
**ASSESSMENT OF GENETIC DIVERSITY OF KENYAN DOLICHOS
BEAN (*Lablab purpureus* L. SWEET) USING SIMPLE SEQUENCE
REPEAT (SSR) MARKERS**

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ABSTRACT

The level of genetic relatedness or dissimilarity of Lablab genotypes used by growers in different regions in Kenya has not been determined. In this study, ninety six (96) Lablab accessions collected from various parts of Kenya were characterized based on simple sequence repeat (SSR) molecular markers. Ten SSR primers were used and detected a mean of 4.3 alleles per primer. Expected heterozygosity was on average 0.38. The average polymorphic information content (PIC) was 0.63. Analysis of molecular variance (AMOVA) revealed 15% genetic variation among populations and 85% variation within populations. Highest Nei's genetic distance of 0.998 was found between Western and Mwingi populations while lowest genetic distance of 0.092 was found between Embu and Meru populations. Lablab purpureus populations exhibited a high level of relatedness as revealed by cluster analysis and dendrogram based unweighted pair group method with arithmetic averages (UPGMA)

Keywords: Accessions, diversity, expected heterozygosity, genetic distance

Introduction

Estimation of genetic diversity in a crop species is prerequisite for its improvement. The use of germplasm with distinct DNA profiles helps to generate breeding populations with broad genetic base (Singh *et al.*, 2012). DNA markers provide an opportunity to characterize genotypes and to

measure genetic relationships precisely than other markers. Conventionally, genetic diversity is estimated by morphological observations recorded on quantitative traits. However, the results of such studies are inconsistent, only relevant for genotypes used and environment involved and cannot be generalized (Singh *et al.*, 2012). Genetic diversity is influenced by selection, mutation, migration, population size, and genetic drift (Hedrick, 2005; Ouborg & Vergeer, 2006). Although morphological markers enable the detection of genetic variation, they are often disguised by factors in the environment, and minimized by a paucity of discernible morphological markers. Significant advancements in molecular biology have shifted the focus of assessment of biodiversity from relying on morphological markers to using isozymes and DNA markers (Konstantinos, 2008).

Lablab is referred to as an 'orphan legume crop' (Varshney *et al.*, 2009). 'Orphan crops' are also referred to as 'underutilized crops' because of their lack of global cultivation and utilization yet have high nutritional qualities, are heat and drought tolerant, and are accessible to less affluent farmers (Khourya *et al.*, 2014). In comparison to major or staples or other economic crops, the 'orphan crops', have often been neglected and are therefore on the verge of extinction in some cases. Albeit late, accessions of the neglected crop gene banks and germplasm institutes have been collected worldwide. However, introduction of the accessions of these crops without their characterization, limits the maximum preservation of their genetic diversity (Bartel, 2009). The assessment of genetic diversity of introductions (accessions) is a pivotal strategy for their successful and efficient preservation, in sites well as ex situ (Van Tienderen *et al.*, 2002). Simple sequence repeats (SSRs) are the most suitable markers for the genetic assessment of germplasm because of their hypervariability, attributable to allelic variations (Ma *et al.*, 2009). In this study SSRs specific for Lablab were used to assess the diversity of Lablab grown in Kenya.

1.1 Advantages of SSR analysis

Simple sequence repeats (SSR) markers have many advantages over the other marker systems. The first advantage is their high reproducibility, which would be the most important in genetic analysis. While reproducibility of the SSR profile is as robust as that with RFLPs, experimental procedures for SSR analysis are much simpler and require only a small amount of template DNA (Boder *et al.*, 2006). Since SSR analysis does not require restriction with enzymes, it can reproduce the same profiles regardless of the state of the template DNA. It also does not require template DNA to be ultra pure, which is a requirement in AFLP analysis. This is a real benefit when one is dealing with specimens that are dry, contaminated, mummified or even in fossilized form in the wild (Boder *et al.*, 2006).

