

**EVALUATION OF ADAPTABILITY POTENTIAL AND GENETIC  
DIVERSITY OF KENYAN DOLICHOS BEAN (*Lablab purpureus* (L.) SWEET)  
GERMPLASM**

**GRACE NYAKONYU KAMOTHO**

**THESIS SUBMITTED TO THE SCHOOL OF AGRICULTURE AND  
BIOTECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT  
FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN SEED SCIENCE  
UNIVERSITY OF ELDORET, KENYA.**

**NOVEMBER, 2015**

**DECLARATION BY CANDIDATE**

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior permission of the author and/ University of Eldoret.

\_\_\_\_\_

Date\_\_\_\_\_

**Kamotho, Grace Nyakonyu****AGR/DPHIL/01/05****University of Eldoret, Eldoret, Kenya****DECLARATION BY SUPERVISORS**

This thesis has been submitted for examination with our approval as University supervisors.

\_\_\_\_\_

Date\_\_\_\_\_

**Prof. Reuben M. Muasya****South Eastern Kenya University, Kitui, Kenya**

\_\_\_\_\_

Date\_\_\_\_\_

**Prof. Miriam G. Kinyua****University of Eldoret, Eldoret, Kenya**

## **DEDICATION**

This thesis is dedicated to my husband Dr. Andrew Chege and my children Zita Wnjiru, Miki Chege and Pucci Gichimu for their support, understanding, patience and encouragement. I also dedicate this thesis to my late parents Nathan Kamotho and Mary Nyokabi for their great interest in my education.

## ABSTRACT

*Lablab purpureus* L., synonym *Dolichos lablab* (L.) Sweet;  $2n = 22$  or  $24$  belongs to the family Leguminosae. It is widely grown in Kenya and popular among many Kenyan communities as a grain legume, animal fodder and green manure. The main growers of *Lablab purpureus* in Kenya are small scale farmers who either grow it as intercrop or pure stand. However, *Lablab purpureus* can be regarded as an under-exploited species. Limited research has been conducted on the species as a food crop in Kenya and consequently information on its adaptability and genetic diversity is scanty. Farmers' choice of type of *Lablab purpureus* to grow is based on seed availability and colour preference. Consequently genotypes are not grown in suitable specific agroecological environments hence low yields are obtained. Cultivars are distinguished by maturity period and morphological traits, and being an under-exploited crop, variety development is still in its infancy and so is the varietal classification. The objectives of the study were: to assess the status of *Lablab purpureus* in Kenya; to evaluate the adaptability potential of *Lablab purpureus* to various agro-ecological conditions in Kenya; to assess the phenotypic diversity of *Lablab purpureus* and to evaluate the genotypic diversity of *Lablab purpureus* using simple sequence repeats (SSRs) molecular marker. To achieve the above objectives, a baseline survey covering major *Lablab purpureus* growing areas of Kenya was conducted. Field experiments on agroecological adaptability were performed using randomized complete block design in three agro-ecological environments. Morphological characterization was conducted using a descriptor from Asian Vegetable Research Development Center (AVRDC). Molecular characterization was conducted by using simple sequence repeat (SSR) molecular markers specific for *Lablab purpureus* and subjecting the DNA to polymerase chain reaction (PCR). Data obtained from the survey was subjected to descriptive statistics (percentages and means) using GenStat statistical software. Analysis of variance (ANOVA), correlation analysis, principal component analysis and cluster analysis were conducted for agroecological adaptability and morphological characterization data using GenStat statistical software. Data from molecular experiment was subjected to summary statistics, analysis of molecular variance (AMOVA), principal coordinate analysis and cluster analysis using GenAlex and Darwin softwares accordingly. Results of the baseline survey indicated the main challenges in *Lablab purpureus* production in Kenya as; pests and diseases, unavailability of good quality seed, low yielding cultivars, cultivars that take long periods to mature and lack of technical knowhow by farmers. Four accessions proved to have high yield potential and of relatively short maturity period in specific agroecological environments and could therefore be considered in future *Lablab purpureus* improvement programmes. Morphological characterization indicated that *Lablab purpureus* germplasm grown in Kenya exhibits a wide variability in quantitative traits and narrow variability in qualitative traits. Molecular characterization revealed that the Kenyan *Lablab purpureus* genotypes have a high degree of relatedness and of narrow genetic base.

## TABLE OF CONTENT

DECLARATION BY CANDIDATE .....	i
DECLARATION BY SUPERVISORS .....	i
DEDICATION .....	ii
ABSTRACT .....	iii
LIST OF FIGURES .....	ix
LIST OF APPENDICES .....	x
ACKNOWLEDGEMENT .....	xii
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1 Overview of thesis structure .....	1
1.2 Background Information.....	3
1.2.1 Taxonomy of <i>Lablab purpureus</i> .....	3
1.2.3 Ecology of <i>Lablab purpureus</i> .....	6
1.2.4 Origin, history and distribution of <i>Lablab purpureus</i> .....	6
1.2.5 Economic Importance .....	7
1.2.6.3 Biochemical markers .....	11
1.3 Problem Statement .....	12
1.4 Objectives .....	13
1.4.1: Broad Objective .....	13
1.4.2: Specific Objective.....	14
1.5 Working Hypothesis .....	14
1.6 Justification.....	14
CHAPTER TWO .....	16
ASSESSMENT OF STATUS OF DOLICHOS BEAN ( <i>Lablab purpureus</i> (L.) )	
GERMPLASM IN KENYA .....	16
2.1 Abstract .....	16
2.2 Introduction.....	17
2.2.1 Distribution of <i>Lablab purpureus</i> in Kenya.....	17
2.2.2 Uses of <i>Lablab purpureus</i> in Kenya .....	17
2.3 Materials and Methods.....	19
2.4 Results.....	21
2.4.1 <i>Lablab purpureus</i> production in areas covered by the survey .....	21
2.4.3 Source of Lablab seed .....	25
2.4.4 Seed harvesting and postharvest handling practices .....	26
2.4.5 Seed sorting and treatment prior to planting.....	29
2.4.6 Technology development in Lablab.....	30
2.5 Discussion .....	30
CHAPTER THREE .....	34
EVALUATION OF ADAPTABILITY POTENTIAL OF THE KENYAN	
DOLICHOS BEAN ( <i>Lablab purpureus</i> (L.) SWEET) UNDER DIFFERENT AGRO-	
ECOLOGICAL ENVIRONMENTS .....	34
3.1 Abstract .....	34
3.2 Introduction.....	35
3.3 Materials and Methods.....	37
3.3.1 Sites for field trials.....	37
3.3.2 Data Collection .....	38
3.3.3 Data analysis .....	40
3.4 Results.....	40
3.4.1 Effect of environment on yield and maturity period of Lablab accessions.....	40
3.4.2 Effect of environment on vegetative characters of Lablab accessions.....	45

3.5 Discussion .....	47
CHAPTER FOUR.....	50
EVALUATION OF PHENOTYPIC DIVERSITY OF THE KENYAN DOLICHOS BEAN ( <i>Lablab purpureus</i> (L.) SWEET) GERMPLASM.....	50
4.1 Abstract .....	50
4.2 Introduction.....	51
4.2.2 Application of morphological markers in crop improvement.....	51
4.3 Materials and Methods.....	53
4.3.1 Seed acquisition and planting .....	53
4.3.2 Morphological characterization .....	53
4.3.3 Data Collection .....	55
4.3.4 Data Analysis .....	55
4.4 Results.....	55
4.4.2 Lablab diversity for colour of flower keel, colour of flower standard and pod colour .....	58
4.4.3 Lablab diversity for yield and yield associated characters .....	60
4.4.4 Lablab diversity for vegetative and seed characters .....	65
4.4.5 Correlation analysis for yield and yield associated characters .....	69
4.4.6a Cluster analysis on reproductive quantitative traits .....	70
4.4.6b Cluster analysis on vegetative quantitative traits.....	74
4.4.7 Principal component analysis (PCA) of Lablab reproductive traits .....	77
4.5 Discussion .....	79
CHAPTER FIVE .....	86
EVALUATION OF GENOTYPIC DIVERSITY OF LABLAB BEAN ( <i>Lablab purpureus</i> (L.) SWEET) USING SIMPLE SEQUENCE REPEAT (SSR) MOLECULAR MARKERS .....	86
5.1 Abstract .....	86
5.2 Introduction.....	87
5.3 Materials and Methods.....	90
5.3.1 Plant materials.....	90
5.3.2 DNA Isolation.....	90
5.3.3 DNA quantification and quality determination.....	92
5.3.6 Selection of SSR primers for diversity .....	94
5.3.7 Grouping of <i>Lablab purpureus</i> into populations .....	95
5.4 Markers' effectiveness in detecting allele availability and polymorphism .....	97
5.4.2 Genetic distance between populations of <i>Lablab purpureus</i> .....	98
5.4.3 Genetic differentiation of <i>Lablab purpureus</i> populations .....	101
5.4.4 Cluster Analysis .....	106
5.5 Discussion .....	107
CHAPTER SIX.....	118
GENERAL DISCUSSION .....	118
6.1 Assessment of the status of <i>Lablab purpureus</i> in Kenya .....	118
6.2 Evaluation of performance of Lablab under different agro-ecological environments.....	119
6.3 Assessment of Lablab phenotypic diversity .....	120
6.4 Evaluation of genotypic diversity of <i>lablab purpureus</i> using simple sequence repeat (SSR) molecular markers .....	121
CHAPTER SEVEN .....	123
CONCLUSION AND RECOMMENDATIONS .....	123
7.1 Conclusion .....	123
7.2 Recommendations.....	124

7.3 Suggestions for further studies.....	126
REFERENCES .....	127
APPENDICES .....	143

## LIST OF TABLES

Table2.1 Places where <i>Lablab purpureus</i> accessions were collected .....	20
Table3.1 Description of locations under which <i>Lablab purpureus</i> accessions were evaluated for erformance. ....	38
Table3.2 Agro-morphological traits used in evaluation of performance of <i>Lablab purpureus</i> accessions in different agro-ecological environments.....	39
Table3. 3 Mean yield and days to maturity of <i>Lablab purpureus</i> accessions across the three environments.....	42
Table3.4 Analysis of variance of seed yield per plant for <i>Lablab purpureus</i> accessions	43
Table3.5 A combined analysis of variance of seed and yield related characters of 45 accessions of <i>Lablab purpureus</i> .....	44
Table3. 6 Effect of environment on mean yield and yield related characters of <i>Lablab purpureus</i> . ....	45
Table3. 7 Combined analysis of variance of vegetative characters of 45 accessions of <i>Lablab purpureus</i> . ....	46
Table3. 8 Effect of site on vegetative characters of <i>Lablab purpureus</i> accessions .....	47
Table4.1 Agro-morphological traits used in characterization of <i>Lablab purpureus</i> ....	54
Table4. 2 <i>Lablab purpureus</i> diversity for seed testa colour, emerging cotyledon colour, hypocotyl colour, main vein colour and growth habit. ....	56
Table4. 3 <i>Lablab purpureus</i> diversity for colour of flower keel, colour of flower standard and pod colour .....	59
Table4. 4 Means of nine quantitative reproductive traits of the forty-five <i>Lablab purpureus</i> accessions. ....	64
Table4. 5 Means of nine vegetative quantitative traits of the forty-five <i>Lablab purpureus</i> accessions .....	68
Table4.6 Correlation matrix on yield and yield related characteristics of <i>Lablab purpureus</i> . ....	70
Table 5.1 List of ten SSR primer pairs (specific for <i>Lablab purpureus</i> ).....	94
Table 5.2 <i>Lablab purpreus</i> populations based on regions of collection .....	95
Table5.3 Characteristics of the 10 <i>Lablab purpureus</i> SSR markers indicating major allele frequency, number of alleles, expected heterozygosity and polymorphism information content (PIC).....	98



Table5. 4a <i>Lablab purpureus</i> pairwise population matrix of Nei genetic identity.....	101
Table5. 4b <i>Lablab purpureus</i> pairwise population matrix of Nei genetic distance.....	101
Table5.5 F-statistics and estimates of differentiation of <i>Lablab purpureus</i> populations for each locus. ....	102
Table5.6 Analysis of molecular variance (AMOVA) for 15 populations of <i>Lablab purpureus</i> and partitioning of the total diversity into population components	103
Table5.7 Mean number of different loci (Na), number of effective loci (Ne) Expected heterozygosity (He), Shannon index (I) across the 15 <i>Lablab purpureus</i> populations. ....	104

## LIST OF FIGURES

Fig.2. 1 Utilization of <i>Lablab purpureus</i> by respondents.....	24
Fig.2. 2 (a) Perception on taste and flavor preference between <i>Lablab purpureus</i> and common bean .....	25
Fig.2. 3 Source of <i>Lablab purpureus</i> seed by respondents.....	26
Fig.2. 4 <i>Lablab purpureus</i> seed sorting and treatment by respondents .....	29
Fig.2. 5 Traits for improvement in <i>Lablab purpureus</i> .....	30
Fig.4. 1 <i>Lablab purpureus</i> diversity for emerging cotyledon colour, hypocotyl colour and main vein colour.....	57
Fig.4. 2 <i>Lablab purpureus</i> diversity for (a) growth habit and (b) colour of seed testa.	58
Fig.4.3 Proportion of <i>Lablab purpureus</i> accessions for (a) colour of flower kill (b) colour of flower standard and (c) pod colour.....	60
Fig.4.4a Dendrogram showing relatedness of <i>Lablab purpureus</i> accessions based on reproductive quantitative traits. ....	74
Fig.4.4b Dendrogram showing relatedness of <i>Lablab purpureus</i> accessions based on Vegetative quantitative traits.....	74
Fig.5. 1 Scatter plot showing the clustering pattern of 15 <i>Lablab purpureus</i> populations represented by different colours and symbols.....	105
Fig.5. 2 Genetic relationship among 15 populations of <i>Lablab purpureus</i> using dendrogram based on Darwin's genetic identity distance. ....	106

## LIST OF APPENDICES

APPENDIX I: Interviewe schedule guide .....	143
APPENDIX II: Map of Kenya showing localities where Lablab bean was collected .....	146
APPENDIX III: Lablab bean accessions collected during the survey .....	147
APPENDIX IV: Photos on Lablab intercropped with maize, pure stand and grown on terraces .....	148
APPENDIX V: Plates showing various activities carried out in the molecular laboratory at KALRO, Biotechnology Institute, Nairobi (source: Author, 2014).....	149
APPENDIX VI: Fischers protected test for means of <i>Lablab purpureus</i> site x accession interaction for days to mature pods, days to flowering, duration flowering and pods per raceme .....	150
APPENDIX VII: Fischers protected test for means of <i>Lablab purpureus</i> site x accession on for number of seeds per pod, 100 seed weight, seed yield per plant and number of racemes per plant .....	154
APPENDIX VIII: Fischers protected test for means of <i>Lablab purpureus</i> site x accession interaction for days to germination, plant height, leaf length and raceme length .....	157
APPENDIX IX: Means of <i>Lablab purpureus</i> site x accession interaction for pod length, pod width, seed length and seed width .....	161
APPENDIX X: Absorbance curve obtained by Nanodrop spectrophotometer.....	165
APPENDIX XI: Agarose gel profiles of SSR polymerase chain reaction (PCR) products ...	166
APPENDIX XII :Allele frequencies by population for codominant data.....	176
APPENDIX XIII: Sample size, number of alleles, number of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index.....	177
APPENDIX XIV:Chart on allelic patterns for co-dominant data across populations .....	181
APPENDIX XV :Allelic co-dominant data across populations.....	182
APPENDIX XVI :Percentages of analysis of molecular variance (AMOVA) for among and within <i>Lablab purpureus</i> populations .....	183
APPENDIX XVII :Principal Coordinates Analysis (PCA).....	184

## LIST OF ABBREVIATIONS

CTAB	- Cetyltrimethylammonium bromide
DNA	- Deoxyribonucleic Acid
dNTP	- Deoxynucleotide Triphosphates (a mixture of dATP, dTTP, dCTP, dGTP)
dATP	- deoxyadenosine Triphosphate
dCTP	- deoxycytidine Triphosphate
dGTP	- deoxyguanine Triphosphate
dTTP	- deoxythymidine Triphosphate
EDTA	- Ethylenediaminetetraacetic acid
GenAlex	- Genetic Analysis in Excel soft ware
GenStat	- GenStat Statistical software
KALRO	- Kenya Agricultural and Livestock Research Organization
ng	- nanogram
OD	- Optical density
PCR	- Polymerase Chain Reaction
PIC	- Polymorphic information content
PVP	- Polyvinylpyrrolidone
RAPD	- Random Amplified Polymorphic DNA
RFLP	- Restriction Fragment Length Polymorphism
Rpm	- Revolutions per minute
TBE	- Tris Boric acid-EDTA
Tm <sup>o</sup> C	- Annealing temperatures
μl	- microlitre
UPGMA	- Unweighted pair group method of arithmetic averages

## ACKNOWLEDGEMENT

I wish to express my appreciation to the following institutions for providing support in the course of my PhD study: Kirk House Trust, UK, for financial support for the survey, field experiments and preliminary laboratory work; Kenya Agricultural Livestock and Research Organization (KALRO)-Njoro, Mabanga Agricultural Training Center-Bungoma and University of Eldoret for allowing me use their farms for my field experiments; KALRO Biotechnology Institute-Nairobi, for the support in molecular characterization work; and, Karatina University for partial financial support and granting me time to complete my doctoral studies.

I am greatly indebted to my supervisors, Professor Miriam Kinyua of University of Eldoret and Professor Reuben Muasya of South Eastern Kenya University for their encouragement, invaluable suggestions, constructive criticism and guidance in every step of the study. I am immensely grateful to Dr. Simon T. Gichuki, Director, KARLO, Biotechnology Institute, Nairobi, for allowing me use the laboratory facilities, reagents and materials required for the molecular characterization experiment.

I extend my heartfelt gratitude to the following for their unfaltering support: Paul Kimani for collecting seeds from farmers in Meru; Eliezah Kamau of KALRO, Thika, for furnishing me with a list of simple sequence repeat (SSR) primers specific for Lablab; Hon. Peris Orocko, MoA Nyamira County and Veronica Ndetu of the Ministry of Agriculture, Kilimo House for providing me with information on Lablab and Cyrus Mugambi, Roy Gitonga, Esther Kimani and Bramwel Wanjala of KALRO Biotechnology Institute, for the invaluable consultations on molecular work and in data analysis. My thanks also go to Professor Mathenge of Karatina University for showing deep interest and concern regarding the completion of my doctoral studies.

My ineffable gratitude goes to my husband Dr. Andrew Chege of Moi University, my daughter Zita Wanjiru, my twin sons Miki Chege and Pucci Gichimu. Your undying love has always been a source of my energy. Thank you for tolerating me when I was away in the field and when I took long hours in the laboratory or in the study room writing this thesis. You are the best family ever.

“The Lord is my Banner” (Exodus 17: 12). Thanks to the many Aarons and Hurs who encouraged me whenever I became desperate and almost gave up. God Bless you all.

To God be all glory and honour.

## **CHAPTER ONE**

### **INTRODUCTION**

#### **1.1 Overview of thesis structure**

This thesis has six chapters as outlined below:

Chapter 1 (General introduction) laid out the background of the study including description of Lablab, its taxonomy, ecology, distribution and economic importance. Genetic diversity and methods used in estimation of genetic diversity in crops were revealed. The chapter also examined the stated problem, objectives, hypotheses and justification of the study.

Chapter 2 documented the findings of a baseline survey carried out on Lablab in regions of the country where the crop is grown. Findings on production practices, challenges in production and utilization of Lablab in Kenya were presented. The chapter also described agro-ecological regions covered by the survey in which samples of germplasm for subsequent studies were collected. Results of the survey were discussed highlighting findings of previous similar studies by other researchers.

Chapter 3 assessed the level of adaptability of Lablab germplasm in different agro-ecological environments in Kenya. For this, forty five accessions of Lablab were grown in different environments and their performance evaluated based on reproductive and vegetative traits. The chapter also revealed literature on effects of environment on crop performance. Descriptive statistics and analysis of variance (ANOVA) were used to determine levels of adaptability of accessions. At the end of the chapter, results were presented, discussed and compared with similar studies previously carried out by other researchers.

Chapter 4 provided an assessment of genetic diversity of Kenyan Lablab germplasm using morphological markers. Morphological characteristics of Lablab using a descriptor from Asian Vegetable Research Development Center (AVRDC) were described. Twenty nine traits were evaluated and dendrograms constructed to display a graphical presentation. Accessions were grouped according to their similarity using cluster analysis. Discussion of results was subsequently presented.

In chapter 5, simple sequence repeat (SSR) molecular marker specific for Lablab was used to assess Kenyan Lablab purpureus diversity. Ten SSRs were used to characterize ninety six Lablab accessions. Results on heterozygosity and polymorphism were presented. Lablab populations were grouped according to their levels of similarity using principal coordinate analysis and cluster analysis. Results were discussed and compared with previous similar studies.

