81 |
| G358B2-D15 | 2.0000 | 1.5201 | 0.3421 | 0.5257 | 0.78 | 0.28 | 82 |
| mBam2co80 | 2.0000 | 1.1638 | 0.1408 | 0.2694 | 0.92 | 0.13 | 97 |
| G180B2-D11 | 2.0000 | 1.4213 | 0.2964 | 0.4728 | 0.82 | 0.25 | 86 |
| G358B3-D15 | 2.0000 | 1.3725 | 0.2714 | 0.4428 | 0.84 | 0.23 | 88 |
| Mean | 2.0000 | 1.5624 | 0.3452 | 0.5235 | 0.76 | 0.28 | 79.83 |

na***** = Observed number of alleles; ne***** = Effective number of alleles [Kimura and Crow (1964)]; h*****= Nei's (1973) gene diversity; I***** = Shannon's Information index [Lewontin (1972)].

**Table 4.** Analysis of molecular variance (AMOVA) for 105 bambara groundnut genotypes.

| Source of variation | df | SS | MS | Variance components | Variation |
|---|---|---|---|---|---|
| Among Accessions | 4 | 15.275 | 3.819 | 0.065 | 2% |
| Within Accessions | 100 | 263.772 | 2.638 | 2.638 | 98% |
| Total | 104 | 279.048 | | 2.703 | 100% |

and Shannon's diversity index of 0.4506.

Analysis of molecular variance (AMOVA) was done on the dataset to partition the total genetic variation among and within the accessions (Table 4). This revealed that the highest proportion of the total variation (98%) was among individuals within accessions. The proportions of variation among the accessions were lower at 2%.

Genetic distance based on Jaccard's similarity coefficient from the SSR marker analysis ranged from 0.08 to 1.17 among the landraces. Accessions from Bungoma county had the least genetic distance (0.41) indicating close genetic relationship while greatest genetic distance was recorded in accessions from Busia county (1.11) indicating genetic distance relatedness. Accessions from the National Genebank of Kenya (1.03) also had a high genetic distance.

## UPGMA, Principal coordinate analyses

A dendogram generated by UPGMA cluster analysis failed to illustrate clear pattern of germplasm clusters based on their places of origin (Figure 1). In most cases, accessions from different regions or counties were clustered with one another. However, it demonstrated that accessions from Busia county and the National Genebank of Kenya tended to agglomerate together in cluster III. All the 105 individual genotypes were grouped into three (I, II, III) main clusters (Figure 1). Except for cluster I all the remaining clusters had sub- clusters. There was a general trend as those accessions from the National Genebank of Kenya and Busia county tended to group together in cluster III while those from Kakamega county tended to cluster together in cluster I. Accessions from Vihiga and Bungoma counties were found in clusters I and III. Two clusters with the highest number of genotypes were cluster II and III with 27 and 58 individual genotypes respectively. Grouping of the genotypes of these landraces into sub- clusters indicated substantial level of intra-landrace polymorphism. Similarly high level of intra-landrace polymorphism can be said of the landraces in cluster II and III all of which had their individual genotypes grouped into more than two sub-cluster units. Cluster I had all the individual genotypes clustered into only one unit suggesting lesser level of intra-landrace polymorphism within the cluster compared to the rest of the landrace clusters.

Principal coordinate analysis (PCoA) (Figures 2 and 3) was performed to reveal genetic relationship among Bambara groundnut accessions. The first three axes accounted for 84.3% of the total variations (Table 5) with each axes explaining 63.58, 12.21 and 8.24% variation in that order. The first three axes accounted for the highest variation (96.81%) for Bungoma county accessions followed by accessions from Busia county (71.4%), Kakamega county (59.59%) and Genebank (59.31%).