This second advantage of the SSR marker system is the polymorphic genetic information

contents. The hyper-variable nature of SSRs produces very high allelic variation seven among very closely related varieties (Wang *et al.*, 2004). The third advantage has to do with the co-dominant nature of SSR polymorphisms. Although homo palsies bands can Bemis leading in scoring SSR profiles, the SSR bands produced from the same set of primers are intuitive lyorthologous. Homoplasly is a phenomenon where in different copies of a locus are identical in stated spite not being identical by descent (Estoup *et al.*, 2002). In SSR analysis, homoplasly can occur if two bands are similar in size but not identical in sequence.

The fourth advantage of the SSR marker system is their abundance and distribution in genomes. As more and more genomic sequences are being identified in various eukaryotic species, it is becoming increasingly evident that SSRs are truly abundant in almost all species, and are well distributed throughout their genomes (Varshney *et al.*, 2005). A fifth advantage of the SSR marker system is that SSRs are preferentially associated with on- repetitive DNA (Varshney *et al.*, 2005).

2. MATERIALS AND METHODS

2.1 Plant Materials

A total of 96 Lablab accessions were collected from the gene bank of Kenya, Rift Valley, Eastern, Coast, Western, Nyanza and Central regions of Kenya. Out of 96 accessions 46 had a brown seed coat of various colour intensities, one was white, one was dotted brown - black and 48 had a black seed coat of various colour intensities. The 96 accessions were planted in a greenhouse at Kenya Agricultural and Livestock Research organization (KALRO), Biotechnology Institute, Nairobi. Seeds were planted on both germination trays and plastic pots to increase the chance of germination so as to obtain leaf for all accessions. After seedling emergence, plants were left to grow for four weeks after which tender leaves were harvested for DNA extraction.

2.2 DNA Isolation

One (1) gramme of leaf tissue was harvested when seedlings developed the first two fully grown leaves (four weeks after seedling emergence). The leaf tissue was placed in eppendorf self-standing tubes each containing two ceramic beads. The tubes with beads and leaf tissue were placed in the geno-grinder machine (Benchtop homogenizer, Fast prep*-24) which was set to run for one minute at 4 up-down movements per second (4M/S). A modification of the cetyl-trimethyl-ammonium bromide(CTAB) method (Kimani *et al.*, 2012) was carried out. Nine hundred micro liters (900µl) of extraction buffer (2% CTAB, 100mM Tris-Hydrochloric acid pH 8.0,

1.4M Sodium chloride (NaCl), 50mM Ethylene-di-amine-tetra-acetic acid (EDTA), 2% Polyvinyl-pyrrolidone (PVP) and 10 μ l of 2% β -mercaptoethanol) was added to the leaf tissue and ground to form a slurry. The slurry was incubated at 65 $^{\circ}$ C for 15 minutes in a water bath with constant shaking. It was then centrifuged at 13000 rpm for five minutes.

Six hundred microliters(600 μ l)of the supernatant were transferred into a fresh append or tube and an equal volume of chloroform: isoamyl alcohol(24:1)was added. The append or tubes were shaken well before separating the contents in a centrifugeat13000 rpmfor5minutes. Five hundred microliters (500 μ l)of the aqueous phase was transferred into a fresh tube and an equal volume of chloroform: isoamylalcohol(24:1)added. The tubes were shaken well and then centrifugedfor5minutesat13000rpm.Four hundred microliters (400 μ l) of the aqueous phase was transferred to a fresh tube and an equal volume of ice-cold isopropanol added and mixed by inverting several times to precipitate the DNA. The tubes were centrifuged at 13000rpm for 5 minutes. The supernatant was decanted leaving the DNA pellet at the bottom of the tube. The pellets were washed using 500 μ l of 70% ethanol and spun for one minute before they were air dried for one hour. The dried pellets were re-suspended in 50 μ l of sterile distilled water. RNA was removed by adding two microliters (2 μ l) of pancreatic rib nuclease A (RNase A) (10mg/ml) and incubating the samples for one hour at 37 $^{\circ}$ C. The samples were stored at minus 20 $^{\circ}$ C.