In chapter 6, conclusion and recommendations derived from the study were made. . Conclusion on baseline survey, Lablab adaptability to different agro-ecological environments, morphological and molecular characterization was arrived at. Recommendations and areas for further research were also provided.

Reference section provided a list of both printed and electronic sources of information that were used in the study. Appendices section covered supplementary materials in form of figures, tables and plates that could not be placed in the body of the thesis.

## 1.2 Background Information

### 1.2.1 Taxonomy of *Lablab purpureus*

*Lablab purpureus* (L.) Sweet, synonym *Dolichos lablab* L.,  $2n = 22, 24$  belongs to the family Fabaceae or Leguminosae, subfamily: Faboideae, tribe: Phaseoleae, subtribe: Phaseolinae. It is also placed in the family Papilionaceae. According to Sheahan, (2012) other names include: *Dolichos benghalensis* Jacq. *Dolichos purpureus* L., *Lablab niger* (Medikus), *Vigna aristata* (Piper) and *Lablab vulgaris* (L.) (Savi). Common names include hyacinth bean, bonavist bean, bonavist pea, country bean, lablab bean, dolichos bean, Egyptian kidney bean, Indian bean, Chinese flowering bean, Pharaoh bean, wild field bean, mouse-ear bean, pig-ears bean, Rongai bean, poor man's bean and Australian pea, retrieved from, [www.tropicalforages.info/key/Forages/Media/.../Lablab\\_purpureus.html](http://www.tropicalforages.info/key/Forages/Media/.../Lablab_purpureus.html). According to Koile and Cheminingwa, (2014), it is also known as 'Gerenge' in Ethiopia, 'Lubia' in Sudan and 'Fiwi' in Zambia. In this study, the scientific name *Lablab purpureus* is used.

Osman *et al.*, (2014) observed that the biosystematics of *Lablab purpureus* and its relatives have recently been reviewed and revised. Formerly, the *Lablab purpureus* was included in the genus *Dolichos* following Linneus but in 1980, Verdcourt assigned the *Lablab purpureus* to the monotypic genus *Lablab* and three subspecies are recognized. Subspecies *uncinatus* is the wild ancestral form distributed mainly in East Africa. Pod size of sub species *uncinatus* is about 40mm x15mm. Subspecies *purpureus* includes a cultivated form with larger pods of 100mm x 40mm. Subspecies *benghalensis* has characteristically longer pods than other subspecies, up to 140mm x 10-25mm (Osman *et al.*, 2014).



### **1.2.2 Plant description**

Lablab is a vigorous perennial herb of either bushy or climbing habit, normally grown as an annual (Valenzuela and Smith, 2002). Wild germplasm is strongly perennial. Stems are thick and can grow up to 3 feet for the determinate type and 25 feet for the climbers. Leaves are large and trifoliate, with the leaflets having a broad ovate-rhomboid shape measuring 7.5 to 15 cm long. The dorsal side of the leaf is smooth with the underside being hairy (Valenzuela and Smith, 2002). Flowers have axillary inflorescences on long-stalked racemes, 30 cm or more in length but shorter in some cultivars. Corolla is normally white, red or purple, in clusters of 4-5, each with two large basal bracts. Pods are variable in shape and colour, normally curved and flattened.

Pods usually contain 3-5 seeds or 6 - 8 seeds in variety *bengalensis*. Seeds are of variable size and colour, normally up to 12.5mm in length and ranging from white, red, brown, black, or speckled, rounded or oval depending on variety (Cook *et al.*, 2005). Hilum is conspicuous and white, approximately 10 mm in length and 7 mm in width. Average weight of 100 seeds ranges from 25 to 40g (Valenzuela and Smith, 2002). Of the two hundred types of Lablab recognized, only two cultivars, Rongai and Highworth, seem to be most popular and available commercially (Sheahan, 2012).

#### **1.2.2.1 Rongai Cultivar**

According to Cameron, (1988) and Oram, (1990) the Rongai cultivar was derived from material from Rongai in Nakuru county of Kenya and was released in New South Wales, Australia in 1962. Rongai is a late maturing white flowering cultivar

that will continue to grow until cut or damaged by frost. In the absence of frost, flowering may continue for several months (FAO, 2012).

#### 1.2.2.2 Highworth Cultivar

The Highworth cultivar originated from Coimbatore, South India and is morphologically similar to Rongai (Cameron, 1988; Oram, 1990). Contrasting with the green foliage, white flowers and light brown seeds of Rongai, foliage of Highworth has a purple band near the leaf axil, purple flowers and black seeds (FAO, 2012). Highworth is an early flowering line with high seed-yielding ability. It is suitable for pulse production and forage uses. It was originally intended for grain production in regions where frost prevented the seeding of Rongai (Cameron, 1988; Oram, 1990).



**Plate 1.1: *Lablab purpureus* in reproductive growth stage in a farm at Murang'a County. (Source: Author, 2006)**

### **1.2.3 Ecology of *Lablab purpureus***

Lablab is sensitive to day length. Most genotypes require short days to initiate flowering, but long-day cultivars exist as well (Cook *et al.*, 2005). The plant is suitable for growing as a rain-fed crop where the average annual rainfall is 600-900 mm. According to Maass *et al.*, (2010) it is more drought tolerant than other similar legumes like common beans (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). In India it is successfully grown commercially, with supplemental irrigation, in areas with a rainfall as low as 400 mm. It requires adequate moisture during the early stages of growth after which its deep roots enable it to exploit residual soil moisture (Cook *et al.*, 2005).

When grown as a market-garden crop for the production of the immature pods it requires watering or frequent rains throughout its growing period (Cook *et al.*, 2005). Seed production can be a problem in regions with high humidity. Locations up to and beyond 2,000 metres above sea level have proved suitable for economic production. It requires a warm climate with average temperatures between 18 and 30°C. Many *Lablab purpureus* types withstand frost for a limited period (Cook *et al.*, 2005). Lablab is both self and cross pollinated, and cooler weather at flowering time can affect seedset. The plant survives on a wide variety of soil types in a pH range of 4.5-7.5 (Cook *et al.*, 2005).

### **1.2.4 Origin, history and distribution of *Lablab purpureus***

Lablab has been regarded as a species of tropical Asian origin, since it is most widely cultivated there (Purseglove, 1974). However, (Cook *et al.*, 2005; Maass *et al.*, 2005; Maass and Usongo, 2007) postulated an East African origin, based on the distribution

of the wild ancestral form, *Lablab purpureus* subspecies *uncinatus*, which is now grown for food throughout much of the world. As early as 1819, seeds of Lablab from Egypt were planted in the botanical gardens in Sydney, New South Wales. However, it was not until after the release of the forage cultivar "Rongai" in 1962, that Lablab became widely used as forage in Australia. Presently, Lablab is common in Africa, extending from Cameroon to Swaziland and Zimbabwe, through Sudan, Ethiopia, Uganda, Kenya and Tanzania (Skerman *et al.*, 1991).

### **1.2.5 Economic Importance**

Lablab has primarily been an ornamental annual vine in the United States of America for generations (Stevens, 2012). The leaves make excellent hay for cattle and goats, but the stem is difficult to dry, and must be mechanically conditioned through crushing (FAO, 2012). Silage made from a mix of Lablab and sorghum raises the protein content of sorghum by roughly 11% with a 2:1 mixture of Lablab: sorghum (FAO, 2012). Lablab is a good source of minerals and vitamins (Basu *et al.*, 2002). In India and China, the complete plant is edible (Kala *et al.*, 2010). Young leaves are eaten raw in salads and older leaves are cooked like spinach. Flowers are eaten raw or steamed. The large starchy root tubers can be boiled and baked. The immature and dried seeds can be boiled and fried (Kala *et al.*, 2010). Both the leaf and seed of Lablab are rich in proteins. Immature pods contain 82% water, 4.5% protein, 2.7-4.2% crude lipid, 10% carbohydrates and 2% cellulose. Mature seeds contain 9.5% water, 20 - 25% crude proteins, 0.8% fat, 63 - 66% carbohydrates and 5 - 7 % dietary fibre (Kala *et al.*, 2010). Koile and Cheminingwa, (2014) observed that in Asia the mature seeds are made into tofu (a curd obtained by compressing the dolichos beans

after soaking, dehulling, partly cooking and adding vinegar) and tempeh (fermented tofu).

Lablab is useful as a cover crop and for forage (FAO, 2012). Its dense green cover during the dry season protects the soil against the action of the sun's rays and decreases erosion by wind or rain. As green manure, it provides organic matter and minerals. It also fixes nitrogen into the soil thereby improving crop yields (Valenzuela and Smith, 2002). Due to its drought tolerance, Lablab grows in a diverse range of environmental conditions. Its multipurpose uses make it an important species globally (Schipper, 2000).

The species is also used for its ethnobotany properties. In the Philippines and China, it is used to reduce fever, flatulence and stimulate digestion. It is also used as an antispasmodic (Stuart, 2011). In Namibia the roots are used to treat heart conditions (Pennachio *et al.*, 2010). In the United States Lablab is commonly used as an ornamental crop in the cut flower industry. It is valued for its colourful flowers and purple peapods (Stevens, 2012).

#### **1.2.6 Genetic diversity in *Lablab purpureus***

The wild Lablab germplasm seems to be always perennial, but over the past few thousand years the landraces have been selected to be mainly annuals. Thus, most Lablab landraces today are true annuals (Cook *et al.*, 2005). In general, landraces are the most diverse populations of cultivated plants (Camacho *et al.*, 2005). The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties and

for direct use by farmers (Mauricio *et al.*, 2015). Classical methods of estimating diversity among groups of plants have relied chiefly upon morphological characters in crop species and their relatives (Gepts, 2006). However, since most of the morphological characters are greatly influenced by environmental factors and the developmental stage of the plant, new techniques which analyze diversity at biochemical or molecular level have been developed and successfully applied in evolutionary and diversity studies of different crops (Biswas *et al.*, 2012).

#### **1.2.6.1 Genetic Markers**

Crop genetic diversity is important for crop adaptation to withstand pests and diseases and it is an important precondition for plant breeders to enhance the progress of traits of economic value such as yield (Parzies *et al.*, 2000). Various methods are available for use in estimating the genetic diversity of crops, such as morphological, biochemical and molecular markers. Measurements of genetic diversity can be generated using conserved accessions in gene banks (Parzies *et al.*, 2000). In the context of *in situ* conservation of landraces, both molecular and morphological marker evaluations are useful for identifying populations for conservation, optimum sites for germplasm collection and ongoing changes in the pattern of diversity in the course of conservation practices (Virk *et al.*, 2000). Few studies have analyzed the pattern of genetic diversity within landraces as compared with that among landraces held in large *ex situ* germplasm collections (Van Zonneveld *et al.*, 2014).

The knowledge and understanding of the genetic structure of plant landraces is important since it may serve as a basis for making decisions related to the selection of sites and populations for *in situ* conservation (Vigouroux, *et al.*, 2011a). The above

information is also valuable for genebank curators in providing a more secure basis on which sampling strategies (number of plants or seeds per sample and pattern of sampling) can be implemented (Engels and Visser, 2000). In this study both molecular and morphological markers were used for characterization of *Lablab purpureus* germplasm grown in Kenya.

#### **1.2.6.2 Morpho-agronomic markers**

In the preliminary characterization of the genotypes, morphological and agronomic traits of the plant are preferred, for being cheaper and easier to assess. The morpho-agronomic description provides information underlying conclusions on the genetic variability of the genotypes, identification of accessions maintained in duplicate, improvement of the data of identification, classification of accessions, regeneration and maintenance of the genetic integrity of genotypes (Chiorato *et al.*, 2005). Among other information that can be obtained from the characterization of germplasm, the determination of the relative importance of the traits used to describe the genetic diversity is noteworthy because in case of limited financial and or human resources, the least relevant traits can be eliminated (Chiorato *et al.*, 2005)

In a strategy to develop what they termed phenotypic similarity index, (PS), Cui *et al.*, (2001), used morphological and agronomic traits to study the phenotypic diversity of Chinese and North American soybean. A total of 47 Chinese and 25 North American cultivars were assessed for 25 characters. Their results showed more phenotypic diversity among the Chinese cultivars, than the North American cultivars and also found clear differences between the two groups. From the use of

morphological markers they managed to come up with a strategic plan to broaden the North American germplasm by the introgression of Chinese cultivars.

### **1.2.6.3 Biochemical markers**

Isozyme analysis was the first technique used in the estimate of genetic variance. Isozymes are protein molecules with different charges, and can be separated by gel electrophoresis based on their molecular sizes, weight and electrical charges (Hedrick, 2005). The use of isozyme is simple and cheap, since no DNA or sequence information, primers and expensive polymerase chain reaction (PCR) machines are needed as in other marker types. Isozyme markers have the advantage of being reproducible, co-dominant, giving them an advantage over other markers such as random amplified polymorphic DNA (RAPD), which are dominant markers (Spooner *et al.*, 2005). The main disadvantage is that there are few isozyme assays per species and the enzymatic loci account for a small and non-random part of the entire genome. Isozyme analysis is also affected by plant tissue and plant developmental stage (Mondini *et al.*, 2009). Different tissues in the same plant can reveal different isozyme variation.

Pasquet *et al.*, (1999) used isozymes to investigate the population structure of bambara groundnut and partitioned the genetic diversity between domesticated and wild forms. A high Nei's genetic identity of 0.948 between the wild and domesticated bambara groundnut landraces lead to a conclusion that the wild bambara groundnut is the progenitor of domesticated landraces. Biochemical markers were later replaced by DNA molecular markers which are more robust as compared to both morphological and biochemical markers.



#### **1.2.6.4 Molecular markers**

Molecular markers are fixed marks in the genome found at specific locations of the genome and are used to identify specific genetic differences (Semagn *et al.*, 2006). In order to precisely identify traits of interest, the marker must be close to the gene of interest so that the allele of both the marker and the gene could be inherited together. DNA markers are passed on from one generation to another through the laws of inheritance (Semagn *et al.*, 2006). Several markers are available for genetic diversity studies. The selection criteria could be based on cost, technical labour, level of polymorphism, reproducibility, locus specificity and genomic abundance (Garcia *et al.*, 2004). Molecular markers are useful in the development of genetic and physical maps, and have increased the efficiency of indirect selection of marker linked traits. Generally, markers are classified into hybridization based DNA markers and PCR-based DNA markers (Gupta *et al.*, 2003). In this study simple sequence repeats (SSRs) molecular marker was used to characterize Lablab germplasm grown in Kenya.

#### **1.3 Problem Statement**

In Kenya, Lablab is grown in different agro-ecological zones ranging from the lowlands of the coastal region to the highlands of Mt. Kenya region. Although the species forms the basis of food security among small-scale farmers where it is grown, it is grossly under-exploited in Kenya. The Ministry of Agriculture categorizes Lablab as a minor crop (MoA, 2014) and, therefore, policy-makers do not accord the crop the attention it deserves. On their part, most researchers have merely viewed the crop as forage and also as a means of soil improvement.

Due to lack of empirical data, information on Lablab adaptability and diversity remains scanty. Therefore, farmer's choice of type of Lablab to grow is purely dependent on seed availability and colour preferences (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). Consequently, genotypes are not grown in suitable agro-ecological environments, leading to low yields obtained from the crop (MoA, 2014).

Previous research has raised a serious alarm over the neglect of Lablab. Maass *et al.*, (2010) argued that the availability of Lablab in Africa was under threat of genetic erosion due to limited research. Tariqul, (2010) asserted that empirical knowledge of genetic diversity within a crop was essential for the long-term success of a breeding programme to maximize the exploitation of germplasm resources. It is therefore, evident that lack of empirical information on adaptability and genetic diversity of Lablab has seriously hindered its improvement. This study, therefore, sought to fill the existing knowledge gap by assessing the adaptability and diversity of Lablab using both morphological and molecular markers and, to generate indepth knowledge on different genotypes that would lead to improvement and conservation of this neglected crop.

## **1.4 Objectives**

### **1.4.1: Broad Objective**

To assess the adaptability potential and genetic diversity of *Lablab purpureus* with a view to identifying distinct genotypes to recommend to researchers and growers for further crop improvement and conservation.

### 1.4.2: Specific Objective

1. To assess the status of *Lablab purpureus* production in Kenya.
2. To evaluate the adaptability potential of *Lablab purpureus* to various agro-ecological environments in Kenya.
3. To assess the phenotypic diversity of *Lablab purpureus* grown in Kenya
4. To evaluate the genotypic diversity of *Lablab purpureus* grown in Kenya using simple sequence repeat (SSR) markers.

### 1.5 Working Hypothesis

- ✓ *Lablab purpureus* production in Kenya has several challenges.
- ✓ *Lablab purpureus* cultivars grown in Kenya are adapted to several agro-ecological environments.
- ✓ *Lablab purpureus* cultivars grown in Kenya have morphological differences
- ✓ *Lablab purpureus* cultivars grown in Kenya have genetic differences.

### 1.6 Justification

In Kenya, Lablab is a minor but important legume which is commonly grown as a grain legume, vegetable, animal fodder and for soil fertility improvement. It is also used as a source of revenue for the farm families as it fetches more revenue per unit quantity than common bean (*Phaseolus vulgaris* L.) in many areas where the two are grown (Kamotho *et al.*, 2010, Kinyua and Kiplagat, 2012). However, as evidenced by the works of Maass *et al.*, (2010), empirical studies on Lablab as a food crop have largely, been neglected by researchers. The Ministry of Agriculture has, equally, not considered Lablab as a priority crop (MoA. 2014). The present study was, therefore,

justifiably significant in several ways. Theoretically, the work constitutes a new addition to the scantily existing corpus of knowledge. The work can hopefully, be a catalyst that would pave the way and interest for further research on other aspects of this neglected crop. The findings and recommendations of the study can guide and inform further research and policy formulation in the Ministry of Agriculture and, as a result, raise the profile and priotisation of Lablab in the country.

## CHAPTER TWO

### ASSESSMENT OF STATUS OF DOLICHOS BEAN (*Lablab purpureus* (L.) ) GERMPLASM IN KENYA

#### 2.1 Abstract

Lablab is a minor but important grain legume in Kenya. It is also used as animal fodder and green manure in mixed crop-livestock systems. Despite its importance, Lablab is a neglected crop with unexploited potential. Very few studies have been conducted in Kenya on the crop. To date the documented information on the status of Lablab production in Kenya is scanty. To assess the status of Lablab production in Kenya, a baseline survey was conducted in the Lablab growing areas of Kenya mainly: Central, Rift Valley, Eastern, and Coastal regions. An interview schedule was administered on 108 respondents. A disproportionate stratified sampling was used in order to obtain a representative sample. The strata used were counties and their agroecological zones. Data was collected on different parameters such as cropping systems, cultural practices, yield, constraints to production and utilization of Lablab. Data was analysed using GenStat where descriptive statistics were obtained. Results obtained from the survey revealed the status of Lablab growing in Kenya with reference to farming practices, seed postharvest handling, utilization and source of seed by growers. Main challenges in Lablab production in Kenya were identified as; pests and diseases, unavailability of good quality seed, low yielding cultivars, cultivars that had a long maturity period and lack of technical knowhow by farmers. This information formed the foundation for further improvement of Lablab production. For instance, improvement of Lablab in Kenya needs to focus on pest and disease resistance, postharvest handling of seed, earliness to maturity and high yields.

## **2.2 Introduction**

In Kenya Lablab, is also known as “njahe” among the Kikuyu, “chabi” among the Meru, Embu and Mbeere, “mbumbu” among the Kamba and Taita, “elikeri” among the Kisiis and “chemakikosor” among the Kalenjini (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). Several other communities who grow and utilize the crop albeit in a limited amount have adopted the name “njahe”. The diversity of names among different communities in Kenya demonstrates the popularity of this crop.

### **2.2.1 Distribution of *Lablab purpureus* in Kenya**

In Kenya, Lablab is grown from near sea level at the coastal region (Lamu), through the dry areas of Esatern Kenya (Mwingi, Machakos, Embu, Mbeere) and Riftvalley region (Nakuru) to the foot of Mount Kenya, largely in Meru, Nyeri, Murang’a and Kiambu counties. Although Lablab is a minor crop in many areas where it is grown, in Lamu county it is a major crop mainly grown as an intercrop with maize where it has effectively replaced common beans both in the field and diet (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012).

### **2.2.2 Uses of *Lablab purpureus* in Kenya**

In Kenya, Lablab is utilized as food. Schippers, (2000) noted that the Kikuyu people in Kenya traditionally consume Lablab during wedding ceremonies. In general, the Meru, Kamba, Mbeere and Kikuyu communities use it in stews and local dishes such as “Githeri” and “Mokimo”. As food, the grains are the most preferred and are presented in a variety of recipes ranging from mixtures with other food stuffs such as potatoes, bananas and various vegetables to exclusive “Njahe” stew. However, leaves

and young pods are also fried and used as vegetables especially during dry months of the year (Kamotho *et al.*, 2010, Kinyua and Kiplagat, 2012).

