

GENETIC DIVERSITY OF DRY BEAN (*Phaseolus vulgaris* L.) ACCESSIONS OF KENYA USING SSR MARKERS

ABSTRACT

Aims: To determine the genetic diversity existing within the Kenyan dry bean using SSR markers.

Place and Duration of Study: this study was conducted in the department of biological sciences, Masinde Muliro University of Science and Technology, Kenya and Bangor University, North Wales United Kingdom, between September 2010 and December 2012.

Methodology: We included 35 marketable dry bean samples collected from farmers, market centers as well as seed stockists in Eastern and Western provinces of Kenya. Data scoring and cluster analysis were performed using simple matching coefficients. SSR allele sizes were visualized the base pair sizes estimated. Gene diversity index, number of polymorphic loci, percent of polymorphic loci and genetic distance for each population was derived using NYTS-pc 2.1 and GenAEx 6.4 software assuming Hardy-Weinberg equilibrium. The similarity coefficients were subjected to unweighted pair group method of arithmetic averages. Analysis of molecular variance was calculated using GenAEx software.

Results: 7 SSR primers were sampled. 5 SSR primers found to be polymorphic were used on 35 bean samples and yielded 49 polymorphic bands. Analysis of molecular variance accredited 8% of the disparity to diversity among the populations while the majority of the diversity (92%), resided within populations. The gene diversity index ranged from 0.1267 in the market population to 0.2377 in the Western province population. The highlands of Eastern province had a gene diversity index of 0.1475 while the dry lands had 0.1991.

Conclusion: There exists a wide variation in the dry bean of Kenya, but it is narrowing. The population genetics of dry beans of Kenya are a possible guide to future bean breeding and germplasm management in Kenya.

Keywords: SSRs, *Phaseolus vulgaris*, dry bean, germplasm characterization, and genetic variation.

1. INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.), is a widely distributed crop of considerable importance in many countries worldwide, being the most important edible food legume in the world representing 50% of grain legumes for direct human consumption [1]. The cultivated bean crop has two distinct gene pools that occurred as a result of domestication in the Andes (Peru and Argentina) and in the Middle America (Mexico, Colombia and Central America). It shows morphological and genetic differences including growth habit, seed size and dormancy [2]. CIAT in focus to crop commitment recognize the bean crop as a source of dietary protein for human consumption, with relatively high amounts of lysine, tryptophan and methionine. In the East and Central Africa, the bean crop is of vital importance in provision of dietary protein for more than 80 million people [3]. Dry beans are cultivated mostly by women for subsistence and the market on more than 5600 hectogram per hectare, accounting for a quarter of global output [3]. Being one of the longest cultivated plants worldwide, it is widely adapted for growth in most agricultural regions of Kenya.

Assessment of genetic diversity among different cultivars enhances introgression of desirable traits like disease resistance, early maturity, and high yielding or drought

resistance in crop [4], [5]. This has the potential to greatly support and expedite selection decisions in any crop breeding. Introgression of desirable characteristics enhances better survival of the crop in the current climatic changes and increasing biotic and abiotic stresses that are threatening to reduce food security. Microsatellites are DNA regions composed of small motifs of 1 to 6 nucleotides repeated in tandem, which are widespread in both eukaryotic and prokaryotic genomes. They are the best markers for assessment of genetic diversity and mapping as they are co- dominant markers, have an advantage of displaying polymorphisms at highly variable loci, can be amplified by polymorphic chain reactions and a well distributed throughout the genome [6], [7]. SSRs were developed from both coding and noncoding regions of the plant genome [8]. Its' analysis involves total extraction of genomic DNA followed by polymerase chain reactions under a specified thermo cyclic program, using SSR primers. The PCR product is later visualized by gel electrophoresis to view the banding patterns.

There are several resources that can be used to search for SSR; Public databases, expressed sequence tags (ESTs) databases [8], [2], DNA libraries such as genomic, genomic-enriched for SSR. In any crop improvement programme, genetic diversity assessment between individuals within a species or between different species or populations is very important to select genetically diverse parental lines to obtain superior recombinants. In common bean, different marker systems namely morphological [9],[10], biochemical [11],[12] and molecular markers namely RFLPs [13][14], RAPDs [10],[14],[15],[16],[17],[18], SSRs [19],[20], AFLPs [21],[22],[23],[24],[25],[26] have been successfully used for genetic diversity analysis. To obtain an accurate specific profile, the use of DNA markers proved to confer significant advantages over morphological and biochemical markers. In this project, a total of 7 dry bean SSR primer pairs ordered from EurofinsTM were used to investigate the genetic diversity in the common bean collection from farmers and market centers to check for polymorphism levels.

