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Molecular Characterization of Common Bean (*Phaseolus vulgaris* L.) Genotypes Using Microsatellite Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ENN and EKM designed the study. Author PJG carried out the laboratory experiments, performed statistical analysis, wrote the first draft of the manuscript. Authors ENN and EKM supervised the laboratory experiments and offered technical advice. All authors read, reviewed and approved the final manuscript.

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ABSTRACT

Common bean (*Phaseolus vulgaris*) is one of the most important legume crops, but the knowledge on genetic diversity of the genotypes grown in Kenya is limited. The objective of this study was to determine the genetic diversity of common bean genotypes from different growing regions (Eastern, Central, Rift Valley, Nyanza and Western) in Kenya using simple sequence repeat (SSR; microsatellites) markers. Using five SSR primers across 40 genotypes, a total of 366 alleles were amplified, with an average of 4.5 alleles per locus. The polymorphism information content (PIC) of the SSR markers ranged from 0.48 to 0.74 with an average of 0.60. The pair wise genetic similarity between common bean genotypes ranged from 0.15 to 1.0 with an average of 0.54. A dendrogram based on the unweighted pair-group method with arithmetic mean (UPGMA) grouped the 40 genotypes into two major clusters. It was notable that the first major cluster was further divided into two-separate sub-clusters, representing genotypes from each of the regions. Principal component analysis (PCA) of the SSR markers showed that the first two principal components (PCs) explained a total of 28.79% of the genetic variation and failed to distinguish significant groupings among the 40 bean genotypes. Analysis of molecular variance (AMOVA) revealed high levels of genetic variation

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(87%) within population, compared to the variation that exists among the populations. This study demonstrated the existence of considerable genetic diversity in common bean genotypes cultivated in Kenya and can be used as a foundation for future breeding programs to produce hybrids of desirable traits. The wider genetic diversity is important for future generations so that it copes with unpredictable climate changes and human needs.

Keywords: Phaseolus vulgaris; simple sequence repeats; genetic diversity; germplasm.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris*) is the most important legume worldwide for direct human consumption, with Uganda, Kenya, Burundi, and Tanzania being the largest producers in Africa [1]. Many people in Africa rely on it for food security, nutrition and income [2]. The crop provides protein-rich food, restores and maintains the soil fertility by fixing atmospheric nitrogen, and also fits well in different cropping systems. Beans are also a rich source of essential vitamins and minerals, soluble fiber, starch and phytochemicals, and are also reported to have low fat content [3-5]. It is the most important plant-based protein source for the people of Kenya, providing 25% of the protein of the local diet [6]. More than half of the farmers in Kenya grow beans and it is widely adapted for growth in most agricultural regions [7]. Despite being an important food crop in Kenya, there has been no focus on understanding the genetic diversity of the genotypes cultivated by farmers.

Characterization of the genetic diversity in the available germplasm provides essential information for its conservation, management of genebanks and utilization in genetic breeding programs needed to meet the demand for future food security [8]. To make the crossing programs effective, parents should belong to different genetic clusters hence the need to know the genetic diversity of the existing genotypes before carrying out any hybridization studies. The narrow genetic base of modern crop cultivars is a challenge in breeding to sustain and improve crop productivity due to the vulnerability of genetically uniform cultivars to potentially new biotic and abiotic stresses [9]. Moreover, assessment of genetic diversity is important to know the source of genes responsible for a particular trait (disease resistance, early maturity, and high yielding or drought tolerance) within the available germplasm [10]. Therefore, it is important to investigate the genetic diversity in common bean germplasm to understand and in the future, broaden the genetic variation available for breeding.

Morphological and agronomic traits are routinely used to assess genetic diversity but are influenced by the environment, development stage and do not correctly reflect genetic relatedness between accessions. To overcome these problems, molecular markers represents a potential tool for effective characterization of genetic diversity and to aid in the management of plant resources [11-14]. These DNA molecular markers, when closely linked to genes of interest can be used to select for desirable allele/s in marker-assisted breeding programs [15]. Genetic diversity in common bean have been studied using different molecular markers such as allozymes [16,17], Amplified Fragment Length Polymorphism, AFLP [18,4], Random Amplified Polymorphism, RAPD [19,20,21], Restriction Fragment Length Polymorphism, RFLP [22], Inter Simple Sequence Repeats, ISSR [4,21] and Simple Sequence Repeats, SSR [23,15]. Of all DNA molecular markers, SSRs have several advantages for genetic fingerprinting, that include being highly polymorphic and reproducible, enormous extent of allelic diversity, frequently co-dominant, strong discriminatory power specific PCR-based assay, randomly and widely distributed in the genome [24,25]. Moreover, these markers are more closely connected with genes of known function [26]. Mutations in the motifs and flanking sequences as well as distribution of SSRs in the genome of a species are exploited to reveal genetic variation and varietal identity. Simple sequence repeat (SSR) marker analysis has been successfully used to evaluate genetic diversity in dry bean genotypes from several countries including Italy, Bulgaria, Nicaragua, Slovenia, Uganda and Ethiopia [21, 4,27,28,15,29].

The objective of this study was to determine the genetic diversity of common bean genotypes in Kenya using SSR markers for use in present and future breeding schemes and conservation programs. This information would contribute to understand the genetic relationship between different genotypes and provide basic information for parental selection of common bean breeding material.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of selected common bean genotypes were obtained from farmers in Central, Nyanza, Eastern, Western and Rift Valley regions of Kenya. A total of 46 genotypes representing a wide spectrum of phenotypic variability were selected for the present study (Fig. 1; Table 1). Three bean seeds per genotype were planted in 2 liter plastic pots filled with a mixture of compost and loamy soil and placed on the bench in a glasshouse. After two weeks of planting, newly opened fresh young leaves of 40 genotypes were collected for DNA extraction. Seeds of 6 genotypes (codes B5, B6, B8, B9, B31 and B32) did not germinate and therefore SSR analysis was not done on these genotypes.

2.2 DNA Extraction

Genomic DNA was extracted using Cetyltrimethylammonium bromide (CTAB) protocol as described by Choudhary et al. [30] with some modifications including the exclusion of the use of liquid nitrogen. The DNA dissolved in 100 µl TE buffer and stored at -20°C until use.

2.3 Quantification and Normalization of DNA Concentration

The purity and quantity of the extracted DNA was assessed by determination of $A_{260/A280}$ and $A_{260/A230}$ absorbance ratio by spectrophotometer (UV–Visible Elico spectrophotometer, India). The integrity of the extracted genomic DNA was verified in 0.8% agarose gel stained with ethidium bromide.