Some farmers use it as a livestock feed where the whole crop is cut at the base. In areas where it is grown, it fetches higher returns per unit quantity as compared to maize and beans (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). The main growers of Lablab in Kenya are small scale farmers who either grow it as intercrop or pure stand (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). In some areas such as Mbeere, Embu, Mwingi, Machakos and Murang'a counties, Lablab plays a major role in soil fertility improvement strategies as it is included in the rotation programme or is intercropped with maize where it forms a good soil cover (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). In such cases, the biennial varieties such as DL1002 and DL1009 are planted. These are known to smother the weeds by the thick canopies they form thereby reducing the number of times the main crop is weeded.

Furthermore, with its deep tap root, Lablab is not only drought hardy, but is able to bring minerals, otherwise not available for annual crops, from the depths to the topsoil (Cook *et al.*, 2005; FAO, 2012). As a legume, the crop is known to provide biological nitrogen fixation which is a process of natural action of converting atmospheric nitrogen into forms available for the plant-soil system which improves productivity in an inexpensive, environmentally friendly manner. This "natural fertilizer" enables small landholders to improve the soil without incurring costs (McDonald *et al.*, 2001). Despite the importance of Lablab in Kenya, low yields are obtained from the crop. A survey was conducted in selected localities in Kenya with the following objectives:-

- ✓ to identify the most commonly grown types of Lablab

- ✓ to establish the farming practices used in Lablab production
- ✓ to establish the constraints in production of Lablab
- ✓ to establish the perception of farmers concerning Lablab in relation to taste and flavour.
- ✓ to determine the utilization of Lablab in Kenya
- ✓ to establish the postharvest handling practices of Lablab seed

### **2.3 Materials and Methods**

A baseline survey was carried out in Lablab growing areas of Kenya. The survey involved examining secondary data at the Ministry of Agriculture county offices, oral interviews with agricultural extension staff and farmers during field visits. After reviewing secondary information on Lablab production in Kenya and discussion with agricultural extension officers of the Ministry of Agriculture, a number of regions were selected (Table 2.1, Appendix 3). The regions are major Lablab growing areas in Kenya. Data was obtained by interviewing farmers in the areas covered by the survey using an interview schedule (Appendix 1).

A disproportionate stratified sampling was used as illustrated by Lohr, (1999) in order to obtain a representative sample. This is a sampling method in which the size of the sample drawn from a particular stratum is not proportional to the relative size of that stratum (Lohr, 1999). The strata used were counties and their agroecological zones. Thus, data from Coastal region of Kenya was taken from Lamu-Mpeketoni which is the major Lablab growing area and falls under agroecological zone II (Lowland). In Eastern region of Kenya data was taken from Mwingi-Central and Mwingi-Migwani, Machakos-Kalama and Machakos-Kathiani, Mbeere-Siakago (agro-cological zone III)



and Meru-Central, Meru-Abothogushi and Meru-Mihiriga-Mieru which is in agro-ecological zone II (Upper highland).

In Central region of Kenya, data was taken from Makuyu, Maragwa-Ridge, Kakuzi and Thika Municipality (agro-ecological zone II (Upper highland)) while in Riftvalley region of Kenya data was taken from Nakuru-Lare, Naivasha and Bahati which are in agro-ecological zone II. Seed and leaf samples were also collected during the survey for preliminary and subsequent studies. A total of one hundred and eight respondents were interviewed. Data obtained from the survey was analyzed using descriptive statistics using GenStat version 12 statistical software (Payne *et al.*, 2009).

**Table 2.1 Places where *Lablab pupureus* accessions were collected**

Region	County	Sub-county	Accessions collected
Coast	Lamu	Mpeketoni	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 (brown)
Eastern	Mwingi	Central	39, 40, 41 (brown)
		Migwani	42, 43, 45 (brown)
	Machakos	Kalama	14, 15, 16, 17 (dark brown)
		Kathiani	18, 19, 20 (dark brown)
		Yatta	21 (black)
	Mbeere	Siakago	29, 30 (brown)
	Meru	Central Abothoguchi East	31 (black)
Mihiriga Mieru East		32 (black)	
Central	Maragwa	Makuyu	22 (black)
		Maragwa-Ridge	23 (black)
	Thika	Kakuzi	24, 25 (black)
		Municipality	26, 27, 28 (black)
Rift Valley	Nakuru	Lare	33, 34, 35 (black)
		Bahati	36 (black)
		Naivasha	37, 38 (black)

## 2.4 Results

### 2.4.1 *Lablab purpureus* production in areas covered by the survey

Table 2.2 indicates that most farmers (84.3%) grew Lablab in small acreage of less than 1.0 acre while 15.7% grew Lablab in acreage more than 1.0 but less than 2.0 acres. The bigger acreage of Lablab was found in Lamu where it has effectively replaced bean in the diet and is commonly grown by a large number of farmers. None of the farmers grew Lablab in acreages above 2.0 acres. This owes to the fact that in areas where Lablab is popular especially in Lamu, farmers have small parcels of land, not more than 2.5 acres per household. Initially each family owned 4.0 hectares (10 acres) but with subdivision by family members, the acreage has reduced. Most respondents (64.8%) preferred growing the black seeded Lablab while 38% and 3.7% grew the brown and dark-brown seeded respectively. The black seeded Lablab is commonly grown and consumed in Central, Riftvalley and Meru regions of Kenya while the brown seeded is commonly grown and consumed in Lamu, Mwingi, Machakos and Mbeere. None of the respondents grew the white and dotted seeded Lablab although they had seen those types with other farmers or in the market.

There were variations in colour shade of Lablab seed coat and description was based on the most prevalent colour shade. Only 44.4% of respondents grew the improved Lablab sourced from the Ministry of Agriculture (cultivars DL1002 and DL1009 from Kenya Agricultural and Livestock Research Organization, Katumani). Where the improved seed was not enough, farmers planted in bulking plots in order to multiply it to meet the demand. However, a big percentage of farmers (56%) still grew the local

Lablab cultivars. The 56% who grew the local Lablab reported that they did not have access to the improved cultivars.

Although all the respondents encountered insect pest problems, only 44% attempted some control options while only 4.6% attempted to control diseases. A few farmers 2.8% used either fertilizer or manure at planting while no farmer top dressed the crop. However, respondents argued that since Lablab is in the family of common beans it was expected to add fertility to soil and therefore it was not necessary to use fertilizers. Most farmers (41.7%) grew Lablab as an intercrop while 34.3% and 25% of the respondents grew it as a pure stand and on terraces respectively. The practice of intercropping Lablab with maize was common in Lamu, Maragwa and Thika. In all the areas visited during the survey, more women (80.6%) than men (19.4%) grew Lablab (Table 2.2).

**Table 2. 2 Farming practices used by respondents**

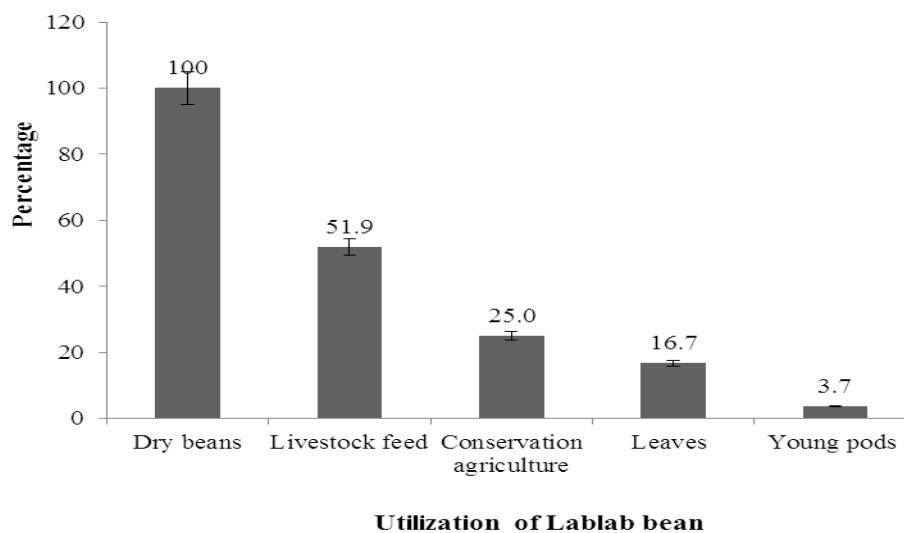
<b>Crop husbandry practices by farmers</b>	<b>No. Resp</b>	<b>% Resp</b>	<b>Crop husbandry practices by farmers</b>	<b>No. Re</b>	<b>% Re</b>
Farmers who grew Lablab bean as pure stand	37	34.3	Farmers who encountered disease incidences	34	31.5
Farmers who grew Lablab bean as an intercrop	45	41.7	Farmers who controlled disease	5	4.6
Farmers who grew Lablab bean on terraces	27	25.0	Farmers who distinguished between insect pests and diseases	3	2.7
Farmers who used fertilizer at planting	2	1.9	Farmers who grew improved Lablab cultivars	48	44.4
Farmers who used manure at planting	1	0.9	Farmers who grew local Lablab cultivar	60	55.6
Farmers who top dressed Lablab bean	0	0	Farmers who grew black coloured Lablab bean	70	64.8
Farmers who encountered insect pest attack on Lablab bean.	108	100	Farmers who grew brown coloured Lablab bean	41	38
Farmers who controlled insect pests	48	44.4	Farmers who grew red coloured Lablab bean	4	3.7
Grew Lablab bean in < 0.5 acres	15.7	14.5	Females who grew Lablab bean	87	80.6
Grew Lablab bean between 0.5 and 1.0 acres	62.3	57.7	Males who grew Lablab bean	21	19.4
Grew Lablab bean between 1.0 and 2 acres	30.02	27.8	Farmers who stored seed	38	35.2
Grew Lablab bean in > 2 acres	0	0	Farmers who did not store seed	70	64.8

**Key: No. Resp – Number of respondents; % Resp – Percentage respondents**

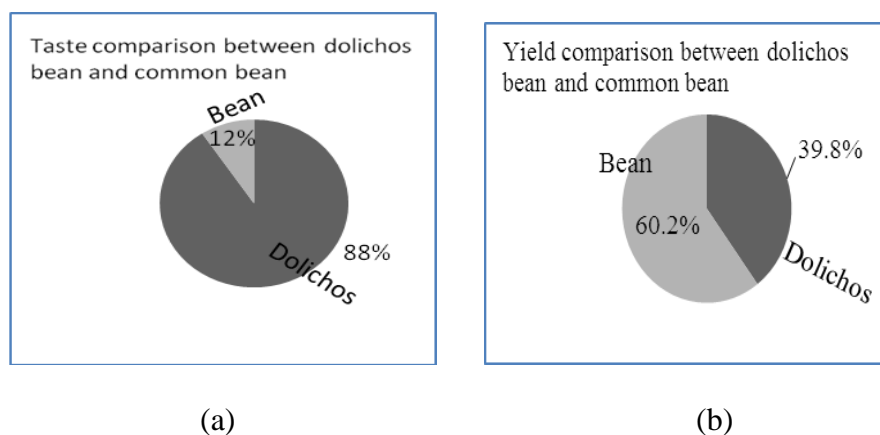
#### **2.4.2 Utilization of *Lablab purpureus* in Kenya**

All the respondents (100%) utilized Lablab dry beans as food (Fig.2.1). Of the respondents, 51.9% utilized it as a livestock feed while 25% used it in conservation

agriculture. The practice of using Lablab in soil erosion control was common in Mbeere, Mwingi and Maragwa regions of Kenya. A few farmers (16.7%) used the leaves as vegetables while only 3.7% consumed the green pods as vegetables especially during the dry seasons. As regards taste and flavor, 88% of the respondents preferred Lablab to common beans. Most respondents (60.2%) observed that they obtained higher yields in common beans than in Lablab per unit area (Fig. 2.2). However, all respondents noted that Lablab fetched more income per unit quantity than common bean. Reasons for low yields in Lablab included use of poor quality seed and attack by pests and diseases.



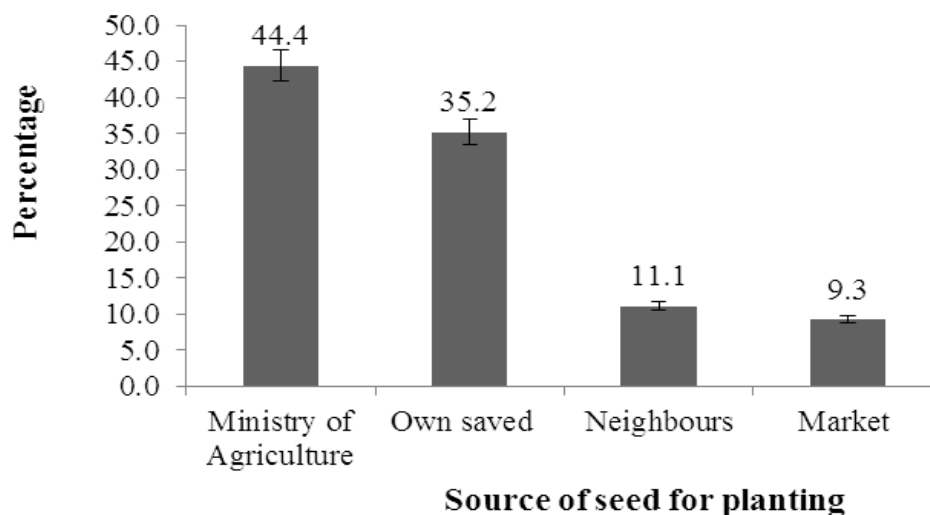
**Fig.2. 1 Utilization of *Lablab purpureus* by respondents**



**Fig.2. 2 (a) Taste and flavor preference between Lablab and common bean and b) Yield comparison between Lablab and common bean.**

#### **2.4.3 Source of Lablab seed**

Only 44.4% of the respondents obtained seeds from the Ministry of Agriculture (Fig. 2.3). This corresponded to number of farmers who used the improved Lablab cultivars (Table 2.2). Other farmers (35.2%) saved seed after harvesting to plant in the following season while 9.3% purchased seed from the market and 11.1% obtained seed from neighbours.



**Fig.2. 3 Source of *Lablab purpureus* seed**

#### **2.4.4 Seed harvesting and postharvest handling practices**

All respondents (100%) used pod colour as an indicator of seed maturity (Table 2.3). Majority of respondents (89.8%) did not separate seed for planting from grain. All respondents dried seed on plant. Pods on same plant and raceme matured at different times. Growers harvested dry pods on piece meal basis as they matured. A few respondents (15.7%) dried the pods further after harvesting while 19.4% dried seeds in the sun after manual threshing. A high percentage of growers (74.1%) dried seed in pods for three to five days after harvesting and further as seed for three to five days.

Seed storage containers included gunny bags, plastics and polythene paper bags and were used by 66.7%, 28.7% and 4.6% of respondents respectively. Bean bruchid and bean weevil were the two insect pests observed in stored *Lablab* seeds. Bean bruchid was a real menace where 73.1% of respondents experienced the damage while bean weevil was reported by 26.9% of the respondents (Table 2.3). Most respondents (64.8%) stored seed only for a short period of upto three months. Most of these

growers sold seed immediately after harvesting or used the grain as food. Moreover, the growers in this group grew Lablab in small land size of less than 1.0 acre thus the harvest was little. A number of growers (31.8%) stored seed for upto six months while a small percentage (3.4%) stored seed for up to one year.

A high percentage of respondents (78.7%) reported high seed germination percentage. However, majority, (89.8%) were not satisfied with quality of seed they planted since they obtained low yields from the crop. The few respondents (21.3%) who reported problems of seed germination had stored the seed for more than 3 months. The 10.2% of respondents who were not satisfied with seed quality cited delayed germination in some instances and infected seedlings observed after germination (Table 2.3).

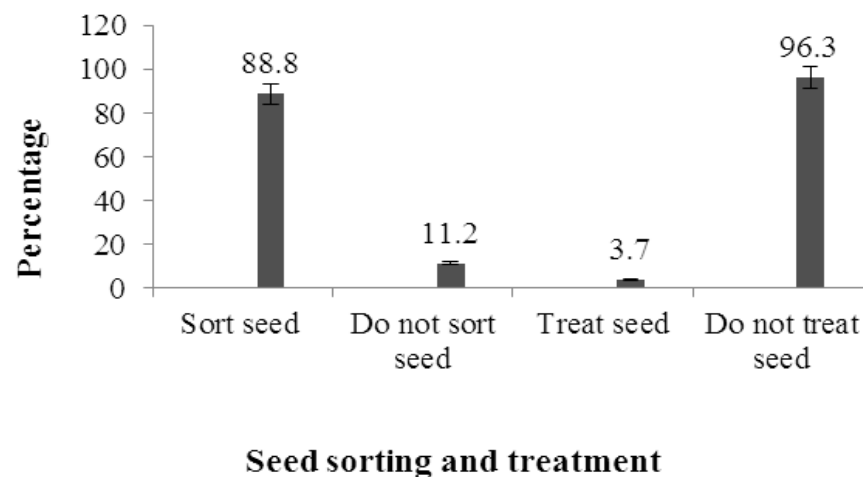


Table 2.3 Seed harvesting and postharvest handling practices of Lablab

Seed harvesting and postharvest handling practices		Percentage of respondents	Seed harvesting and postharvest handling practices	Percentage of respondents	
<b>Seed maturity indicators</b>			<b>Seed storage period</b>		
Pod colour		100	0 – 3 months	64.8	
Seed colour		0	3 – 6 months	31.8	
Leaf colour		0	6 – 12 months	3.4	
<b>Seed harvesting</b>			>12 months	0	
Separate from grain	Yes	10.2	<b>Quality of seed planted by farmers</b>		
	No	89.8	Satisfied with quality of seed planted		
			Yes	10.2	
			No	89.8	
<b>Seed drying</b>			<b>Seed germination problems</b>		
On plant		100	Encountered Problems in seed germination	Yes	21.3
As pod		15.7		No	78.7
As grain		19.4	<b>Criterion for seed sorting prior to planting</b>		
On plant, as pod and as grain		74.1	Size	14.8	
<b>Seed storage containers</b>			Colour	5.6	
Pots		0	Shape	10.2	
Plastics		28.7	Disease and insect pest damage	58.2	
Gunny bags		66.7	Do not sort seed	11.2	
Polythene paper bags		4.6	<b>Seed treatment options</b>		
<b>Major insect pests in storage</b>			Chemicals	3.7	
Bean bruchid		73.1	Traditional methods	78.7	
Bean weevil		26.9	Sanitation	11.6	

#### 2.4.5 Seed sorting and treatment prior to planting

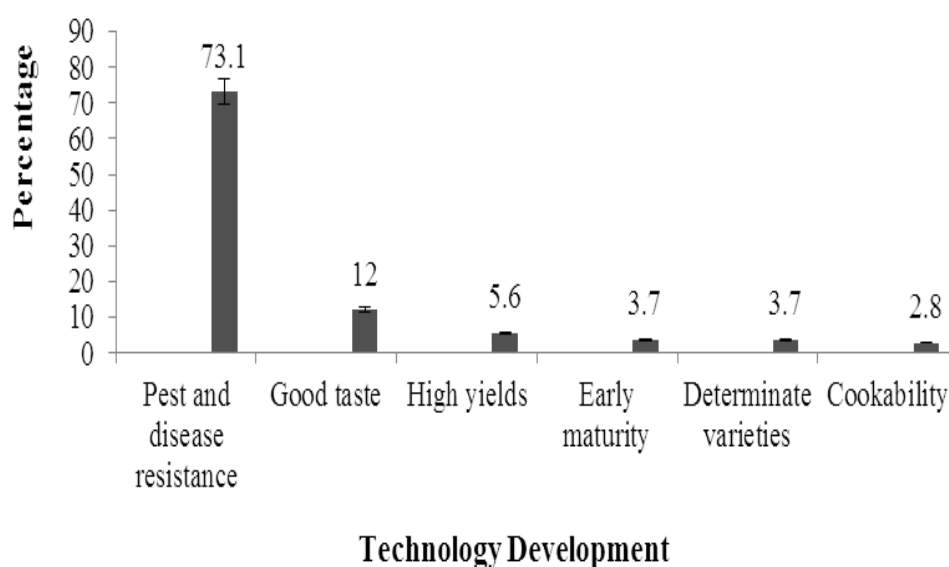
A high percentage of respondents (88.8%) sorted seed for planting (Fig. 2.4). Seed sorting was based on size by 14.8% of respondents, colour by 5.6% and shape by 10.2% while 56.2% sorted on the basis of disease and insect pest damage (Table 2.3). The only disease that respondents could easily identify was brown spots on seeds. A few growers (11.2%) reported that they did not sort seed for planting. Only 3.7% of the respondents treated seed before planting while 96.3% did not (Fig. 2.4). Seed treatment options included chemicals that were used by a small percentage of respondents (2.8%), while traditional methods such as use of wood-ash was used by a higher percentage (78.7%) and sanitation was used by 18.5% of respondents (Table 2.3).



