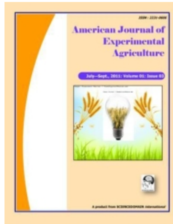
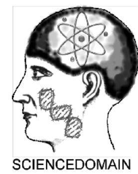


American Journal of Experimental Agriculture
5(4): 306-319, 2015, Article no.AJEA.2015.030
ISSN: 2231-0606

SCIENCEDOMAIN ***international***
www.sciencedomain.org



Genetic Diversity of Dry Bean (*Phaseolus vulgaris* L.) Accessions of Kenya Using SSR Markers

Nyakio Kithinji Maryrose[1], Katherine Andrea Steele[2] and Valerie A. P. Palapala[3*]

[1]Biological Sciences Department, Masinde Muliro University of Science and Technology, P.O. Box 190, Kakamega, Kenya.
[2]Bangor University and Centre for Advanced Agricultural Research International and Development (CARIAD), Wales.
[3]School of Science and Engineering, Rongo University College, P.O. Box 103, Rongo, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study. Author NKM performed data collection, statistical analysis and wrote the first draft of the manuscript. Author KAS oversaw the sample preparation for SSR analysis of the study in the laboratory and looked into manuscript correction. Author VAPP managed the literature searches and correction of the manuscript as the corresponding author. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/AJEA/2015/10284
Editor(s):
(1) Lanzhuang Chen, Laboratory of Plant Biotechnology, Faculty of Environment and Horticulture, Minami Kyushu University, Miyazaki, Japan.
Reviewers:
(1) Anonymous, Universidade Federal de Lavras, Brazil.
(2) Anonymous, University of Zagreb Faculty of Agriculture, Croatia.
(3) R. Najafzadeh, Department of Horticultural Sciences, Faculty of agriculture, Tarbiat Modares University (TMU), Tehra, Iran.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=737&id=2&aid=6511

Original Research Article

Received 22nd March 2014
Accepted 13th August 2014
Published 11th October 2014

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 Western Kenya and Bangor University, North Wales, between September 2010 and December 2012.
Methodology: Thirty five (35) marketable dry bean samples collected from farmers, market centers as well as seed stockists were subjected to SSR analysis. Data generated was subjected to analysis with the GenAlEx 6.4 software assuming Hardy-Weinberg equilibrium to determine gene diversity index, number of polymorphic loci and alleles, genetic distances, analysis of molecular

**Corresponding author: Email: valeriepalapala@ymail.com;*

variance (AMOVA) and principal components analysis (PCA). NYTS-pc 2.1 software was used to construct an unweighted pair group method arithmetic averages (UPGMA) dendogram using the generated similarity coefficients.

Results: Of the 7 SSR primers tested, 5 SSR primers were found to be polymorphic and used to screen the bean samples. The 5 primer combinations generated 49 polymorphic bands in 35 samples. 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. Cluster analysis segregated the bean samples into 9 clusters.

Conclusion: There exists considerable variation in the dry bean of Kenya that is narrowing. There is need to intensify efforts to broaden the bean variation for sustainability. 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]. It originated and was domesticated in the new world in two centers of origin (Mesoamerica and Andes). Dry beans show 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]. 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. 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].

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. 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 [10,14-18], SSRs [19,20], AFLPs [21-26] 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 study, a total of 7 dry bean SSR primer pairs were used to investigate the genetic diversity in the common bean collection from farmers and market centers to check for polymorphism levels.

2. MATERIALS AND METHODS

2.1 Germplasm Collection and Genomic DNA Extraction

A total of 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 market centres. All seeds were planted in aseptic conditions using sterilized soil in a greenhouse for a period of three weeks. Total genomic DNA from 300gms of fresh tissue obtained from three weeks old dry bean seedling was extracted following instructions from Qiagen™ with few modifications. The quality of DNA was checked by DNA quantification on a Thermo scientific Nanodrop™ spectrophotometer.

2.2 SSR Markers and PCR Amplification

Seven SSR primer pairs (Promega Corporation, USA) were selected on the basis of the published dry bean microsatellite framework map for the genetic diversity analysis [27,28]. Primers that revealed polymorphic banding patterns were selected while primers that showed monomorphic banding patterns were excluded from the study. Five microsatellite primers which revealed high polymorphism levels were selected and used for final polymerase chain reaction (PCR) amplification. 7 SSR primer pairs [27] ordered from Eurofins were used to investigate the genetic diversity in the dry bean collection from Kenya. A final volume of 20 µl for the PCR reaction mix included 25 ng of genomic DNA, 0.1 pM of forward and reverse primers, 10 mM Tris HCL (pH 7.2), 50mM KCl, 1.5 - 2.5 mM $MgCl_2$, 250 µM total dNTPs and 1 unit of Taq polymerase. The temperature profile in the thermocycler was a hot start at 94ºC for 5 minutes, two cycles of 94ºC for 1 minute, 57ºC for 1 minute and 72ºC for 2 minutes; two cycles of 94ºC for 1 minute, 57ºC for 1 minute and 72ºC for 2 minutes; two cycles of 94ºC for 1 minute, 56ºC for 1 minute and 72ºC for 2 minutes; five cycles of 94ºC for 1 minute, 55ºC for 1 minute and 72ºC for 2 minutes; 26 cycles of 94ºC for 1 minute, 54ºC for 1 minute and 72ºC for 2 minutes and a final extension of 72ºC for 5 minutes.