2.4 Identification and Selection of SSR Primers

Five simple sequence repeat markers (Table 2) were used for characterizing common bean genotypes. Primer selection was based on previous studies, which showed high amplification patterns and polymorphic characteristics [11,31]. Among the markers used, two (Bmd2 and Bmd17) were specific for common beans [11] and 3 (Vm71, Vm74 and Vm94) were specific for cowpea [31]. Their names, repeat types, predicted fragment length, base sequences and melting temperatures are presented in Table 2.

2.5 Polymerase Chain Reaction (PCR)

SSR-PCR amplifications were performed in 20 µl reaction volumes containing 1X GoTaq Green Master Mix (Promega Corporation, Madison, USA), 10 µM of each of the forward and reverse primers, 50 ng template DNA and nuclease-free water up to 20 µl. All the PCR reactions were carried out in 200 µl thin-walled PCR tubes. Amplifications were performed in a MJ Mini™ Thermal Cycler (Bio-Rad, Singapore) as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 46 - 61°C depending on the primer pair) for 60 seconds, extension at 72°C for 2 minutes with a final extension at 72°C for 7 minutes. The samples were then maintained at 10°C. The PCR reaction for each SSR primer was performed at least twice using DNA from independent extractions and only clear and reproducible bands were used in data evaluation.

Fig. 1. Phenotypic diversity of common bean genotypes collected in Kenya

Table 1. Local names, region of acquisition and characteristics of common bean genotypes used in the present study

No.	Code	Local name	Geographical region	100-seed weight $^{-1}$ (g)	Seed size	Seed colour
1	B1	Kikuyu1	Central (Muranga)	28.8	Medium	Brown
2	B2	Kikuyu2	Central (Maragua)	24.5	Small	Brown
3	B3	Kikuyu3	Central (Kiambu)	30.2	Medium	Cream with brown specks
4	B4	Amini	Nyanza (Keroka)	42.2	Large	Brown
5	B5	Yellow kidney	Nyanza (Kisii)	27.3	Medium	Yellow
6	B6	Makueni1	Eastern (Makueni)	31.1	Medium	Brown
7	B7	Makueni2	Eastern (Makueni)	47.8	Large	Light brown
8	B8	GLP 24	Eastern (Makueni)	49.0	Large	Dark brown
9	B9	Red haricot	Eastern (Makueni)	33.0	Medium	Brown
10	B10	Sugar1	Western (Kakamega)	39.6	Medium	Cream with red specks
11	B11	Makueni7	Eastern (Makueni)	32.2	Medium	Creamy
12	B12	Kiboko1	Eastern (Makueni)	29.8	Medium	Brown
13	B13	Masaku	Eastern (Machakos)	39.4	Medium	Cream with red specks
14	B14	Kibwezi1	Eastern (Makueni)	19.9	Small	Cream with red strips
15	B15	Rose coco	Eastern (Embu, Mbeere, Meru)	54.9	Large	Brown
16	B16	Royoo	Nyanza (Kisii)	23.1	Small	Dark brown
17	B17	Rose coco	Rift Valley (Cherangani)	33.6	Medium	Red
18	B18	Super Rose Coco	Eastern (Embu)	42.0	Large	Brown
19	B19	Mwitemania	Western (Kakamega)	46.7	Large	Cream with brown specks
20	B20	GLP 2	Central (Kiambu)	34.3	Medium	Red
21	B21	Unknown3	Central (Limuru)	33.9	Medium	Cream with brown specks
22	B22	GLP 24	Rift Valley (Kitale)	48.2	Large	Red
23	B23	New Rose Coco	Central (Kirinyaga)	33.5	Medium	Maroon
24	B24	Enyoro	Nyanza (Nyamira)	21.9	Small	Dark brown
25	B25	Nyaibu	Nyanza (Keumbu)	55.8	Large	Black
26	B26	Unknown1	Rift Valley (Njoro)	24.1	Small	Red
27	B27	Zaire	Nyanza (Mosocho)	22.9	Small	Light brown
28	B28	Bunda	Nyanza (Rongo)	65.5	Large	Black
29	B29	Unknown4	Western (Vihiga)	22.9	Small	Red
30	B30	Unknown7	Western (Vihiga)	19.0	Small	Maroon
31	B31	Wairimu	Central (Mwea)	33.3	Medium	Light brown
32	B32	Unknown11	Central (Mwea)	46.7	Large	Brown

No.	Code	Local name	Geographical region	100-seed weight $^{-1}$ (g)	Seed size	Seed colour
33	B33	Kakamega1	Western (Kakamega)	31.8	Medium	Maroon
34	B34	Kakamega2	Western (Kakamega)	27.7	Medium	Maroon
35	B35	Kakamega3	Western (Kakamega)	35.8	Medium	Brown with red strips
36	B36	Morogi	Nyanza (Kisii)	20.0	Small	Black
37	B37	Canadian wonder	Eastern (Embu, Meru, Mbeere)	53.9	Large	Red
28	B38	Kisii3	Nyanza (Kisii)	31.4	Medium	Brown with red strips
39	B39	Morogi	Nyanza (Kisii)	20.6	Small	Black
40	B40	Migori1	Nyanza (Migori)	43.2	Large	Brown
41	B41	Raila	Nyanza (Migori)	32.4	Medium	Red
42	B42	GCP 004	Eastern (Machakos)	43.2	Large	Brown with white specks
43	B43	Yellow kidney	Eastern (Mbeere, Machakos)	40.8	Large	Yellow
44	B44	KAT 56	Eastern (Machakos)	48.4	Large	Red
45	B45	KAT B9	Eastern (Machakos)	48.2	Large	Red
46	B46	KAT 69	Eastern (Machakos)	40.1	Large	Red

Seed size = 100-seed weight^{-1}; Small = <25 g, Medium = 25 - 40 g, Large = >40 g.

Table 2. Simple sequence repeat markers used in the molecular diversity studies of Kenyan common bean genotypes

Primer name	Repeat	Primer sequence (5'-3')		Size range (bp)	Tm°C	Reference
		Forward	Reverse			
Vm71	(AG012(AAAG)3	TCGTGGCAGAGAATCAAAGACAC	TGGGTGGAGAAAACAAACC	100 - 250	58	Isemura et al., 2012
Vm74	(AC)8(A)5	CTGCTACACCTTCCATCATTC	CCTTTGCGTTGTGGTGGTTT	100 – 400	55	Isemura et al., 2012
Vm94	(CA)12(AAAG)3	TCGAACTTTGGCTTGAGG	TGTCGTTTTGTCCCCCATTA	100 - 350	61	Isemura et al., 2012
Bmd2	(CGG)8	AGCGACAGCAAGAGAACCTC	CAACGTTTTGTCCCCCATTA	50 - 400	50	Blair et al., 2006
Bmd17	(CGCCAC)6	GTTAGATCCCGCCCAATAGTC	CAACAAACGGAAGGGCGTGGTTT	100 - 900	46	Blair et al., 2006

2.6 Separation of Amplified PCR Products by Agarose Gel Electrophoresis

The amplified products were analyzed on a 2% agarose gel in 1X Tris-Acetate EDTA (TAE) buffer. The gel was then stained with ethidium bromide (0.5 µg/ml) and viewed under UV transilluminator (Herolab RH-5.1, Germany). Estimation of SSR allele/band sizes were based on the migration of the amplicons/fragments through the gel in comparison to that of 100 bp DNA ladder (Bioneer, South Africa).