2. MATERIAL AND METHODS

Bean germplasm

Thirty five bean accessions and varieties were selected from a collection of 150 dry bean seeds that had been collected from farmers and market centers in a field survey conducted in Eastern and Western provinces of Kenya (table 1). The accessions represented four populations, Meru and Embu, Machakos and Mbeere, Western Kenya and markets.

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Table 1. Germplasm description of 35 bean samples used in genetic diversity studies using SSRs in Kenya.

Accession Number	Accession Code	Local name	Seed source	Characteristics i.e. color, seed size small or large etc	Longitude	Latitude
4.	Mw01	Mwitmania	Farmer seed	Mwitmania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.26589°	037.75072°
9.	Ro02	Rose coco	Farmer seed	Rose coco; Variegated, purple on cream, medium sized,	00.26836°	037.66205°

14.	GA03	Gasele	Farmer seed	globular. Canadian wonder; white, to light grey, medium to large sized, oblong	00.31809°	037.66199°
24.	Mw04	Mwiternania	Farmer seed	Mwiternania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.33229°	037.64431°
32.	Gas05	Gasele	Farmer seed	Canadian wonder; white to light grey medium to large sized, oblong	00.04799°	037.65575°
34.	Can06	Canadian wonder	Farmer seed	Canadian wonder; Purple- black, medium to large sized, oblong	00.12636°	037.56236°
41.	Wai07	Wairimu	Farmer seed	Red haricot: small to medium sized, oblong.	00.56743°	037.49013°
46.	Can08	Canadian wonder	Farmer seed	Canadian wonder; Purple- black, medium to large sized, oblong	00.60266°	037.51808°
52.	Rmw09	Red Mwiternania	Farmer seed	Mwiternania; resembles Pinto bean, variegated, red–grey on cream, small to medium sized globular.	00.49228°	037.46354°
60.	Roc10	Rose coco	Farmer seed	Rose coco; Variegated, purple on cream, medium sized, globular.	00.40642°	037.62504°
64.	Mw11	Mwiternania	Farmer seed	Mwiternania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.39233°	037.60790°
69.	Rmw12	Red Mwiternania	Farmer seed	Mwiternania; resembles Pinto bean, variegated, red – grey on cream, small to medium sized globular.	00.38413°	037.58708°
70.	Ny13	Nyayo	Farmer seed	Rose coco; variegated with large red flecks on cream, medium sized, and oblong.	01.56349°	037.25222°
72.	Kb14	KATB1	Farmer seed	Pale yellow, medium sized, oblong.	01.56406°	037.25496°
78.	Kik15	Kikuyu	Farmer seed	Rose coco; Variegated, red on cream, medium sized, globular.	01.53483°	037.26243°
80.	Roc16	Rose coco	Farmer seed	Rose coco; Variegated, purple on cream, medium sized, globular.	00.12736°	034.73612°
85.	KKf17	KK15	Farmer seed	Black seed coat, medium sized, oblong	00.05426°	034.74751°
88.	Rh18	Red Haricot	Farmer seed	Red haricot: small to medium sized, oblong.	00.06007°	034.72225°
90.	Sugo19	Sugar 1	Farmer seed	Rose coco; variegated with large red flecks on light brown, medium to large sized, oblong.	00.12375°	034.78494°
95.	KKt20	KK 20	Farmer seed	Rose coco; variegated with large red flecks on cream, medium to large sized, oblong.	00.11862°	034.80309°
97.	Rosc02	Rose coco	Farmer seed	Rose coco; Variegated, purple	00.28283°	034.72213°