2.3 DNA Quantification and Quality Determination

The quantity and quality of genomic DNA was examined by comparing the template DNA isolated from samples with a DNA ladder (gene ruler) of one kilo base (1 kb)ina0.8%agarosegelusing1xTBEbuffer and viewed in a gel box (G: Box, Syngene). The concentration and quality was further determined at optical density (OD) readings of 260 nm and 280 nm using a Nano-drop spectrophotometer (Thermo Scientific Nano-Drop 2000C). The concentrations were used to guide the normalization of DNA of each sample at a concentration of 20ng/ μ L. Additionally, the ratio of OD 260/280 was provided by the Nano-drop and this gave an indication of purity of the samples. Pure DNA has OD260/OD280 value of 1.8 and a deviation from this signifies the presence of contaminants that inhibit PCR reaction.

2.4 PCR Optimization

PCR optimization was carried out using four selected DNA samples. A pre-mix containing dNTPs (dATPs, dCTPs, dGTPs and dTTPs), MgCl₂, Tris-HCl (pH 9.0), KCl and TaqDNA was used. A master mix containing 2 μ L of sterile distilled water, 0.5 μ L of 10pmoles forward primer, 0.5 μ L of 10pmoles reverse primer, 5 μ L premix and 2 μ L of template DNA was prepared. Amplification was carried out in a Thermocycler machine (Techne-TC 412, Applied Bio systems Veriti systems) programmed with the following regime and 35 cycles: initial denaturation at

95°C for 5 minutes; denaturation at 95°C for 1 minute; annealing at 57°C for 1 minute; extension at 72°C for 1 minute; final extension at 72°C for 10 minutes and final hold at 4°C.

The diluted DNA samples were subjected to polymerase chain reaction (PCR) amplification using simple sequence repeat (SSR) markers. A total of 34 SSR markers specific for Lablab were screened. PCR products were examined by comparing the template DNA with a DNA ladder (gene ruler) of one kilo base (1 kb) in a 1.5% agarose gel using 1x TBE buffer and viewed in a gel box (G: Box, Syngene).

2.5 Gel Electrophoresis of PCR Products

Four samples per marker were separated on 1.5% agarose gel at 80V for 40 minutes. Agarose powder was dissolved in Tris-borate EDTA (1x TBE) buffer by slowly boiling in a microwave oven. The agarose was allowed to cool and 1mg/ml concentration of ethidium bromide was added to the gel. The warm agarose solution was then poured into the gel tray in which combs were inserted to form sample wells. The gel was allowed to solidify for 30 minutes before immersing in the electrophoresis tank containing 100ml TBE buffer. The samples were run alongside 1.0µL 1kb DNA ladder at 80 volts for 40 minutes. The amplified products were viewed under UV light in a gel box (G: Box, Syngene). Twenty six of the primers showed amplification at various degrees while eight primers did not amplify at all. Among the 26 primers that showed amplification, ten best were selected to amplify 96 accessions of *Lablab purpureus*.

2.6 Selection of SSR primers for diversity

Selection of SSR primers was based on polymorphic bands observed on agarose gel. SSR primers that gave clear polymorphism with minimum absent and faint bands were selected. All the 34 Lablab SSR primers were screened using four DNA samples. The ten selected as polymorphic simple sequence repeat (SSR) primers were used to amplify 96 Lablab accessions considered in this study. DNA fragment analysis was performed by comparing the bands' base pair sizes with that of the DNA ladder and also by using information from previous study on the same markers. The number of alleles and frequency per marker were obtained using the GeneticAnalysisinExcel (GenAlEx) version 6.2 software (Peakall, & Smouse, 2006).

2.7 Grouping of *Lablab purpureus* into Populations

The 96 Lablab accessions were grouped into 15 populations based on regions of collection. Lablab accessions collected from different regions varied in colour of seed coat although black and brown seed coats were more popular. Genebank collection from Eastern region of Kenya, Makueni and Nairobi populations exhibited a higher diversity in seed coat colour where whitish-brown, white and dotted seed coats were found.