2.7 Scoring and Analysis of Microsatellite Data

The PCR reaction for each SSR primer was performed at least twice. Only clear and reproducible bands were used in data evaluation. The alleles/bands were scored as presence (1) and absence (0) and were recorded in a data matrix table as discrete variables. The summary of the statistics including the observed number of alleles per locus (na), number of effective alleles (ne), gene diversity (h), Shannon's information index (I), and gene differentiation (Gst) were determined using GenAlEx6 software [32]. The polymorphism information content (PIC) value was calculated following the formula described by [33]. For genetic distance analysis based on SSR results, the allelic size data were transformed to binary data: presence (1) versus absence (0) of each allele. A similarity matrix was generated using the Nei's genetic distance [34]. Similarity data were processed through the unweighted pair group method using arithmetic mean (UPGMA) cluster analysis conducted using MVSP 3.1 program. To assess further the genetic relationships of common bean accessions as individual plants, a principal component analysis (PCA) was conducted based on the SSR variation patterns using MVSP 3.1 software. Analysis of molecular variance (AMOVA) among and within populations was performed using GenAlEx 6.5 program [32]. Degree of freedom (Df), was determined by using the formula Df = N – 1, where N is the number of values in the data set.

3. RESULTS

3.1 Polymorphism and Diversity Parameters Revealed by SSR Markers

All forty genotypes were successfully amplified with the five microsatellite primer pairs. A total of 366 reproducible and scorable alleles (a band represents an allele) were amplified with the 5 SSR primer pairs among the 40 genotypes. The number of alleles produced by each SSR primer ranged from 3 (Vm71, Vm74 and Vm94) to 5 (Bmd17) with an average of 4.5 alleles per locus (Table 3). A sample amplification pattern of the primer Bmd2 is shown in Fig. 2. An average of 73.3 alleles were amplified with the highest number of alleles amplified being observed for marker Bmd17. Least number of alleles (29) was amplified by marker Vm94. The number of observed alleles ranged from 1.60 for (Vm71, Vm74 and Vm94) to 2.0 for Bmd17 with a mean of 0.8069 (Table 3). The number of effective allele values ranged from 1.1875 to 1.5502 with a mean value of 1.3530. It was observed that marker Vm71 had the lowest values while marker Bmd2 had the highest value.

For all the genotypes, the PIC values for the SSR loci ranged from 0.4818 for Vm94 to 0.7439 for Vm71, with an average PIC value of 0.5958 (Table 3). The mean Nei's (1973) gene diversity (h) of the loci producing polymorphic bands in the 40 common bean genotypes ranged from 0.1215

Table 3. Diversity parameters for 5 SSR loci used to analyze genetic diversity of common bean germplasm in Kenya

Locus	No. of alleles	Total no. of alleles	na*	ne*	h*	Ho	PIC	I*
Vm71	3	66	1.6000	1.1875	0.1215	0.5800	0.4918	0.1988
Vm74	3	62	1.6000	1.2332	0.1445	0.6250	0.5547	0.2282
Vm94	3	52	1.6000	1.2568	0.1715	0.5536	0.4818	0.2729
Bmd2	4	91	1.8000	1.5502	0.3212	0.7350	0.7069	0.4718
Bmd17	5	95	2.0000	1.5375	0.3150	0.7750	0.7439	0.4811
Mean	**3.6**	**73.2**	**1.7200**	**1.3530**	**0.2149**	**0.6537**	**0.5958**	**0.3306**
St. Dev	**0.9**	**18.8**	**0.4583**	**0.3497**	**0.1878**	**0.0969**	**0.1222**	**0.2653**

** 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)]; Ho = observed heterozygosity*

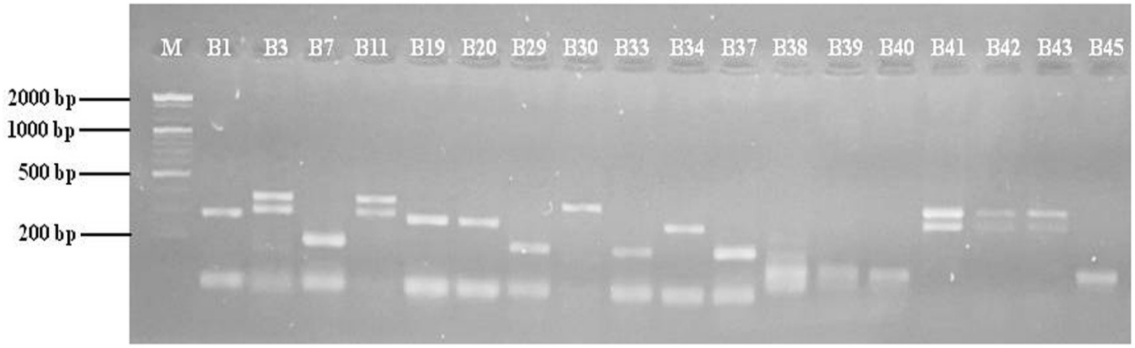

Fig. 2. Electrophoretic SSR marker profile of 18 common bean genotypes generated by primer Bmd2

to 0.3212 with a mean value of 0.2129. Markers Vm71 and Bmd2 had the lowest and the highest values of 0.1215 and 0.3212 respectively. This observation was also confirmed by Shannon's information index at locus Bmd17 ($p = 0.4811$), which had the highest value as compared to the lowest value of $p = 0.1988$ at locus Vm71. The observed heterozygosity (Ho) calculated for each primer ranged from 0.5536 (Vm71) to 0.7750 (Bmd17) with a mean of 0.6537 (Table 3).

3.2 Similarity Coefficient among the 40 Common Bean Genotypes

Genetic similarity matrix among all studied genotypes was obtained from fragments amplified with 5 SSR markers using Jaccard similarity coefficients. The similarity coefficient among the 40 bean genotypes ranged from 0.15 to 1.0 with an average of 0.54, which suggested that there was an abundant genetic diversity in the common bean accessions grown in Kenya. The highest similarity coefficient was 1.00 between code B19 and B21 (Table 4). It indicated that the two genotypes were genetically similar and there was no genetic distance (GD). However, the genetic similarities (0.15) between two genotypes B22 and B37 were the smallest. The low values of genetic similarity obtained indicated a high genetic diversity among the common bean genotypes.