2.3 Electrophoretic Separation and Visualization of Amplified Products

Superfine agarose (3%) gel preparation involved the addition of 300ml of chilled 1 x TAE buffer (pH 8.0) to a flask with 9g of superfine agarose powder. 15µl of safeview™ was added to the gel and cast on a tray to set at room temperature before chilling it in the fridge for 20 minutes. The gel was then carefully placed in a tank with plenty of 1x TAE buffer and into each well, 12µl of the sample was carefully loaded with the first and last well having 7µl of DNA ladder mix (44µl buffer 1x TAE, 6µl DNA ladder and buffer 10µl loading buffer). The gel electrophoresis was run for 4 hrs at 90 volts. The bands were visualized under an ultra violet transilluminator.

2.4 SSR Data Analysis

The capacity of each primer to distinguish among the genotypes studied was evaluated by; % GC content, number of fragments amplified, number of polymorphic and % polymorphic fragments generated. The means of number of fragments amplified, number of polymorphic fragments and % polymorphic fragments were computed. Visualization of the amplified bands on the super fine agarose gel was repeated to validate reproducibility The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of Hyper ladder V. Clear bands were considered as markers and scored. The position of the band on the gel was determined and base pair sizes estimated using the hyper (V) DNA ladder. Since SSRs are co-dominant markers and beans are diploid, the banding patterns were translated to either homozygous or heterozygous genotypes at each locus (as codes 1 to 4) and the allelic structure for each sample was derived and scored. The total number and percentage of polymorphic markers (%P) were determined for each primer pair utilized. For Pv ag 001 code 1(125 pb), code 2 (150 bp), code 3 (175bbp), code 4 (200 bp). Pv at 008 code1 (125bp) code 2 (175 bp), code 3 (200 bp) and code 4(210 bp). Pv ggc 001 code 1 (250 pb), code 2 (210 bp), code 3 (300 bp) code 4 (305 bp). Pv ag 004 code 1 (175bp), code 2 (200bp), code 3 (250 bp), code 4 (300bp). For each primer set, the band patterns derived were used to generate a binary data matrix of 1 for present or 0 for not present. Each polymorphic SSR band was considered as a locus with two alleles: presence and absence. The data set was used directly in comparisons of genetic similarity between accessions using genetic analysis software.

The generated data set was subjected to analysis with the GenAlEx 6.4 software to determine population genetic measures for each population including the total of alleles across all populations (Na); the mean number of different alleles (Ne); Shannon Index of genetic diversity (I) applied for obtaining partition of genetic variability within and between populations;

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

Accession number	Accession code	Local name	Seed source	Population	Characteristics i.e. color, seed size small or large etc	Longitude	Latitude
4.	Mw01	Mwitemania	Farmer seed	Eastern Kenya (Meru and Embu)	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.26589°	037.75072°
9.	Ro02	Rose coco	Farmer seed	Eastern Kenya (Meru and Embu)	Rose coco; Variegated, purple on cream, medium sized, globular.	00.26836°	037.66205°
14.	GA03	Gasele	Farmer seed	Eastern Kenya (Meru and Embu)	Canadian wonder; white, to light grey, medium to large sized, oblong	00.31809°	037.66199°
24.	Mw04	Mwitemania	Farmer seed	Eastern Kenya (Meru and Embu)	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.33229°	037.64431°
32.	Gas05	Gasele	Farmer seed	Eastern Kenya (Meru and Embu)	Canadian wonder; white to light grey medium to large sized, oblong	00.04799°	037.65575°
34.	Can06	Canadian wonder	Farmer seed	Eastern Kenya (Meru and Embu)	Canadian wonder; Purple- black, medium to large sized, oblong	00.12636°	037.56236°
41.	Wai07	Wairimu	Farmer seed	Eastern Kenya (Meru and Embu)	Red haricot: small to medium sized, oblong.	00.56743°	037.49013°
46.	Can08	Canadian wonder	Farmer seed	Eastern Kenya (Meru and Embu)	Canadian wonder; Purple- black, medium to large sized, oblong	00.60266°	037.51808°
52.	Rmw09	Red Mwitemania	Farmer seed	Eastern Kenya (Meru and Embu)	Mwitemania; resembles Pinto bean, variegated, red–grey on cream, small to medium sized globular.	00.49228°	037.46354°
60.	Roc10	Rose coco	Farmer seed	Eastern Kenya (Meru and Embu)	Rose coco; Variegated, purple on cream, medium sized, globular.	00.40642°	037.62504°
64.	Mw11	Mwitemania	Farmer seed	Eastern Kenya (mbeere and machakos	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	00.39233°	037.60790°
69.	Rmw12	Red Mwitemania	Farmer seed	Eastern Kenya (mbeere and machakos	Mwitemania; resembles Pinto bean, variegated, red – grey on cream, small to medium sized globular.	00.38413°	037.58708°
70.	Ny13	Nyayo	Farmer seed	Eastern Kenya (mbeere and machakos	Rose coco; variegated with large red flecks on cream, medium sized, and oblong.	01.56349°	037.25222°
72.	Kb14	KATB1	Farmer seed	Eastern Kenya (mbeere and machakos	Pale yellow, medium sized, oblong.	01.56406°	037.25496°
78.	Kik15	Kikuyu	Farmer seed	Eastern Kenya (mbeere and machakos	Rose coco; Variegated, red on cream, medium sized, globular.	01.53483°	037.26243°
80.	Roc16	Rose coco	Farmer seed	Eastern Kenya (mbeere and machakos	Rose coco; Variegated, purple on cream, medium sized, globular.	00.12736°	034.73612°

