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ASSESSMENT OF THE GENETIC DIVERSITY OF CULTIVATED COMMON BEANS (*Phaseolus vulgaris* L.) FROM CAMEROON AND KENYA USING ALLOZYMES MARKERS

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ABSTRACT

In order to provide essential information for plant breeding and crop improvement programs, genetic diversity assessment is a prerequisite. In this study, starch gel electrophoresis of 9 enzyme systems encoding 19 putative loci was used to assess allozyme diversity of 13 cultivated common bean (*Phaseolus vulgaris*) accessions from Cameroon and 21 from Kenya. Results of genetic variation among the studied accessions revealed 26.32% as proportion of polymorphic loci (P), 1.263 as average number of alleles per locus (A), 0.007 as observed heterozygosity (H_o) and 0.079 as the total expected heterozygosity (H_T). The level of genetic diversity within and among the various accessions were $H_S = 0.014 \pm 0.003$ and $D_{ST} = 0.065 \pm 0.013$ respectively. *G*-statistics estimation indicated a high level of genetic heterogeneity between accessions ($G_{ST} = 0.825 \pm 0.021$), suggesting that cultivated *P. vulgaris* maintains about 82.5 % of its genetic variation among accessions. This distribution was more pronounced in Kenyan accessions (89.2 %) compared to Cameroonian accessions (69.9%). Important deviation from Hardy-Weinberg expectations was reported in this study ($F_{IT} = 0.903 \pm 0.018$). This genetic disequilibrium is likely due to either high genetic differentiation between accessions ($F_{ST} = 0.861 \pm 0.023$) or significant nonrandom mating within accessions ($F_{IS} = 0.233 \pm 0.096$). There was no significant gene flow among cultivated *P. vulgaris* accessions in Cameroon ($N_m = 0.058 \pm 0.029$) and in Kenya ($N_m = 0.045 \pm 0.041$).

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1 Introduction

Phaseolus Vulgaris L. is a diploid plant species with $2n = 2x = 22$ that consist of wild and domesticated forms (Velasquez & Gepts, 1994). Among the five cultivated species of the Genus *Phaseolus* (*P. vulgaris*, *P. dumosus*, *P. coccineus*, *P. lunatus* and *P. polyanthus*), *P. vulgaris* is the primarily cultivated crop with a broad commercial importance (Caicedo et al., 1999). It was first domesticated in Central and Southern America and is now widely cultivated in the tropics, subtropics and temperate regions (Gepts & Debouck, 1991; Burle et al., 2010). World over, common bean has an annual production of about 18.3 million tons (FAOSTAT, 2009). It is an annual legume representing a major protein source and providing essential vitamins, minerals and fibers to millions of people in both urban and rural areas of many developing countries (Messina, 1999; Dursun et al., 2010). These characteristics confer this crop as an excellent candidate for feeding people and their livestock.

Classically, *P. vulgaris* is a predominantly self-pollinating crop with an occasional occurrence of cross-pollination by pollinators such as bumblebee (Wells et al., 1988). Although being an autogamous plant with high self-reproduction rate, *P. vulgaris* however shows wide variations in the growth habit, many differences in pod and seed pigmentations, sizes, shapes and colours (Leakey, 1988; Singh, 1989). These variations in phenotypic traits reflect the evolution of the crop in various environmental conditions.

The exploitation of local plant genetic resources in Africa is likely a way to accomplish the objective of food security, particularly with an exponentially growing population. This requires preservation and availability of a high level of genetic diversity of crop resources (Given, 1987). Also in order to adjust and adapt themselves for pathogens and the ever changing environments, crop plants have to rely on a broad genetic base of variation. Characterization of crop germplasm using genetic markers provides estimates of genetic diversity, information which is essential for a rational utilization of genetic resources in breeding programs. Conventionally, plant genetic diversity is estimated using variations in morpho-agronomics traits such as yield, resistance, tolerance, colour and size (Szilagyi et al., 2011; Silva et al., 2016). Recently, biochemical markers such as allozymes (Kouam et al., 2012; Pasquet, 1999) and DNA based markers (Coulibaly et al., 2002; Semagn et al., 2006; Adesoye & Ojobo, 2012) have been more frequently used. Morphological and agronomics markers are highly influenced by the environment and generally shows low level of polymorphism compared to biochemical and molecular markers (Duminil & Michele, 2009).