3.3 Genetic Relationships among Common Bean Genotypes

The similarity coefficient matrix was used for UPGMA cluster analysis. The dendrogram constructed based on genetic similarities between genotypes showed that the 40 genotypes formed two major groups (A and B) (Fig. 3, Table 5). The genotypes did not form specific groups according to geographic regions of acquisition/collection (Table 5). Group A was the largest and the most diverse consisting of 38 genotypes from all the geographical regions. This group was further divided into 2 clusters; clusters I and II containing 7 and 31 genotypes, respectively. Both clusters I and II were further divided into two subclusters each (1 and 2 for cluster I; 3 and 4 for the cluster II). Group B includes two genotypes from Rift Valley and Nyanza regions.

The genetic relationships among genotypes were also confirmed by scatter plot derived through principal component analysis (PCA). Principal component analysis based on allele frequencies generated using 5 SSR markers failed to detect significant grouping among the 40 common bean genotypes (Fig. 4). The first and second principal components comprised 16.0% and 12.79% of the total variation, respectively.

3.4 Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was used to estimate the partitioning of genetic variance among and within populations (Table 6). AMOVA results based on SSR data revealed that the vast majority of the total genetic variance was due to within population variation (87%) and only 13% of the genetic variation was among the five (Central, Eastern, Western, Nyanza and Rift valley) populations. Most of the genetic diversity of *P. vulgaris* resides within the populations. Both the diversity between and within populations was statistically significant at $p<0.01$.

Table 4. Pairwise genetic similarity index among 40 common bean genotypes based on SSR data

	B1	B2	B3	B4	B7	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24
B1	1	0.67	0.43	0.58	0.33	0.42	0.42	0.31	0.50	0.58	0.39	0.46	0.50	0.46	0.58	0.70	0.58	0.38	0.46	0.50
B2		1	0.40	0.43	0.31	0.50	0.39	0.29	0.46	0.43	0.36	0.31	0.46	0.42	0.54	0.50	0.54	0.20	0.42	0.36
B3			1	0.79	0.57	0.77	0.77	0.64	0.50	0.79	0.71	0.47	0.85	0.69	0.79	0.64	0.79	0.31	0.47	0.71
B4				1	0.50	0.69	0.69	0.57	0.43	0.85	0.64	0.62	0.77	0.62	0.85	0.83	0.85	0.33	0.50	0.77
B7					1	0.58	0.36	0.73	0.42	0.50	0.67	0.39	0.67	0.64	0.62	0.46	0.62	0.18	0.50	0.67
B10						1	0.67	0.54	0.50	0.69	0.62	0.46	0.75	0.73	0.69	0.54	0.69	0.27	0.36	0.62
B11							1	0.43	0.39	0.69	0.50	0.36	0.62	0.46	0.57	0.67	0.57	0.27	0.27	0.62
B12								1	0.50	0.47	0.62	0.46	0.75	0.58	0.69	0.54	0.69	0.17	0.46	0.62
B13									1	0.43	0.58	0.55	0.58	0.55	0.54	0.50	0.54	0.50	0.31	0.46
B14										1	0.53	0.62	0.64	0.62	0.71	0.69	0.71	0.33	0.40	0.64
B15											1	0.54	0.83	0.67	0.77	0.62	0.77	0.36	0.54	0.83
B16												1	0.54	0.50	0.62	0.58	0.62	0.44	0.39	0.54
B17													1	0.82	0.92	0.75	0.92	0.36	0.54	0.83
B18														1	0.75	0.58	0.75	0.44	0.39	0.67
B19															1	0.83	1.00	0.33	0.62	0.77
B20																1	0.83	0.40	0.46	0.75
B21																	1	0.33	0.62	0.77
B22																		1	0.18	0.36
B23																			1	0.54
B24																				1

Table 4. Continued

	B25	B26	B27	B28	B29	B30	B33	B34	B35	B36	B37	B38	B39	B40	B41	B42	B43	B44	B45	B46
B1	0.46	0.31	0.57	0.50	0.46	0.50	0.33	0.27	0.39	0.39	0.39	0.31	0.46	0.30	0.40	0.50	0.50	0.40	0.67	0.56
B2	0.42	0.29	0.33	0.46	0.42	0.36	0.18	0.25	0.36	0.36	0.36	0.29	0.42	0.27	0.25	0.46	0.46	0.50	0.46	0.50
B3	0.57	0.64	0.31	0.62	0.69	0.60	0.39	0.54	0.71	0.85	0.60	0.64	0.69	0.46	0.43	0.71	0.40	0.54	0.40	0.54
B4	0.50	0.57	0.33	0.54	0.62	0.77	0.42	0.46	0.64	0.77	0.53	0.57	0.62	0.50	0.46	0.64	0.43	0.46	0.54	0.46
B7	0.64	0.73	0.18	0.55	0.50	0.54	0.40	0.46	0.82	0.67	0.54	0.58	0.8	0.50	0.60	0.82	0.55	0.46	0.42	0.33
B10	0.58	0.54	0.27	0.64	0.58	0.50	0.36	0.42	0.75	0.75	0.62	0.67	0.73	0.60	0.42	0.75	0.39	0.70	0.39	0.42
B11	0.36	0.43	0.27	0.39	0.46	0.50	0.25	0.31	0.50	0.62	0.40	0.43	0.46	0.46	0.42	0.50	0.20	0.42	0.29	0.42
B12	0.46	0.67	0.17	0.50	0.58	0.50	0.36	0.55	0.62	0.75	0.62	0.67	0.73	0.46	0.42	0.62	0.39	0.42	0.29	0.42