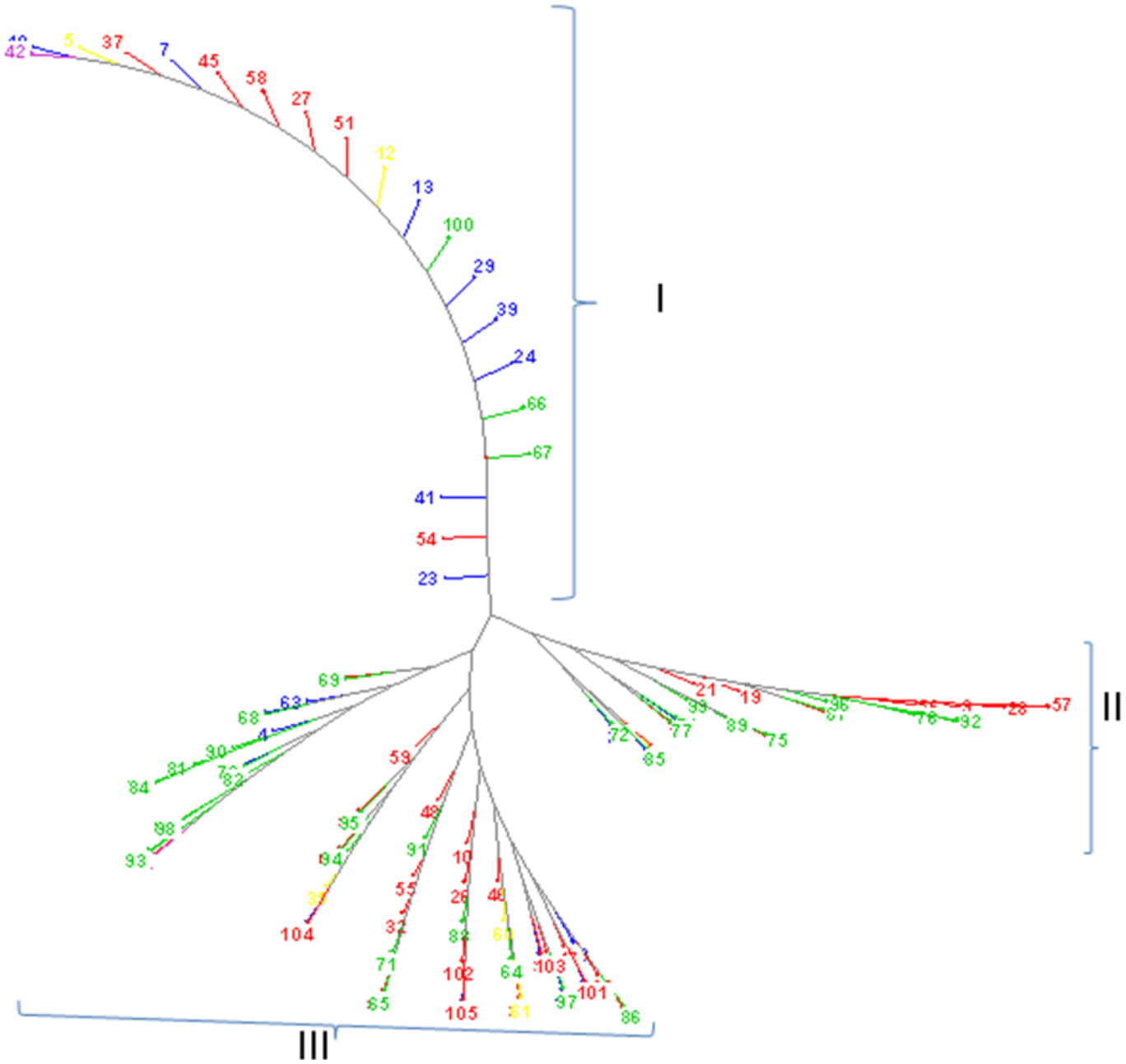


**Figure 1.** Genetic relationships generated by Jaccard's similarity coefficients among 105 Bambara groundnut accessions. Accessions given in red were from Busia, blue from Kakamega, purple from Vihiga, yellow from Bungoma and green from Genebank.

Principal component analysis failed to differentiate accessions according to their area of origin. Most of the accessions overlapped demonstrating close genetic relationships. This suggests that these accessions could have originated from the same source. From the PCoA plot of the accessions (Figure 3), principal axes 1 and 2 showed that KE/BN/34/1, KE/BN/13/5, KE/BN/16/3 from Busia county and GBK/050668 from the National Genebank of Kenya were the most distinct from the other accessions.