Accession number	Accession code	Local name	Seed source	Population	Characteristics i.e. color, seed size small or large etc	Longitude	Latitude
85.	KKf17	KK15	Farmer seed	Western Kenya	Black seed coat, medium sized, oblong	00.05426°	034.74751°
88.	Rh18	Red Haricot	Farmer seed	Western Kenya	Red haricot: small to medium sized, oblong.	00.06007°	034.72225°
90.	Sugo19	Sugar 1	Farmer seed	Western Kenya	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	Western Kenya	Rose coco; variegated with large red flecks on cream, medium to large sized, oblong.	00.11862°	034.80309°
97.	Rosc021	Rose coco	Farmer seed	Western Kenya	Rose coco; Variegated, purple on cream, medium sized, globular.	00.28283°	034.72213°
101.	Rosc022	Rose coco	Farmer seed	Western Kenya	Rose coco; Variegated, purple on cream, medium sized, globular.	00.28405°	034.70447°
103.	Sugf23	Sugar 5	Farmer seed	Western Kenya		00.28305°	034.72014°
108.	Wai24	Wairimu	Farmer seed	Western Kenya	Red haricot: small to medium sized, oblong.	00.29542°	034.54762°
112.	Okuo25	Okuodho	Farmer seed	Western Kenya	Canadian wonder; Purple- black, medium to large sized, oblong	00.29649°	034.54858°
114.	Kik26	Kikuyu	Farmer seed	Western Kenya	Rose coco; Variegated, red on cream, medium sized, globular	00.29649°	034.54858°
126.	Rai27	Raila	Farmer seed	Western Kenya	Rose coco; variegated with large red flecks on cream, medium sized and oblong.	00.56354°	034.57097°
138.	Katv28	Kat. Variety	Farmer seed	Western Kenya	Canadian wonder; Purple- black, medium to large sized, oblong	00.25069°	034.75063°
141.	Gi3029	Gasele	Farmer seed	Western Kenya	Canadian wonder; white to light grey, medium to large sized, oblong	00.24770°	034.75199°
144.	Okuo30	Okuodho	Farmer seed	Western Kenya	Canadian wonder; Purple- black, medium to large sized, oblong	00.33535°	034.47865°
150	Mwitemania	Mwitemania	Kenya seed company, Kakamega branch	Market centre	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	--------------	-------------
165	Mwezi moja	APC	Kenya seed company, Kakamega branch	Market centre	Mwezi moja; many fine purple spots, medium to large sized, oblong.	--------------	-------------
171	Mwitemania	Mwitemania	Market- Chogoria	Market centre	Mwitemania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	--------------	-------------
177	Raco4C	Rose coco	Market- Kakamega	Market centre	Rose coco; Variegated, purple on cream, medium sized, globular.	--------------	-------------
189	Gac03C	**********	Market-Mumias	Market centre	Canadian wonder; white to light grey, medium to large sized, oblong	--------------	-------------

Heterozygosity index based on Nei's genetic diversity index (*H)* and calculated assuming Hardy-Weinberg equilibrium, q = (1- band Freq.) 0.5 *and* $p = 1 - q$. ions (=2 * p * q) [28]); The genetic distance (D) for each population was derived using GenAlEx 6.4 software assuming Hardy-Weinberg equilibrium where: $q = (1\text{- band frequency})^{1/2}$ and $p = (1\text{-}q)$; *"p"* is frequency of visual alleles and *"q"* is the frequency of null alleles. To compare the populations' actual genetic structure with expected genetic structure, it was assumed that there was no genetic drift, no gene flow between populations, from migration or transfer of gametes, negligible mutations, individuals were mating randomly and that natural selection was not operating on the populations. NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

2.4.1 Analysis of Molecular Variance (AMOVA)

The SSR data polymorphic loci generated was loaded into spreadsheets. AMOVA [29] was determined using GenAlEx 6.4 software [30]. AMOVA is a method used to estimate population differentiation directly from molecular data [29] and was conducted to examine the distribution of genetic variation at all hierarchical levels among populations, and among regions. The index measured the genetic differentiation of the four populations (Eastern – Meru & Embu; Eastern Mbeere and Machakos; Western and Markets).

2.4.2 Principal Components Analysis (PCA)

PCA was calculated by the GenAlEx 6.4 [30] to test the relationships between accessions by distance and genetic diversity. PCA is a statistical technique with wide ranging applications [31]. The main goal of PCA is to reduce the dimensionality by decomposing the total variances observed in an original data set. Thus, it is used to transform a set of original variables into a set of new and uncorrelated variables. The mathematic principle of PCA method lies in coordinate conversion. Cluster analyses were implemented by Unweighted Pair Group Method of Arithmetic averages (UPGMA method), and the corresponding dendrogram was constructed. This was done by subjecting the data matrix to similarity coefficients to UPGMA and a dendogram was generated with the help of NTSYS- PC software version 2.1 (Execter software, New York, USA).