	1			on cream, medium sized, globular.		
101.	Rosc02 2	Rose coco	Farmer seed	Rose coco; Variegated, purple on cream, medium sized, globular.	00.28405°	034.70447°
103.	Sugf23	Sugar 5	Farmer seed		00.28305°	034.72014°
108.	Wai24	Wairimu	Farmer seed	Red haricot: small to medium sized, oblong.	00.29542°	034.54762°
112.	Okuo25	Okuodho	Farmer seed	Canadian wonder; Purple- black, medium to large sized, oblong	00.29649°	034.54858°
114.	Kik26	Kikuyu	Farmer seed	Rose coco; Variegated, red on cream, medium sized, globular	00.29649°	034.54858°
126.	Rai27	Raila	Farmer seed	Rose coco; variegated with large red flecks on cream, medium sized and oblong.	00.56354°	034.57097°
138.	Katv28	Kat. Variety	Farmer seed	Canadian wonder; Purple- black, medium to large sized, oblong	00.25069°	034.75063°
141.	Gi3029	Gasele	Farmer seed	Canadian wonder; white to light grey, medium to large sized, oblong	00.24770°	034.75199°
144.	Okuo30	Okuodho	Farmer seed	Canadian wonder; Purple- black, medium to large sized, oblong	00.33535°	034.47865°
150	Mwitem ania	Mwitemania	Kenya seed company, Kakamega branch	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	-----	-----
165	Mwezi moja	APC	Kenya seed company, Kakamega branch	Mwezi moja; many fine purple spots, medium to large sized, oblong.	-----	-----
171	Mwitem ania	Mwitemania	Market- Chogoria	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	-----	-----
177					-----	-----
	Raco4C	Rose coco	Market- Kakamega	Rose coco; Variegated, purple on cream, medium sized, globular.		
189	Gac03C	*****	Market-Mumias	Canadian wonder; white to light grey, medium to large sized, oblong	-----	-----

65 DNA extraction

66 Total genomic DNA from 300gms of fresh tissue obtained from three weeks old dry bean
67 seedling was extracted following instructions from Qiagen™ with few modifications.
68 Quantification was done on DNA quantification and its' purity was analyzed on a
69 NanoDrop™ 1000 spectrophotometer.

70 SSR analysis: 25 ng of the quantified genomic DNA was used for the SSR marker analysis
71 of genetic diversity. 7 SSR primer pairs [27] were ordered from Eurofins and used to
72 investigate the genetic diversity in the dry bean collection from Kenya. A final volume of 20
73 µl for the PCR reaction mix included 25 ng of genomic DNA, 0.1 pM of forward and reverse
74 primers, 10 mM Tris HCL (pH 7.2), 50mM KCl, 1.5 -2.5 mM MgCl₂, 250 µM total dNTPs and

1 unit of Taq polymerase. The temperature profile in the thermocycler was a hot start at 94 °C for 5 minutes, two cycles of 94 °C for 1 minute, 57 °C for 1 minute and 72 °C for 2 minutes; two cycles of 94 °C for 1 minute, 57 °C for 1 minute and 72 °C for 2 minutes; two cycles of 94 °C for 1 minute, 56 °C for 1 minute and 72 °C for 2 minutes; five cycles of 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 2 minutes; 26 cycles of 94 °C for 1 minute, 54 °C for 1 minute and 72 °C for 2 minutes and a final extension of 72 °C for 5 minutes. Following amplification, 12 µl PCR product was carefully loaded to 3% superfine agarose gel stained with 15 µl of safeview™ with the first and last well having 7 µl of DNA ladder mix (44 µl buffer 1x TAE, 6 µl DNA ladder and 10 µl loading buffer). Agarose gel was run for 4 hrs at 90 volts. The bands were visualized under an ultra violet transilluminator.