The aptitude to detect allelic variation at allozyme loci has modernized research in the field of evolution, biochemical and population genetics (Wendel & Weeden, 1989; Weir, 1996).

These variations are useful to examine genetic evaluation processes and to ascertain genetic diversity in many plant species (Hamrick & Godt, 1990).

Estimation of plant genetic diversity is important in conservation, maintenance and use of genetic resources of plant species (Hodgkin et al., 1995; Karp et al., 1997). Genetic diversity survey is a prerequisite for phylogenetic and taxonomic studies (Spooner et al., 2007; Killan et al., 2007; Labate et al., 2009; Kwak & Gepts, 2009). Genetic diversity can also be applied in ecological studies to unveil the origin, distribution and the domestication centre of a given crop (Bhullar et al., 2009; Szilagyi et al., 2011). Genetic diversity of common bean have been studied by using different biochemical markers such as allozymes (Koenig & Gepts, 1989; Singh et al., 1991; Santalla et al., 2002); Molecular markers such as AFLP (Lioi et al., 2005; Svetleva et al., 2006), RAPD (Ocampo et al., 2005; Martins et al., 2006; Marotti et al., 2007), RFLP (Nodari et al., 1992), ISSR (Svetleva et al., 2006; Marotti et al., 2007) and Simple sequence repeats (SSRs) (Khaidizar et al., 2012; Fisseha et al., 2016).

Despite the economic importance of cultivated common bean seeds in Cameroon and the whole Sub-Saharan Africa, knowledge of its genetic diversity and differentiation is very little and is lacking particularly in Cameroon. Therefore, it appears important to collect and estimate the genetic diversity of accessions using genetic markers. The objective of this study was to characterize and estimate the genetic diversity indices of collections of cultivated common bean from Cameroon and Kenya using allozyme markers. Because of their co-dominance, these markers are particularly relevant to identify the heterozygosity which justifies their use in the present study.

2 Materials and Methods

2.1 Plant materials

Thirty four cultivated common bean accessions were screened for allozyme diversity (Table 1). Among these accessions, 13 genotypes were collected from Cameroon while 21 from Kenya. These accessions were obtained from Kenya Agricultural Research Institute (KARI) in Kenya, local market in Kenya and Cameroon. Because seeds of the different accessions showed differences in size, weight of 100 seeds, seed length and width were estimated for the studied accessions.

2.2 Enzyme electrophoresis and staining

Five healthy seeds per accession were sampled. These seeds were nicked and imbibed in water overnight to initiate germination and enzyme expression.

Table 1 Assessment of diversity of the collected accessions of *Phaseolus vulgaris* by various morphological seed traits .