	B25	B26	B27	B28	B29	B30	B33	B34	B35	B36	B37	B38	B39	B40	B41	B42	B43	B44	B45	B46
B13	0.55	0.50	0.50	0.60	0.55	0.46	0.30	0.36	0.46	0.46	0.46	0.64	0.55	0.40	0.50	0.58	0.46	0.50	0.46	0.67
B14	0.50	0.47	0.33	0.54	0.50	0.64	0.42	0.36	0.64	0.64	0.53	0.57	0.62	0.50	0.46	0.64	0.43	0.46	0.54	0.36
B15	0.82	0.91	0.36	0.73	0.82	0.83	0.46	0.64	0.69	0.69	0.47	0.62	0.67	0.55	0.64	0.83	0.58	0.64	0.58	0.64
B16	0.50	0.46	0.30	0.55	0.50	0.67	0.56	0.46	0.43	0.43	0.43	0.58	0.50	0.50	0.46	0.54	0.42	0.46	0.55	0.33
B17	0.67	0.75	0.36	0.73	0.82	0.69	0.46	0.64	0.69	0.83	0.57	0.62	0.82	0.55	0.50	0.83	0.46	0.64	0.46	0.64
B18	0.64	0.58	0.44	0.70	0.64	0.54	0.40	0.46	0.67	0.67	0.54	0.58	0.80	0.50	0.46	0.82	0.42	0.60	0.42	0.46
B19	0.62	0.69	0.33	0.67	0.75	0.77	0.42	0.58	0.64	0.77	0.53	0.57	0.75	0.50	0.46	0.77	0.54	0.58	0.54	0.58
B20	0.46	0.54	0.40	0.50	0.58	0.75	0.36	0.42	0.50	0.62	0.40	0.43	0.58	0.46	0.55	0.62	0.39	0.42	0.50	0.55
B21	0.62	0.69	0.33	0.67	0.75	0.77	0.42	0.58	0.64	0.77	0.53	0.57	0.75	0.50	0.46	0.77	0.54	0.58	0.54	0.58
B22	0.30	0.27	0.60	0.33	0.30	0.36	0.29	0.22	0.25	0.25	0.15	0.27	0.30	0.25	0.38	0.36	0.20	0.22	0.33	0.38
B23	0.50	0.58	0.18	0.42	0.50	0.54	0.27	0.46	0.43	0.43	0.33	0.27	0.39	0.15	0.23	0.54	0.55	0.33	0.55	0.46
B24	0.67	0.75	0.36	0.58	0.67	0.83	0.46	0.50	0.69	0.69	0.47	0.50	0.67	0.55	0.64	0.83	0.46	0.50	0.58	0.50
B25	1	0.73	0.44	0.89	0.80	0.67	0.56	0.60	0.67	0.54	0.54	0.58	0.64	0.50	0.60	0.82	0.70	0.78	0.70	0.60
B26		1	0.27	0.64	0.73	0.75	0.36	0.55	0.62	0.62	0.40	0.54	0.58	0.46	0.55	0.75	0.5	0.55	0.50	0.55
B27			1	0.50	0.44	0.36	0.29	0.22	0.25	0.25	0.25	0.27	0.30	0.25	0.38	0.36	0.33	0.38	0.50	0.57
B28				1	0.89	0.58	0.63	0.67	0.58	0.58	0.58	0.64	0.70	0.56	0.50	0.73	0.60	0.88	0.60	0.67
B29					1	0.67	0.56	0.78	0.54	0.67	0.54	0.58	0.64	0.50	0.46	0.67	0.55	0.78	0.55	0.78
B30						1	0.46	0.50	0.57	0.57	0.38	0.50	0.54	0.55	0.64	0.69	0.58	0.50	0.73	0.50
B33							1	0.71	0.46	0.46	0.46	0.50	0.56	0.57	0.50	0.46	0.44	0.50	0.44	0.33
B34								1	0.50	0.64	0.50	0.55	0.60	0.44	0.40	0.50	0.50	0.56	0.36	0.56
B35									1	0.83	0.69	0.75	0.82	0.55	0.64	0.83	0.58	0.50	0.46	0.39
B36										1	0.69	0.75	0.82	0.55	0.50	0.69	0.46	0.50	0.36	0.50
B37											1	0.75	0.67	0.42	0.39	0.57	0.46	0.50	0.36	0.39
B38												1	0.73	0.60	0.55	0.62	0.50	0.55	0.39	0.42
B39													1	0.67	0.60	0.82	0.55	0.60	0.42	0.46
B40														1	0.63	0.55	0.40	0.63	0.40	0.30
B41															1	0.64	0.50	0.40	0.50	0.40
B42																1	0.58	0.64	0.58	0.50
B43																	1	0.50	0.78	0.50
B44																		1	0.50	0.56
B45																			1	0.50
B46																				1

Table 5. Number of genotypes and their corresponding groupings based on 5 SSR loci data

Group	Cluster	Sub-cluster	No. of genotypes in each cluster	Genotypes	Geographic region of collection
A	I	1	4	B1, B2, B13, B46	Central and Eastern
		2	3	B23, B43, B45	Central and Eastern
				B3, B36, B19, B21, B17, B10, B7, B35, B42, B39, B18, B37, B38, B12, B11, B20, B4, B14,	Central, Eastern, Rift Valley, Nyanza and Western
		3		B24, B26, B15, B30, B25, B26,	
			29	B29, B40, B41, B16, B44,	
		4	2	B33, B34	Western
	II				
B			2	B22, B27	Rift Valley and Nyanza