3. RESULTS

3.1 Level of Polymorphism

Out of the seven SSR primers screened for polymorphism, their reproducibility and aptitude to differentiate 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). A total of 49 polymorphic amplicons (bands) were generated from 5SSR combinations of forward and reverse primers in 35 dry bean samples, with an average of 9.8 polymorphic loci/ SSR primer (Table 2). All primers produced variations in polymorphism (Fig. 1). The greatest visible polymorphism was resolved by primer Pv- ag 004 (Fig. 1D) and followed by primer Pv- ggc 001 and primer Pv- at 008. Primer Pv- ag001 showed the least visible polymorphism. The primers generated different band sizes of amplified products as follows: Pv ag 001 revealed code 1(125 pb), code 2 (150 bp), code 3 (175bbp), code 4 (200 bp). Pv at 008 code1 (125bp) code 2 (175 bp), code 3 (200 bp) and code 4(210 bp). Pv ggc 001 code 1 (250 pb), code 2 (210 bp), code 3 (300 bp) code 4 (305 bp). Pv ag 004 code 1 (175bp), code 2 (200bp), code 3 (250 bp), code 4 (300bp).

Individual clustering analysis by NTSYS pc divided individuals into individual clusters. The fractional member- ship of each dry bean individual to genetic clusters is displayed in Fig. 2. A dendrogram based on the genetic distance of all genotypes analyzed showed genetic relatedness of dry bean individuals sampled. The dry bean varieties in the study clustered into nine groups. This study showed genetic distances ranging from 0.64 to 1.00 revealing that there were more differences between populations. Cluster 1 included 7 individuals (20%) of dry bean varieties from the slopes of Mt. Kenya of Eastern province (Meru and Embu) population, none from the Western, Eastern (Machakos and Mbeere) and Market populations. Cluster 2 composed of one individual (2.8%) from Western province population. Cluster 3 was representation of samples from three populations; Eastern province, Western province and Markets centers. We attribute this to gene flow and transfer of seed samples through market distribution in the country. Cluster 4 had a collection of dry bean samples from Western

province and Eastern market centers. Cluster 5 had one variety from Eastern province population. Cluster 6 had individuals from the dry lands. Cluster 7 had 5 (14.3%) of individuals still from all populations. Cluster 8 had 5 varieties (14.3%) from Western province population.

The hierarchical analysis of molecular variance was performed with the four populations used in this study. Partitioning of the entire species diversity using analysis of molecular variance accredited 8% of the disparity to diversity among the populations while the majority of the diversity (92%), resided within populations (Table 3). Though both the diversity separation component i.e. between and within populations was statistically significant at ≤ 0.05, the figures suggest that most of the genetic diversity of *P. vulgaris* resides within the populations.

Evaluation of the four populations used in this study by principal component analysis revealed 68% of the total variation existence, with the first principal component displaying 48.02% and the second 20.22% variation (Fig. 3). The PCA for these SSR markers clearly segregated the samples into distinct identifiable sub-groups and thereby described the population diversity by adequately discriminating between populations. It was clearly evident that most of the samples were duplicated in the collection and should belong to same genetic individuals. The results also illustrated that genetically similar bean germplasm was being cultivated in different regions of the country under different local names and for classification purposes should be treated as duplicate copies of the same cultivars or accessions. For example accessions Katv028 from Eastern and Roc10 from Western region of the country are the genetically similar and should be identified by one name. However, the analysis was able to classify accessions from same region together. For example, accessions Mw004 Ga05, Can06 Wa07 all from Eastern were grouped together.

The SSR marker therefore estimated high diversity indices in *P .vulgaris* based on gene diversity index (H) [32]. Among the four populations, the gene diversity index ranged from 0.1267 in the market population to 0.2377 in the Western province population (Table 4). 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.