**Fig.2. 4 *Lablab purpureus* seed sorting and treatment**

#### 2.4.6 Technology development in Lablab

Majority of the respondents (73.1%) preferred a variety that is insect pest and disease resistant since this is the main challenge in Lablab production. However, 12% of the respondents preferred a variety that has a better taste. Other respondents (5.6%) preferred a variety that is high yielding while 3.7% of growers favoured a variety that matures early and a determinate type. A small percentage (2.8%) observed that they preferred a variety that takes a short period to cook.



**Fig.2. 5 Traits for improvement in *Lablab purpureus***

#### 2.5 Discussion

From the responses on production practices, it was evident that the low Lablab yield obtained by farmers was as a result of lack of disease and insect pest control, lack of fertilizer usage at planting and top dressing stages, use of poor quality seed, lack of seed dressing at planting and use of low yielding local cultivars. Main reason given by farmers for not controlling insect pests and diseases was the high cost of chemicals and ignorance (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). Many farmers

admitted they did not know the difference between disease and insect pests attack symptoms. Thus wrong control measures could have been used for control of either disease or insect pests. These results are similar to findings from other studies, for instance, Katungi, (2010) reported low yields in common bean (*Phaseolus vulgaris*) as a result of lack of disease control, use of poor quality seed and poor cultural practices such as late weeding by farmers in Ethiopia and Eastern Kenya. Chemining'wa *et al*, (2014) reported constraints of navy bean production in Kenya as lack of good quality seed and lack of technical skills.

The study found out that all respondents who grew Lablab utilized it as food, others used it as a livestock feed and in such places as Mbeere, Mwingi and Maragwa it was used in conservation agriculture where it was commonly grown on bench terraces. Maass *et al.*, (2010) observed that Lablab is grown as a pulse crop in Africa, Asia and the Caribbean. The crop is also used as a source of revenue as it fetches high prices than common bean per unit quantity. Ngailo *et al.*, (2003) conducted a market survey of Eastern Africa and found a good demand and subsequently a high price for Lablab in Kenya. The study shows that in Kenya Lablab is popularly used as dry beans for food while in India and China edible pods are more popular (Saraswat, 1986).

The level of adoption of new varieties was particularly encouraging as 44.4% of the respondents planted seeds supplied by the Ministry of Agriculture (DL1002, or DL1009) from KARLO, Katumani. However, 20.4% of growers obtained seed from neighbours and market (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). Similar results in different crop species have been reported by other workers for instance, in Botswana (Brink *et al.*, 1996) found that while most of the farmers' preferred to use

the previous season's harvest as their seed stock, they also exchanged bamba groundnut seeds with friends and family members.

The efforts that farmers put in trying to preserve their own seed were also noted as 35% of respondents used their own saved seed. However this practice was limited by ineffective pest control methods employed such as dressing the seed with wood-ash. In Kenya the practice of treating common beans (*Phaseolus vulgaris*) before storage using botanicals such as neem plant parts and wood ash is common with some farmers, retrieved from, [www.infonet-biovision.org](http://www.infonet-biovision.org). Although a high number of respondents sorted seed based on seed characteristics such as colour, size, shape or presence of pest and or disease infection, a few growers still ate good quality seed but inadvertently planted the deformed or diseased seed (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). On whether farmers treated seeds before planting, the results indicated a likelihood of disease and pest transmission especially seed borne diseases which could easily be conveyed from one farm to the other or one season to the next. This is particularly worrying given that 96% of the respondents did not treat seed prior to planting.

A greater percentage of the respondents wanted a technology that reduced the cost of production with regard to pest and disease management. Farmers complained about the high cost of pesticides, reduced efficacy of the chemicals used as well as the time spent spraying given that some varieties are perennial. Besides, regular spraying is not only expensive, but also hazardous to the environment. It was also noted during the survey that there was a problem with identification of pests or diseases as many farmers confused the two hence the chances of inappropriate control strategies

(Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). The challenges identified in Lablab production in Kenya are similar to those reported by other workers for instance, Mogbo *et al.*, (2014) reported that insect pests were a major constraint in production and storage of cowpea seed in Nigeria. He further observed that studies on resistant varieties were conducted where resistance genes from wild cowpea species were successfully transferred into cultivated cowpea varieties including resistance to the storage weevil (*callosobrochus maculate*), leaf hopper and aphids.

## CHAPTER THREE

### EVALUATION OF ADAPTABILITY POTENTIAL OF THE KENYAN DOLICHOS BEAN (*Lablab purpureus* (L.) SWEET) UNDER DIFFERENT AGRO-ECOLOGICAL ENVIRONMENTS

#### 3.1 Abstract

A genotype or variety is considered to be more adaptive if it has high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments. In Kenya, there are no known *Lablab* genotypes for specific environments. Choice of *Lablab* genotypes to grow is based on colour preference and seed availability thus low yields are obtained. The study was undertaken to evaluate the performance of *Lablab* genotypes under diverse environmental conditions. Field trials of forty five (45) accessions of *Lablab* collected from Rift Valley, Eastern, Coast and Central regions of Kenya were established in three locations with different agro-ecological environments; (Nakuru - Kenya Agricultural and Livestock Research Organization-Njoro farm, Uasin Gishu - University of Eldoret farm and Bungoma - Mabanga Agricultural Training Center. The forty five accessions and three environments were factorially combined and replicated three times in a randomized complete block design (RCBD). Significant differences among accessions were found for seventeen quantitative traits related to vegetative, reproductive and yield and its components. Environmental effects were significant for all traits evaluated. Accessions 22, 23 and 36 proved to have high yield potential and took a relatively short period to mature in site 1 and 2 while accession 7 was the best performer in site 3. The four accessions could therefore be adopted as suitable genotypes in the respective agro-ecological environments.

### 3.2 Introduction

Crop genotypes grown in different environments frequently encounter significant fluctuations in yield performance, particularly when the growing environments are distinctly different (Shi *et al.*, 2009). The fluctuation of crop performance with changing environments technically termed as genotype x environment (G x E) interaction, potentially presents limitations on selection and recommendation of varieties for target set of environments (Navabi *et al.*, 2006). Genotype x environment interaction, defined as the differential phenotypic response of genotypes to environmental changes, can be quantified using several procedures, all of which are based on evaluation of genotypes under multiple environments (Vargas *et al.*, 2001). Such tests enable quantification of not only the average performances of crop genotypes across environments but also to assess the magnitude and pattern of cultivar performance fluctuation or consistency across a range of environments.

The differential phenotypic response of genotypes to environmental changes cannot be explained by the genotype and the environment main effect unless and otherwise it is considered along with G x E interaction effects (Reza *et al.*, 2007). Understanding the extent and pattern of G x E interaction effect can also help to effectively design appropriate breeding strategies, optimize varietal selection vis-a-vis the target production environments, and to define suitable areas of recommendation domain, where a given cultivar can be better adapted (Yan and Hunt, 2001). Based on magnitude and pattern of G x E interaction effects, breeders must either decide whether to exploit specific adaptation by selecting superior genotype for the target environments or to minimize the interaction effects by selecting stable genotypes widely adapted to a wide range of environments (Yan and Hunt, 2001).



It is widely accepted that genotype (G), growing conditions (E) and their interaction (G x E) are key factors in optimization of phenotypic traits in agricultural crops. Adaptability of a genotype over diverse environments is usually tested by the degree of its interaction with different environments under which it is planted. A genotype or variety is considered to be more adaptive or stable if it has high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments (Muhammad *et al.*, 2003). Genotype by environment interactions for various traits have previously been studied by different researchers in various crops including chickpeas (Muhammad *et al.*, 2003), dry beans (Balasubramanian *et al.*, 1999), hard winter wheat (Moore *et al.*, 2006) and sunflowers (Leon *et al.*, 2003). However, information on influence of environment and genotype on seed yield of Lablab is extremely limited.

Yield is a complex trait due to gene action and interaction with the environment, that is, different reactions of genotypes on changeable environmental conditions (Moghaddam and Pourdad, 2011). The yield of a certain genotype in a specific environment consists of genotype main effects, environment main effects, and genotype by environment interaction. Seed yield per plant is one of the main yield components which is greatly influenced by genotype, environment and complex genotype-by-environment interactions. There are also highly significant and positive correlations of seed yield per plant with seed weight (Ali *et al.*, 2003). In crop production, yield stability is considered the most important socio-economic category, especially in extreme environmental conditions (Moghaddam and Pourdad, 2011).

### **3.3 Materials and Methods**

#### **3.3.1 Sites for field trials**

Field trials of forty five (45) accessions of Lablab collected from Rift Valley, Eastern, Coast and Central regions of Kenya (Appendix 3) were established in three locations with different agro-ecological environments; Nakuru-Kenya Agricultural and Livestock Research Organization (KALRO)-Njoro farm, Bungoma-Mabanga Agricultural Training Center (ATC), and Uasin Gishu-University of Eldoret farm. The forty five accessions and three environments were factorially combined and replicated three times in a randomized complete block design (RCBD) in gross plot sizes of 2.25m X 1.8m at spacing of 90cm x 75cm (6 plants per accession per replication). Planting was done in July 2008 and harvesting was completed in January 2009. The experimental plots were kept clean throughout the period of the study and plants were scouted for pests and diseases which were controlled accordingly.

Site 1 (Nakuru-KALRO, Njoro) receives bi-modal pattern of rainfall with long rains from March to July and short rains from August to October. It has fertile mollic-andosol, well- drained, deep to very deep, dark reddish brown in color, consisting of heavy textured friable silty clay to sandy loam soils. Site 2 (Uasin Gishu-University of Eldoret farm) has one rainfall season per year, from April to October and has rhodic-ferrasols. Site 3 (Bungoma-Mabanga Agricultural Training Center (ATC)) has a two-season rainfall regime. The long rains cover March to July while the short rains start in August to October. It is endowed with well-drained, rich and fertile arable soils.

**Table3.1 Description of locations under which Lablab accessions were evaluated for performance.**

Location	Geographical Position		Altitude (m.a.s.l)	Rainfall (mm)	T°C	
	Latitude	Longitude			Min	Max
Site1	0 <sup>0</sup> 20'2328 <sup>0</sup> S	35 <sup>0</sup> 563048' E	2423	1800-2000	12	22
Site2	0 <sup>0</sup> 32 <sup>0</sup> N.	35 <sup>0</sup> 17' E	2134	900-1300	10	23
Site3	0 <sup>0</sup> 625482°N	34 <sup>0</sup> 693649'E	1500	1250-1800	15	30

KEY: Site 1- Nakuru-KALRO, Njoro; Site 2- Uasin Gishu-University of Eldoret; Site 3- Bungoma-Mabanga Agricultural Training Center (ATC); m.a.s.l- meters above sea level; mm- millimeters; T°C- Temperature; Min - Minimum; Max - maximum

### 3.3.2 Data Collection

Data on quantitative characters of Lablab was taken from the three sites. Traits considered included; days to germination, days to 50% flowering, duration of flowering, days to 90% mature pods, plant height, leaf length, leaf width, number of pods per raceme, number of racemes per plant, number of pods per plant, seeds per pod, pod length, pod width, seed length, seed width, 100 seed weight and seed yield per plant. Measurements were taken with a tape measure in centimeters, counting and weighing accordingly. Overall, seventeen characters were considered in this study and data was collected as indicated in Table 3.2.

**Table3.2 Agro-morphological traits used in evaluation of performance of Lablab accessions in different agro-ecological environments.**

<b>Trait</b>	<b>Description</b>
Plant height	In centimeters, from cotyledon scar to tip of plant on 10 randomly selected mature plants
Leaflet length , measured on the terminal leaflet of third trifoliolate leaf from pulvinus to leaf tip	3=5-7, 5=9-11, 7=13-15 10 randomly selected mature plants
Leaflet width, measured on the terminal leaflet of third trifoliolate leaf on the widest part of leaf	3=2-6, 5=7-10, 7=11-15 10 randomly selected mature plants
Days to maturity	Days from emergence to stage when 90% of pods are ripe
Days to flowering	From emergence to stage when 50% of plants have begun to flower
Duration of flowering	From first flowers to stage where 50% of plants have finished flowering
Pod length	In centimeters, average of 10 randomly chose mature pods. If pods are curved, measure straight line from base to tip of pods
Pod width	In centimeters, of the largest width from 10 randomly chosen mature pods
Number of seeds per pod	Average from 10 randomly chosen ripe pods
Number of pods per raceme	Average of 10 randomly chosen racemes
Number of racemes per plant	Average of 10 randomly chosen 3 mature plants
Number of pods per plant	Average of 10 randomly chosen 3 mature plants when 90% of pods are ripe
Seed length	In millimeters, average of 10 ripe seeds chosen at random
Seed width	In millimeters, average of 10 ripe seeds chosen at random In millimeters, average of 10 ripe seeds chosen at random
Seed weight	Weight of 100 seeds in milligrams, moisture content of 12-14%
Seed yield	Weight of all seeds harvested from 3 randomly chosen plants
Seed germination	Days taken for 90% shoot emergence in field

### 3.3.3 Data analysis

Descriptive statistics was employed to analyze the mean values of the agromorphological traits according to sites. Data was also subjected to analysis of variance (ANOVA) using GenStat statistical version 12 software (Payne *et al.*, 2009).

## 3.4 Results

### 3.4.1 Effect of environment on yield and maturity period of Lablab accessions

The three sites were significantly different in seed yield per plant (Table 3.3). However, accessions collected from same geographical region recorded yields that had no significant differences. Seed yield per plant varied from 17.7g to 203.5g. The least seed yield per plant was recorded in Bungoma-Mabanga ATC farm while the highest yield per plant was realized in Nakuru-KARLO, Njoro farm. In general, Nakuru recorded higher yields in all the accessions while Bungoma gave the least yield per plant in all the accessions. Uasin-Gishu ranked number two in yield per plant. The best accession in nakuru was 36 with a seed yield of 203.5g/plant. In Uasin-Gishu, the best accession was also 36 with a seed yield of 157.05g/plant while in Bungoma, accession 7 had the highest seed yield with 95.07g/plant.

Generally, accessions collected from Lamu performed better in Bungoma than other accessions and recorded an average yield of 95g/plant (Table 3.3). However, accession 36 also recorded a high seed yield of 92.85g/plant in Bungoma. Accession 36 was the best performer across all the sites. Other accessions that recorded high seed yield per plant across the three sites were 22 and 23 that recorded a seed yield of 188.1g/plant and 188.0g/plant respectively in Nakuru. In Uasin-Gishu, both accessions (22 and 23) obtained a seed yield of 124.19g/plant. In Bungoma accession

22 recorded 85.28g/plant while 23 obtained 85.25g/plant. Accession 36 was collected from Nakuru-Bahati while the two accessions 22 and 23 were sourced from Maragwa-Makuyu.

All accessions irrespective of places of collection recorded a shorter period of maturity in Bungoma while in Nakuru, all accessions took a long period to mature (Table 3.3). In Uasin-Gishu accessions were not significantly different from Nakuru in maturity period. In general, accessions collected from Lamu had the least days to maturity with a mean range of 117.8 days to 118.0 days. Accessions 36 (collected from Nakuru-Bahati), 21 (collected from Machakos-Yatta) and accessions 39, 40, 41, 42, 43, 44 and 45 (collected from Mwingi) also recorded a short period to mature of about 120 days. Accessions 22 and 23 (collected from Murang'a-Makuyu), 24, 25, 26, 27 and 28 (collected from Thika) ranked third as regards maturity period which was about 147 days (Table 3.3).

Accessions 37 and 38 collected from Nakuru-Naivasha were the poorest yielders with a yield of 22.8 and 23.0 g/plant in Nakuru and 19.15 and 19.14g/plant in Uasin-Gishu respectively. In Bungoma the two accessions recorded 17.69 and 17.70g/plant respectively. Other accessions that performed poorly across the three sites were 14, 15, 16 and 17 (collected from Machakos-Kalama). Accessions 18, 19 and 20 (collected from Machakos-Kathiani) were also poor performers across the three sites. Accession 37 and 38 (collected from Nakuru-Naivasha) took the longest period to mature with a mean of 189.6 and 188.2 days respectively. Accessions collected from Machakos-Kalama (14, 15, 16, and 17) and Machakos-Kathiani (18, 19, and 20) also had a long maturity period with a mean range of 186.1 to 186.6 days (Table 3.3).

**Table3. 3 Mean yield and days to maturity of Lablab accessions across the three environments.**

Accession	Seed yield per plant				Days to maturity			
	Nakuru	Uasi-Gishu	Bungoma	Mean across sites	Nakuru	Uasin-gishu	Bungoma	Mean across sites
1	138.1	112.31	95.05	115.2	123.3	122.3	108.7	118.1
2	138.0	112.29	95.05	115.1	123.3	122.7	108.3	118.1
3	138.1	112.28	94.99	115.1	123.7	122.3	108.7	118.2
4	138.0	112.29	94.98	115.0	123.3	122.7	108.3	118.1
5	138.5	112.28	95.04	115.1	123.1	122.4	108.3	117.9
6	138.3	112.27	95.01	115.3	123.3	122.3	108.7	117.8
7	138.2	112.29	95.07	115.2	123.7	122.1	108.7	117.9
8	138.1	112.28	94.98	115.1	123.4	122.3	108.3	118.0
9	138.1	112.28	95.03	115.1	123.3	122.7	108.7	118.2
10	138.0	112.27	95.05	115.0	123.7	122.3	108.7	118.2
11	138.0	112.28	94.99	115.0	123.4	122.3	108.3	118.0
12	138.1	112.28	95.04	115.1	123.3	122.4	108.7	118.1
13	138.0	112.29	95.01	115.0	123.3	122.3	108.3	118.0
14	63.2	51.62	35.06	50.0	192.6	191.3	176.3	186.4
15	63.1	51.59	35.03	49.9	192.7	190.7	176.7	186.2
16	63.2	51.41	35.01	49.8	192.5	190.7	176.3	186.2
17	63.1	51.61	35.02	49.9	192.3	191.3	176.7	186.4
18	56.0	40.88	30.04	42.3	192.1	191.3	176.3	186.2
19	56.1	40.87	30.22	42.4	192.3	190.7	176.3	186.1
20	56.0	40.88	29.98	42.3	192.7	190.3	176.7	186.6
21	75.1	63.51	41.42	60.0	126.3	125.7	111.3	121.1
22	188.1	124.19	85.28	132.5	153.3	152.7	136.7	147.4
23	188.0	124.19	85.25	132.5	153.7	152.3	136.3	147.5
24	181.3	121.30	77.95	126.8	153.3	152.7	136.7	147.6
25	181.4	121.30	77.94	126.9	153.7	152.3	136.7	147.7
26	181.3	121.29	77.92	126.9	153.7	152.3	136.3	147.4
27	181.3	121.28	77.93	126.6	153.3	152.3	136.7	147.4
28	181.4	121.30	77.93	126.8	153.7	152.7	136.3	147.6
29	173.7	113.77	68.42	118.7	158.3	157.7	141.7	152.2
30	173.8	113.77	68.43	118.6	158.7	157.7	141.7	152.4
31	181.5	120.98	85.61	129.4	158.3	157.3	141.3	152.0
32	181.4	120.98	85.66	129.3	158.3	157.3	141.3	152.0
33	153.6	105.81	73.69	111.1	172.7	172.3	157.7	167.6
34	153.7	105.80	73.70	111.1	173.3	172.7	157.3	167.6
35	153.6	105.81	73.71	111.0	172.7	172.3	157.3	167.4
36	203.5	157.05	92.85	151.1	125.7	124.7	111.7	120.0
37	23.1	19.14	17.70	19.9	197.3	195.7	172.7	189.6
38	23.0	19.15	17.69	19.7	196.7	195.3	172.7	188.2
39	119.2	96.37	64.84	93.5	126.3	125.7	111.7	120.9
40	119.2	96.10	64.49	93.3	125.7	125.3	111.3	120.4
41	119.1	96.07	64.50	93.2	126.3	125.7	111.6	120.9
42	119.2	96.06	64.48	93.3	126.7	125.3	111.3	120.8
43	119.1	96.05	64.50	93.4	126.3	125.7	111.6	120.9
44	119.2	96.04	64.49	93.3	125.7	125.3	111.3	120.4
45	119.1	95.98	64.72	93.3	126.3	125.3	111.7	120.8
Range	180.5	138.2	77.4	107.2	74.2	73.6	64.4	68.6
Min	23.0	19.0	17.7	19.7	123.1	122.1	108.3	117.8
Max	203.5	157.2	95.1	126.9	197.3	195.7	172.7	186.4
Mean	129.54	96.93	70.60	99.02	149.06	147.80	133.51	143.45

Key:

Seed yield per plant: LSD ( $P \leq 0.05$ ) = 2.17; Grand mean = 99.02; SED = 0.110;

Days to maturity: LSD ( $P \leq 0.05$ ) = 2.93CV% = 0.1; Grand mean = 143.45; SED = 0.473.