3.0 RESULTS

3.1 Markers' effectiveness in detecting allele availability and polymorphism

A total of 43 alleles were detected and all were polymorphic (Table 1). The number of alleles detected per primer pair ranged from 4 to 5 with an average of 4.3 alleles. The highest number of alleles amplified products was observed in LabT2, LabT3 and LabT7. LabT6 was more frequent while LabT1 was the least frequent in the Lablab germplasm studied. The highest PIC value of 0.67 was observed in SSR primers LabT3, LabT7 and LabT33 while the lowest PIC value of 0.58 was observed in primer LabT6. The higher the PIC value, the more informative is the SSR marker. Hence, all SSR primers were found to be highly informative in revealing the genetic diversity among the Lablab populations. The expected heterozygosity at each polymorphic locus ranged from 0.23 (LabT6) to 0.46 (LabT1) and on average was 0.38. In general the expected heterozygosity was low (Table 1).

Table 1 Characteristics of the 10 Lablab SSR markers indicating major allele frequency, number of alleles, expected heterozygosity and polymorphism information content (PIC).

Marker	Major Allele Frequency	Allele Number	Expected Heterozygosity	PIC
LabT1	0.3177	4.0000	0.4583	0.6530
LabT2	0.5000	5.0000	0.2292	0.6400
LabT3	0.3958	5.0000	0.4688	0.6715

LabT6	0.5313	4.0000	0.2083	0.5763
LabT7	0.3594	5.0000	0.4271	0.6701
LabT14	0.3698	4.0000	0.3021	0.6416
LabT24	0.4427	4.0000	0.4688	0.6133
LabT25	0.4115	4.0000	0.4271	0.6371
LabT28	0.4115	4.0000	0.4896	0.5838
LabT33	0.3698	4.0000	0.3229	0.6735
Mean	0.4109	4.3000	0.3802	0.6360

3.2 Genetic distance between populations of *Lablab purpureus*

The level of relatedness between the 15 populations of *Lablab* was established through a genetic distance matrix (Table 2) derived from the proportion of shared (common) loci (Nei, 1983), using GeneticAnalysisinExcel(GenAlEx)version6.2software(Peakall&Smouse,2006).Pairwise comparison of Nei's unbiased genetic distance among the 15 populations ranged from a low of 0.092 between Embu and Meru populations to a high of 0.998between Mwingi and Western populations (Table 2). Other populations that exhibited high genetic distances were Nakuru and Western with a Nei's genetic distance of 0.966. In addition, populations that had a low Nei'sgenetic distance were Murang'a and Nyeri that had a Nei's genetic distance of 0.121 and between genebank collection from Eastern region of Kenya and those collected from Murang'a with a Nei's genetic distance of 0.138. Similarly, genebank collection from coastal region of Kenya was closely related to population collected from Nairobi with a Nei's genetic distance of 0.160.

Table 2 . *Lablab purpureus* pairwise population matrix of Nei genetic distance

EM	GC	GE	LM	MC	MK	ME	MU	MW	NA	NK	NY	RV	TH	WE	
0.000															EM
0.357	0.000														GC
0.348	0.414	0.000													GE
0.437	0.498	0.293	0.000												LM
0.614	0.712	0.272	0.317	0.000											MC
0.435	0.341	0.223	0.271	0.370	0.000										MK
0.092	0.343	0.312	0.352	0.549	0.346	0.000									ME
0.402	0.459	0.138	0.342	0.235	0.200	0.371	0.000								MU
0.536	0.678	0.247	0.298	0.572	0.351	0.557	0.332	0.000							MW
0.373	0.160	0.326	0.434	0.577	0.169	0.280	0.351	0.506	0.000						NA
0.348	0.521	0.478	0.527	0.863	0.520	0.485	0.430	0.318	0.426	0.000					NK
0.384	0.532	0.193	0.386	0.413	0.194	0.225	0.121	0.382	0.255	0.464	0.000				NY
0.275	0.422	0.344	0.229	0.656	0.395	0.227	0.513	0.234	0.294	0.339	0.360	0.000			RV
0.649	0.577	0.250	0.382	0.357	0.446	0.638	0.297	0.654	0.468	0.682	0.390	0.676	0.000		TH
0.483	0.542	0.446	0.496	0.627	0.358	0.336	0.553	0.998	0.409	0.966	0.487	0.651	0.588	0.000	WE