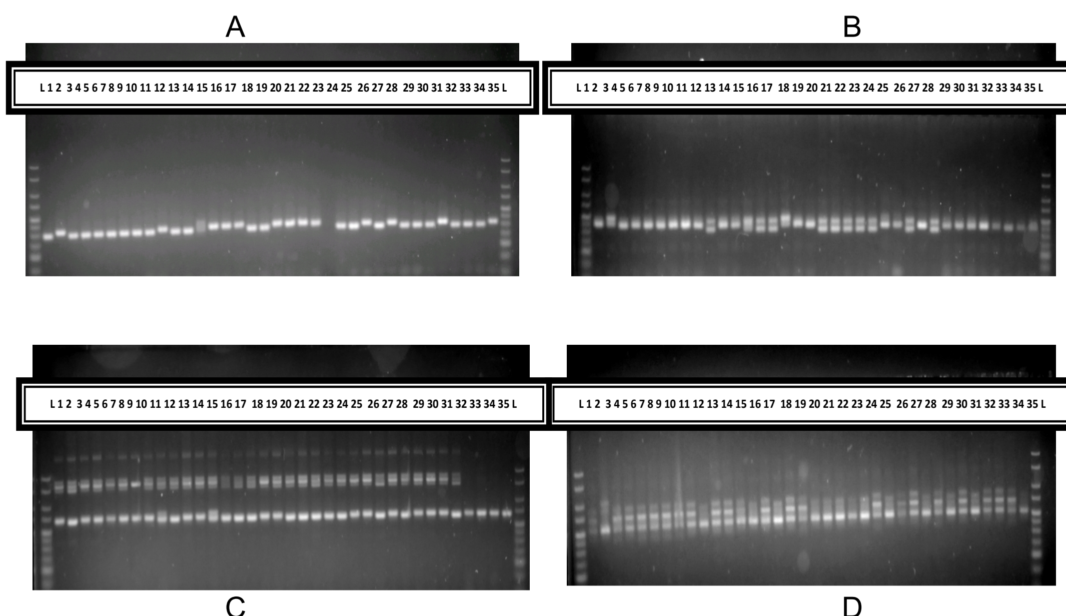


Fig. 1. Separation of alleles on 3% superfine agarose gel stained with 15 µl of safeview^{TM}. The PCR products were amplified using SSR primers: A - Pv- ag001; B - Pv- at 008; C - Pv- ggc 001; D - Pv-ag 004. The lanes marked L with band sizes is the DNA ladder marker hyper (V). All the 35 dry bean accessions represent the four bean population; Eastern (Meru and Embu), Eastern (Machakos and Kitui), Western and Markets

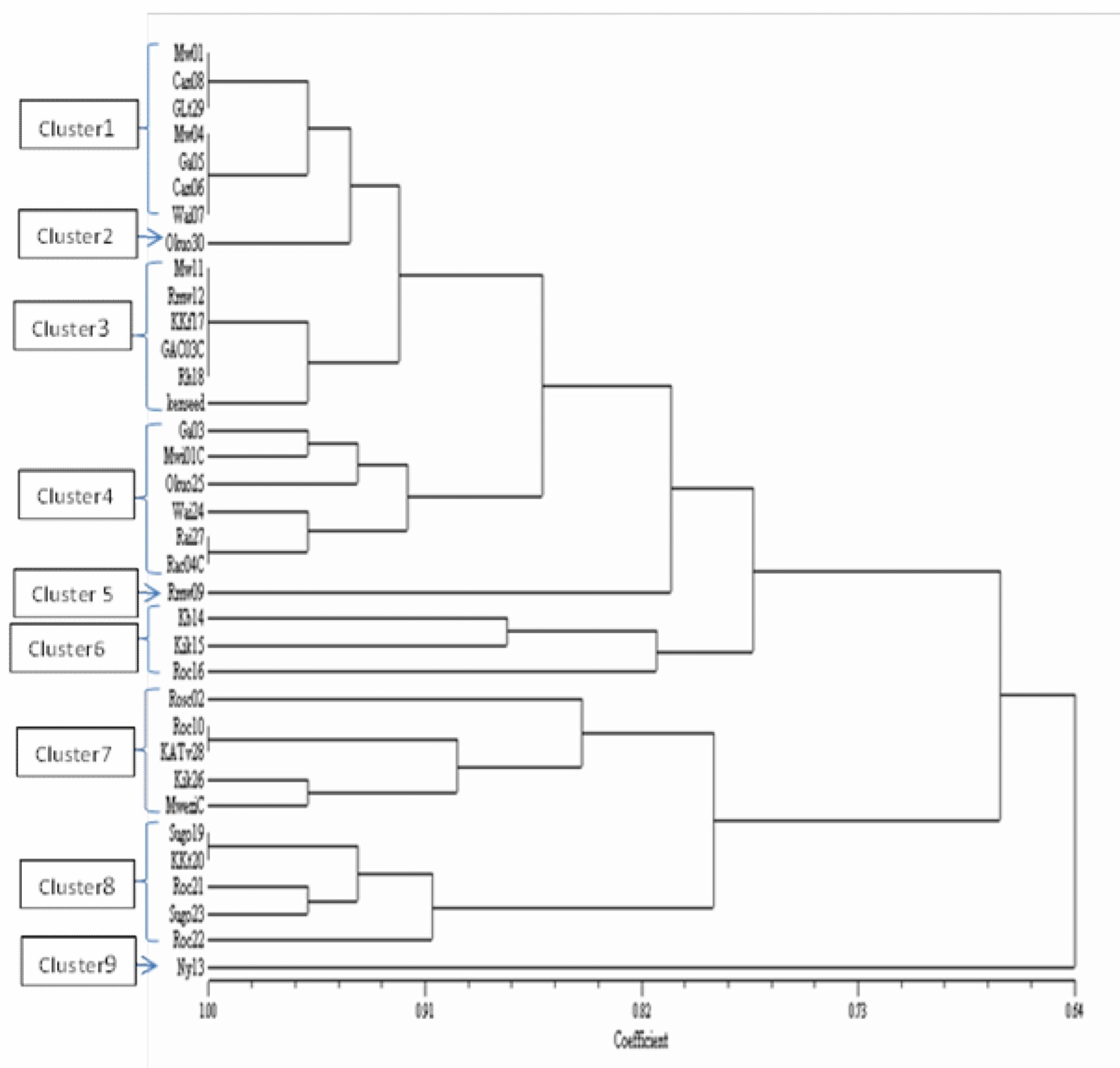


Fig. 2. Phylogenetic tree of Dry beans based on genomic DNA fingerprinting with 49 polymorphic bands generated from five primer combinations of SSR. The dendogram was drawn by NTSYS-PC software version 2.1 (Execter software NY, USA)