## DISCUSSION

Genetic analysis of diversity is very critical as it gives more accurate measure of polymorphism compared to morphological characterizations. In the present study, extent and organization of genetic diversity within 105 accessions of Bambara groundnut from Western Kenya and the National Genebank of Kenya was assessed using 12 polymorphic SSR bands. The 12 SSR markers revealed the availability of polymorphism among the

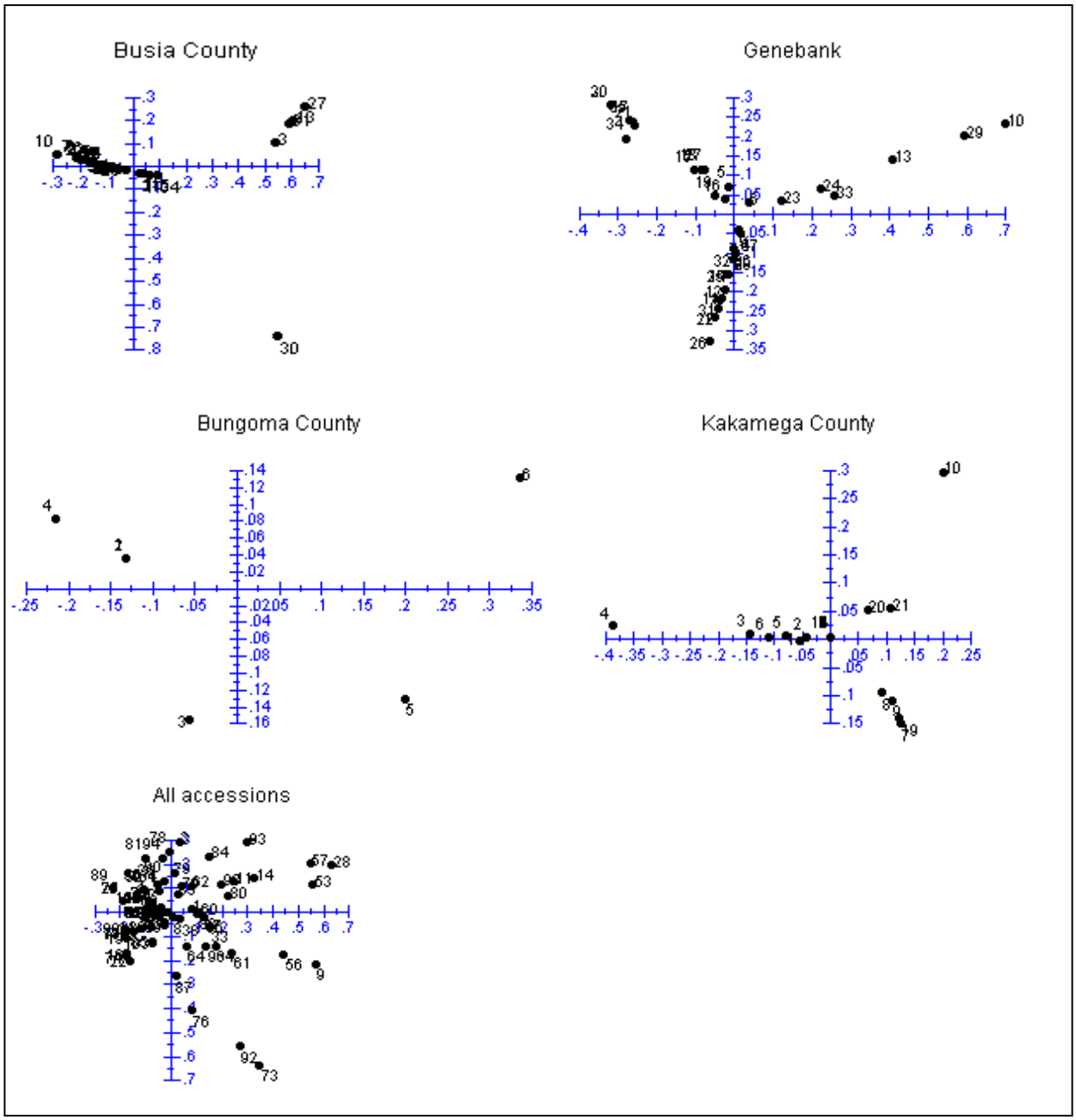


**Figure 2.** PCoA of axes 1 and 2 based on dissimilarity of 12 SSR markers across 105 Bambara groundnut landraces from different regions.