Table 3.4 shows that the three sources of variation were highly significant. In the analysis of variance, the sum of squares for accession main effect represented 59.37% of the total variation, and this factor had the highest effect on seed yield per plant. The differences between sites explained 31.82% of the total yield variation, while the effect of accession by site (G x E) interaction explained 8.81%.

**Table 3.4 Analysis of variance of seed yield per plant for *Lablab purpureus* accessions**

Source of variation	Sum of Squares	df	Mean Square	F	Sig.	Variability Explained (%)
Rep	0.01	2	0.01	0.000	1.000	
Site	235420.03	2	117710.02	6488975.451	0.000	31.82
Accession	439259.09	44	9983.16	550339.653	0.000	59.37
Accession * Site (GxE)	65166.28	88	740.53	40822.822	0.000	8.81
Site * Rep	0.22	4	0.01	0.000	1.000	
Accession * Rep	1.38	88	0.02	0.000	1.000	
Accession * Site *Rep	3.48	176	0.03	0.000	1.000	
Error	4.90	270	0.02			
Total	4711087.01	405				
Corrected Total	739850.30	404				

There were significant environmental (site) effects for all characters evaluated. Similarly, there were significant genotype (accession) effects on all the characters studied. The combined analysis of variance further indicates that there was significant genotype (G) x environmental (E) interaction effects on all the characters except for seed length and seed width (Table 3.5).



**Table 3.5 A combined analysis of variance of seed and yield related characters of 45 accessions of *Lablab purpureus*.**

Source of variation	df	DaF	DuF	DMP	PR	RP	PP
Rep (Block)	2	0.07	0.31	0.13	0.02	0.03	0.08
Site	2	4400.82*	600.13*	12400*	62.1*	253.04*	42100*
Accessions	44	1314.83*	547.70*	6460*	23.2*	20.65*	68900*
Accession x Site (GxE)	88	1.85*	0.53	1.83*	1.13*	1.67*	134*
Error	268	0.33	0.33	0.34	0.01	0.04	0.42
C.V%		0.6	2.4	5.4	0.9	2.4	0.7

**Table 3.5 Continued**

Source of variation	df	SP	100 SW	SY	SL	SW
Rep (Block)	2	0.002	0.013	0.007	0.002	0.040
Site	2	19.626*	11.9*	118000*	0.046*	0.811*
Accessions	44	0.308*	91.9*	9980*	0.014*	0.006*
Accession x Site (GxE)	88	0.096*	0.045*	741*	0.003	0.001
Error	268	0.004	0.009	0.018	0.004	0.003
C.V%		2.0	0.4	0.1	5.6	6.0

Key:\*Significant at  $P \leq 0.05$  level of probability; df-degrees of freedom; DaF-Days to flowering; DuF-Duration of flowering; DMP-Days to mature pods; PR- Pods per raceme; RP-Raceme per plant; PP-Pods per plant; SP- Seeds per pod; 100 SW -100 Seed weight; SY- Seed yield per plant; SL-Seed length; SW-Seed width.

Environment in which Lablab was grown had a significant effect on yield and yield related characters such as days to flowering, duration of flowering, days to mature pods, pods per raceme, racemes per plant, pods per plant and seeds per pod (Table 3.6). Nakuru was overall the best in terms of Lablab accessions performance. The least in performance was Bungoma which recorded the lowest values for all characters evaluated. Nakuru and Uasin-Gishu were not significantly different in days to flowering, duration of flowering and days to mature pods.

**Table 3.6 Effect of environment on mean yield and yield related characters of Lablab.**

Site	DaF(days)	DuF(days)	DMP	PR	RP
Nakuru	102.126a	25.637a	149.06a	8.674a	11.305a
Uasin-Gishu	102.037a	25.652a	147.80a	7.944b	9.694b
Bungoma	92.193b	21.993b	133.51b	7.319c	8.582c
Grand mean	98.79	24.42	143.53	8.00	9.86
LSD (0.05)	0.14	0.14	2.93	0.02	0.50
C.V (%)	0.6	2.4	0.4	0.9	2.4

**Table 3.6 Continued**

Site	PP	SP	100SWT(g)	SY(g)
Nakuru	113.374a	3.727a	26.225a	129.54a
Uasin-Gishu	91.704b	3.187b	25.721b	96.93b
Bungoma	78.367c	2.990c	26.243c	70.60c
Grand mean	94.48	3.30	26.06	99.02
LSD (0.05)	0.16	0.017	0.62	2.17
C.V (%)	0.7	2	0.4	0.1

Key: Columns having same letters are not significantly different at  $P \leq 0.05$ ; Site 1- Nakuru-KALRO, Njoro; Site 2- Uasin Gishu-University of Eldoret; Site 3- Bungoma-Mabanga Agricultural Training Center (ATC); DaF- Days to flowering; DuF - Duration of flowering; DMP - Days to mature pods; PD - Pods per raceme; RP - Raceme per plant; PP - Pods per plant; SP - Seeds per pod; 100SWT - 100 Seed weight; SY - Seed yield per plant; g-grams.

### 3.4.2 Effect of environment on vegetative characters of Lablab accessions

All the vegetative characters studied were significantly affected by environment (site) (Table 3.7). There were also significant genotype (accessions) and genotype (accession) x environment (site) effects on all the vegetative characters studied except for mature pod width.

**Table 3.7 Combined analysis of variance of vegetative characters of 45 accessions of *Lablab purpureus*.**

Source of variation	df	DG	PH(cm)	LL(cm)
Rep (Block)	2	0.0321	1.19	0.13
Site	2	1505.14*	56900*	127.39*
Accessions	44	16.90*	51400*	16.07*
Accession x Site (G x E)	88	1.61*	83.1*	0.36*
Error	268	0.39	3.57	0.024
C.V%		5.4	0.9	1.5

**Table 3.7 Continued**

Source of variation	df	RL(cm)	PL(cm)	PW(cm)
Rep (Block)	2	0.12	0.0001	0.0003
Site	2	3115.87*	8.99*	0.2865*
Accessions	44	81.78*	0.645*	0.0035
Accession x Site (G x E)	88	9.13*	0.0622*	0.0027
Error	268	0.16	0.004	0.0036
C.V%		1.4	1.3	2.7

Key: \*Significant at  $P \leq 0.05$ ; df - degrees of freedom; DG-Days to germination; PH - Plant height; LL- Leaf length; RL - Raceme length; PL- Pod length; PW - pod width

There were significant differences between sites in days to germination, plant height, leaf length, raceme length and pod length (Table 3.8). Accessions planted in Bungoma took the shortest time to germinate (7.84 days) while those planted in Nakuru, took the longest time to germinate (14.36 days). Generally, Lablab accessions planted in Nakuru performed better than in the other two sites. However, there were no significant differences between Nakuru and Bungoma in pod width, seed length and seed width. Uasin-Gishu was significantly different and recorded poor performance for these traits (Table 3.8).

**Table 3. 8 Effect of site on vegetative characters of Lablab accessions**

Site	DG (days)	PH (cm)	LL (cm)	RL (cm)
Nakuru	14.36a	236.59a	11.511a	33.94a
Uasin-Gishu	12.39b	195.58b	9.57b	24.42b
Bungoma	7.84c	217.94c	10.65c	28.09c
Grand mean	11.528	216.695	10.579	28.815
LSD (0.05)	0.150	0.453	0.038	0.097
C.V (%)	5.4	0.9	1.5	1.4

**Table 3.8 Continued**

Site	PL (cm)	PW (cm)	SL (cm)	SW (cm)
Nakuru	5.16a	2.18a	1.107a	0.932a
Uasin-Gishu	4.73b	2.15b	1.057b	0.821b
Bungoma	4.95c	2.18a	1.096a	0.930a
Grand mean	5.024	2.194	1.0983	0.9069
LSD (0.05)	0.015	0.014	0.0148	0.0129
C.V (%)	1.3	2.7	5.6	6.0

Key: Columns having same letters are not significantly different at  $P \leq 0.05$ ; DG - Days to germination; PH - Plant height; LL - Leaf length, RL - Raceme length; PL - Pod length; PW - Pod width; SL - Seed length; SW - Seed width

### 3.5 Discussion

A genotype or variety is considered to be more adaptive or stable if it has high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments (Muhammad *et al.*, 2003). Measuring GxE interaction is important to determine an optimum breeding strategy for releasing cultivars with an adequate adaptation to target environments (Fan *et al.*, 2007). Some genotypes have high adaptation; however, some have specific adaptability (Yan and Hunt, 2001). Analysis of variance for the forty-five Lablab accessions evaluated over the three sites (environments) showed strong evidence that genotype, environment and

genotype x environment interaction had significant effects on yield. All the traits studied were significantly affected by environment and genotype.

Different accessions were affected differently by production environments. These variations could be attributed to different climatic and edaphic conditions at the different sites. Among the tested Lablab accessions, number 36 (collected from Nakuru-Bahati) had the highest yield in Nakuru and Uasi-Gishu while accession 7 (collected from Lamu) gave the highest yield in Bungoma. Accession 36 also ranked highly in Bungoma. Accessions 23 and 24 collected from Murang'a-Makuyu had also good performance in the three sites. The significantly higher number of pods per plant recorded by accession 36, 23 and 24 across sites could indicate that they were more efficient in partitioning photo-assimilates into pods and consequently into seeds. It also implies that the accessions were not affected negatively by the three environments and could be well adapted to the three locations.

The presence of genotype x environment interactions has been reported by several workers for instance, Gemechu *et al.*, (2005) found that genotype by environment effect was almost four times higher than that of genotype effect on performance of field pea genotypes on number of primary branches, seed size, number of seeds and grain yield. Asfaw *et al.*, (2008) studied adaptability of small red bean elite lines in Ethiopia and observed a significant genotype by environment effect on grain yield. In a study on performance of cowpea in two agroecological zones in Ghana, Addoye-Quaye *et al.*, (2011) reported a strong environmental effect on genotype performance for number of pods per plant, 1000 seed weight and grain yield. Similarly, Mrdja *et al.* (2012) in a study on sunflower seed quality in relation to production environment

found that seed germinability, 100 seed weight and seed yield per plant were affected by interaction between genotype and environment. Kumaresan and Nadarajan, (2010) reported a significant genotype by environment effects on 1000 seed weight and seed yield in 64 sesame genotypes evaluated in three environments. Similarly, Casquero *et al.*, (2006) found a significant genotype by environment interaction effect on seed weight, seed width and seed yield in common bean.

The environmental effects and the interactions between genotype and environment highlight the different response of genotypes to environmental conditions. This means that the best genotype for one environment is not the best for another. Therefore, it is important that specific types of genotypes are developed to overcome the interaction of genotype by environment (Agbogidi and Ofuoko, 2005). This study has also demonstrated that different Lablab genotypes performed differently in the different environments where accessions 22, 23 and 36 performed highly in Nakuru and Uasin-Gishu and accession 7 was the best performer in Bungoma.

## CHAPTER FOUR

### EVALUATION OF PHENOTYPIC DIVERSITY OF THE KENYAN

### DOLICHOS BEAN (*Lablab purpureus* (L.) SWEET) GERMPLASM

#### 4.1 Abstract

The morphological method is the oldest and considered the first step in description and classification of germplasm. There is much disagreements as to names and varieties of Lablab. It is not uncommon to find that morphologically similar cultivars do not bear the same name while cultivars bearing the same name may not be identical morphologically. This ambiguity in names necessitated the need to carry out a study on morphological characterization of Lablab. Forty five (45) accessions of Lablab collected from farmers fields in Rift Valley, Eastern, Coast and Central regions of Kenya were planted at Kenya Agricultural and Livestock Research Organization (KALRO), Nakuru-Njoro farm. A descriptor from Asian Vegetable Research Development Center was used as a guide. Eight qualitative and twenty quantitative traits were used to characterize the forty five (45) Lablab accessions. Results on means separation showed a high level of variability in quantitative traits and a low level of variability in qualitative traits. Eigen vectors derived from principal component analysis indicated that seed yield per plant, number of pods per plant, plant height and days to 90% mature pods contributed highly to total diversity in Lablab. In conclusion, Lablab germplasm grown in Kenya is morphologically diverse in quantitative traits where different genotypes are distinctly dissimilar.

## 4.2 Introduction

Morphological characterization is the first step that should be done before more profound biochemical or molecular studies are carried out (Hoogendijk and Williams, 2001; Hedrick, 2005). Morphological characterization is traditionally the most common method used and many different crops have been studied (González *et al.*, 2002). Some of the most important advantages of using morphological characterization are that they are simple to identify and do not need specialized labour, published descriptor lists are readily obtainable for most major crop species, it can be carried out *in situ*, is relatively low-cost and easy to perform. However, morphological estimations are more dependent on environment and are more subjective than other measurements (Li *et al.*, 2009). Morphological variability depends on a limited number of genes, and may not access much of the potential variability for the agronomic traits present in a crop (Mayes *et al.*, 2009). The use of morphological and agronomic traits is a standard way of assessing genetic variation for many species, especially under-researched crops (Azam-Ali *et al.*, 2001).

### 4.2.2 Application of morphological markers in crop improvement

Morphological markers have been used for phenotypic diversity studies in a number of crops. Several numerical taxonomic techniques have been successfully employed to classify and measure the patterns of genetic diversity in the germplasm collection by other researchers working on crops such as black gram (*Vigna mungo*) and Mungbean (*Vigna radiata*) (Ghafoor *et al.*, 2001) and wheat (*Triticum aestivum*) (Bechere *et al.*, 1996). Agronomic and morphological characters have been used to identify traits contributing to yield in crops like bambara groundnut (Makanda *et al.*, 2009) and soybean (Malik *et al.*, 2007).



Differentiation between cooking and dessert bananas was done based on morphological, physical and chemical characteristics of 23 unripe cultivated varieties of Colombian Musaceae (Gibert *et al.*, 2009). Morphological characterization of mandarin fruits from the Citrus germplasm active bank of Centro de Citricultura Sylvio Moreira/IAC was done using 38 fruit morphological description characters and large phenotypic variation in most of the analyzed characters was observed (Domingues, 1999). Morphological characterization of cashew (*Anacardium occidentale* L.) in four populations in Malawi detected that variation between accessions could be attributed to genetic history, eco-geographic origin and selection for desired agronomic traits by farmers (Chipojola *et al.*, 2009). A preliminary selection of 19 mango accessions and cultivars from a collection at the Umbeluzi Research Station in Mozambique was done (Ascenso *et al.*, 1981). The study focused on colour, size, shape, weight and volume of fruit, number of embryos per seed, peel thickness, adherence, flavour, texture, fibre content, juice, soluble solids, sugars, acidity, pH and ratio of soluble solids to acidity. As a result, the five most desirable varieties were selected (Ascenso *et al.*, 1981).

Although molecular characterization is increasingly being used, morphological characterization continues to be a useful component that enhances the power of molecular methods (Hedrick, 2005). According to Opong-Konadu *et al.*, (2003) in Lablab, it is not uncommon to find that morphologically similar cultivars do not bear the same name while cultivars bearing the same name may not be identical morphologically. Morphological characterization is thus imperative in order to distinguish the different genotypes and identify certain characteristics that would be useful as references in Lablab breeding programmes.

### **4.3 Materials and Methods**

#### **4.3.1 Seed acquisition and planting**

Seeds of forty five (45) accessions of Lablab were obtained from Rift Valley, Eastern, Coast and Central regions of Kenya (Appendix 3). The forty five (45) accessions of Lablab were planted on gross plots of 2.25m x 1.8m at spacing of 90cm x 75cm (6 plants per accession per replication) in Kenya Agricultural and Livestock Research Organization (KALRO), Njoro farm. Randomized complete block design (RCBD) was used and each block was replicated three times. The experimental plots were kept clean throughout the period of the study. Plants were scouted for pests and diseases which were controlled accordingly.

#### **4.3.2 Morphological characterization**

Morphological study was carried out through characterization of the reproductive and vegetative characters of each accession from the time the seeds germinated. Data were taken on qualitative characters of the accessions by visual scoring of variations in emerging cotyledon color, hypocotyl color, pod colour, vein color of fully developed primary leaves, growth habit, colour of flower keel, colour of flower standard and seed coat colour of mature seeds (Table 4.1). Quantitative data was taken on, hypocotyl length, leaflet length and width, plant height, days to flowering, duration of flowering, raceme length, pod length and width, number of pods per raceme, racemes per plant, number of nodes per raceme, days to maturity, seed length and width, 100 seed weight and seed yield per plant. Each trait was described and scored as indicated in Table 4.1.

**Table 4.1 Agro-morphological traits used in characterization of Lablab accessions**

<b>Trait</b>	<b>Description</b>
Emerging cotyledon color	1=white, 2=green, 3=purple
Hypocotyl color	1=green, 2=purple
Hypocotyl length	In centimeters from the soil surface to cotyledon scar
Vein color of fully developed primary leaves (on inner face)	1=green, 2=purple
Plant height	In centimeters, from cotyledon scar to tip of plant on 10 randomly selected mature plants
Leaflet length measured on the terminal leaflet of third trifoliate leaf from pulvinus to leaf tip	3=5-7, 5=9-11, 7=13-15 10 randomly selected mature plants
Leaflet width measured on the terminal leaflet of third trifoliate leaf on the widest part of leaf	3=2-6, 5=7-10, 7=11-15 10 randomly selected mature plants
Growth habit	1=determinate bush, 2=intermediate semi-climber, 3=indeterminate climber, 4=others
Days to maturity	Days from emergence to stage when 90% of pods are ripe
Days to flowering	From emergence to stage when 50% of plants have begun to flower
Colour of flower keel	1=greenish, 2=tinged (pink or purple)
Colour of flower standard (upper part of inner side)	1=white, 3=light pink, 5=deep pink, 7=violet
Raceme length	In centimeters, one raceme from each of 10 plants at pod filling period; if determinate type, one terminal raceme; if indeterminate type, one lateral raceme- 6 <sup>th</sup> from apex
Duration of flowering	From first flowers to stage where 50% of plants have finished flowering
Pod length	In centimeters, average of 10 randomly chose mature pods. If pods are curved, measure straight line from base to tip of pods
Pod width	In centimeters, of the largest width from 10 randomly chosen mature pods
Pod colour of mature pods	1=light green 2=green 3=green with purple suture 4=purple
Number of seeds per pod	Average from 10 randomly chosen ripe pods
Number of pods per raceme	Average of 10 randomly chosen racemes
Number of racemes per plant	Average of 10 randomly chosen 3 mature plants
Number of nodes per raceme	Average of 10 randomly chosen racemes
Number of pods per plant	Average of 10 randomly chosen 3 mature plants when 90% of pods are ripe
Seed coat colour of ripe seeds	1=white, 2=green, 3=brown, 4=purple
Seed length	In millimeters, average of 10 ripe seeds chosen at random
Seed width	In millimeters, average of 10 ripe seeds chosen at random
Seed weight	Weight of 100 seeds in milligrams, moisture content of 12-14%
Seed yield	Weight of all seeds harvested from 3 randomly chosen plants
Seed germination	Days taken for 90% shoot emergence in field

### **4.3.3 Data Collection**

Data for morphological studies were generated from plant materials obtained from each accession picked at random from the three blocks (replicates). Morphological characters were determined by measurement in centimeters, counting, weighing and visual determination. A descriptor from Asian Vegetable Research Development Center (AVRDC) was used as a guide for morphological characterization (Table 4.1). Overall, twenty eight characters were considered in this study.

### **4.3.4 Data Analysis**

Descriptive statistics was employed to analyze the mean values of the agromorphological traits. The multivariate statistical methods employed were principal component analysis (PCA) and cluster analysis (CA). The principal component analysis produced vector loadings for variables on principal component (PC) axes while cluster analysis produced a cluster grouping in the form of a dendrogram. Pearson correlation analysis was employed to identify the dependence of characters on each other.