Accession name	Location of Origin	Local name	100 Seed Weight (gm)	Seed length (mm)	Seed width (mm)
CMR (13)					
KEB-CB070	Baham, Cameroon		44.80	12.93	6.54
KEB-CB071	Dschang, Cameroon		23.10	10.48	6.04
KEB-CB072	Baham, Cameroon		26.90	10.86	6.00
KEB-CB073	Baham, Cameroon		65.00	15.68	7.29
KEB-CB074	Bafoussam, Cameroon		24.00	10.40	6.13
KEB-CB075	Baham, Cameroon		23.10	9.79	5.88
KEB-CB076	Dschang, Cameroon		22.20	13.09	4.55
KEB-CB077	Baham, Cameroon		31.80	11.51	6.06
KEB-CB078	Baham, Cameroon		50.00	12.11	7.18
KEB-CB079	Dschang, Cameroon		12.80	9.86	3.81
KEB-CB080	Baham, Cameroon		20.00	8.50	5.33
KEB-CB081	Baham, Cameroon		26.10	10.19	6.24
KEB-CB082	Dschang, Cameroon		20.00	11.08	5.01
Mean $\pm$ SE (CMR)			29.98 $\pm$ 4.05*	11.27 $\pm$ 0.51*	5.85 $\pm$ 0.27*
KEN (21)					
KEB-CB091	Ukunda, Kenya	Canadian	60.00	12.60	7.70
KEB-CB092	Ukunda, Kenya		30.40	10.74	7.05
KEB-CB093	Ukunda, Kenya		50.00	12.50	8.20
KEB-CB094	Ukunda, Kenya		60.00	13.70	7.40
KEB-CB095	Ukunda, Kenya	Climber	83.30	14.72	8.97
KEB-CB096	Ukunda, Kenya	Rost Cocoa	49.00	15.83	7.30
KEB-CB097	Ukunda, Kenya	Soja green	48.00	12.03	6.93
GLP 004	KARI, Kenya		37.55	15.40	6.10
GLP 2	KARI, Kenya		43.80	15.05	6.60
KEB-CB098	Ukunda, Kenya	saitoti	41.60	13.39	7.09
KEB-CB099	Ukunda, Kenya	Nyayo	45.80	14.60	7.20
KEB-CB100	Ukunda, Kenya	SuraMbaya small	26.20	10.99	6.46
KEB-CB101	Ukunda, Kenya	SuraMbaya big	35.30	11.41	7.86
KAT B1	KARI, Kenya		36.45	11.33	5.93
KAT B9	KARI, Kenya		46.15	11.73	7.27
KAT X56	KARI, Kenya		51.75	14.80	6.50
KAT X69	KARI, Kenya		41.15	14.85	6.95
KEB-CB102	Ukunda, Kenya	Wairimu	28.40	11.03	6.13
KEB-CB103	Ukunda, Kenya		21.33	10.80	6.35
KEB-CB104	Ukunda, Kenya		55.83	15.35	7.80
KEB-CB105	Ukunda, Kenya		59.50	17.10	8.80
Mean $\pm$ SE (KEN)			45.31 $\pm$ 3.08*	13.33 $\pm$ 0.43*	7.17 $\pm$ 0.18*
Mean $\pm$ SE (ALL)			39.45 $\pm$ 2.74	12.54 $\pm$ 0.37	6.67 $\pm$ 0.19

*: Significantly different ($P < 0.010$) between Cameroonian and Kenyan accessions.

The imbibed seed was crushed and homogenized with the extraction buffer consisting of 9.866mM Tris-HCl, 0.990mM EDTA and 0.004% NADP at pH 6.80 (Pasteur et al., 1987) using a porcelain mortar and pestle. Obtained seeds extracts were applied on a horizontal 14% (w/v) starch gel with the gel buffer consisting of 5mM L-histidine mono HCL, 2.5mM NaCl titrated to pH 6.0 with 5M NaOH (Second & Trouslot, 1980). The electrode buffer for enzyme electrophoresis was composed of 0.41 M citrate, trisodium salt titrated to pH 6.0. Nine enzymes were investigated for this study. Their staining

was carried out from the method given by Wendel & Weeden (1989) with some modifications, the detail is as follows: (a) Alcohol dehydrogenase [β -NAD (10 mg), Ethanol (0.5 ml), Tris-HCl 0.5M pH 8.50 (10 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), H₂O (62.5 ml)]; (b) Formate dehydrogenase [Formic acid (150 mg), β -NAD (10 mg), Tris-HCl 0.5M pH 8.50 (20 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), H₂O (60ml)]; (c) Fluorescent esterase [4-methylumbelliferyl acetate (10 mg), Acetone (3 ml), Sodium acetate 0.05M pH 5.0 (15ml), Agar (2%) (15 ml)]; (d) Glucose -6-Phosphate

dehydrogenase [D-glucose 6-phosphate (30 mg), β -NADP (5 mg), Tris-HCl 0.5M pH 8.50 (12 ml), MgCl_2 (50 mg/ml) (1 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), Agar (2%) (15ml)] (e) Isocitrate dehydrogenase [DL-isocitric acid (100 mg), β -NADP (5 mg), Tris-HCl 0.5M pH 8.50 (12 ml), MgCl_2 (50 mg/ml) (1 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), Agar (2%) (15ml)]; (f) Malate dehydrogenase [Malate 1M, pH 8.0 (3 ml), β -NAD (10 mg), Tris-HCl 0.5M pH 8.50 (10 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), H_2O (69ml)]; (g) Phosphoglucosomerase [Fructose-6-Phosphate di-sodium salt (20 mg), β -NADP (5 mg), Tris-HCl 0.5M pH 8.50 (12 ml), MgCl_2 (50 mg/ml) (1 ml), Glucose 6-Phosphate dehydrogenase (50 μ l), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), Agar (2%) (15ml)]; (h) Phosphoglucosomutase [α -D glucose 1-Phosphate (90 mg), β -NADP (5 ml), Tris-HCl 0.5M pH 8.50 (12 ml), MgCl_2 (50 mg/ml) (1 ml), Glucose 6-Phosphate dehydrogenase (50 μ l),

NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), Agar (2%) (15ml)]; (i) Shikimate dehydrogenase [Shikimic acid (30 mg), β -NADP (5 ml), Tris-HCl 0.5M pH 8.50 (12 ml), MgCl_2 (50 mg/ml) (1 ml), NBT (10 mg/ml) (1 ml), PMS (1 mg/ml) (1 ml), Agar (2%) (15ml)]. Patterns of each enzyme and its loci were analyzed and scored.

2.3 Gel scoring

Gel scoring was carried out by the procedure give by Pasquet (1999) for each enzyme, the designation '1' was for the presumed loci encoding the most anodally migrating isozymes. Additional loci were numbered sequentially in order of decreasing electrophoretic mobility. For each isozyme, the most common allozyme and the inferred allele were designated '100'. The other allozymes were measured in millimeters in relation to that standard.

Table 2 Enzyme system essayed on seed of common bean (*Phaseolus vulgaris*) in Cameroon and Kenya.

Enzyme name	Abbreviation	E.C Number*	Number of locus	Locus designation	Migration Direction	Alleles observed	Polymorphism	Number of alleles
Alcohol dehydrogenase	<i>Adh</i>	E.C. 1.1.1.1	3	Adh-1	Anode	100	No	1
				Adh-2	Anode	100	No	1
				Adh-3	Anode	100	No	1
Formate dehydrogenase	<i>Fdh</i>	E.C. 1.2.1.2	1	Fdh	Anode	100	No	1
Fluorescent esterase	<i>Fle</i>	E.C.3.1.1*	1	Fle	Anode	100, 105	Yes	2
Isocitrate dehydrogenase	<i>Idh</i>	E.C. .1.1.42	2	Idh-1	Anode	100	No	1
				Idh-2	Anode	100, 104	Yes	2
Malate dehydrogenase	<i>Mdh</i>	E.C. .1.1.37	3	Mdh-1	Anode	100, 107	Yes	2
				Mdh-2	Anode	100	No	1
				Mdh-3	Anode	100	No	1
Phosphoglucosomerase	<i>Pgi</i>	E.C. 5.3.1.9	3	Pgi-1	Anode	100	No	1
				Pgi-2	Anode	100	No	1
				Pgi-3	Anode	100	No	1
Glucose 6-phosphate dehydrogenase	<i>G6pd</i>	E.C. .1.1.49	2	G6pd-1	Anode	100	No	1
				G6pd-2	Anode	100	No	1
Phosphoglucose mutase	<i>Pgm</i>	E.C. 5.4.2.2	2	Pgm-1	Anode	100, 102	Yes	2
				Pgm-2	Anode	100	No	1
Shikimate dehydrogenase	<i>Sdh</i>	E.C. .1.1.25	2	Sdh-1	Anode	100	No	1
				Sdh-2	Anode	100, 103	yes	2
N = 9	N = 9		N = 19	N = 19		N = 24	P = 26.32%	A = 1.263

*: Nomenclature of the International Union of Biochemistry and Molecular Biology; **: Not specific enzyme; N = Total number; P = Proportion of polymorphic loci; A = mean number of alleles per locus

Table 3 Genetic diversity, F-statistic and gene flow estimates in common bean (*Phaseolus vulgaris*).