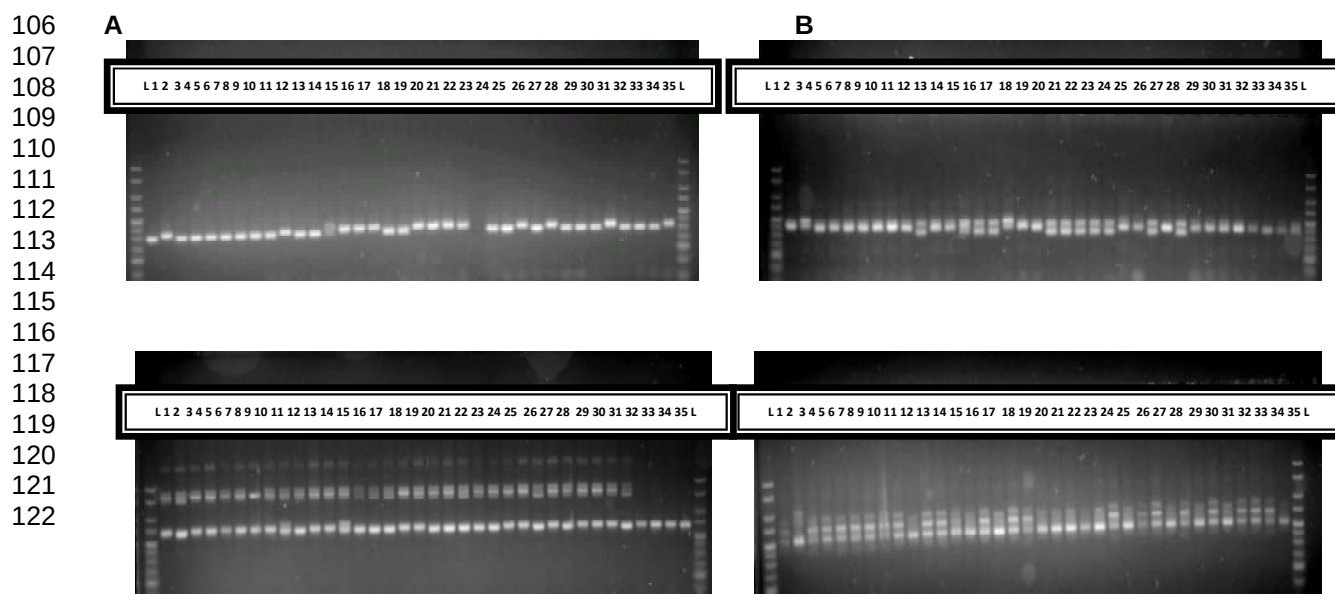
Data scoring and cluster analysis

Data scoring and cluster analysis were performed using simple matching coefficients. SSR allele sizes were visualized the base pair sizes estimated using the hyper (V) DNA ladder. Nei's (1978) gene diversity index (H), number of polymorphic loci (N), percent of polymorphic loci (P), observed number of alleles (A) and genetic distance (D) for each population was derived using NYTS-pc 2.1 and GenAEx 6.4 software assuming Hardy-Weinberg equilibrium. The similarity coefficients was subjected to unweighted pair group method of arithmetic averages (UPGMA) and a dendrogram was generated with the help of NTSYS-PC software version 2.1 (Exeter software, New York, USA). The data matrix was subjected to analysis of molecular variance (AMOVA) to partition the genetic variation into within and among the populations' components using GenAEx software.

3. RESULTS

3.1 Level of polymorphism

Out of the 7 SSR primers screened for polymorphism, their reproducibility and aptitude to differentiate among four selected samples drawn randomly from the vast population collection of *P. vulgaris*, 5 SSR primers were chosen for further analyses of all samples used in this study (table 2). All primers produced variations in polymorphism (Figs: 1A-D). The greatest visible polymorphism was resolved by primer Pv-ag 004 (Fig. 1D). The 5 SSR primers amplified a total of 49 polymorphic bands with an average of 9.8 polymorphic loci/SSR primer. The product sizes ranged from 150 base pairs for primers Pv-ag001 and Pv-at 008 to 305 base pairs for primer Pv-ggc001 and Pv-ag004.



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C **D**
Fig. 1 A. PCR product of primer Pv- ag001, **B** PCR product of primer Pv- at 008, **C** PCR product of Pv- ggc 001, **D** Pv-ag 004 for 35 dry bean samples from Kenya

Table 2. Primer codes, nucleotide sequence, GC content, number of fragments amplified, number of polymorphic fragments and percentage of polymorphic fragments for each SSR primer used to evaluate *P. vulgaris* accessions of Kenya

Primer Code	Nucleotide sequence	GC Content (%)	No. of fragments amplified	No. of polymorphic fragments	(%) polymorphic fragments
Pv- ag 001	CAATCCTCTCTCTCATTTCC AATC	42%	35	13	37
Pv-ag 004	TTGATGACGTGGATGCATTGC	48%	84	14	17
Pv- ggc001	CTGAAGCCCGAATCTTGCGA	55%	37	10	27
Pv- ag 002	CCTCTCTCCCGAACTTATTCAT CTC	48%	35	1	17
Pv- at 008	AGTCGCCATAGTTGAAATTTAG GTG	40%	50	11	22
Mean			48.2	9.8	24

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137 Population genetic structure: Analysis of molecular variance with the SSR marker:
138 Partitioning of the entire species diversity using analysis of molecular variance accredited
139 8% of the disparity to diversity among the populations while the majority of the diversity
140 (92%), resided within populations (table 3). Though both the diversity separation component
141 i.e. between and within populations was statistically significant at ≤ 0.05 , the figures suggest
142 that most of the genetic diversity of *P. vulgaris* resides within the populations.