## **4.4 Results**

### **4.4.1 Lablab diversity for emerging cotyledon colour, hypocotyl colour, vein colour and growth habit**

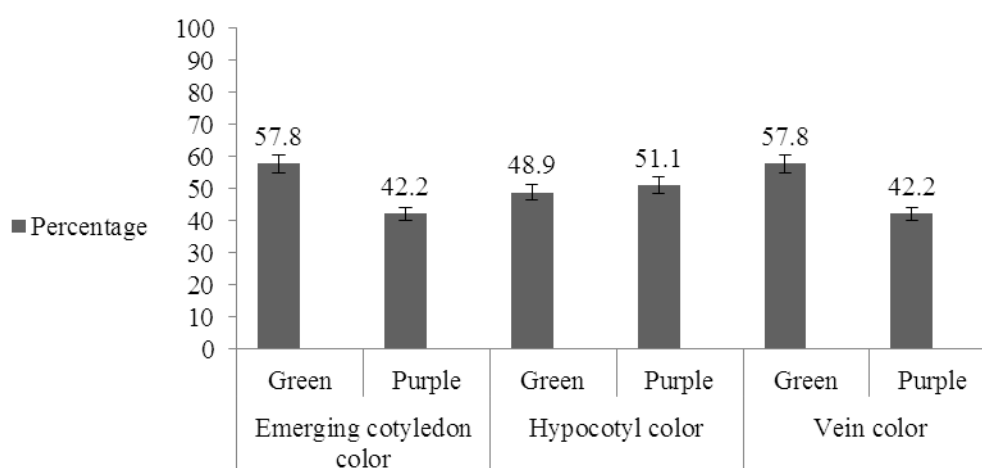
Table 4.2 shows that Lablab accessions collected from Lamu, Mbeere and Mwingi had a green emerging cotyledon colour, green hypocotyl colour and green main vein colour. Accessions collected from Machakos, Murang'a, Thika, Meru, Nakuru-Naivasha had purple emerging cotyledon colour, purple hypocotyl colour and purple main vein colour. Accessions collected from Lamu and Nakuru-Bahati, exhibited a

determinate bush growth habit. Accessions collected from Machakos (Kalama and Kathiani) and Nakuru-Naivasha exhibited an indeterminate growth habit while accessions collected from Machakos-Yatta, Maragwa, Thika, Meru, Nakuru-Lare) and Mwingi were intermediate semi-climbers.

**Table4.2 Lablab diversity for seed testa colour, emerging cotyledon colour, hypocotyl colour, main vein colour and growth habit.**

Accession	Place collected	Seed testa colour	Emerging cotyledon colour	Hypocotyl colour	Vein colour	Growth habit
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	Lamu	Brown	green	green	green	Determinate bush
14, 15, 16	Machakos-Kalama	Dark-Brown	purple	purple	Purple	Indeterminate climber
17, 18, 19, 20	Machakos-Kathiani	Dark-Brown	purple	purple	purple	Indeterminate climber
21	Machakos-Yatta	Black	purple	purple	purple	Intermediate semi-climber
22, 23	Murang'a-Makuyu	Black	purple	purple	purple	Intermediate semi-climber
24, 25, 26, 27	Thika-Kakuzi	Black	purple	purple	purple	Intermediate semi-climber
28	Thika-Municipality	Black	purple	purple	purple	Intermediate semi-climber
29, 30	Mbeere-Siakago	Brown	green	green	green	Intermediate semi-climber
31	Meru-Abothoguchi	Black	purple	purple	purple	Intermediate semi-climber
32	Meru-Mihiriga-Mieru	Black	purple	purple	purple	Intermediate semi-climber
33, 34, 35	Nakuru-Lare	Black	purple	purple	purple	Intermediate semi-climber
36	Nakuru-Bahati	Black	purple	purple	purple	Determinate bush
37, 38	Naivasha-Maragushu	Black	purple	purple	purple	Indeterminate climber
39, 40, 41, 44, 45	Mwingi-Central	Brown	green	green	green	Intermediate semi-climber
42, 43	Mwingi-Migwani	Brown	green	green	green	Intermediate semi-climber

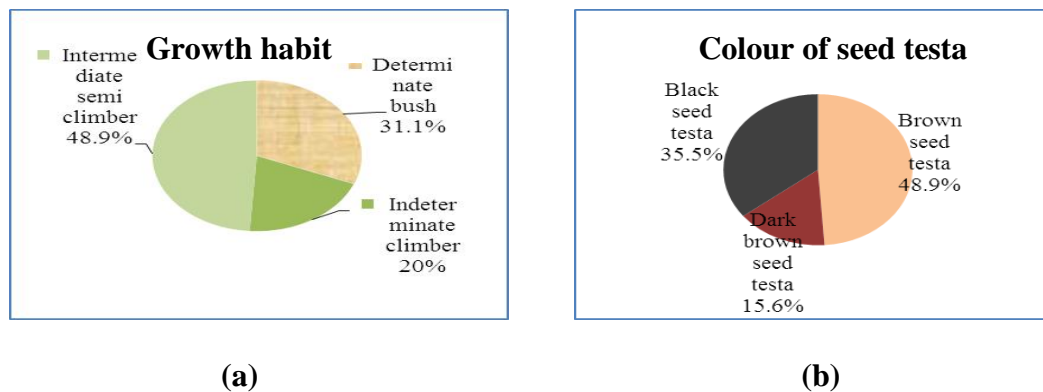
Figure 4.1 indicates that 57.8% of the Lablab accessions had green colour of emerging cotyledon while 42.2% had purple colour. Out of the 45 Lablab accessions 48.9% had green hypocotyl colour while 51.1% had purple colour. All Lablab accessions that had green emerging cotyledon colour had also a green vein colour (48.9%) while accessions that had purple emerging cotyledon colour had a purple vein colour (51.1%).



#### Lablab morphological diversity

**Fig.4. 1 Lablab diversity for emerging cotyledon colour, hypocotyl colour and main vein colour.**

The forty five Lablab accessions could be grouped into one of the three growth habit categories, that is, determinate bush, indeterminate climber and intermediate semi-climber (Fig.4.2a). Accessions that had a determinate growth habit comprised of 31.1% while 20% were indeterminate climbers and 48.9% were intermediate semi-climbers. Figure 4.2b, shows that Lablab accessions considered in this study had three seed testa colour variations, that is, black (35.5%), brown (48.9%) and dark brown (15.6%).



**Fig.4. 2 *Lablab purpureus* diversity for (a) growth habit and (b) colour of seed testa**

#### **4.4.2 *Lablab* diversity for colour of flower keel, colour of flower standard and pod colour**

*Lablab* accessions collected from Lamu, Mbeere and Mwingi had a greenish colour of flower keel, white colour of flower standard and green pod colour (Table 4.3). Accessions that had green pod colour were either white or light pink in colour of flower standard while those that had pod colour that was green with purple suture had purple colour of flower standard. Accessions collected from Machakos (Kalama and Kathiani) had a dark tinged pink colour of flower keel, light pink colour of flower standard and green pod colour. Accessions collected from Machakos (Yatta), Murang'a, Thika, Meru, Nakuru and Naivasha had tinged purple colour of flower keel, purple colour of standard and green with purple suture pod colour.

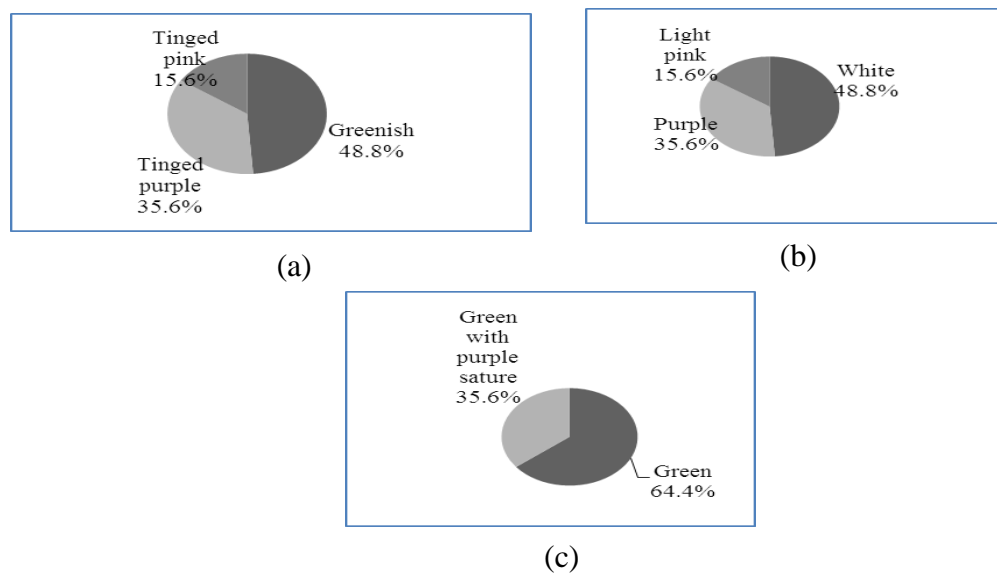
**Table 4.3 Lablab diversity for colour of flower keel, colour of flower standard and pod colour**

<b>Accession</b>	<b>Colour of flower Keel</b>	<b>Colour of flower standard</b>	<b>Pod colour</b>
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	greenish	white	green
14, 15, 16	Tinged (pink)	Light-pink	green
17, 18, 19, 20	Tinged (pink)	Light-pink	green
21	Tinged (purple)	purple	Green with purple suture
22, 23	Tinged (purple)	purple	Green with purple suture
24, 25, 26, 27	Tinged (purple)	purple	Green with purple suture
28	Tinged (purple)	purple	Green with purple suture
29, 30	greenish	white	green
31	Tinged (purple)	purple	Green with purple suture
32	Tinged (purple)	purple	Green with purple suture
33, 34, 35	Tinged (purple)	purple	Green with purple suture
36	Tinged (purple)	purple	Green with purple suture
37, 38	Tinged (purple)	purple	Green with purple suture
39, 40, 41, 44, 45	greenish	white	green
42, 43	greenish	white	green

The colour of flower standard and the colour of flower keel seemed to have a strong association where all accessions with white colour of flower standard had greenish colour of flower keel. Those that had purple colour of flower standard had a tinged purple colour of flower keel while Lablab accessions with light pink colour of flower standard had also a tinged colour of flower keel (Table 4.3). Figure 4.3 indicates that out of the forty five accessions studied, 48.8% had greenish colour of flower keel,



35.6% had tinged purple colour while 15.6% had tinged pink colour. The 45 Lablab accessions depicted three colours of flower standard, that is, white (48.8%), purple (35.6%) and light pink (15.6%). The forty five accessions considered in the study could be grouped into two pod colours, that is, green pod colour that comprised of 64.4% and green with purple sature pods that were 35.6% (Fig. 4.3).



**Fig.4.3 Proportion of *Lablab purpureus* accessions for (a) colour of flower keel (b) colour of flower standard and (c) pod colour.**

#### 4.4.3 Lablab diversity for yield and yield associated characters

Lablab accessions varied significantly in days to 50% flowering (Table 4.4). However, accessions collected from Machakos (15, 16, 17, 18, 19 and 20) and those collected from Naivasha (37 and 38) were not significantly different. Similarly, significance in days to 50% flowering was also depicted in accessions 22 and 23 (collected from Murang'a) and 24, 25, 26, 27 and 28 (collected from Thika). Accessions 29 and 30, collected from Mbeere were not significantly different from

accessions 31 and 32 collected from Meru. Generally there were distinct differences in days to flowering among accessions collected from different regions. Accessions collected from Lamu took the shortest period (88.7days) from planting to flowering.

In general, there were significant differences in duration to flowering among Lablab accessions (Table 4.4). However, accessions 22 and 23 (collected from Makuyu), 24, 25, 26, 27 and 28 (collected from Thika), 29 and 30 (collected from Mbeere) and 31, 32 (collected from Meru) were not significantly different. Similarly, accessions collected from Lamu, Machakos-Yatta and Nakuru-Bahati were not significantly different and these accessions recorded the shortest duration of flowering (Table4.4).

Lablab accessions differed significantly on days to maturity (Table 4.4). For instance, accessions collected from Lamu (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12 and 13) were significantly different from all other accessions and similarly, accessions collected from Nakuru (33, 34, 35 and 36) and those collected from Mwingi (39, 40, 41, 42, 43, 44 and 45) were also significantly different from accessions collected from other regions. However, accessions 22 and 23 (collected from Murang'a) 24, 25, 26, 27 and 28 (collected from Thika) were not significantly different. Similarly, accessions collected from Meru (31 and 32) and those collected from Mbeere (29 and 30) were not significantly different in days to 90% mature pods. Number of pods per raceme categorized Lablab accessions according to regions of collection. Accessions collected from different regions recorded significant different values for number of pods per raceme.

Number of racemes per plant was significantly different among Lablab accessions (Table 4.4). However, accessions collected from Lamu and Mwingi were not significantly different. Similarly, accessions collected from Murang'a, Thika, Meru and Nakuru-Bahati were not significantly different in number of racemes per plant. Accessions collected from Mbeere and Nakuru-Lare had the same number of racemes per plant. Accessions 36, collected from Nakuru-Bahati and accessions 25 and 26, collected from Thika recorded the highest number of racemes per plant. In general, accessions differed in number of seeds per pod (Table 4.4). Nevertheless, accessions collected from Lamu and Mwingi were not significantly different while accessions collected from Thika, Meru and Nakuru were also not significantly different in number of seeds per pod. Highest number of pods was recorded for accessions collected from Thika, Meru and Nakuru.

Lablab accessions collected from different regions were significantly different in number of pods per plant (Table 4.4). Accession 36 collected from Nakuru-Bahati had the highest number of pods per plant seconded by accessions 24, 25, 26, 27, 28 (collected from Thika), 31 and 32 collected from Meru. Lowest number of pods per plant was recorded with accession number 37 and 38 collected from Naivasha while accessions 18, 19 and 20 collected from Machakos-Kathiani were the second lowest in number of pods per plant. Generally accessions grouped according to regions of collection based on 100 seed weight (Table 4.4). Accessions collected from different regions differed significantly in 100 seed weight. However, accessions collected from Thika, Meru and Nakuru-Lare were not significantly different. Accessions collected from Murang'a registered the highest seed weight while accessions collected from Naivasha had the lowest seed weight.

Accessions collected from same region were not significantly different in seed yield per plant but differed significantly with accessions collected from other regions (Table 4.4). However, accessions collected from Thika and Meru were not significantly different. Accession 36 collected from Nakuru-Bahati recorded the highest seed yield per plant, seconded by accessions 22 and 23 collected from Murang'a. Accessions 37 and 38 recorded the lowest seed yield per plant while the second lowest were accessions 18, 19 and 20 collected from Machakos-Kathiani. Generally, accessions collected from Machakos performed poorly in seed yield per plant.

**Table 4. 4 Means of nine quantitative reproductive traits of Lablab accessions.**

Acc	DaF	DuF	DaM	PR	RP	SP	PP	100 SW	SY
1	88.7a	16.1a	123.3a	8.8a	11.2a	3.63a	122.9a	24.5a	138.1a
2	88.7a	16.2a	123.3a	8.8a	11.1a	3.60a	122.9a	24.5a	138.2a
3	88.8a	16.3a	123.7a	8.7a	11.2a	3.57a	123.1a	24.6a	138.1a
4	88.7a	16.1a	123.3a	8.7a	11.1a	3.58a	123.2a	24.5a	138.0a
5	88.7a	16.3a	123.1a	8.8a	11.2a	3.60a	122.9a	24.6a	138.1a
6	88.6a	16.2a	123.3a	8.7a	11.1a	3.57a	123.0a	24.5a	138.2a
7	88.7a	16.3a	123.7a	8.8a	11.1a	3.60a	123.1a	24.5a	138.2a
8	88.8a	16.1a	123.4a	8.7a	11.2a	3.58a	123.3a	24.6a	138.1a
9	88.7a	16.2a	123.3a	8.8a	11.1a	3.57a	122.8a	24.5a	138.1a
10	88.8a	16.3a	123.7a	8.7a	11.2a	3.60a	122.9a	24.5a	138.0a
11	88.7a	16.1a	123.4a	8.6a	11.1a	3.57a	123.2a	24.6a	138.0a
12	88.6a	16.3a	123.3a	8.7a	11.2a	3.61a	123.0a	24.5a	138.1a
13	88.7a	16.2a	123.3a	8.7a	11.1a	3.60a	123.1a	24.6a	138.2a
14	121.3b	35.3b	192.6b	5.6b	9.8b	3.87b	65.8b	23.0b	63.2b
15	121.7b	35.7b	192.7b	5.6b	9.7b	3.85b	65.9b	23.1b	63.1b
16	121.3b	35.3b	192.5b	5.7b	9.8b	3.87b	65.8b	23.0b	63.2b
17	121.7b	35.3b	192.3b	5.6b	9.7b	3.47c	65.9b	23.1b	63.1b
18	121.3b	35.7b	192.1b	5.4b	9.8b	3.46c	60.3c	23.1b	56.0c
19	121.7b	35.3b	192.3b	5.5b	9.7b	3.47c	60.6c	23.0b	56.1c
20	121.7b	35.7b	192.7b	5.7b	9.8b	3.47c	60.3c	23.1b	56.0c
21	93.6c	16.5a	126.3c	6.6c	10.6c	3.83b	89.7d	22.0c	75.1d
22	98.7d	25.6c	152.3d	9.6d	13.3d	4.11d	135.7e	32.5d	188.1e
23	98.5d	25.7c	152.8d	9.7d	13.3d	4.11d	135.4e	32.6d	188.0e
24	98.3d	25.1c	152.7d	10.9e	13.4d	4.18d	146.4f	29.3e	181.2e
25	98.7d	25.3c	152.8d	10.8e	13.4d	4.20d	146.7f	29.4e	181.3e
26	98.3d	25.7c	152.7d	10.9e	13.2d	4.19d	146.9f	29.3e	181.2e
27	98.7d	25.2c	152.8d	10.8e	13.1d	4.19d	146.7f	28.4e	181.2e
28	98.7d	25.3c	152.7d	10.9e	13.1d	4.20d	146.4f	29.4e	181.3e
29	102.4e	25.6c	158.3e	9.5f	12.6e	3.88b	125.4g	28.6f	173.7f
30	102.7e	25.7c	158.7e	9.4f	12.6e	3.87b	125.6g	28.7f	173.8f
31	102.4e	25.2c	158.3e	10.7g	13.1d	4.16d	146.4f	29.3e	181.3e
32	102.5e	25.3c	158.3e	10.8g	13.1d	4.20d	146.3f	29.4e	181.4e
33	113.7f	31.1d	172.7f	10.1h	12.6e	4.10d	133.8h	29.3e	153.6g
34	113.4f	31.3d	173.3f	10.2h	12.5e	4.12d	133.6h	29.4e	153.7g
35	113.7f	31.1d	172.7f	10.2h	12.6e	4.13d	133.4h	29.3e	153.6g
36	91.3g	16.3a	125.7c	12.1i	13.4d	4.17d	162.6i	29.8g	203.5h
37	121.3b	44.7e	197.3g	4.7j	5.4f	2.83e	34.4j	19.7h	23.1i
38	121.2b	44.6e	196.7g	4.8j	5.4f	2.87e	34.5j	19.6h	23.0i
39	103.3e	27.7f	126.3c	9.2k	12.4e	3.60a	128.6k	27.7i	119.2j
40	103.1e	27.3f	125.7c	9.1k	12.3e	3.59a	128.5k	27.8i	119.2j
41	103.3e	27.6f	126.3c	9.2k	12.4e	3.60a	128.4k	27.7i	119.1j
42	103.2e	27.7f	126.6c	9.1k	12.3e	3.63a	128.5k	27.8i	119.2j
43	103.1e	27.5f	126.3c	9.1k	12.4e	3.62a	128.6k	27.7i	119.1j
44	103.2e	27.4f	126.5c	9.2k	12.3e	3.62a	128.4k	27.8i	119.2j
45	103.3e	27.7f	126.3c	9.1k	12.4e	3.63a	128.5k	27.8i	119.1j
Ra	36.0	31.0	66.0	7.4	6.87	1.4	128.4	13.0	180.5
Min	88.0	15.0	123.0	4.7	5.43	2.8	34.3	19.6	23.0
Max	124.0	46.0	189.0	12.1	13.30	4.2	162.7	32.6	203.5
GM	102.5	26.16	143.45	8.67	11.31	3.72	115.37	26.22	129.54
CV%	0.6	2.2	0.4	1.00	1.7	1.9	0.3	0.3	0.1

Key:

Means with similar letters are not significantly different at  $p \leq 0.05$ ; Acc - Accession; DaF - Days to 50% flowering; DuF - Duration of flowering; DM - Days to maturity; PR - Number of pods per raceme; RP - Number of racemes per plant; SP - Number of seeds per pod; PP - Number of pods per plant; 100 SW - 100 seed weight; SY - Seed yield; Ra - Range; GM - Grand mean.

#### **4.4.4 Lablab diversity for vegetative and seed characters**

Lablab accessions considered in this study differed significantly in days taken to germination in the field (Table 4.5). Germination in this case was considered as shoot emergence. Data was taken when each accession had 90% of seedlings having emerged. The forty-five accessions could be grouped into three categories according to days taken by seeds to germinate. Seed germination ranged between 11.7days to 17.7days. Accessions collected from Lamu, Machakos-Yatta, Mbeere and Mwingi were not significantly different in days taken by seeds to germinate. Similarly, accessions collected from Machakos (Kalama and Kathiani) and Nakuru-Naivasha were not significantly different in days to germination. Accessions collected from Machakos (Kalama and Kathiani) and Nakuru-Naivasha took the longest period of time to germinate. The third group comprised of accessions collected from Murang'a, Thika, Meru and Nakuru-Lare. These were also not significantly different in days taken by seeds to germinate. Accession 36 collected from Nakuru-Bahati was significantly different from all others and took the shortest time to germinate. In general, seed germination seemed to have a strong association with growth habit and colour of seed testa where accessions that exhibited an indeterminate growth habit took longer period to germinate. Similarly, accessions that had brown or dark brown seed testa took longer period to germinate as compared to accessions with black seed testa.