		Genetic diversity estimates					F-Statistics estimates			Gene flow estimates
	Polymorphic loci	H _O	H _S	H _T	D _{ST}	G _{ST}	F _{IS}	F _{IT}	F _{ST}	N _m
CMR (13)	<i>Fle</i>	0.000	0.000	0.142	0.142	1.000	1.000	1.000	1.000	0.000
	<i>Idh-2</i>	0.038	0.169	0.336	0.166	0.496	0.733	0.885	0.568	0.190
	<i>Mdh-1</i>	0.000	0.000	0.260	0.260	1.000	1.000	1.000	1.000	0.000
	<i>Pgm-1</i>	0.077	0.200	0.262	0.062	0.238	0.556	0.705	0.335	0.496
	<i>Sdh-2</i>	0.038	0.077	0.482	0.405	0.840	0.429	0.920	0.860	0.041
	Mean over loci ± SE	0.008 ± 0.004	0.023 ± 0.007	0.078 ± 0.031	0.055 ± 0.022	0.699 ± 0.078	0.592 ± 0.035	0.904 ± 0.017	0.735 ± 0.041	0.058 ± 0.029
KEN (21)	<i>Fle</i>	0.024	0.019	0.024	0.005	0.192	-0.333	-0.012	0.241	0.788
	<i>Idh-2</i>	0.071	0.086	0.487	0.401	0.824	0.077	0.853	0.841	0.047
	<i>Mdh-1</i>	0.000	0.000	0.091	0.091	1.000	1.000	1.000	1.000	0.000
	<i>Pgm-1</i>	0.000	0.029	0.387	0.358	0.926	1.000	1.000	0.938	0.016
	<i>Sdh-2</i>	0.024	0.019	0.418	0.399	0.954	-0.332	0.943	0.957	0.011
	Mean over loci ± SE	0.006 ± 0.003	0.008 ± 0.003	0.074 ± 0.012	0.066 ± 0.010	0.892 ± 0.116	0.103 ± 0.044	0.757 ± 0.100	0.826 ± 0.079	0.045 ± 0.041
ALL (34)	<i>Fle</i>	0.015	0.012	0.071	0.059	0.834	-0.333	0.792	0.844	0.046
	<i>Idh-2</i>	0.059	0.118	0.448	0.330	0.737	0.429	0.869	0.770	0.075
	<i>Mdh-1</i>	0.000	0.000	0.161	0.161	1.000	1.000	1.000	1.000	0.000
	<i>Pgm-1</i>	0.029	0.094	0.344	0.250	0.727	0.636	0.914	0.765	0.077
	<i>Sdh-2</i>	0.029	0.041	0.485	0.443	0.915	0.200	0.939	0.924	0.021
	Mean over loci ± SE	0.007 ± 0.002	0.014 ± 0.003	0.079 ± 0.016	0.065 ± 0.013	0.825 ± 0.021	0.233 ± 0.096	0.903 ± 0.018	0.861 ± 0.023	0.021 ± 0.016

2.4 Genetic diversity analysis

Based on allele frequencies and compositions at the different loci, we calculated using G-Statistics from Nei (1978), the total genetic diversity (H_T), the diversity distributed within accession (H_S) and between accessions (D_{ST}), related by the expression $H_T = H_S + D_{ST}$. The genetic heterogeneity among accessions (G_{ST}), calculated as the ratio D_{ST}/H_T were calculated according to Nei (1978) and using GenAlEx 6.5 software (Peakall & Smouse, 2012). Genetic diversity indices that include the proportion of polymorphic loci (P), the mean number of allele per locus (A) and the observed heterozygosity (H_O) were also calculated.

F-statistics using Fstat (Goudet, 1995) was used to estimate the inbreeding coefficients (F_{IT} and F_{IS}) and appreciate the deviation from Hardy-Weinberg expectations. Genetic differentiation (F_{ST}) and gene flow (N_m) were also estimated. Nei's (1987) genetic distance (D) was calculated between accessions and was used to construct a dendrogram using the Unweighted paired group method with arithmetic average (UPGMA) with the help of MEGA software program.

3 Results

3.1 Allozyme diversity

For all the 34 analyzed accessions, weight of 100 seeds, seed length and seed width were 39.45 ± 2.74 [Range: 12.80 (KEB-CB079) to 83.30 (KEB-CB095)]; 12.54 ± 0.37 [Range: 8.50 (KEB-CB080) to 17.10 (KEB-CB105)]; and 6.67 ± 0.19 [Range: 3.81 (KEB-CB079) to 8.97 (KEB-CB095)]; respectively (Table 1). Using student t-test, these three characters were significantly higher in Kenyan accession compared to Cameroonian accessions ($P < 0.010$, Table 1).