Table 2. Descriptive information of the primers, amplification and polymorphism generated by SSR analysis of dry beans

Primer code	Nucleotide sequence (Forward primer Sequence (5′→3′)	GC Content (%)	No. of fragments amplified	No. of polymorphic fragments	(%) polymorphic fragments
Pv- ag 001	CAATCCTCTCTCTCTCATTTCC 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

Table 3. Analysis of molecular variance (AMOVA) based on 124 polymorphic loci of SSR analysis

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	

d.f – degree of freedom; SS – sum of squared observations; MS – mean sum of squared observations; Est. Var. estimated variance; % percentage; p≤0.01

Table 4. Descriptive statistics analysis of polymorphic SSR loci (GenAlEx 6.4) of dry beans from various populations

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 (%P)
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

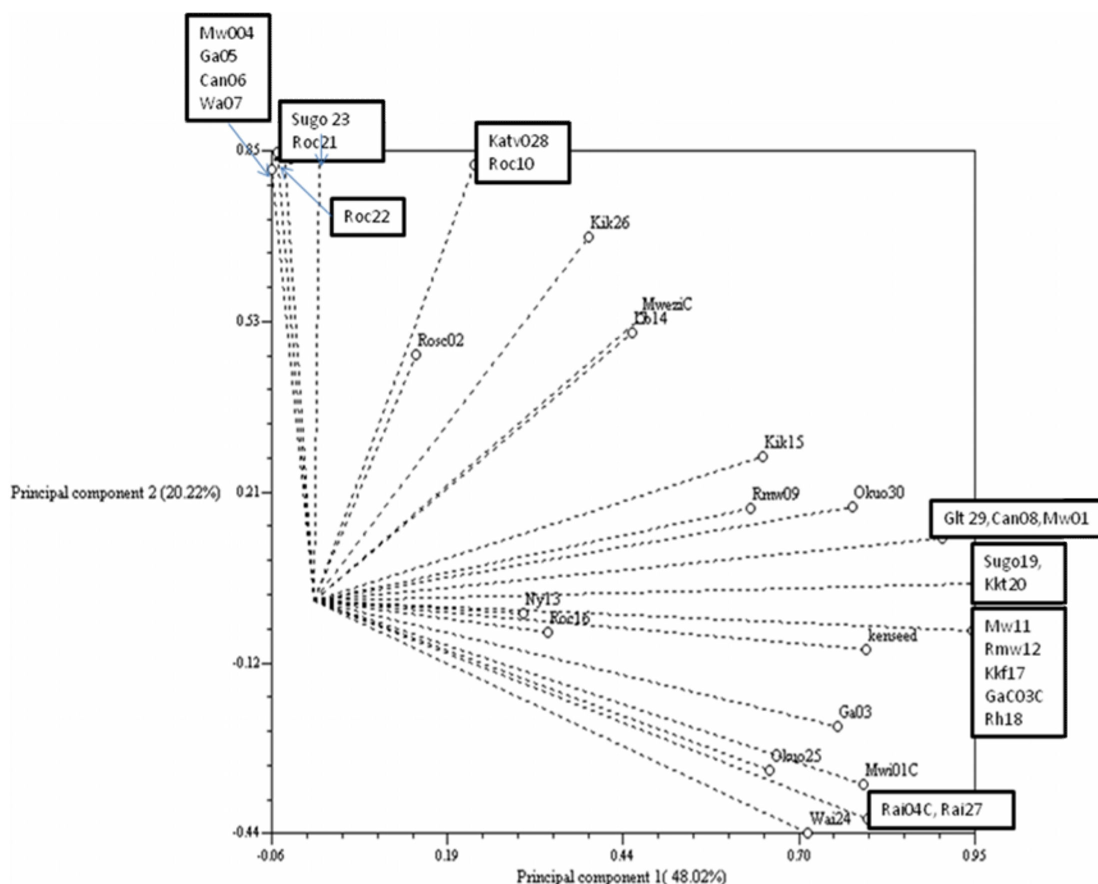


Fig. 3. Principal component analyses of 35 dry bean samples using SSR markers

4. DISCUSSION

Evaluation of genetic diversity of any food crop is an essential component in germplasm characterization and as a pre requisite in conservation prospects. This ranges from identification of genes as genetic resources for breeding purposes, to combine or to rapidly verify breeding material [33]. This aids researchers in identification of potential parents with desirable genes for introgression into local cultivars for improved crop productivity. Morphological and seed traits have long been the means of studying taxonomy and variability among plant species. DNA markers have been used for purposes such as diversity, genome mapping, and varietal identification. Molecular markers differ from biochemical markers because they are not affected by the environment. Microsatellites are among the most widely used molecular markers. The importance of microsatellites in plant genetic diversity is well established and documented [34]. 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 [27,28]. Other markers like AFLP which reveal many loci per experiment are usually preferred over SSRs [6].