landraces of bambara groundnuts as evidenced in genetic distances and the cluster analysis (Figure 1). Previous studies by Massawe et al. (2002) based on AFLP molecular marker analysis revealed extensive genetic diversity between 12 African Bambara groundnut landraces from diverse origin. Amadou et al. (2001) also reported considerable genetic diversity among 25 African Bambara groundnut accessions from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, using random amplified polymorphic DNA (RAPD) markers. They demonstrated two main groups of accessions mainly along the lines of their geographic origin. Similarly Somta et al. (2011) reported high genetic diversity among 240 Bambara groundnut accessions from Africa and Southeast Asia using SSR markers as did Olukolu et al. (2012) and Aliyu et al. (2013).

In contrast, based on isozyme analysis, Pasquet et al. (1999) reported that both wild and domesticated bambara groundnuts were characterized by low genetic diversity, indicating that wild Bambara groundnut is the progenitor

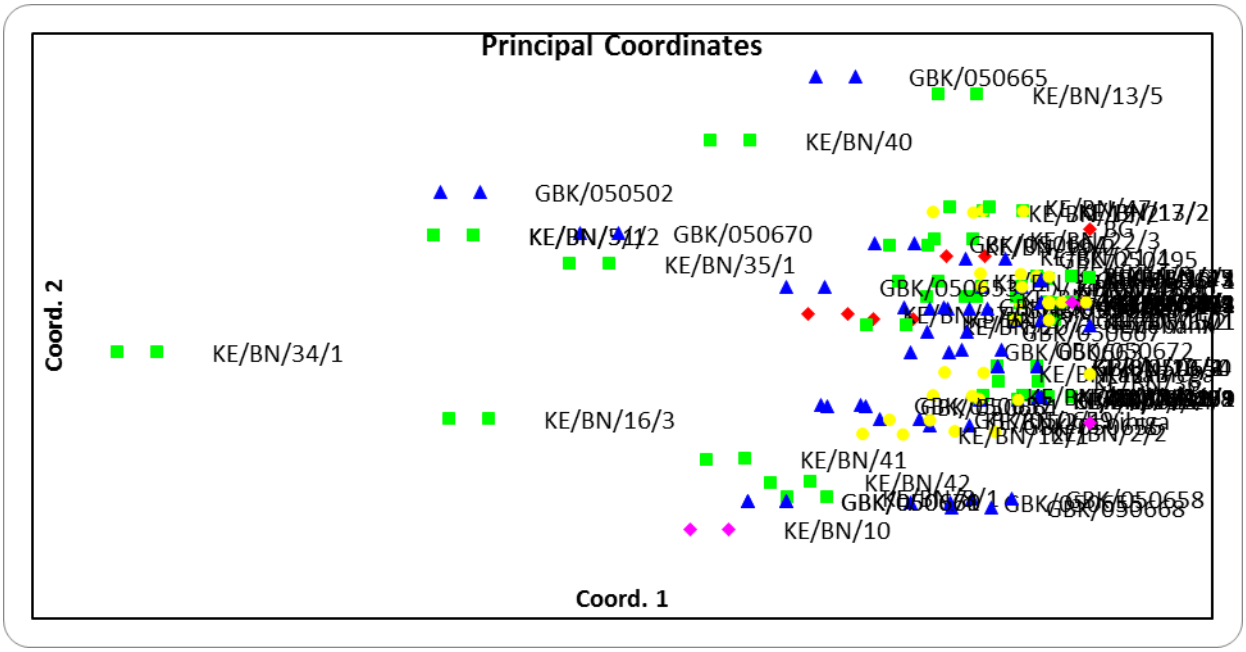


**Figure 3.** Configuration of bambara groundnut accessions under principal component axis 1 and 2. BG- Bungoma county.

**Table 5.** Eigen value and percentage of total variation accounted for by the first three component axes.

| Axis | Eigen value | Proportion (%) | Cumulative (%) |
|---|---|---|---|
| 1 | 29.21 | 63.58 | 63.85 |
| 2 | 5.612 | 12.21 | 76.06 |
| 3 | 3.786 | 8.24 | 84.30 |

of the domesticated form. This is expected as isozymes are generally limited by the low levels of polymorphism detectable and may fail to discriminate cultivars differing only slightly in genetic make up.