Cultured accessions of *P. vulgaris* showed allozyme variation in five of the nine enzyme systems analyzed in the study. The monomorphic enzyme systems consisted of *Adh*, *Fdh*, *G6pd* and *Pgi* (Table 2). The nine enzyme systems showed nineteen bands of activity. From these nineteen analyzed enzyme loci, five were polymorphic (*Fle*, *Mdh-1*, *Idh-2*, *Pgm-1* and *Sdh-2*), corresponding to a proportion of polymorphic loci of (p) of 26.32%. The remaining fourteen did not reveal any variation of their electrophoretic mobility, each displaying a unique allele.

Table 4 Allele frequencies in cultivated common bean (*Phaseolus vulgaris*) from Cameroon and Kenya accessions.

Locus	Alleles	Total (N=34)	CMR (13)	KEN (21)
<i>Adh-1</i>	100	1	1	1
<i>Adh-2</i>	100	1	1	1
<i>Adh-3</i>	100	1	1	1
<i>Fdh</i>	100	1	1	1
<i>Fle</i>	100	0.963	0.923	0.988
	105	0.037	0.077	0.012
<i>Idh-1</i>	100	1	1	1
<i>Idh-2</i>	100	0.662	0.788	0.583
	104	0.338	0.212	0.417
<i>Mdh-1</i>	100	0.912	0.846	0.952
	107	0.088	0.156	0.048
<i>Mdh-2</i>	100	1	1	1
<i>Mdh-3</i>	100	1	1	1
<i>Pgi-1</i>	100	1	1	1
<i>Pgi-2</i>	100	1	1	1
<i>Pgi-3</i>	100	1	1	1
<i>G6pd-1</i>	100	1	1	1
<i>G6pd-2</i>	100	1	1	1
<i>Pgm-1</i>	100	0.779	0.847	0.738
	102	0.221	0.153	0.262
<i>Pgm-2</i>	100	1	1	1
<i>Sdh-1</i>	100	1	1	1
<i>Sdh-2</i>	100	0.588	0.404	0.702
	103	0.412	0.596	0.298

Two different allelic variants were found for each of these polymorphic loci (Table 2). Differences in allele frequencies were not evident between accessions from the two countries.

Table 4 gives the frequency of each allele for each country. All alleles were reported from both geographical regions. The accessions from Kenya exhibited reduced amount of genetic diversity. Two of the five polymorphic loci (*Fle* and *Mdh-1*) had the frequency of the most common allele over 90% for Kenyan accessions while this is only one (*Fle*) for the Cameroonian accessions. We found twenty-four distinct alleles overall across the nineteen loci. The mean number of alleles per locus was 1.263 at the species level, ranging from 1 to 2 across loci (Table 2). Gene diversity or expected heterozygosity for the species was somewhat low: $H_T = 0.078$ for Cameroonian accessions and $H_T = 0.074$ for Kenyan accessions and $H_T = 0.079$ overall for the entire accessions analyzed (Table 3); in other words, only 7.9 % of individuals are genetically expected to be heterozygous at a given locus under random mating conditions. Observed levels of heterozygosity was $H_0 = 0.008$ for Cameroonian accessions and it was 0.006 for Kenyan accessions. Observed heterozygosity was generally lower than Hardy-Weinberg expectations for all the loci studied. The average of heterozygosity was $H_0 = 0.007 \pm 0.002$. It ranges from 0 (Locus *Mdh-1*) to 0.059 (Locus *Idh-2*) (Table 3). Very little gene diversity was observed within accessions ($H_s = 0.014$) while moderate genetic diversity was detected between accessions ($D_{ST}=0.065$) (Table 3). The coefficient of gene

differentiation ($G_{ST} = D_{ST}/H_T$) was 0.825. This was more pronounced to Kenyan accessions ($G_{ST} = 0.892$) compared to Cameroonian accessions ($G_{ST} = 0.699$) (Table 3).