Key: EM- Embu; GC-Genebank Coast; GE-Genebank Eastern; MC-Machakos; MK-Makueni; ME-Meru; MU-Murang'a; MW-Mwingi; NA-Nairobi; NK-Nakuru; NY-Nyeri; RV-Rift Valley; TH-Thika; WE-Western

3.3 Analysis of molecular variance (AMOVA)

Population diversity components were partitioned using analysis of molecular variance (AMOVA) (Table 3). The AMOVA denoted that most (85%) of the molecular variation in Lablab bean accessions was partitioned within populations, with lesser amounts (15%) partitioned among populations. The estimation of the variance components among and within populations using analysis of molecular variance (AMOVA) was significant ($P \leq 0.01$).

Table 3 Analysis of molecular variance (AMOVA) for 15 populations of *Lablab purpureus* and partitioning of the total diversity into population components.

Source of variation	df	TSS	MSS	Estimated Variance	Percent molecular variance (%)	P	PhiPT (ΦPT)
Among Populations	14	258.445	18.460	1.567	15%	0.010	0.153
Within Populations	81	701.274	8.658	8.658	85%		
Total	95	959.719		10.225	100%		

Key: PhiPT(ΦPT) - the estimate of population genetic differentiation based on permutation across the full data set; df - degree of freedom; TSS - total sum of squares; MSS - Mean sum of squares.

3.4 Cluster Analysis

Dendrogram was constructed based on the similarity matrix data by applying un weighted pair group method with arithmetic averages (UPGMA) cluster analysis using the Genetic Analysis in Excel (GenAIEx) version 6.2 software (Peakall, & Smouse, 2006). Based on genetic distances, the UPGMA neighbour-joining tree method generated two distinct clusters (A and B) for the 15 *Lablab* populations. According to the resultant dendrogram, fourteen (14) populations were clustered into one cluster (B) while population number 15 (Western) which is comprised of accessions from Bungoma and Kisumu separated on its own cluster A (Fig. 1). Cluster B further separated into sub-clusters B1 and B2. Sub-cluster B1 was composed of Thika and Machakos populations while sub-cluster B2 comprised of Lamu, Rift Valley, Mwingi, Nakuru, Makueni, Nyeri, Murang'a, Genebank Eastern, Nairobi, Genebank Coast, Meru and Embu populations (Fig.1).