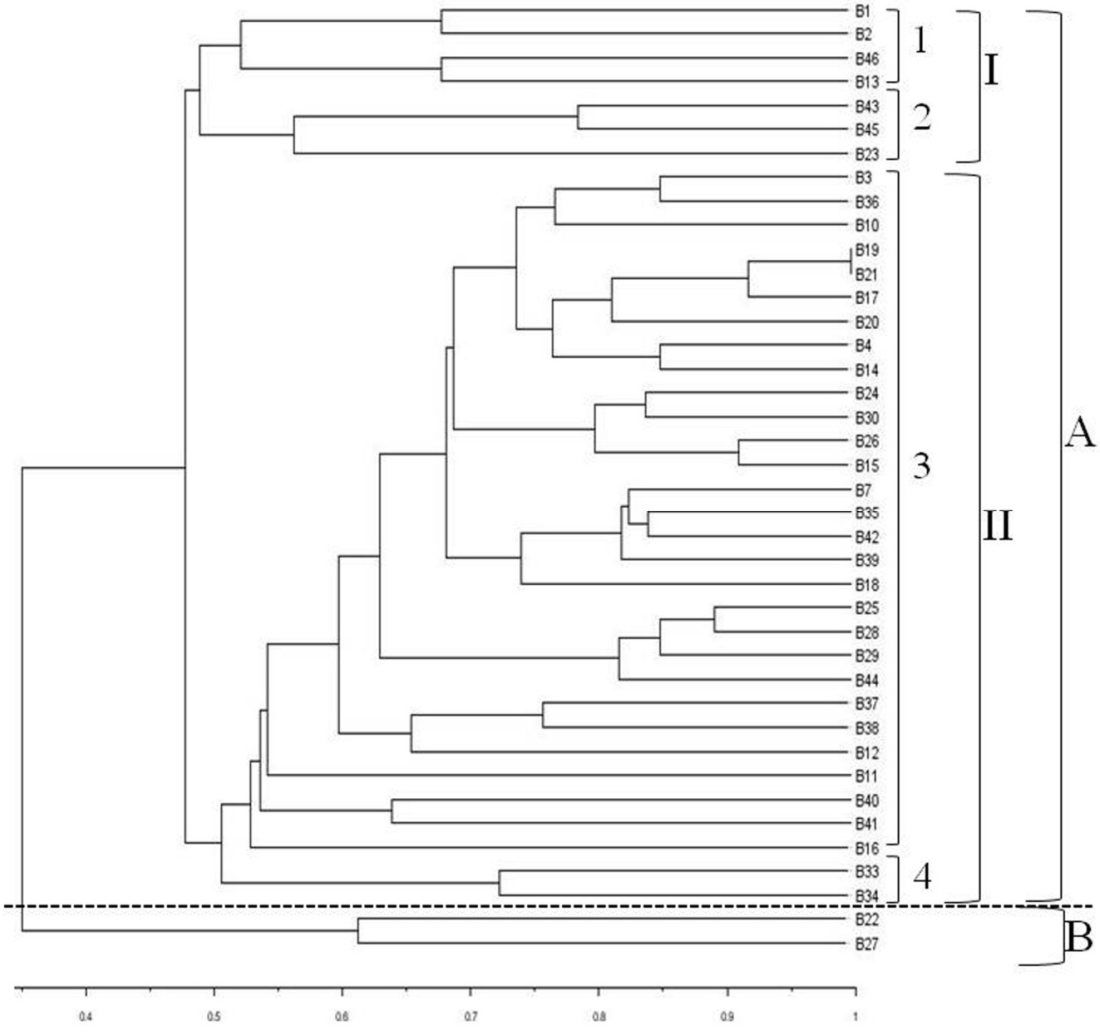

Fig. 3. Dendrogram showing genetic relationship among 40 genotypes of common beans by SSR data using UPGMA

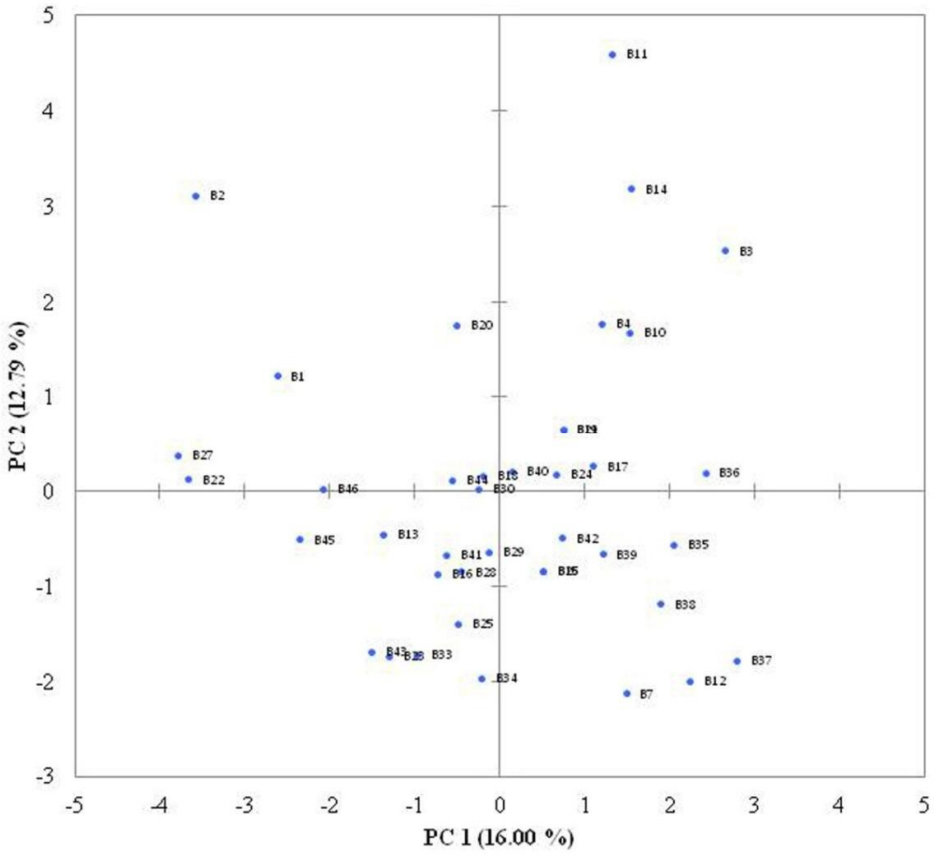

Fig. 4. Principal component analysis (PCA) of 40 common bean genotypes based on 5 SSR data. PC 1 and PC 2 refer to the first and second principal components, respectively. The numbers in parentheses refer to the proportion of variance explained by the corresponding axes

Table 6. Analysis of molecular variance (AMOVA) of 40 common bean genotypes based on 5 SSR markers

Source of variation	Df	SSD	MSD	VC	TVP (%)	P-value
Among populations	4	24.475	4.895	0.378	13%	< 0.01
Within populations	35	83.000	2.441	2.441	87%	< 0.01
Total	39	107.475		2.819	100%	

Degrees of freedom (df), sum of squares (SSD), mean squares (MSD), variance component (VC) and total variance percentage (TVP), p< 0.01

4. DISCUSSION

Knowledge of genetic diversity is a crucial determinant of germplasm utilization in crop improvement strategies to meet the demand for future food security. Germplasm with high level of genetic diversity is a valuable resource for broadening the genetic base in any breeding program. Limited genetic diversity poses a threat to the survival of a species as this limits ability to respond to changes in climate, pathogen populations and agricultural practices [35]. Hence, evaluating different sets of genetic materials with appropriate tools would be useful for identifying diverse genotypes to be incorporated in different breeding programs. A number of tools including morphological and molecular markers have been used to reveal genetic diversity in crop plants. Using morphological markers is not an easy task because these traits can be affected by environmental factors and cultivation conditions, which reduce the accuracy of the results [36]. SSR markers are considered a powerful

molecular tool for the characterization of genetic variability in common bean and other legumes [8, 12,13,37].

In the present study, SSR markers were successfully used to determine genetic diversity among 40 common bean genotypes grown in Kenya. The three SSR primers specific to cowpea produced amplification products in common bean showing that a considerable level of sequence conservation exists within the primer regions flanking the microsatellite loci. This was the first time that SSRs developed for *V. unguiculata* were used in the species *P. vulgaris*. The five SSR markers were able to discriminate between the different genotypes. Studies have shown that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci are employed [24]. A total of 366 alleles were amplified with 4.5 alleles per SSR loci. The average number of alleles per locus (4.5) was higher compared to previous reports using AFLP (1.45) and SSAP markers (1.68) [38,39]. This suggests that SSR markers are very suitable tool for assessing genetic diversity of common bean. Asfaw et al. [23] found 389 alleles with an average of 10 alleles per locus using 38 SSR markers from a collection of 192 common bean collections from East Africa. Blair et al. [12] reported 301 alleles with an average of 10 alleles across 30 SSR markers in 365 common bean genotypes from Central Africa. Okii et al. [15] also found 423 alleles with an average of 19 alleles per locus using 22 SSR markers in 100 common bean genotypes from Uganda. The marked differences of alleles recorded in this study and other previous studies in common bean can be attributed to the differences in the number and type of polymorphic markers used, sample sizes, collection sites and geographical origin of the genotypes.