3.2 Genetic structure

Allele frequencies that govern the genetic structure are given in Table 4. The estimation of genetic structure by means of Nei's genetic diversity is presented in Table 3. The mean total heterozygosity (H_T) and the within population genetic diversity (H_S) were 0.079 and 0.014, respectively. The inter-population genetic diversity (D_{ST}) varied from 0.059 (*Fle*) to 0.443 (*Sdh-2*) with a mean at 0.065 and the coefficient of genetic differentiation among populations (G_{ST}) varied from 0.727 (*Pgm-1*) to 1.000 (*Mdh-1*) with a mean at 0.825, indicating that about 83% of the genetic variation can be attributed to variation among accessions. The inbreeding coefficients (F_{IS} and F_{IT}) for all the five polymorphic loci were significantly different from zero (Table 3). The mean estimates of these inbreeding coefficients were significant ($F_{IS}= 0.233$ $P < 0.001$; $F_{IT}= 0.903$ $P < 0.001$), reflecting that observed levels of heterozygosity was smaller than would have been expected in case of random sexual reproduction. These deviation from Hardy-Winberg expectations are more pronounced in Cameroonian accessions ($F_{IS} = 0.592$; $F_{IT} = 0.904$, table 3) than Kenyan accessions ($F_{IS} = 0.108$; $F_{IT} = 0.757$, table 3). High F_{ST} values for all the polymorphic loci were found (Table 3). The mean $F_{ST} = 0.861 \pm 0.023$, $P < 0.001$.

Figure 1 An UPGMA clustering tree based on Nei's (1972) genetic distance calculated for 19 allozyme loci in 34 accessions of cultivated *P. vulgaris*

This high value indicates significant genetic differentiation in accessions of cultivated *P. vulgaris*. A UPGMA dendrogram illustrating genetic relationships among accessions were constructed (Figure 1). Gene flow estimation indicate low levels of pollen migration among *P. vulgaris* accessions. Wright's (1951) model on gene flow gave an estimate of $Nm = 0.021 \pm 0.016$ (Table 3).

4 Discussions

In the present study, few morphological seed traits (100-seed weight, seed length and width) and allozyme markers were combined with different statistical data analysis in order to characterize thirty four accessions of cultivated *P. vulgaris* from Cameroon and Kenya. The aim was to increase the understanding of the patterns of genetic diversity of this crop in these regions. In general, this approach of estimating the levels of germplasm diversity is of crucially important in order to avoid its loss and maximize its potential utilization.

The 100-seed weight, seed length and seed width were analyzed to assess difference of common bean accessions from

Cameroon and Kenya. A significant difference (t-test: $P < 0.01$) between 100-seed weight means from Cameroon (29.98 ± 4.05) and Kenya (45.31 ± 3.08) was observed. In Kenyan accessions, Seed length (13.33 ± 0.43) and seed width (7.17 ± 0.18) were significantly high ($P < 0.01$) compared to Cameroon accessions seed length (11.27 ± 0.51) and seed width (5.85 ± 0.27) (Table 1). These results ranges *P. vulgaris* accessions from Kenya as large seed with 100-seed weight $> 40g$ and those from Cameroon as medium size ($25 g < 100$ -seed weight $< 40g$) (Singh et al. (1991). According to Voysest (1983), Gepts (1988) and Singh et al. (1991), large size seeds correspond to bean of Andean origin and medium size seeds are associated with landraces from Mesoamerican gene pool. In this study, dominance of bean seeds with large size in Kenyan accessions and medium size seeds in Cameroon accessions was reported. This indicates the possible Andean origin for Kenyan *P. vulgaris* and Mesoamerican origin for Cameroon accessions.