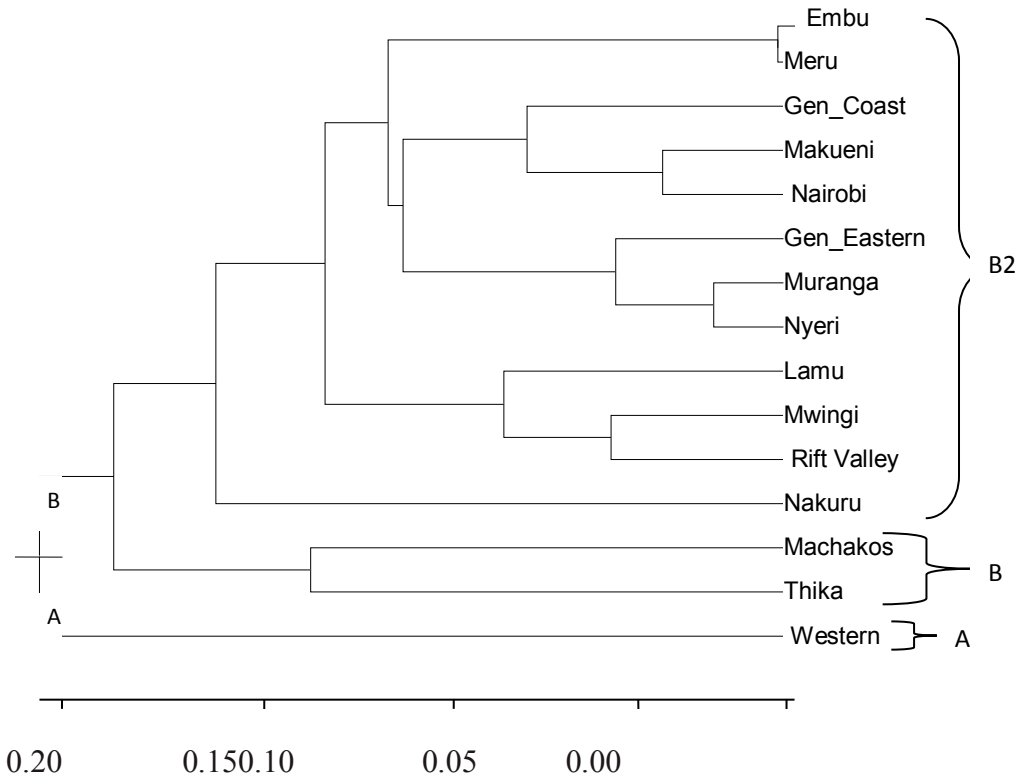


Fig.1 Genetic relationship among 15 populations of *Lablab purpureus* using dendrogram based on Darwin’s genetic identity distance.

4. DISCUSSION

4.1 Markers’ effectiveness in detecting allele availability and polymorphism

In this study, a total of 43 alleles were detected and all were polymorphic. The number of alleles at a marker locus is related to the genetic diversity that can be revealed by a particular marker. The more alleles at a locus, the higher the degree of diversity that can be revealed and the more efficiently closely related genotypes can be distinguished (Nagy *et al.*, 2012). SSR markers are locus-specific and generally amplify one locus (Gupta and Varshney, 2000). Genetic diversity evaluation within a population is indispensable for characterizing of germplasm and offers insight into the evolutionary characteristic, management, exploitation and establishment of breeding approaches for breeders (Li *et al.*, 2011). According to Shibairo *et al.*, (2015),

heterozygosity is considered low if it is less than 0.4, moderate (0.4 - 0.7) and high when greater than 0.7. The expected heterozygosity at each polymorphic locus ranged from 0.23 (LabT6) to 0.46 (LabT1) and on average was 0.38 indicating a low heterozygosity in Lablab accessions studied. However, the level of heterozygosity obtained in this study was relatively high compared to a mean heterozygosity value of 0.189 obtained by Kimani *et al.*, (2012) on fifty Kenyan Lablab accessions using amplified fragment length polymorphism (ALFP).

The low heterozygosity is expected considering Lablab is a self-pollinated crop. It could also be attributed to subsequent loss of unexploited genetic potential. Similar results have been reported in other legumes for instance, in common bean (*Phaseolus vulgaris*), Masi *et al.*, (2003) analysed 264 genotypes based on 30 SSR markers and identified an average of 4.3 alleles per locus and low heterozygosity. Using 18 microsatellites Lazrek *et al.*, (2009) investigated the genetic diversity of 136 lines of *Medicago truncatula* populations from Tunisia and detected an average of 4.2 alleles per locus. Diouf & Hilu, (2005) identified an average of 5.3 alleles per locus in 11 cowpea (*Vigna unguiculata*) varieties in Senegal, using 30 SSR markers.

Polymorphic information content (PIC) provides an estimate of discriminatory power of a locus by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles (Nagy *et al.*, 2012). The highest PIC value of 0.67 was observed in SSR primers LabT3, LabT7 and LabT33 while the lowest PIC value of 0.58 was observed in primer LabT6. In contrast, Asare *et al.*, (2010) reported a low mean PIC value of 0.38 and an average of 3.8 alleles per loci in 141 cowpea accessions collected from nine geographic regions of Ghana. Diaz *et al.*, (2010) observed a PIC value of 0.54 in 92 common bean landraces. Similarly Benchimol *et al.*, (2007) studied genetic diversity of dry beans with 87 SSR loci and found a PIC value range of 0.05 to 0.83, with a mean of 0.45.