143 **Table 3. Analysis of molecular variance using SSR marker across four populations in**
144 ***P. vulgaris* samples**

Source	d.f	SS	MS	Est. Var.	%	P – value
Among Pops	3	13.295	4.432	0.226	8	0.01
Within Pops	31	79.562	2.567	2.567	92	0.01
Total	34	92.857		2.792	100	

155 Genetic identity (I) and genetic distance (D) among the different varieties *P. vulgaris* of
156 Kenya [28]: The four different populations had different dry bean varieties in terms of
157 morphological and genetic aspect. They were grouped in relation to their morphological

resemblance in terms of seed size and colour for identification purposes. The analysis revealed genetic relatedness (figure 2) among the dry bean samples and the actual genetic distances are shown. The variety 'rose coco' is evidently genetically different from the rest of the varieties used in the study with a genetic distance of 0.1359 from white haricot and 0.0759 from 'Mwiternia variety. The genetic distance between the red and white haricot was the least at 0.0034 (table 4).

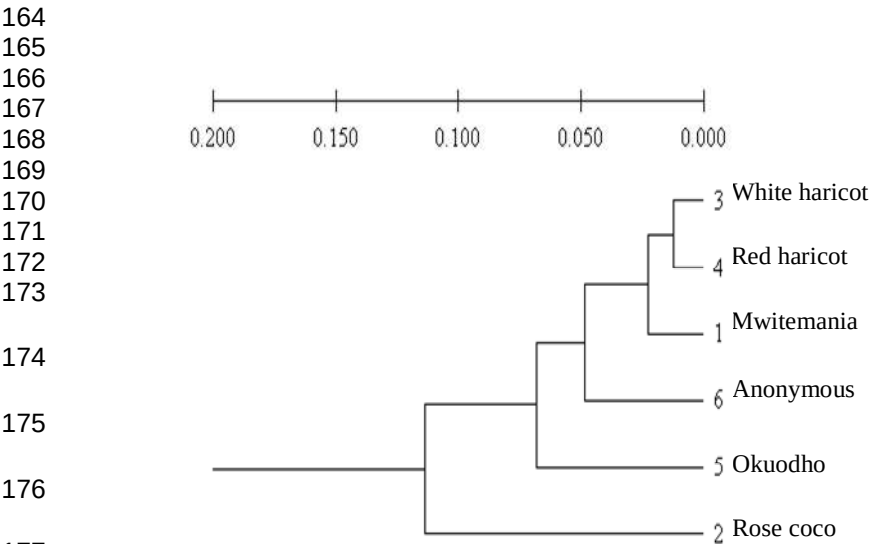


Fig 2. Neighbor-joining dendrogram based on Nei's (1978) genetic distances

Table 4. Actual genetic distance based on 35 OTUs and 124 loci of common bean samples

Variety	1	2	3	4	5	6
1	0.0000					
2	0.0759	0.0000				
3	0.0169	0.1359	0.0000			
4	0.0036	0.0765	-0.0034	0.0000		
5	0.0627	0.1322	0.0356	0.0372	0.0000	
6	0.0178	0.0559	0.0281	0.0060	0.0477	0.0000

Key: 1: Mwiternia, 2: Rose coco, 3: White haricot, 4: Red haricot, 5: Okuodho, 6: Anonymous

The pair wise matching coefficients among *P. vulgaris* generated a dendrogram with the similarity coefficient values ranging from 0.64 to 1.00 (Figure 3). The tree generated two

major groups, Group 1 and group 2, with the group1 generating two sub-clusters (1a and 1b). The first major group had all samples from the markets showing a closer relationship within samples from the markets and those from farmers. The relationship among cultivars evaluated using principal component analysis displayed 68% of the total variation, with the first principal component displaying 48.02% and the second 20.22% (Figure 4). The SSR marker estimated high diversity indices in *P. vulgaris* based on gene diversity index (H) [28]. The gene diversity index ranged from 0.1267 in the market population to 0.2377 in the Western province population (table 5). The highlands of Eastern province (Meru and Embu population) had a gene diversity index of 0.1475 while the dry lands (Mbeere and Machakos population) had 0.1991. The total population mean for gene diversity was 0.17775.

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Table 5. Neis' gene diversity index (H) and percentage of polymorphic loci (P) among four populations of *P. vulgaris* based on SSR markers

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Population	Sample size (N)	Mean observed number of alleles (NA)	Shannon's information index (I)	Nei's gene diversity index (H)	Number of polymorphic loci	% polymorphic loci
Eastern (Meru & Embu)	10	1.5417	0.2363	0.1475	13	54.17
Eastern (Mbeere& Machakos)	6	1.5833	0.3039	0.1991	14	58.33
Western Kenya	14	1.5833	0.3455	0.2377	14	58.33
Market centers	5	1.3333	0.1884	0.1267	8	33.33