In this work, genotypes were clustered into three clusters (I, II and III) with clusters II and III forming sub-clusters. There was substantial intra-landrace polymorphism as two of the three clusters had sub-clusters with distinct genotypes though from different regions. The high level of intra-landrace polymorphism could be attributed to seed exchange between farmers as well as the geographical proximity of the areas. Contrary to the higher intra-polymorphism of most of the landraces, genotypes in cluster I appeared less heterogeneous. Accessions from Kakamega, Busia counties and the National Genebank of Kenya tended to form a clear group (cluster III). This was elucidated further by AMOVA, which partitioned the total genetic variation among and within accessions. This showed that majority of genetic variation observed in the germplasm (98%) was due to the variation among individuals instead of being between specific accession groups. Divergent accessions may have good breeding value, which may be utilized for direct selection and as parents of crosses with accessions from different clusters. The mixture of accessions in cluster I, II and III mainly from the counties of Busia, Kakamega and National Genebank of Kenya indicated that Bambara groundnut accessions in this group constituted a more heterogenic group, with variable genetic backgrounds. This can also be explained by the high frequency of bambara groundnut seed exchange by farmers over wide geographic-ethnic regions as well as the different informal names given to landraces from one region to another which may give room for genotype duplications as was suggested by Hudu and Saaka (2011).

The low level of genetic diversity revealed in this work could be supported by the fact that small scale farmers in Eastern Africa generally tend to exchange seeds frequently. A farmers field survey (Ntundu, 2002) indicated that at least 44% of farmers in Tanzania obtain their bambara groundnut seeds from others farmers within (39%) and outside (5%) of their regions, annually. In their survey on seed market assessment in Dodoma, Iringa and Morogoro regions in Tanzania, Ashimogo and Rukulantile (2000) reported that 35.4% of farmers obtained maize (*Zea mays* L.) seeds from their fellow farmers, while 60.1% use only their own seeds. Similar practice has been reported to be common among growers of cucurbits (*Cucurbita moschata* Duch) in Zambia where at least 40% of the farmers obtain their seeds from other farmers (Gwanama and Nichterlein, 1995) within their neighbouring growing regions. Further studies showed that sources of seed for planting of Bambara groundnut in Ghana include farmer saved seed,

exchange and market purchase (Berchie et al., 2010).

Principal component analysis (PCA) is a descriptive technique which reveals the pattern of character variation among genotype (Aremu et al., 2007). PCA failed to group accessions according to their areas of origin. This could have lead to a generally low coefficient of variation observed in Bambara groundnut accessions, an indication of a high level of uniformity. This suggested that the source of these accessions could be same due to seed exchange among the farmers.

## Conclusion

Our study reported herein shows that Bambara groundnut landraces from Kenya, form a genetically diverse population and SSR markers can be effectively employed to assess genetic diversity and to measure the extent of genetic relationship among accessions. Knowledge of the degree of genetic relationships between bambara groundnut accessions will be of importance for crop improvement and may help to establish a core collection as part of the germplasm collection management to sample a maximum of genetic variation of accessions. This study reveals that Bambara groundnut accessions from Western Kenya and the National Genebank of Kenya constitute three major genetic clusters. The study revealed a low genetic variability among the accessions but a high genetic variability within them.

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### REFERENCES

Aliyu S, Festo JM (2013). Macrosatellites based marker molecular analysis of Ghanian bambara groundnut (*Vigna subterranean* (*L.*) Verdc.) landraces alongside morphological characterization. Gen. Resour. Crop Evol. 60:777-787.

Amadou HI, Bebeli PJ, Kaltsikes PJ (2001). Genetic diversity in bambara groundnut (*Vigna subterranea* L.) germplasm revealed by RAPD markers, *Genome* 44:995-999.

Aremu CO, Adebayo MA, Ariyo OJ, Adewale BB (2007). Classification of genetic diversity and choice of parents for hybridization in cowpea *Vigna unguiculata* (L.) Walp for humid savanna ecology. Afr. J. Biotechnol. 6 (20):2333-2339.