Polymorphic information content values range from zero (which is an indicative of monomorphism) to one (very high discriminative power with many alleles in equal frequencies) and the higher the PIC value, the more informative is the SSR marker (Nagy *et al.*, 2012). Hence, primer LabT2, LabT3 and LabT7 were found to be highly informative in revealing the genetic diversity among the Lablab populations and may be useful in future genetic diversity analysis.

4.2 Genetic distance between populations of *Lablab purpureus*

Pairwise comparison of Nei's unbiased genetic distance among the 15 populations ranged from a low of 0.092 between Embu and Meru populations to a high of 0.998 between Mwingi and Western populations. Other populations that exhibited high genetic distances were Nairobi and

Western with a Nei's genetic distance of 0.966. Low genetic distances were also observed between populations Murang'a and Nyeri with a Nei's genetic distance of 0.121. This implies that the Embu/Meru populations and Murang'a/Nyeri populations are closely related. This could be attributed to the nearness of these geographical regions hence there could be higher chances of local communities sharing Lablab accessions as seed stock.

Genetic distance is the difference between two entities that can be described by allelic variation or the extent of gene difference between populations or species that is measured by some numerical quantity (Nei, 1987). According to Beaumont *et al.*, (1998), genetic distance is any quantitative measure of genetic difference be it at the sequence level or the allele frequency level, that is calculated between individuals, populations or species. It calculates the allelic substitutions per locus which have occurred during separate evolution of two populations or species. The calculation of a genetic distance between two populations gives a relative estimation of the time that has passed since the populations have survived as single cohesive units (Nei, 1983). The genetic distances displayed in this study have revealed the level of genetic similarity between Lablab genotypes found in different regions in Kenya. The identified genetically distinct populations, for instance Mwingi and Western, could be potentially important sources of germplasm for further improvement programme in the Lablab genotypes. Hybridizing selected members from the two populations could probably result to genotypes with high heterosis. According to Schnable, (2013) heterosis arises in crosses between genetically distinct individuals as a result of a diversity of mechanisms.

4.3 Analysis of molecular variance (AMOVA)

The AMOVA denoted that most (85%) of the genetic variation in Lablab accessions was partitioned within populations with lesser amounts (15%) partitioned among populations. Estimated variance among populations was 1.567 and within population was 8.65 out of 10.225. Similar observations have been made in several studies for instance Kimani *et al.* (2012) reported a 99% variation within and 1% variation among Lablab populations using amplified fragment length polymorphism (AFLP) markers. Kushwaha *et al.*, (2013), found 86% variation within populations and 14% variation among populations in lentils. The low level of genetic variation among Lablab populations could be as a result of gene flow (introduction and migration of alleles or genotypes) from one region to another through seed trade or accidental transportation of both seed and pollen. Since Lablab is predominantly self-pollinating, the high

level of variability within populations could be attributed to genotype mixture of great diversity held by farmers.

The high gene diversity found in Nairobi could be attributed to the fact that it is a business hub region where traders of Lablab from all over the country converge. Some Lablab accessions found in Nairobi could also have come from other countries such as Tanzania and Uganda due to cross border trade. Although Lablab is predominantly a self-pollinating crop which shows little inbreeding depression, significant levels (6-10%) of natural cross pollination occurs (Gnanesh *et al.*, 2006; Kukade & Tidke, 2014). Consequently, Lablab landraces grown by small scale farmers are mixtures of great diversity as indicated by various colour shades of seed testa (Kamotho *et al.*, 2010; Kinyua & Kiplagat, 2012). Furthermore, the low level of diversity among populations could be attributed to gene flow as a result of exchange of germplasm by farmers across regions. Additionally, there could be directional selection where growers select genotypes with desirable characteristics and maintain them as seed stocks. This selection leads to a state of adaptation in a progressively changing environment. Accordingly, Lablab genotypes grown in Kenya are basically of narrow genetic base.