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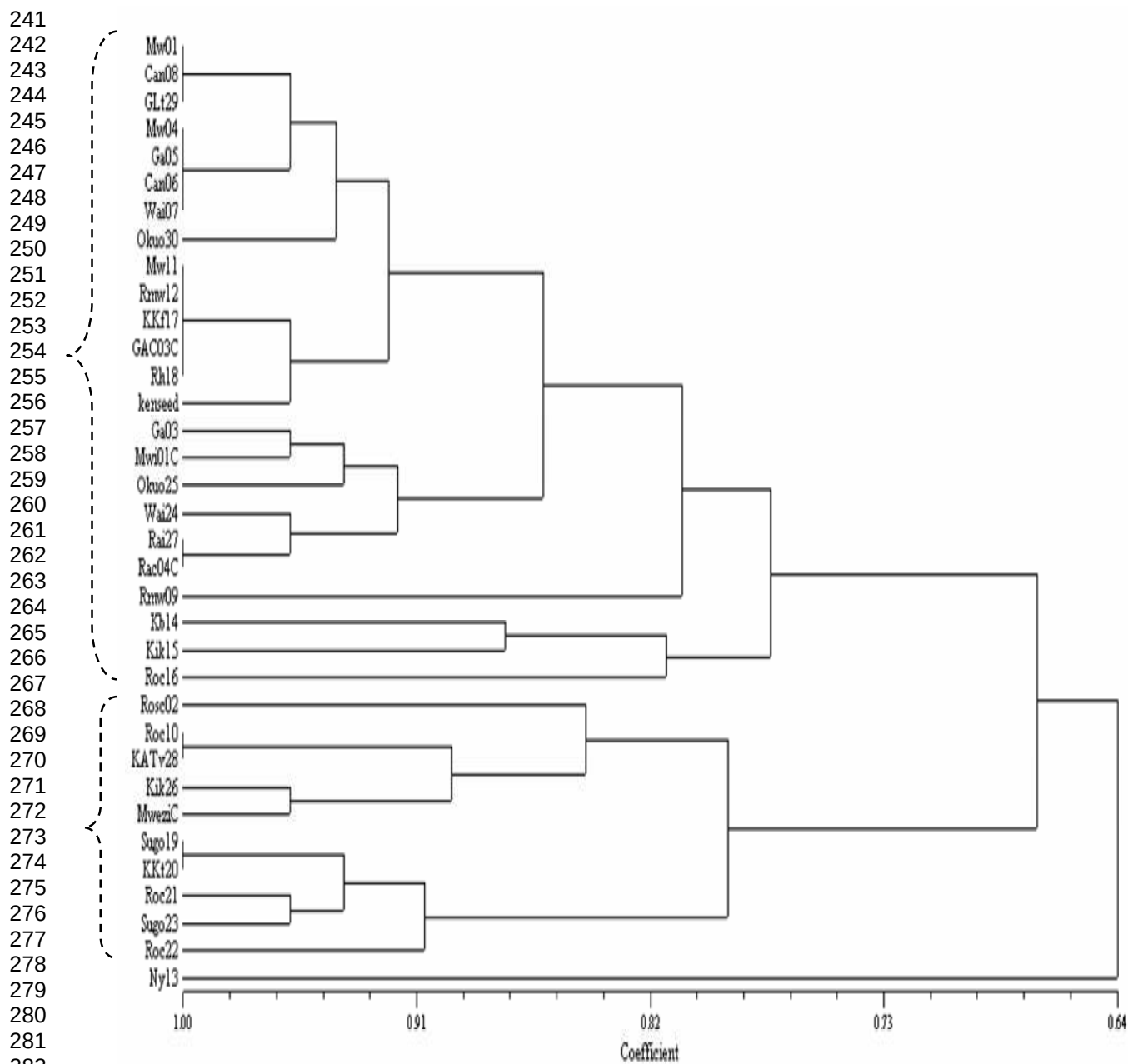


Fig. 3 UPGMA dendrogram representing the associations among four populations of common bean of Kenya using SSR markers