Ariyo OJ (1990). Measurement and classification of genetic diversity in okro *Abelmoschus Esculentus.* Annals Appl. Biol. 116:335-341.

Baryeh EA (2001). Physical properties of bambara groundnuts. J. Food Eng. 47:321-326.

Berchie JN, Adu-Dapaah HK, Dankyi AA, Plahar WA, Nelson- Quarety F, Haleegoah J, Asafu-Agyei JN, Addo JK (2010). Practices and constraints in bambara groundnut production, marketing and consumption in the brong ahafo and upper-East regions of Ghana. J. Agron. 9(3):111-118.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Human Gen. 32:314-331.

Cattan-Toupance I, Michalakis Y, Neema C (1998). Genetic structure of wild bean populations in their South-Andean centre of origin. Theor. Appl. Gen. 96:844-851.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An intergrated software package for population genetic data analysis. Evol. Bioinform. Online 1:47-50.

Goli AE, Begemann F, Ng NQ (1997). Characterization and evaluation of IITA's bambara groundnut collection. *In* Proceedings of the workshop on conservation and improvement of bambara groundnut (*Vigna subterranea* (L.) Verdc.). Eds Heller J, Begemann F, Mushonga J, Institute of Plant Genetics and Crop Plant Research, Gatersleben/Department of Research and Specialist Services, Harare/International Plant Genetic Resources Institute, Rome, Italy, pp. 101-118.

Hepper FN (1963). Plant of the 1957-58 West Africa expedition II: The bambara groundnut (*Voandzeia subterranea*) and Kersting's groundnut (*Kerstingiella geocarpa*) wild in West Africa. Kew Bulletin 16:39-407.

Hudu BA, Saaka SJB (2011). Characterization of bambara groundnut landraces and their evaluation by farmers in the upper West Region of Ghana. J. Dev. Sust. Agric. 6:64-74.

Liu K, Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinform. 21:2128-2129.

Massawe FJ, Dickinson M, Robert JA, Azam-Ali SN (2002). Genetic diversity in bambara groundnut (*Vigna subterranea* (L.) Verdc) landraces revealed by AFLP markers, Genome 45:1175-1180.

Mosiwa O. Odireleng (2012). Genetic diversity and population structure analysis of bambara groundnut (Vigna subterranean (L) Verdc.) lanraves using morpho-agronomic characters and SSR markers.Msc Thesis, The University of Nottingham Sutton, Leicestering, Uk

Olukolu BA, Mayes S, Stadler F, Ng QN, Fawole I, Dominique D, Azam-Ali SN, Abott GA, Kole C (2012). Genetic diversity in bambara groundnut (*Vigna subterranea* (L.) Verdc.) as revealed by phenotypic descriptors and DArT marker analysis. Gen. Resour. Crop Evol. 59:347-358.

Pasquet RS, Schwede S, Gepts P (1999). Isozyme diversity in bambara groundnut, Crop Sci. 39:1228-1236.

Perrier X, Flori A, Bonnot F (2003). Data analyses methods, in: Hamon, P, Seguin, M, Perrier, X, Glaszmann, JC (Eds.). Genetic diversity of cultivated tropical plants, Enfield, Science Publishers, Montpellier, France. pp. 43-76.

Perrier X, Jacquemoud-Collet JP (2006). DARwin software.

Rungnoi O, Suwanprasert J, Somta P, Srinives P (2012). Molecular genetic diversity of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) revealed by RAPD and ISSR marker analysis. *SABRAO.* J. Breed. Gen. 44 (1):87-101.

Saitou N, Nei M (1987). The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Somta P, Chankaew S, Rungnoi O, Srinives P (2011). Genetic diversity of the Bambara groundnut (*Vigna Subterranea* (L.) Verdc.) as assessed by SSR markers. Genome. 54:898-910.

Suwanprasert J, Toojinda T, Srinives P, Chanprame S (2006). Hybridization technique for Bambara groundnut. Breed. Sci. 56:125-129.

Trethowan RM, Mujeeb-Kazi A (2008). Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Sci. 48:1255-1265.

Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM (2000). POPGENE 32, Microsoft window-based Freeware for Population Genetic Analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.