Lablab accessions were significantly different in plant height, leaf length and leaf width (Table 4.5). Plant height ranged from 122.7cm to 374.7cm while leaf length varied between 9.1cm and 13.1cm and leaf width ranged between 8.5cm and 11.6cm. Accessions collected from same localities were not significantly different. Accessions

collected from Nakuru-Naivasha recorded the highest plant height. However, these accessions did not record the highest leaf length and width. Accession 36 collected from Nakuru-Bahati recorded the shortest plant height and was also among the accessions with large leaf length and width. Accessions differed significantly in raceme length. However, accessions collected from Lamu, Machakos-Yatta, Murang'a, Thika, Meru and Nakuru-Bahati did not differ significantly. The longest raceme length was recorded for accessions collected from Machakos (Kalama and Kathiani) while the shortest was recorded for accessions collected from Nakuru-Naivasha. Accessions collected from Mbeere and Mwingi did not significantly differ in raceme length.

Lablab accessions differed significantly in pod length. Pod length ranged from 1.8 to 2.3cm (Table 4.5). However, accessions collected from Murang'a, Thika and Meru were not significantly different. Similarly, accessions collected from Mbeere and Meru were not significantly different in pod length. Shortest pod length was recorded for accessions 37 and 38 collected from Nakuru-Naivasha while the highest pod length was recorded for accession 36 collected from Nakuru-Bahati. Generally, there were no significant differences in pod width. Conversely, accessions collected from Nakuru-Naivasha were significantly different from all others and had the lowest pod width.

Based on seed length, Lablab accessions could be grouped into four classes, that is, those that had a seed length of 1.2cm, 1.1cm, 1.0cm and the smallest with 0.8cm. Accessions collected from Lamu and Machakos (Kalama and Kathiani) did not differ significantly in seed length. Similarly, accessions collected from Machakos-Yatta and Mwingi did not significantly differ and likewise those collected from Thika, Meru,

Mbeere and Nakuru (Lare and Bahati) did not significantly differ in seed length. Accessions collected from Murang'a recorded the highest seed length and differed significantly from all others. On the other hand, accessions collected from Nakuru-Naivasha recorded the lowest seed length and differed significantly from all the others.

Seed width ranged from 0.7cm to 0.9 cm (Table4.5). In general, there were no significant differences among accessions in relation to seed width. However, accessions 21 (collected from Machakos-Yatta) 37 and 38 collected from Nakuru-Naivasha had the shortest seed width of 0.7mm and were significantly different from all other accessions.



**Table 4. 5 Means of nine vegetative quantitative traits of Lablab accessions**

Ac	DG	PH	LL	LW	RL	NR	HL	PL	PW	SL	SW
1	14.3a	157.7a	9.2a	8.7a	32.9a	11.8a	3.8a	5.3a	2.2a	1.1a	0.8a
2	14.3a	160.3a	9.3a	8.6a	33.4a	11.6a	3.7a	5.4a	2.2a	1.1a	0.8a
3	14.7a	157.1a	9.1a	8.5a	33.0a	11.7a	3.8a	5.4a	2.2a	1.1a	0.8a
4	14.3a	159.2a	9.3a	8.6a	33.2a	11.6a	3.8a	5.3a	2.1a	1.1a	0.8a
5	14.3a	161.1a	9.2a	8.6a	33.2a	11.8a	3.9a	5.4a	2.2a	1.1a	0.8a
6	14.7a	156.3a	9.1a	8.5a	33.1a	11.5a	3.8a	5.3a	2.1a	1.1a	0.8a
7	14.3a	156.1a	9.2a	8.6a	33.4a	11.3a	3.7a	5.4a	2.2a	1.1a	0.8a
8	14.7a	160.0a	9.3a	8.7a	33.3a	11.6a	3.8a	5.3a	2.2a	1.1a	0.8a
9	14.3a	158.5a	9.1a	8.5a	33.1a	11.7a	3.7a	5.4a	2.2a	1.1a	0.8a
10	14.7a	159.7a	9.3a	8.6a	32.9a	11.4a	3.8a	5.4a	2.2a	1.1a	0.8a
11	14.3a	160.4a	9.1a	8.7a	33.2a	11.5a	3.7a	5.3a	2.2a	1.1a	0.8a
12	14.3a	160.0a	9.1a	8.6a	33.3a	11.6a	3.7a	5.3a	2.1a	1.1a	0.8a
13	14.7a	159.4a	9.2a	8.5a	33.2a	11.4a	3.8a	5.4a	2.1a	1.1a	0.8a
14	17.7b	351.1b	13.1b	11.5b	44.2b	9.6b	4.6b	4.6b	2.2a	1.1a	0.8a
15	17.5b	350.6b	12.9b	11.6b	44.1b	9.2b	4.7b	4.5b	2.2a	1.1a	0.8a
16	17.7b	348.8b	13.1b	11.6b	44.3b	9.7b	4.7b	4.6b	2.1a	1.1a	0.8a
17	17.4b	349.6b	12.9b	11.5b	44.0b	9.3b	4.6b	4.5b	2.2a	1.1a	0.8a
18	17.7b	347.3b	12.9b	11.4b	43.7b	9.7b	4.7b	4.4b	2.1a	1.1a	0.8a
19	17.5b	348.2b	13.0b	11.5b	43.8b	9.1b	4.6b	4.3bc	2.2a	1.1a	0.8a
20	17.7b	349.7b	12.9b	11.5b	43.8b	9.3b	4.7b	4.4b	2.2a	1.1a	0.8a
21	14.3a	159.1a	9.2a	8.5a	32.8a	11.7a	3.9a	4.3bc	2.2a	1.0b	0.7b
22	12.3c	225.7d	11.6c	9.3c	32.9a	11.2a	4.4c	5.5a	2.3ab	1.2c	0.9c
23	12.7c	222.3d	11.7c	9.3c	33.2a	11.3a	4.3c	5.6ad	2.3ab	1.2c	0.9c
24	12.3c	222.0d	11.8c	9.2c	32.9a	12.6c	4.2c	5.6ad	2.2a	1.1a	0.8a
25	12.0c	224.9d	11.8c	9.2c	33.0a	12.5c	4.3c	5.5a	2.2a	1.1a	0.8a
26	12.7c	222.3d	11.7c	9.1c	33.3a	12.5c	4.4c	5.5a	2.2a	1.1a	0.8a
27	12.3c	224.5d	11.9c	9.2c	33.2a	12.6c	4.2c	5.5a	2.1a	1.1a	0.8a
28	12.3c	224.8d	11.8c	9.1c	32.8a	12.7c	4.3c	5.5a	2.2a	1.1a	0.8a
29	14.3a	272.7e	12.6d	10.2d	29.8c	11.9a	4.7d	4.9e	2.2a	1.1a	0.8a
30	14.7a	273.2e	12.7d	10.3d	29.6c	11.8a	4.6d	5.0e	2.1a	1.1a	0.8a
31	12.7c	273.3e	12.7d	10.1d	32.9a	12.4c	4.5d	5.5a	2.2a	1.1a	0.8a
32	12.3c	271.4e	12.6d	10.3d	33.0a	12.3c	4.4d	5.6a	2.2a	1.1a	0.8a
33	12.3c	336.3f	12.8d	10.2d	36.7d	12.5c	4.5d	5.0e	2.1a	1.1a	0.8a
34	12.3c	335.9f	12.7d	10.2d	37.0d	12.4c	4.5d	5.0e	2.2a	1.1a	0.8a
35	12.0c	337.8f	12.8d	10.1d	37.3d	12.4c	4.4d	4.9e	2.2a	1.1a	0.8a
36	11.7d	122.7g	12.7d	10.2d	33.2a	13.8d	4.2e	5.5a	2.1a	1.1a	0.8a
37	17.7b	374.7h	12.3e	11.1e	25.3e	6.1e	4.8f	4.2bc	1.9c	0.8d	0.7b
38	17.7b	374.6h	12.3e	11.2e	25.2e	6.2e	4.9f	4.1bc	1.8c	0.8d	0.7b
39	14.0a	205.5i	12.7d	11.5b	29.8c	11.6a	4.4d	4.9e	2.2a	1.0b	0.8a
40	14.3a	203.6i	12.8d	11.6b	30.3c	11.7a	4.5d	4.8e	2.2a	1.0b	0.8a
41	14.0a	202.1i	12.9d	11.5b	30.4c	11.5a	4.4d	4.9e	2.1a	1.0b	0.8a
42	14.3a	200.6i	12.7d	11.5b	29.8c	11.7a	4.5d	4.8e	2.1a	1.0b	0.8a
43	14.3a	201.8i	12.8d	11.6b	30.1c	11.4a	4.5d	4.9e	2.2a	1.0b	0.8a
44	14.3a	201.6i	12.9d	11.4b	30.2c	11.6a	4.4d	4.9e	2.2a	1.0b	0.8a
45	14.0a	202.2i	12.8d	11.5b	30.3c	11.5a	4.5d	4.8e	2.2a	1.0b	0.8a
Ra	6.0	252.0	4.0	3.1	19.1	7.7	1.2	1.3	0.5	0.4	0.2
Mn	11.67	122.7	9.1	8.5	25.2	6.1	3.7	4.3	1.8	0.8	0.7
Mx	17.67	374.7	13.1	11.6	44.3	13.8	4.9	5.6	2.3	1.2	0.9
Gm	14.36	236.6	11.5	10.0	33.9	11.1	4.3	5.2	2.2	1.1	0.8
CV%	4.1	1.2	1.5	1.3	1.6	1.8	2.7	1.4	3.1	5.3	5.8

Key:

Means with similar letters are not significantly different at  $p \leq 0.05$ . Ac - Accessions, DG - Days to germination, PH - Plant height, LL - Leaf length, LW - Leaf width, RL - Raceme length, L - Pod length, PW - Pod width, SL - Seed length, SW - Seed width, Ra - range, Mn - minimum value, Mx - maximum value, Gm - Grand mean.

#### 4.4.5 Correlation analysis for yield and yield associated characters

Correlation components for seed yield and yield associated characters were determined for forty five Lablab accessions. A Pearson product-moment correlation was run to determine the relationship between the various agro-morphological components for seed yield and yield associated characters. Table 4.6 indicates that there was a significant positive correlation between seed yield per plant and yield associated traits such as pods per raceme, ( $r = 0.950$ ,  $p < 0.01$ ), racemes per plant ( $r = 0.873$ ), pods per plant ( $r = 0.937$ ), 100 seed weight ( $r = 0.912$ ), and seeds per pod ( $r = 0.741$ ). Other traits that correlated strongly were pods per plant and pods per raceme ( $r = 0.973$ ), racemes per plant and pods per raceme ( $r = 0.848$ ), 100 seed weight and pods per raceme ( $r = 0.853$ ), pods per plant and raceme per plant ( $r = 0.901$ ), seeds per pod and racemes per plant ( $r = 0.774$ ), 100 seed weight and pods per raceme ( $r = 0.853$ ), 100 seed weight and racemes per plant ( $r = 0.767$ ), 100 seed weight and pods per plant, 100 seed weight and seeds per pod ( $r = 0.641$ ).

There was a positive strong correlation at  $p < 0.01$  between seed yield per plant with number of nodes per raceme ( $r = 0.791$ ), pod length ( $r = 0.568$ ) and seed length ( $r = 0.438$ ). Seed yield per plant correlated positively with seed width ( $r = 0.196$ ) at  $p < 0.05$ . There was also strong positive correlation between plant height and hypocotyl length ( $r = 0.837$ ,  $p < 0.01$ ), leaf length and hypocotyl length ( $r = 0.914$ ,  $p < 0.01$ ) and leaf length and plant height ( $r = 0.712$ ,  $p < 0.01$ ). Days to germination, hypocotyl length, plant height, leaf length, leaf width and raceme length had a strong negative correlation with seed yield per plant. Both pod length and pod width correlated negatively with hypocotyl length, plant height and leaf length. Pod width and pod length had a strong positive correlation ( $r = 0.229$ ) at  $p < 0.01$  (Table 4.6).

**Table 4.6 Correlation matrix on yield and yield related characteristics of Lablab.**

	DaF	DuF	DM	PR	RP	PP	SP	Wt	SY
DaF	1								
DuF	0.933**	1							
DM	0.902**	0.833**	1						
PR	-0.607**	-0.433**	-0.540**	1					
RP	-0.568**	-0.515**	-0.457**	0.848**	1				
PP	-0.715**	-0.580**	-0.599**	0.973**	0.901**	1			
SP	-0.199*	-0.243**	-0.027	.604**	0.774**	0.641**	1		
Wt	-0.236**	-0.113	-0.220*	0.853**	0.767**	0.788**	0.667**	1	
SY	-0.501	-0.374**	-0.359**	0.950**	0.873**	0.937**	0.741**	0.912**	1

**Table 4.6 Continued**

	DG	HL	PH	LL	LW	RL	NR	PL	PW	SL	SW	SY
DG	1											
HL	0.302**	1										
PH	0.488**	0.837**	1									
LL	0.087	0.914**	0.712**	1								
LW	0.281**	0.876**	0.669**	0.928**	1							
RL	0.407**	0.195*	0.460**	0.177*	0.070	1						
NR	-0.706**	-0.407**	0.469**	-0.223**	-0.441**	-0.070	1					
PL	-0.398**	-0.750**	-0.707**	-0.696**	0.746**	-0.045	0.499**	1				
PW	0.030	-0.119	-0.102	-0.191*	-0.191*	0.079	0.027	0.229**	1			
SL	-0.516**	0.265**	0.011	0.414**	0.277**	-0.218*	0.278**	0.056	0.033	1		
SW	-0.098	-0.089	-0.134	-0.005	-0.075	0.145	0.220*	0.194*	0.053	-0.004	1	
SY	-0.875**	-0.368**	-0.538**	-0.221**	-0.458**	-0.339**	0.791**	0.568**	0.079	0.438**	0.196*	1

Key:

\*\* . Correlation is significant at the 0.01 level; \* . Correlation is significant at the 0.05 level; DaF - Days to flowering; DuF - Duration of flowering; DM - Days to mature pods; PR - Pods per raceme; RP - Racemes per plant; PP - Pods per plant; SP - Seeds per pod; Wt - 100 seed weight; DG - Days to germination; HL - Hypocotyl length; PH - Plant height; LL - Leaf length; LW - Leaf width; RL - Raceme length; NR - Nodes per raceme; PL - Pod length; PW - Pod width; SL - Seed length; SW - Seed width; SY - Seed yield.

#### 4.4.6a Cluster analysis on reproductive quantitative traits

The taxonomic dissimilarity matrix of reproductive quantitative traits for the 45 Lablab genotypes was employed for cluster analysis and a dendrogram was constructed (Figure 4.4a) using GenStat Statistical version 12. The 45 accessions were

largely divided into two clusters, A and B at dissimilarity coefficient 0.84. Cluster A comprised of 9 accessions and cluster B had 36 accessions. Cluster A was further divided into two sub-clusters A1 and A2 at dissimilarity coefficient 0.93 (Table 4.7a, Fig.4.4a). Sub-cluster A2 subdivided further into A2a and A2b at dissimilarity coefficient 0.99 to separate accessions collected from Machakos-Kalama (14, 15, 16, 17) in A2a and accessions collected from Machakos-Kathiani (17, 18, 19, 20) in A2b. Similarly, cluster B was further divided into sub-clusters B1 and B2 at dissimilarity coefficient 0.935. Sub-cluster B2 further subdivided into two sub-clusters (a, and b) at dissimilarity coefficient 0.95. Generally, accessions with similar traits were grouped together irrespective of regions of collection in the larger clusters but with further subdivisions, several accessions grouped according to places of collection.

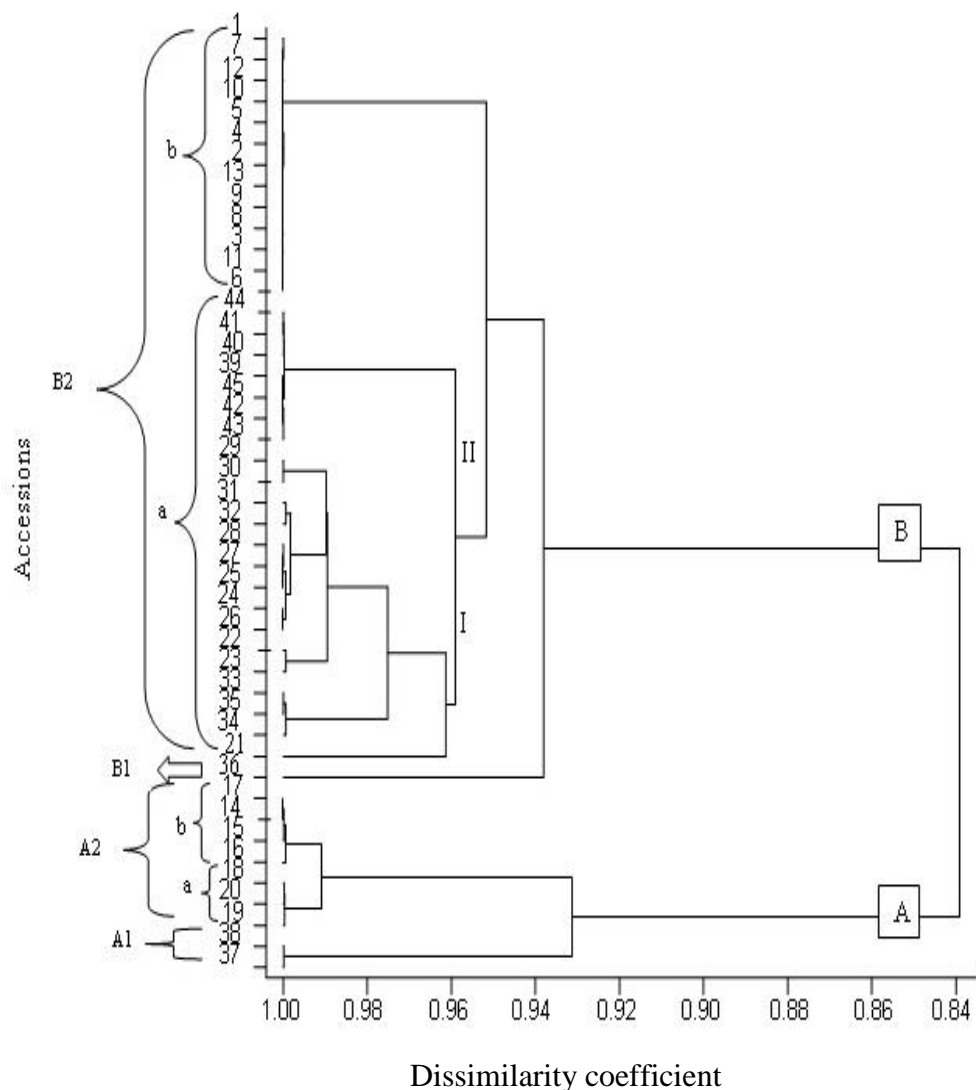
Cluster A comprised of accessions collected from Machakos-Kalama (14, 15, 16 and 17), Machakos-Kathiani (18, 19 and 20) and Naivasha (37 and 38). The Naivasha accessions had small sized seeds that had a black seed testa and produced purple flowers. They were the poorest performers for most traits considered in this study but had the highest plant height. They took long to germinate and mature and had the least yield per plant. Lablab accessions collected from Machakos (Kalama and Kathiani) had distinct pink flowers and dark brown seed testa. They took relatively long to mature and had low yield per plant (Table 4.4) compared to other accessions and were second poorest performers in the reproductive quantitative traits considered in this study. Cluster B1 was composed of one accession, 36, collected from Nakuru-Bahati. This accession had a black seed testa and purple flowers. It was the best performer in most of the traits evaluated. It recorded the highest yields per plant and it ranked second in time to germinate and mature.

Cluster B2 was the largest with 35 accessions of Lablab collected from Lamu (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13), Machakos-Yatta (21), Maragwa (22 and 23), Thika (24, 25, 26, 27 and 28), Mbeere (29 and 30), Meru (31 and 32), Nakuru-Lare (33, 34 and 35) and Mwingi (39, 40, 41, 42, 43, 44 and 45). Accessions collected from Lamu, Mbeere and Mwingi had brown seed testa and white flowers. Accessions collected from Machakos-Yatta, Murang'a, Thika, Meru and Nakuru-Lare had black seed testa and purple flowers. Accessions in this cluster performed above average in all traits under evaluation. This cluster further sub-divided into two, B2a and B2b (Table 4.7a, Fig. 4.4a).