The 96 Lablab accessions assayed exhibited low genetic diversity as indicated by low expected mean heterozygosity (H_e) of 0.38. The expected heterozygosity accounts for the occurrence of the different types of alleles or loci in a population (Mohammadi & Prasanna, 2003). In previous studies, Kimani *et al.*, (2012) used amplified fragment length polymorphism (AFLP) markers to assay 50 Kenyan Lablab accessions and reported low diversity among them. Maassetal.,(2005) used AFLP markers to determine the sources of diversity in cultivated and wild Lablab accessions from Angola, Egypt, Ethiopia, Kenya, Mozambique, Malawi, Nigeria, Sudan, Tanzania, Uganda, South Africa, Zambia, Zimbabwe and Asia. Moderate genetic diversity was displayed for the landraces from Africa and Asia. Accessions clustered according to their sub specific taxonomic organization and also as cultivated and wild forms. The Kenyan Lablab revealed that the existing variations in cultivated form had no geographic basis. Indeed, clustering of the accessions was not dependent on the geographical area of collection. Maassetal.,(2005) suggested that continuous exchange and selection from a narrow set of landraces may have resulted in a reduction in the genetic base of the crop in Indian and African continents. Conversely, great diversity has also been reported for the wild forms from Africa (Maassetal., 2005). The wild and cultivated forms can be crossed to produce variability of high vigour hybrids in the Kenyan Lablab genotypes. A large agro-morphological diversity of Lablab has been reported in South Asia (Maassetal., 2010), and these can also be included in the breeding programs to expand the genetic base of the Kenyan Lablab genotypes.

4.4 Cluster Analysis

Cluster analysis groups individuals or objects based on characteristics they possess so that individuals with similar descriptions are mathematically gathered into the same cluster (Hair *et al.*, 1995). Clustering is the classification of objects into different groups, or to reduce the amount of data by categorizing or grouping similar data items together. There are distance based methods, in which a pair-wise distance matrix is used as an input for analysis by a specific clustering algorithm, leading to a graphical representation such as a dendrogram in which clusters may be visually identified (Mohammadi & Prasanna, 2003). Clustering pattern indicated a narrow genetic base of Lablab accessions. A dendrogram constructed on the basis of genetic distance matrix and by unweighted paired group method with arithmetic averages (UPGMA), using GenAIEx version 6.2 software (Peakall, & Smouse, 2006) resolved the 15 Lablab populations into two distinct clusters. According to the resultant dendrogram, fourteen populations were clustered into one cluster (B) while population number 15 (Western) which comprised of accessions from Bungoma and Kisumu separated on its own cluster (A).

The results of cluster analysis indicate a narrow genetic base for Kenyan Lablab similar to that of India and China (Yaming *et al.*, 2013). Narrow genetic variation of genotypes may result during the long cultivation history of species as an adaptation to the local agro-climatic conditions (Seehalak *et al.*, 2006). In the long run, this could have been the case in locally adapted Lablab genotypes. Farmers continuously select good seed for planting based on desirable agromorphological traits such as yield, disease resistance, drought tolerance and earliness in maturity. This agricultural practice could maintain and also probably contribute to the genetic uniqueness by strengthening the specific adaptations obtained by the landraces (Seehalak *et al.*, 2006). Molecular markers are scattered through out the genome and their association with various agronomic traits is influenced by the cultivator under selection pressure induced by domestication. Probably the Lablab genotypes used in this study could have been selected over the years for specific agronomic traits thus the reason for clustering most of the population in one cluster. Therefore, the need to design breeding programs with the aim of broadening the genetic base of Kenyan Lablab is of paramount importance. This could be achieved by introgressing genotypes from the wild and also from different regions in Africa, Australia, and Asia.

5. CONCLUSION

Results of this study indicate that Lablab germplasm assayed by SSR markers is of narrow genetic base. The SSR analysis was successful in the estimation of genetic diversity among Lablab

change no types. The study found that Lablab bean populations from Mwingi and Western are distantly related and therefore selected genotypes of desirable agronomic traits from the two populations could be hybridized to produce genotypes with high heterosis. These results are expected to benefit Lablab bean breeding efforts in Kenya as well as aid in conservation of Lablab germplasm.

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