4.1 Genetic Distance-Based Analysis

Microsatellite genetic distance refers to the genetic variance among populations, which can be estimated by a variety of parameters in relation to the frequency of a particular trait. The UPGMA-based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar types clustered together. The present investigation addresses the utilization of 5 microsatellite markers to reveal genetic polymorphism existing within 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 [35]. Similar studies of genetic diversity of dry beans with 87 SSR loci [36], 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 results of SSR analysis also gave the better demonstration that the minimum genetic distance between dry bean varieties from the same geographic location as the distance approached zero, which meant homogeneous proximity. The genetic distance within the same phenotype of dry bean samples used in the study was less than that between different phenotypes; the variation range of the genetic distance within the same phenotype cluster was larger. This suggests that there was a great potential genetic difference within the same cluster.

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 [20,27,28] 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 [37] 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.

4.2 Genetic Diversity among the Populations

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 dry bean samples from the market centers and western province of Kenya. A small percentage of the variation from analysis of variance could be attributed to differences between populations within clusters, while differences between populations within "subspecies" accounted for a much greater percentage of the variation.

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 unguiculata*, 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 [38]. 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 [36]. 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 [39]. 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 [36]. 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 were evident [40] 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. Dendogram 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 [41] 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.

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. 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.

5. CONCLUSION

The outcomes of this study suggest that molecular improvement innitiaves, in particular SSR offers a reliable and effective means of assessing genetic diversity within and between dry bean populations of Kenya. Variation of DNA finger- prints among accessions within dry bean cultivars in Kenya in regard to the AMOVA revealed a high within population variation than among populations. However, authors suggest the inclusion of additional number of SSR markers for a similar study and 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.

Simple sequence repeat markers offer a relatively valuable tool for grouping of germplasm and are a good complementation to field trials for identifying groups of genetically similar cultivars. 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. Field trials for identification of perfect heterotic patterns can be planned more efficiently based on findings from SSR analyses. 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 offer a scope for bean breeding programs to comprehend the genetics of the bean crop in Kenya and help generate new superior cultivars in the future.

ACKNOWLEDGEMENTS

The authors acknowledge the support from CAPACITATE East Africa project that gave financial aid to making the work a success. We gratefully acknowledge the assistance from CARIAD office, Bangor University for the opportunity to conduct this research work at the biotechnology laboratory and for technical support. We would like to express our appreciation to dry bean farmers of Kenya and seed stockists for their cooperation in providing the seed samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. McClean P, Kami J, Gepts P. Genomics and genetic diversity in common bean. In: Legume crop genomics. AOCS Press, Champaign. 2004;60–82.
2. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (*Phaseolus* ssp.)- Model food legumes. Plant Soil. 2003;252:55-128.
3. FAOSTAT (Food and Agriculture Organization of the United Nations). Statistics Division; 2012. Available: http://faostat.fao.org/site/291/default.aspx Accessed 7/6/2014.
4. Baenziger PS, Russell WK, Graef GL. Campbell BT. Improving lives: 50 years of crop breeding, genetics, and cytology (C-1). Crop Science. 2006;46:2230–2244.
5. Weising K, Nybom H, Wolf K, Kahl G. DNA finger printing in plants. 2 ed. CRC Press, Taylor & Francis. 2005;444.
6. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. The comparison of RFLP, RAPD, AFLP, and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding. 1996;2:225–238.
7. Hedrick PW, Parker KM, and Lee RN. Using microsatellite and MHC variation to identify species, ESUs, and MUs in the endangered Sonora topminnow. Molecular Ecology. 2001;10:1399-1412.
8. Scotti I, Magni F, Fink R, Powell W, Binelli G, and Hedley PE. Microsatellite repeats are not randomly distributed within Norway spruce (*Picea abies* K.) expressed sequences, Genome. 2000;43:41–46.
9. Singh SP, Gutie´rrez JA, Molina A, Urrea C, Gepts P. Genetic diversity in cultivated common bean. II. Marker-based analysis of morphological and agronomic traits. Journal of Crop Science. 1991a;31:23–29.
10. Galvan MZ, Mene´ndez-Sevillano MC, De Ron AM, Santalla M, Balatti PA. Genetic diversity among wild common beans from Northwestern Argentina based on morpho - agronomic and RAPD data. Genetic Resources and Crop Evolution. 2006;53:891–900.
11. Koenig R, Gepts P. Allozyme diversity in wild *Phaseolus vulgaris*: Further evidence for two major centers of genetic diversity. Theoretical and Applied Genetics. 1989;78:809–817.
12. Singh SP, Nodari, R, Gepts, P. Genetic diversity in cultivated common bean: Allozymes. Journal of Crop Science. 1991b;31:19–23.
13. Becerra V. Gepts P. RFLP diversity of common bean (*Phaseolus vulgaris* L.) in its centers of origin. Genome.1994;37:256–263.
14. Me´tais I, Aubry C, Hamon B, Jalouzot R, Peltier D. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). Theoretical

and Applied Genetics. 2000;101:1207–1214.