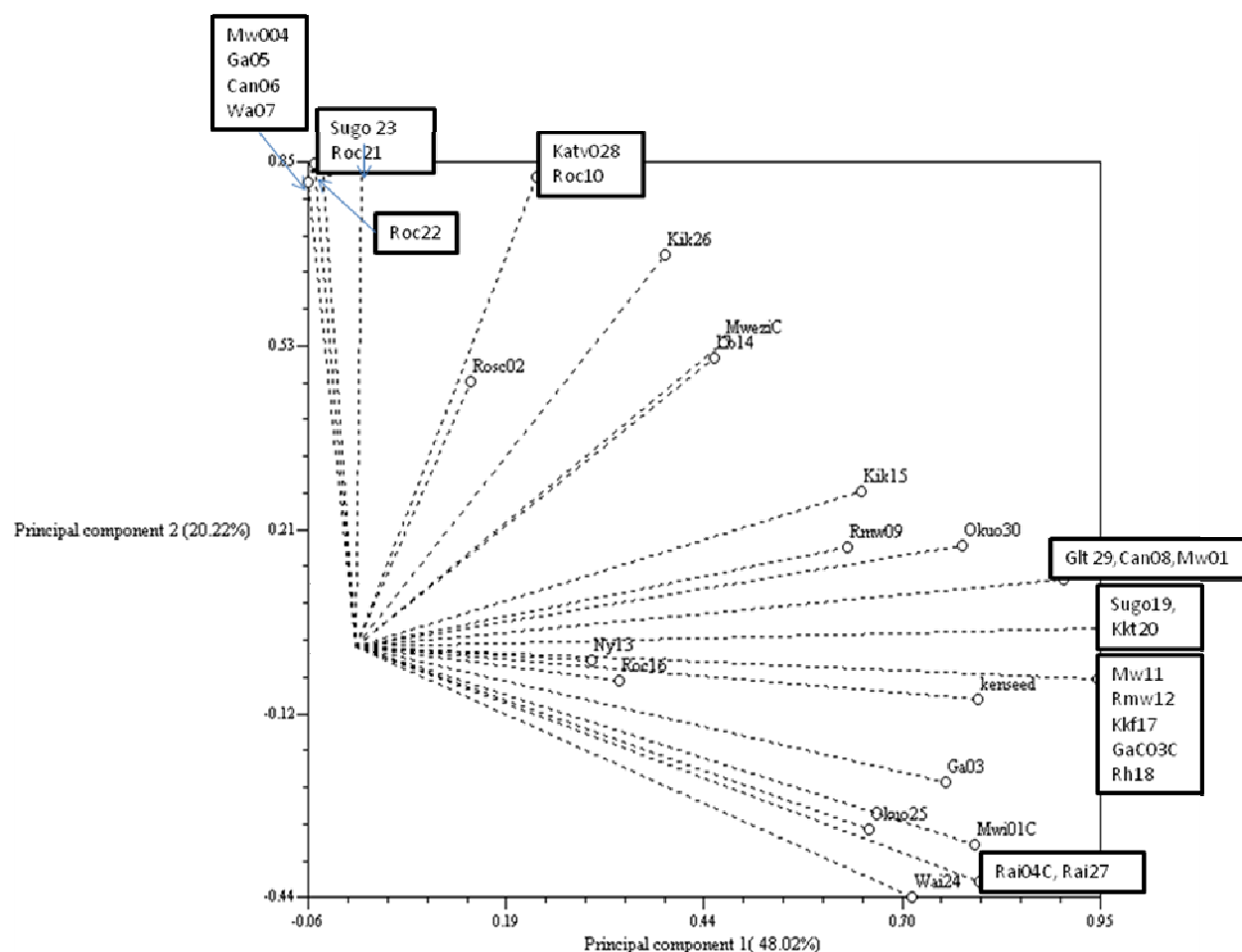


Fig. 4 principal component analyses of 35 dry bean samples using SSR markers

4. DISCUSSION

Genetic variability information of genotypes is a pre requisite in conservation prospects, which range from identification of genes as genetic resources for breeding purposes, which predict the ability to combine or to rapidly verify breeding material [29]. Variability studies are well thoughtout prebreeding initiatives towards efforts of developing superior cultivars. The importance of microsatellites in plant genetic diversity is well established and documented [30]. The high cost of their development and low number per loci per experiment restricts their use in analysis of a large number of individuals particularly of commercial significance. A range of molecular markers can be employed to study genetic diversity [27],[31]. Other markers like AFLP which reveal many loci per experiment are usually preferred over SSRs [6].

Microsatellites have described the genetic variation that exists in the Kenyan dry bean. It is a codominant marker thus a more efficient marker to use. Their advantages for diversity studies include uniform genome coverage, easy to implement, high levels of polymorphism and specific PCR-based assay [32]. Similar studies of genetic diversity of dry beans with 87 SSR loci [33], found PIC values from 0.05 to 0.83, with a mean of 0.45. There exists

variation within the market class of dry bean and it's clear from the above results that the Kenyan dry bean genetic base is narrowing, presenting a bottleneck in future bean breeding. The hyper variability observed at SSR loci was expected because of the unique mechanism of replication slippage that occurs more frequently by which this variation is generated. On the basis of high polymorphism information content [20][27][31] SSRs have successfully helped describe the level of genetic diversity existing within the market class of common beans in Kenya. Similar studies have reported 17 of 21 elite dry bean cultivars [34] fell within the similarity distances 0.03-0.33. Similarly, approximately 90% of observed total dissimilarity in our study was concentrated within the interval 0.79- 1.00 under this study.