The polymorphic information content (PIC) demonstrates the informativeness of the SSR loci and their potential to detect differences among the varieties based on their genetic relationships [40]. In this study, the PIC values for the SSR loci ranged from 0.4818 for Vm94 to 0.7439 for Bmd17, with an average PIC value of 0.5958, which confirms that SSR markers used in this study were highly informative, because PIC values higher than 0.5 indicate high polymorphism [41]. The high level of polymorphism is due to diverse genotypes and more variation of SSR loci used in the present study. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus [41]. Benchimol et al. [42] assessed the genetic diversity of 20 common bean genotypes with SSRs and found PIC values ranging from 0.05 to 0.83. Perseguini et al. [43] obtained PIC values varying from 0.03 to 0.70 for a set of 60 common bean genotypes, suggesting that PIC is strongly influenced by the number and diversity of the genotypes under evaluation. Lower PIC value may be the result of closely related genotypes and the high values of PIC indicate that the markers used showed that the varieties were highly diverse. In addition, the number of alleles amplified by a primer and its PIC values depends upon the repeat number and the repeat sequence of the microsatellite sequence [44,12].

Gene diversity or expected heterozygosity can be used as a general indicator of the amount of genetic variability in a population [45]. The mean Nei's (1973) gene diversity (h) of the loci producing polymorphic bands in this study ranged from 0.1215 to 0.3212 with a mean value of 0.2129. Marker Vm71 had the lowest value while marker Bmd2 had the highest value, suggesting that Bmd2 loci could be useful in revealing genetic diversity of common bean genotypes in Kenya. This observation was also confirmed by Shannon's information index at locus Bmd17 ($p = 0.4811$), which had the highest value as compared to the lowest value of $p = 0.1988$ at locus Vm71. The observed heterozygosity (Ho) calculated for each primer ranged from 0.5536 (Vm71) to 0.7750 (Bmd17) with a mean of 0.6537. The genetic similarity coefficients ranged from 0.15 to 1.0 with an average of 0.54, which indicate substantial diversity (0 to 85%) among the genotypes used in the present study. These results reveal an abundance of genetic diversity in the common bean genotypes cultivated in Kenya.

The genetic diversity of a population in a species is affected by a number of factors, including the seed dispersal, gene flow, natural selection, geographic range, and the diversity center [46]. In the present study, the dendrogram constructed using UPGMA method suggested occurrence of two major clusters. The UPGMA cluster analysis of the genotypes based on the SSR data illustrated no clear grouping of genotypes by geographical region. The observed low divergence of common bean genotypes from different growing regions could be explained by the high gene flow rate or the extensive

germplasm exchange within Kenya and in most cases farmers grow common beans, either from seeds collected in their neighborhood or from seeds purchased at the market. Principal component analysis (PCA) is a widely used tool in analyzing genetic variation among plant accessions and provides information about associations between genotypes, which are useful in formulating better strategies for breeding [47]. The common bean genotypes did not cluster into distinct groups on the scatter plots. In addition, there was no obvious relationship between geographical origin and distribution of the genotypes on the scatter plot. In the PCA scatter plot, the distances among the genotypes reflected the genetic distances among them, hence varieties that clustered close together were interpreted to be closely related and sharing similar genetic traits whereas those that clustered far apart were distantly related. Clustering of the bean genotypes by UPGMA and PCA methods revealed that there was no association in the observed pattern of variations with their geographical origin. Such non-congruence between the clustering pattern and geographical origin could be due to exchange of germplasm among the different geographical regions. Therefore, the artificial transfer of genotypes from one region to another resulted in a false determination of the geographic origin.

5. CONCLUSION

Genetic variability is important for the development of new and improved cultivars. The findings of this study demonstrate the existence of a considerable amount of genetic diversity among common bean genotypes grown in different regions of Kenya. This indicates the potential application of such genotypes in common bean breeding programs by exploiting molecular markers for selection of specific traits. The cluster analysis results can still be used by bean breeders to guide crossings and to evaluate the need to incorporate greater genetic variability in their breeding programs. The results of the current study show that SSR markers can be reliably used for common bean genetic diversity studies, which is key in conducting breeding programs in order to obtain new biotic and abiotic-tolerant common bean varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAOSTAT (Food and Agriculture Organization of the United Nations) Statistics Division; 2015. Available:http://faostat.fao.org/
2. Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA. Phenotyping common bean for adaptation to drought. Frontiers in Physiology; 2013. DOI: 10.3389/fphys.2013.00035
3. Nyombaire G, Siddiq M, Dolan K. Effect of soaking and cooking on the oligosaccharides and lectins of red kidney beans (*Phaseolus vulgaris* L.). Bean Improvement Cooperative. 2007;50:31-32.
4. Svetleva D, Pereira G, Carlier J, Cabrita L, Leitao J, Genchev D. Molecular characterization of *Phaseolus vlugaris* L genotypes included in Bulgarian collection by ISSR and AFLP analyses. Scientia Horticulturae. 2006;109:198-206.
5. Beebe SE, 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.
6. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (*Phaseolus* spp.) – model food legumes. Plant Soil. 2003;252:55-128.
7. Katungi E, Horna D, Gebeyehu S, Sperling L. Market access, intensification and productivity of common bean in Ethiopia: A microeconomic analysis. African Journal of Agricultural Research. 2011;6(2):476-487.
8. Arunga EE, Kinyua M, Ochuodho J, Owuoche J, Chepkoech E. Genetic diversity of determinate French beans grown in Kenya based on morpho-agronomic and simple sequence repeat variation. Journal of Plant Breeding and Crop Science. 2015;7(8):240-250.
9. Abdurakhmonov IY and Abdukarimov A. Application of association mapping to understanding the genetic diversity of plant germplasm resources. International Journal of Plant Genomics. 2008;92(6): 478-487.
10. Nyakio KM, Katherine AS, Valerie AP. Genetic diversity of dry bean (*Phaseolus vulgaris* L.) accessions of Kenya using SSR markers. American Journal of Experimental Agriculture. 2015;5(4):306-319.

11. Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE. Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics. 2006;113(1):100-109.
12. Blair MW, Hurtado N, Chavarro CM, Munoz-Torress CM, Giraldo MC, Pedraza F, Tomkins J, Wing R. Gene-based SSR markers for common bean (*Phaseolus vulgaris* L.) derived from root and leaf tissue ESTs: An integration of the BMc series. Plant Biology; 2011.
DOI: 10.1186/1471-2229-11-50
13. Grisi MC, Brondani RP, Blair M, Gepts P, Brondani C, Pereira PA. Genetic mapping of a new set of microsatellite markers in a reference common bean (*Phaseolus vulgaris*) population BAT93 X JALO EEP558. Genetics and Molecular Research. 2006;891-900.
14. Laurentin H. Data analysis for molecular characterization of plant genetic resources. Genetic Resources and Crop Evolution. 2009;56:277-292.
15. Okii D, Tukamuhabwa P, Odong T, Namayanja A, Mukabaranga J, Paparu P, Gepts P. Morpholgical diversity of tropical common bean germplasm. African Crop Science Journal. 2014;59-67.
16. Singh SP, Nodari R, Gepts P. Genetic diversity in cultivated common bean: I. Allozymes. Crop Science. 1991;331:19-23.
17. Santalla M, Rodino AP, De Ron AM. Allozyme evidence supporting southwestern Europe as a secondary centre of genetic diversity for common bean. Theoretical and Applied Genetics. 2002;104:934-944.
18. Lioi L, Piergiovanni AR, Pignone D, Puglisi S, Santantonio M, Sonnante G. Genetic diversity of some surviving on-farm Italian common bean (*Phaseolus vulgaris* L) landraces. Plant Breeding. 2005;124:576-581.
19. Ocampo CH, Martin JP, Sanchez-Yelamo MD, Ortiz JM, Toro O. Tracing the origin of Spanish common bean cultivars using biochemical and molecular markers. Genetic Resources and Crop Evolution. 2005;52:33-40.
20. Martins SR, Vences FJ, Miera LE, Barroso MR, Carnide V. RAPD analysis f genetic diversity among and within Portugese landraces of common white bean (*Phaseolus vulgaris* L). Scientia Horticulturae. 2006;108:133-142.
21. Marrotti I, Bonetti A, Minelli M, Catizone P, Dinelli G. Characterization of some Italian common bean (*Phaseolus vulgaris* L) landraces by RAPD, semirandom and ISSR molecular markers. Genetic Resources and Crop Evolution. 2007;54: 175-188.
22. Nodari RO, Koinange EM, Kelly JD, Gepts P. Towards an integrated linkage map of common bean: 1. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theoretical and Applied Genetics. 1992;84:186-192.
23. Asfaw A, Blair MW, Almekinders C. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) landraces from the East African highlands. Theoretical and Applied Genetics. 2009; 120:1-12.
24. 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.
25. Pejic I, Ajmone-Marsane P, Morgane M, Kozumplick V, Castaglioni P, Taramino G, Motto M. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, SSRs and AFLPs. Theoretical Applied Genetics. 1998;97: 1248-1255.
26. Ellis JR, Burke JM. EST-SSRs as a resource for population genetic analyses. Heredity. 2007;99:125-132.
27. Gomez OJ, Blair MW, Frankow-Lindberg BE, Gullberg U. Comparative study of common bean (*Phaseolus vulgaris* L.) landraces conserved *ex situ* in genebanks and *in situ* by farmers. Genetic Resources and Crop Evolution Journal. 2005;52:371-380.
28. Maras M, Sustar-Vozlic J, Javornik B, Meglic V. The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (*Phaseolus vulgaris* L) Acta Agric Slov. 2008;91:87-96.
29. Fisseha Z, Tesfaye K, Dagne K, Blair MW, Harvey J, Kyallo M, Gepts P. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) germplasm of Ethiopia as revealed by microsatellite markers. African Journal of Biotechnology. 2016;15(52):2824-2847.
30. Choudhary K, Mathur N, Choudhary OP, Pillai U. Protocol for isolation of genomic

DNA from dry and fresh leaves of *Vigna* Species suitable for RAPD and restriction digestion. Advances in Biological Research. 2008;2(5-6):83-86.
31. Isemura T, Kaga A, Tabata S, Somta P, Srinives P, Shimizu T, Jo U, Vaughan DA, Tomooka N. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS ONE. 2012;7(8): e41304.
32. Peakall R and Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics. 2012;28(19): 2537-2539.
33. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 1980;32(3):314-331.
34. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA. 1979;76(10):5269-5273.
35. Manifesto MM, Schlatter AR, Hopp HE. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Science. 2001;41(3):682–690.
36. Lee M. DNA markers and plant breeding programs. Adv. Agron. 1995;55:265-344.
37. Varshney RK, Graner A, Sorrells ME. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology. 2005;23:48.
38. Liu Y, Hou W. Genetic diversity of Faba bean germplasms in Qinghai and core germplasm identified based on AFLP analysis. Legume Genomics & Genetics. 2010;1:1.
39. Ouji A, Bok SE, Syed NH, Abdellaoui R, Rouaissi M, Flavell AJ, Gazzah ME. Genetic diversity of faba bean (*Vicia faba* L.) populations revealed by sequence specific amplified polymorphism (SSAP) markers. African Journal of Biotechnology. 2012;11(9):2162-2168.
40. Al-Badeiry, NA, Al-Saadi AH, Merza, TK. Analysis of genetic diversity in maize (*Zea mays* L.) varieties using simple sequence repeat (SSR) Markers. Pure and Applied Science. 2014;6:1768-1774.
41. DeWoody JA, Honeycutt RL, Skow LC. Microsatellite markers in white-tailed deer. Journal of Heredity. 1995;86:317–319.
42. Benchimol LL, Campos T, Carbonell SAM, Colombo CA, Chioratto AF, Formighieri EF, Souza AP. Structure of genetic diversity among commo bean (*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. Genetic Resources and Crop Evolution. 2007;54:1747-1762.
43. Perseguini JM, Chioratto AF, Zucchi MI, Colombo CA, Carbonell SA, Mondego JM, Gazaffi R, Garcia AA, de Campos T, de Souza AP, Rubiano LB. Genetic diversity in cultivated carioca common bean based on molecular marker analysis. Genetics and Molecular Biology Journal. 2011;34(1): 88–102.
44. Blair MW, Paulo I, Astudillo C, Grusak MA. A legume biofortification quandary: variability and genetic control of seed coat micronutrient accumulation in common beans. Frontiers in Plant Science; 2013. DOI: 10.3389/fpls.2013.00275
PMCID: PM3725406.
45. Nassiry MR, Javanmard A, Tohidi R. Application of statistical procedures for analysis of genetic diversity in domestic animal populations. Americal Journal of Animal and Veterinary Sciences. 2009; 4(4):136-141.
46. Hamrick JL, Godt MJW. Allozyme diversity in plant species. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (Eds) Plant Population Genetics, Breeding and Genetic Resources. Sinauer Press, Sunderland, Mass. 1989;43–63.
47. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics. 2006;38:904–909.

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