15. Duarte MJ, Bosco dos Santos J, Cunha Melo L. Genetic divergence among common bean cultivars from different races based on RAPD markers. Genetics and molecular Biology. 1999;22:419–426.
16. Beebe S, Skroch PW, Tohme J. Duque MC. Pedraza F, Nienhuis J. Structure of genetic diversity among common bean landraces of Mesoamerican origin based on correspondence analysis of RAPD. Crop Science. 2000;40:264-273.
17. Maciel FI, Gerald LTS, Echeverrigaray S. Random amplified polymorphic DNA (RAPD) markers variability among cultivars and landraces of common bean (*P. vulgaris* L.) of South Brazil. Euphytica. 2001;120:257–263.
18. Tiwari M, Singh N, Rathore M, Kumar N. RAPD markers in the analysis of genetic diversity among common bean germplasm from Central Himalaya. Genetic Resources in Crop Evolution. 2005;52:315–324.
19. Me´tais I, Hamon B, Jalouzot R, Peltier D. Structure and level of genetic diversity in various bean types evidenced with microsatellite markers isolated from a genomic enriched library. Theoretical and Applied Genetics. 2002;104:1346–1352.
20. Blair MW, Giraldo MC, Buendı´a HF, Tovar E, Duque MC, Beebe SE. Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theoretical Applied Genetics. 2006;113:100–109.
21. Tohme J, Gonza´lez OD, Beebe S, Duque C. AFLP analysis of gene pools of a wild bean core collection. Crop Science. 1996;36:1375–1384.
22. Papa R, Gepts P. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theoretical and Applied Genetics. 2003;106:239–250.
23. Beebe S, Rengifo J, Gaitán-Solís E, Duque MC, Tohme J. Diversity and origin of Andean landraces of common bean. Crop Science. 2001;41:854–862.
24. Rosales-Serna R, Hernandez-Delgado S, Gonzalez-Paz M, Acosta-Gallegos JA, Mayek-Perez N. Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. Journal of Crop Science. 2005;45:1951–1957.
25. Fabio LM, Sergio E, Lee TSG, Flipe GG. Genetic relationships and diversity among Brazilian cultivars and land races of common beans (*Phaseolus vulgaris* L.) revealed by AFLP markers. Genetic Resources and Crop Evolution. 2003;50:887–893.
26. Pallottini L, Garcia E, Kami J, Barcaccia G, Gepts P. The genetic anatomy of a patented yellow bean. Journal of Crop Science. 2004;44:968–977.
27. Yu K, Park SJ, Poysa V, Gepts P. Integration of SSR markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). Heredity. 2000;91:429–434.
28. Yu K, Park SJ, Poysa V. Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). Genome.1999;42:27-34.
29. Excoffier L, Smousse PE, Quattro JM. Analysis of molecular variance inferred from metric distances aong DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics. 1992;131:479-491.
30. Peakall R, Smouse PE. GENEALEX 6: Genetic analysis in excel. Population genetic software for teaching and research.. Molecular Ecology Notes: 2006;6:288-295.
Available: http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x
31. Zhao X, Yonglei T, Yang R, Feng H, Qingjian O, Tian Y, Zhongyang T, Mingfu L, Niu Y, Jianhui J, Shen G, and Ruqin Y. Coevolution between simple sequence repeats (SSRs) and virus genome size. BMC Genomics. 2012;13:435-446.
32. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics.1978;89:583–590.
33. Vicente MC, Guzman FA, Engels J, Ramanatha Rao V. Genetic characterization and its use in decision making for the conservation of crop germplasm. The Role of Biotechnology, Villa Gualino, Turin, Italy, 5-7 March. 2005;122-128.
34. Singh SP. Broadening the genetic base of common bean cultivars views. Crop Science. 2001;41:1659–1675.
35. Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castaglioni P, Taramino G, Motto M. Comparative analysis of genetic similarity among maize inbred lines

detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet.1998;97:1248-1255.

36. Benchimol LL, de Campos T, Carbonell SAM, Colombo CA, Chioratto AF, Formighieri EF, Gouvea LRL, de Souza AP. Structure of genetic diversity among common bean (*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. Genetic Resources and Crop Evolution. 2007;54:1747–1762.
37. Alzate-Marin AL, Costa MR, Sartorato A, Del Peloso MJ. Genetic variability and pedigree analysis of Brazilian common bean elite genotypes. Sci. Agri. 2003;60:290.
38. Somata P, Sommanas W, Srinives P. Molecular diversity assessment of AVRDC - the world vegetable center elite-parental mung beans. Breed. Sci. 2009;59:149-157.
39. Lioi L, Piergiovanni, AR, Pignone, D, Puglisi, S. Genetic diversity of some surviving on-farm Italian common bean (*Phaseolus vulgaris* L.) landraces. Plant Breeding. 2005;124:576-581.
40. Gaitan-Solis E, Duque MC, K.J. Edwards KJ, Tohme J. Microsatellite repeats in common bean (*Phaseolus vulgaris*): Isolation, characterization, and cross-species amplification in *Phaseolus* ssp. Crop Sci. 2002;42:2128-2136.
41. Loarce Y, Gallego R, Ferrer EA. Comparative analysis of the genetic relationship between rye cultivars using RFLP and RAPD markers. Euphytica. 1996;88:107-115.

© 2015 Maryrose et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=737&id=2&aid=6511