Populations from Eastern Mbeere and Machakos and Western Kenya had a relatively higher value for the expected mean heterozygosity. Interestingly, the greatest heterozygosity and percentage polymorphism within populations is observed in these two populations that are spatially distinct latitudinally (the Western province and Eastern province, Mbeere and Machakos). Distance estimates based on the simple matching coefficient support the low estimates of mean heterozygosity. These were typically high within populations indicating that genetic variation among the populations was narrow. Gene flow through transference of seed to different regions is responsible for low heterozygosity between common bean samples from the market centres and western province of Kenya.

An overlap was observed in varieties Rh18, GAC03C, KKf17, Rmw12 and Mw11. This means that this specific marker was not robust enough to bring out the discrepancy there exists between this specific bean samples, even though they showed variations morphologically. Evaluation of divergence between 39 lines of *Vigna uniculata*, using 48 pairs of SSR primers, observed that the first two principal components accounted for 21.74% of the variation, 14.18% by the first and 7.56% by the second [35]. Evaluation of divergence among 29 genotypes of dry beans using 87 SSR primer pairs revealed 45% of the total variation, an analysis whose findings resembled our findings [33]. Evaluation of genetic diversity of 33 dry bean populations with SSR and amplified fragment length polymorphism (AFLP) markers, observed that both types of markers grouped the genotypes into Andean and Mesoamerican centers of origin [36]. In a study of the genetic diversity of 20 dry bean genotypes, using SSR markers, two large groups were formed, one containing majorly the Andean-origin genotypes and the other majorly the Mesoamerican-origin genotypes [33]. SSR markers have been proved to have the capacity to separate genotypes by centers of origin, supporting the conclusions that we made in our study.

Adequate discrimination of common bean genotypes where evident [37] with similar successful amplifications of expected allele sizes. The results of this study point toward SSR analysis being successfully used for the estimation of genetic diversity among common bean genotypes. Dendograms showed clustering within populations, supporting the above analyses of existence of high genetic variation within the populations, but the genetic base was narrow. Breeding programs use genetic variability within a species [38] for production and selection of new cultivars of high production potential. The increased diversity of SSR marker is highly variable, suggesting that co-evolution processes could have led to an increase in diversity in genes depending on a specific locus. Altogether, these data advocate that in addition to selection by farmers, adaptation to diverse environments and interactions with biotic factors, the diversity of local bean varieties are maintained. Genetic variation within and between cultivated and wild genotypes can yield more valuable information on the presence of a wider genetic base, presence of useful alleles that can be introgressed into the common bean of market class hence aid the breeder in efforts to address the decreasing food security.

5. CONCLUSION

In the present study SSR provided good insight of genetic diversity available in Kenyan dry bean accessions. SSR marker does not require very high quality and quantity of DNA (25 ng), therefore can be employed in situations where obtaining high quality DNA is almost unfeasible. Majority of the samples used were local land races from farmers and market places. However, authors suggest the inclusion of more local land races and wild types for the selection of useful alleles to expedite and facilitate the integrated genetic improvement of the dry bean. Germplasm from international programs in the tropics and subtropical regions of the world should also be included in different breeding programmes to broaden the genetic base of dry bean cultivars. The need for the conservation of plant genetic resources has been widely accepted, with reference to the thinning genetic resources available for upgrading of dry bean breeding programs.

Emphasis should also be given to the comparative utility of EST (expressed sequence tag)-SSRs and cDNA AFLP/mRNA-AFLP which score only the expressed region of the genome that can detect variation in the articulated portion of the genome, so that gene tagging give a "perfect" marker-trait associations. SNPs and DArTs could also be used in a similar study to contribute more information on the level of genetic diversity that exists in the Kenyan dry bean. Germplasm characterization and evaluation complimented by molecular studies generate the information base for more proficient utilization of these valuable resources by gene bank managers, plant breeders and research scientists to focus on achievement of more desirable traits. Utilization of plant genetic resources following a network approach, with effective connections between gene banks and plant breeders should be encouraged. The present study aimed to genetically characterize the dry bean of Kenya using molecular makers. The results may offer scope for bean breeding programs to comprehend the genetics of the bean crop in Kenya and help generate new superior cultivars in the future.

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