To avoid extinction and promote the long-term survival of a plant species, genetic diversity is essential. The loss of genetic variation is thought to decrease adaptability of populations in

variable and changing environments (Hamrick, 1994; Young et al., 1996). Estimation of genetic diversity parameters include proportion of polymorphic loci, P ; mean average number of allele per locus, A ; and total gene diversity H_T . This study shows that genetic diversity parameters in cultivated common bean ($P = 26.32\%$; $A = 1.263$; $H_T = 0.079$) is close and comparable to the means for selfing species ($P = 20.0\%$; $A = 1.31$; $H_T = 0.074$) (Hamrick & Godt, 1990). Total genetic diversity obtained in this study ($H_T = 0.079$) is lower than that found in wild *P. vulgaris* ($H_T = 0.132$) (Koenig & Gepts, 1989). These results are acceptable since cultivated common beans are derived from the wild and should express less amount of genetic diversity. Moreover, the geographical range and number of enzymes examined by Koenig & Gepts (1989) were larger. The breeding system of a crop species is an important determinant of variability at both the species and population levels. Plant species that are self-pollinated and insect pollinated have a mixed breeding system. This combination is well known to be associated with low level of genetic diversity compared to obligatory out-crossed plant species (Hamrick & Godt, 1990). Further, Koenig & Gepts (1989) describe common bean as a highly self-pollinated crop species. Although selfing would lead to individual homozygosity, this study showed that cultivated *P. vulgaris* display substantial allozyme variation. This agrees with the report of Allard et al. (1968) saying that selfing species are not necessarily nil in allelic variants. The mean within accession genetic diversity ($H_s = 0.014$) is very small, indicating no significant genetic diversity within accessions of cultivated *P. vulgaris*. These results agree with those reported by Loveless & Hamrick (1984), indicating that annual self-pollinated plants have fewer alleles per locus.

The levels and patterns of genetic diversity can allow making inferences about the demographic history of a plant population (Milligan et al., 1994). The low percentage of polymorphic loci and numbers of alleles per locus obtained in this study indicate that cultivated common bean has a history of important or long-lasting population bottlenecks that have caused the loss of genetic diversity. The genetic heterogeneity between populations (G_{ST}) measures the proportion of genetic variation between populations in relation to the total species genetic diversity (H_T). Results of this study indicate that significant level of genetic heterogeneity among accessions is consistent with the selfing and with the annual nature of cultivated common bean, whose seeds are primarily dispersed by gravity (Loveless & Hamrick 1984). Also, the isolation of a plant population for a longer period for selection is susceptible to cause important genetic differentiation (Ellstrand, 1992).

The level of genetic differentiation is largely a function of gene flow among populations via pollen and seed dispersal (Loveless & Hamrick 1984). Estimates of genetic differentiation indices very high ($F_{ST} = 0.861$, $P < 0.001$; $G_{ST} = 0.825$, $P < 0.001$) indicate significant degree of differentiation among common bean accessions. Since the species of concern is a tropical inbred and bees pollinated legume (Koenig & Gepts, 1989), these results likely fit with the general

observation that inbreeding species maintain high and significant level of population differentiation (Hamrick & Godt 1990). Several other studies on other tropical inbred crop species using allozyme concluded the presence of significant genetic differentiation: $F_{ST} = 0.646$ for wild cowpea (Kouam et al., 2012), $F_{ST} = 0.712$ in bambara groundnut (Pasquet et al., 1999) and $F_{ST} = 0.519$ for wild lima bean (Bi et al., 2003). Gene flow is defined as the movement of gene within and among populations. It has a significant influence on the distribution of the genetic variation (Hamrick, 1989). Indirect population genetics statistics estimates gene flow as the number of individual migrating from one population to the other and per generation (Nm). The estimate of Nm was based on the level of population differentiation (Wright, 1951) and was very low: $Nm = 0.021$. According to population genetics theories, Nm less than one suggest that local differentiation of populations will result (Wright, 1951). This study reveals significant and positive inbreeding coefficients ($F_{IS} = 0.233$ and $F_{IT} = 0.903$). These values were much higher in Cameroonian accessions (Table 3) but, either in Cameroonian or Kenya accession, both indicate significant deficit of heterozygous individuals. Many previous allozyme studies carried out on Common bean and other self-pollinated plant species reported deficiency of heterozygotes regardless of the examined enzyme loci and the number of analysed accessions (Bi et al., 2003; Kouam et al., 2011). A key feature of the floral biology of *P. vulgaris* is the position of stigma and anther that are in contact (Koenig & Gepts, 1989). This disposition reduces cross-pollination and promotes high reproductive assurance even in the absence of pollinators and this will definitely lead to positive and significant inbreeding coefficients as observed in this study.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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