Sub-cluster B2a was composed of accessions collected from Machakos-Yatta (21), Murang'a (22 and 23), Thika (24, 25, 26, 27 and 28), Mbeere (29 and 30), Meru (31 and 32), Nakuru-Lare (33, 34 and 35) and Mwingi (39, 40, 41, 42, 43, 44 and 45). Sub-cluster B2b was comprised of accessions collected from Lamu (1,2,3,4,5,6,7,8,9,10,11,12 and 13). B2a accessions were better performers in most traits as indicated in Table 4.4 but sub-cluster B2b had the shortest germination and maturity period. Sub-cluster B2a further divided into two distinct groups (I) and (II) at dissimilarity coefficient of 0.96 (Table 4.7a, Fig. 4.4a). Sub-cluster B2a (I) was composed of accessions 21, (collected from Machakos-Yatta), 33, 34, 35 (collected from Nakuru-Lare), 22, 23 (collected from Murang'a), 24, 25, 26, 27, 28 (collected from Thika), 31, 32 (collected from Meru) and 29, 30 (collected from Mbeere). The sub-cluster further subdivided at dissimilarity coefficient 0.99 to place the various accessions to their places of collection. Sub-cluster B2a (II) was composed of accessions 39, 40 41, 42, 43, 44 and 45 that were collected from Mwingi.

**Table4.7a Cluster distribution of Lablab accessions based on reproductive quantitative traits.**

Cluster	Sub-cluster	Dissimilarity coefficient	Number of entries	Accessions in cluster
A		0.84	9	14, 15, 16, 17, 18, 19, 20, 37, 38
	1	0.93	2	37, 38
	2	0.93	7	14, 15, 16, 17, 18, 19, 20
	2a	0.99	3	18, 19, 20
	2b	0.99	4	14, 15, 16, 17
B		0.84	36	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45
	1	0.935	1	36
	2	0.935	35	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45
	2a	0.95	22	21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45
	2b	0.95	13	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
	2aI	0.96	15	21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35
	2aII	0.96	7	39, 40, 41, 42, 43, 44, 45



**Fig.4.4a Dendrogram showing relatedness of *Lablab purpureus* accessions based on reproductive quantitative traits.**

#### **4.4.6b Cluster analysis on vegetative quantitative traits**

The relationship between the 45 *Lablab purpureus* accessions based on their vegetative quantitative traits was determined by cluster analysis and a dendrogram constructed (Figure 4.4b) using GenStat version 12 Statistical software. *Lablab purpureus* accessions distinctly separated into two clusters, A and B, at dissimilarity coefficient 0.825 (Table 4.7b, Figure 4.4b). Cluster A was composed of accessions 37 and 38 collected

from Nakuru-Naivasha. These accessions were indeterminate in growth habit, had purple flowers and black seed testa. The two accessions had the largest plant height, fewest seeds per pod, shortest pod length, and smallest seeds as indicated by seed length and width (Table 4.4). The rest of the accessions grouped together irrespective of localities of origin in cluster B. However, cluster B further subdivided into sub-clusters B1 and B2 at dissimilarity coefficient 0.850 (Table 4.7b, Figure 4.4b).

Cluster B1 was composed of accessions collected from Machakos-Kalama (14, 15, 16 and 17), and Machakos-Kathiani (18, 19 and 20). These accessions ranked second in plant height and had the longest racemes but with fewest number of nodes per raceme (Table 4.5). The accessions in this cluster were semi-climbers in growth habit, had distinct pink flowers and dark brown seed testa (Table 4.2).

Cluster B2 had 36 accessions collected from Lamu, Mwingi, Murang'a, Thika, Meru, Nakuru (Lare and Bahati) and Mbeere. The accessions were similar in pod width and seed width. Conversely, they subdivided further into two groups B2a and B2b at dissimilarity coefficient of 0.910 (Table 4.7b, Figure 4.4b). B2a was comprised of accessions collected from Murang'a (22 and 23), Thika (24, 25, 26, 27 and 28), Mbeere (29 and 30), Meru (31 and 32), Nakuru-Lare (33, 34 and 35) and Nakuru-Bahati (36). All these accessions apart from 36 were semi-climbers in growth habit. Accession from Mwingi, Mbeere and Lamu had a brown seed testa and white flowers while those from Murang'a, Thika, Meru and Nakuru-Lare had seeds with black seed testa and purple flowers. Though they had significant variability in most of the vegetative traits (Table 4.5) these accessions had similar seed length and seed width.

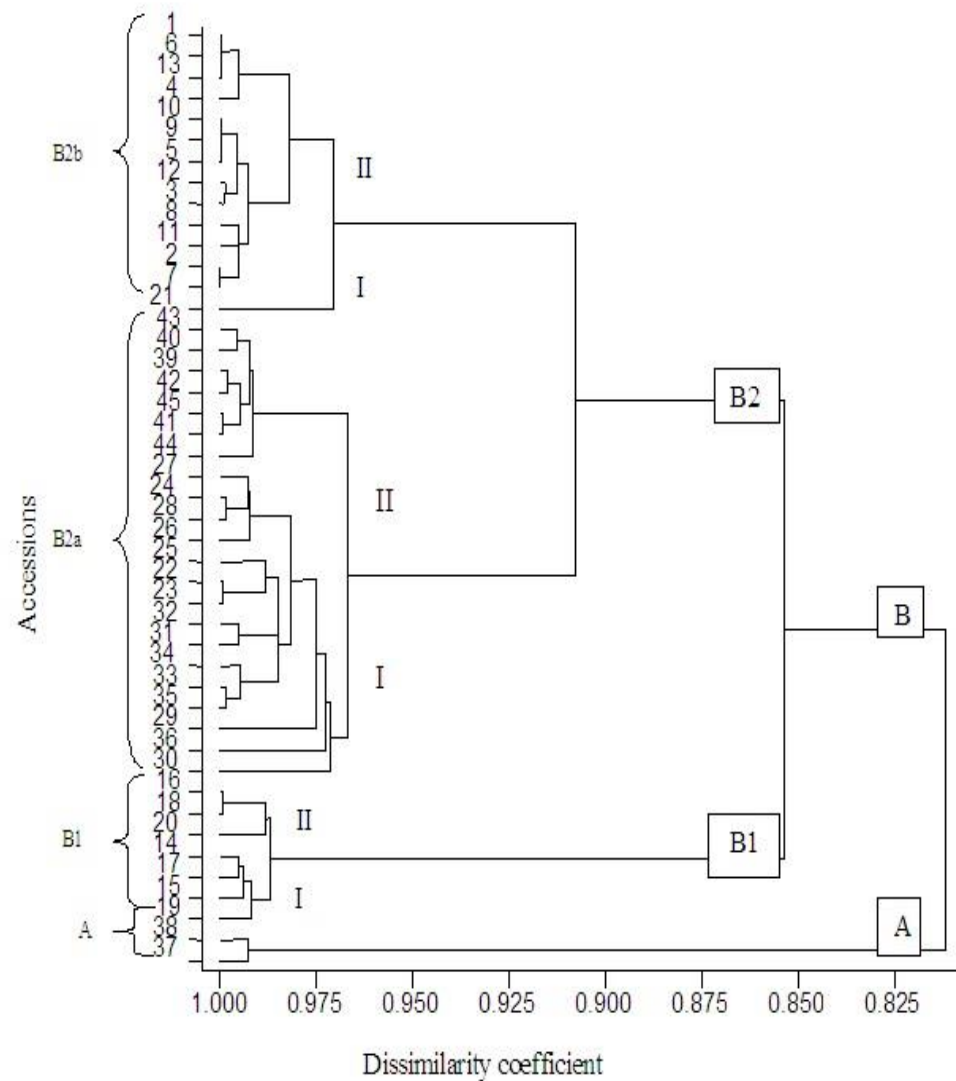
B2b was composed of accessions collected from Machakos-Yatta and Lamu.



Accession 21 from Machakos-Yatta had a black seed testa and purple flowers while accessions from Lamu had brown seed testa and white flowers. These accessions had similar plant height, leaf size, raceme length, number of nodes per raceme and length of hypocotyls (Table 4.5).

**Table 4.7b Cluster distribution of *Lablab purpureus* accessions based on reproductive quantitative traits.**

Cluster	Sub-cluster	Dissimilarity coefficient	Number of entries	Accessions in cluster
A		0.825	2	37, 38
B		0.825	43	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45
	1	0.850	7	14, 15, 16, 17, 18, 19, 20
	2	0.850	36	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45
	2a	0.910	22	22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45
	2b	0.910	13	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21
	2aI	0.970	15	22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35
	2aII	0.970	7	39, 40, 41, 42, 43, 44, 45
	2bI	0.975	1	21
	2bII	0.975	13	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13



**Fig.4.4b Dendrogram showing relatedness of Lablab accessions based on vegetative quantitative traits.**

#### 4.4.7 Principal component analysis (PCA) of Lablab reproductive traits

Four principal component (PC) axes made a substantial contribution to the total variation of the forty five Lablab accessions considered in this study. The first two PC axes described 98.67% of the total variation for the traits evaluated (Table 4.8). The first Eigen vector explained 85.23% of the total variation while the second described

13.44%. Three traits, that is, seed yield per plant, pods per plant and plant height loaded PC1. Days to 90% ripe pods contributed highly to variation in PC2 while raceme length, days to 50% flowering and duration of flowering loaded PC3. Two traits (seed yield per plant and pods per plant) that contributed greatly to PC1 re-featured in PC4.

**Table4.8 Eigen vectors and percentage variance of Lablab reproductive morphological traits.**

	Principal Component Axes			
	PC1	PC2	PC3	PC4
Eigen value	4.67	0.74	0.04	0.02
(%) variance per PC axis	85.23	13.44	0.78	0.47
% cummulative variance across PC axes	85.23	98.67	99.45	99.92
	<b>Eigen vector loadings for the traits</b>			
Days to 50% Flowering	-0.15	0.30	0.68	-0.02
Days to 90% ripe pods	-0.28	0.86	-0.33	0.22
Duration of flowering (days)	-0.09	0.19	0.54	-0.27
Pods per raceme	0.03	0.01	0.08	0.03
Pods per plant	0.53	0.05	0.26	0.79
Racemes per plant	0.03	0.02	0.02	0.06
Seed yield per plant	0.78	0.35	-0.11	-0.49
100 seed weight(g)	0.04	0.06	0.23	-0.12
Days to germination	0.01	-0.03	0.06	0.54
Leaf length	0.01	0.01	-0.06	-0.83
Plant height	0.92	0.39	-0.02	0.02
Pod length	0.00	0.01	0.02	0.09
Pod width	0.00	0.00	0.00	0.01
Raceme length	0.03	0.00	1.00	-0.08

## 4.5 Discussion

### Lablab diversity for agro-morphological traits

Germplasm characterization is an important component of breeding programmes for an effective and efficient management and utilization of plant genetic resources. Morphological markers have been used for assessment of relationships among plant genotypes and for estimating genetic diversity among germplasm lines (Rai *et al.*, 2010). In this study, Lablab accessions exhibited a wide variability in quantitative morphological traits such as plant height, days to flowering, duration of flowering, days to maturity, number of pods per plant, 100-seed weight and seed yield per plant. However, there was low variability for qualitative morphological traits such as growth habit, colour of main vein, hypocotyl colour, colour of flower keel, colour of flower standard, and colour of seed testa where the 45 accessions could be grouped into two or three classes.

Lablab accessions varied significantly in days to 50% flowering where the range was 36 days while minimum days to flowering was 88 and the accession with the longest period to flower took 124 days. The findings are in agreement with Savitha *et al.*, (2008) who reported a high phenotypic coefficient of days to 50% flowering in Lablab but contradicts with results by Basavarajappa and Byre Gowda, (2004) who reported a low phenotypic coefficient for the trait. Morphological characterization is affected by environmental influence and also subjectivity by the researcher. According to Singh *et al.*, (2012) the results of such studies are inconsistent, only relevant for genotypes used and environment involved and cannot be generalized. Therefore, contradictory and inconsistent results are expected. Wide variability was also observed in days to maturity where the range was 66 days and the accession that

had shortest period to mature took 123 days while the accession with the longest maturity period took 197 days. Seed yield was also variable among the accessions. The range was 180.7 grammes and the accession that recorded the least yield per plant had 22.8 grammes while the highest yielder had 203.5 grammes.

Wide variability was observed in number of pods per plant and 100 seed weight. The accession with least number of pods per plant recorded 34.3 and the highest number of pods per plant was 162.7 while the range was 128.4 and the mean was 113.4. The 100 seed weight ranged from 19.6 to 32.6 grammes with a mean of 26.2. Savitha *et al.*, (2008) reported low phenotypic coefficient of variability in dry seed weight and high phenotypic coefficient in number of pods per plant. Similar results were also obtained by Basavarajappa and Byre Gowda (2004). Savitha *et al.*, (2008) observed that wide variability in traits gives an ample opportunity to select genotypes according to the requirement or type of breeding programmes. A number of Lablab accessions used in this study could be selected for certain desirable traits.

Seed length and seed width showed little variability among accessions used in this study. Savitha *et al.*, (2008) observed a moderate phenotypic variability in seed width but a low phenotypic variability in seed length. The forty five Lablab accessions exhibited a wide variability in pod length but low variability in pod width. In a study with 114 accessions of Lablab collected from India, Savitha *et al.*, (2008) obtained a low variability in pod length and pod width. However, Mohan and Aghora (2006), observed high variability for Lablab pod characteristics.

Lablab accessions were variable in number of racemes per plant and raceme length.

The minimum mean number of racemes per plant was 5.4 and the maximum number was 13.3 while the range was 6.9 and a mean of 11.3. Raceme length varied from 25.2cm to 44.3cm with a range of 19.1 and a mean of 33.9. These results agree with Ali *et al.*, (2005) and Savitha *et al.*, (2008) who observed a high phenotypic coefficient for number of racemes per plant and moderate phenotypic coefficient for raceme length.

### **Correlation of yield and other agro-morphological traits**

A basic knowledge of interrelationship of certain plant characters with yield and correlation among themselves is an important topic for breeder to improve a complex character such as yield. Yield is an important and complex trait difficult to manipulate for crop improvement (Shi *et al.*, 2009), however traits such as seed number per plant, seed yield, pods number per plant and 100-seed weight could be correlated to other characters (Ozie, 2012). This will then allow an indirect selection of yield based on those characters. Seed yield is a final product of several components determined at different growth stages (Savitha *et al.*, 2008). In the current study, characters that exhibited a positive significant correlation with seed yield per plant were pods per raceme, pods per plant, racemes per plant, 100 seed weight, pod length, seed length and seeds per pod. Similar findings were also reported in pigeon pea (Bhadru *et al.*, 2010). In a study to evaluate eight cowpea varieties in Nigeria, Agbogidi and Egho, (2012) found strong association between plant height, leaf area and grain yield.

Other traits that associated strongly were pods per plant and pods per raceme, racemes per plant and pods per raceme, 100 seed weight and pods per raceme, racemes per plant and pods per plant, seeds per pod and racemes per plant, and 100 seed weight

and racemes per plant. Ouedraogo, *et al.*, (2008) found positive but low correlation between seed yield per plant and days to flowering ( $r = 0.06$ ) and 100 seed weight ( $r = 0.257$ ) on 310 accessions of bamba groundnut from Burkina Faso. Jonah *et al.*, (2010), reported high positive correlation between seed yield per hectare and pod yield per plant ( $r = 0.87$ ), seed yield per hectare and seed yield per plant ( $r = 0.91$ ) and between seed yield per plant and plant height ( $r = 0.77$ ) in 12 accessions of bamba groundnuts from Nigeria. They also identified a high correlation between pod length and pod width at ( $r = 0.89$ ) and seed length and seed width at ( $r = 0.82$ ), traits which are potentially useful for selecting genotypes with bigger seeds.

Seed yield per plant was negatively correlated with days to 50% flowering and days to 90% mature pods. This was in contrast with Sevin *et al.*, (2008) who reported a positive correlation between these traits. Other traits that exhibited negative association with seed yield per plant were duration of flowering, plant height, raceme length, leaf length and leaf width. In a study involving 48 Indian Lablab genotypes Rai *et al.*, (2011) reported that correlations between morphological traits showed that several seed traits were in significant positive correlation with pod characteristics. They also reported a positive correlation between seed yield per plant and number of seeds, number of pods, number of branches and 100 seed weight in Indian Lablab. Ali *et al.*, (2005) reported a positive correlation between seed yield per plant with pod width and a significant positive correlation between seed yield per plant with pod length in 20 Lablab from Bangladesh.

In the current study, seed yield correlated positively with pod width and significantly with pod length. Selection of best Kenyan Lablab accessions could be based on a

combination of traits such as days to maturity, seed yield per plant, 100 seed weight, number of pods per raceme, number of pods per plant, number of seeds per pod, number of racemes per plant, pod length, seed length and seed width. Similarly, Savitha *et al.*, (2008) reported that traits for consideration in the selection of best Indian Lablab genotypes were number of racemes per plant, number of pods per plant, seed width, seed weight and seed yield.

#### **Cluster analysis on quantitative traits**

Although cluster analysis grouped the 45 Lablab accessions used in this study into two distinct clusters with sub-clusters per each group, the accessions were found to differ significantly in one or more individual traits. Clustering was irrespective of localities of collection where genotypes collected from one locality also fell into other separate clusters. One cluster was composed of nine Lablab genotypes that had recorded high plant height, low number of pods per raceme, low number of racemes per plant, few seeds per pod and low seed yield per plant. This group also exhibited a semi-climber growth habit but accessions varied in seed testa colour and colour of flowers. The accessions were collected from different localities. The other cluster grouped thirty-six Lablab accessions together. This group generally was a better performer in most of the quantitative morphological traits evaluated. However, there were further sub-clustering to separate the genotypes on commonness in performance. Accession 36 separated from the group of thirty-six accessions to form a sub-cluster of its own. This accession recorded the highest in a number of traits such as seed yield per plant, number of pods per raceme and number of raceme per plant. The other sub-cluster was composed of 35 accessions where accessions 22 and 23 were the best



performers in terms of seed yield per plant. The two accessions recorded the highest 100 seed weight and seed length.

Rai *et al.*, (2010) observed two clusters of 30 Indian Lablab genotypes where 4 genotypes were placed in one cluster and 26 genotypes into another. The cluster with 26 was again divided into groups with 20 and 6 genotypes and the 20 genotypes were divided further into two groups of 11 and 9 genotypes. Tariqul, (2010) reported seven clusters of 88 Lablab accessions from Bangladesh where one accession was placed on its own cluster. The present study agrees with findings in other studies that accessions collected from the same geographic region may not group together (Sultana *et al.*, 2010; Tariqul, 2010).

### **Principal component analysis**

Principal component analysis showed that the first two principal component axes (PC1 and PC2) with a proportion of 85.23% and 13.44% respectively contributed more to the total variation of Lablab. In a study on characterization of common bean breeding lines, Atila *et al.*, (2010) reported that the first four principal component axes explained 81% of variability. According to Chahal and Gosal, (2002) characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with absolute values closer to zero.

In this study, characters having higher values in the first and second principal components were seed yield per plant that had an Eigen vector loading of 0.78, pods per plant with an Eigen vector loading of 0.53, plant height and days to 90% mature pods that had an Eigen vector loading of 0.92 and 0.86 respectively. The four traits contributed highly to the total diversity and were responsible for the differentiation of

clusters. Atila *et al.*, (2010) reported that seed number per pod, pod number per plant and pod length contributed to diversity in common bean lines. Agbolade *et al.* (2013) reported that in a study on morpho-vegetative variability of 24 miscellaneous legume species, the first and second principal components contributed 55.39% of the total variability. They further reported that number of stems per branch, internode length, rachis length and terminal leaflet length contributed highly to the variability.

## CHAPTER FIVE

### EVALUATION OF GENOTYPIC DIVERSITY OF LABLAB BEAN (*Lablab purpureus* (L.) SWEET) USING SIMPLE SEQUENCE REPEAT (SSR) MOLECULAR MARKERS

#### 5.1 Abstract

Estimation of genetic diversity in a crop species is prerequisite for its improvement. DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships precisely than other markers. Information on genetic diversity on Kenyan Lablab is scanty. Exchange of seed stock among farmers is a common practice in Lablab production. However, the level of genetic relatedness or dissimilarity of Lablab genotypes used by growers in different regions 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. Characterization was carried out in Biotechnology Institute, Kenya Agricultural and Livestock Research Organization (KALRO) molecular laboratory, Nairobi. Ten SSR primers were used and a total of 43 alleles were generated with a mean of 4.3 alleles per primer. Expected heterozygosity ranged from 0.23 to 0.46 and on average was 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 1.081 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 Nei's genetic identity and dendrogram based on unweighted pair group method with arithmetic averages (UPGMA).

## 5.2 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 and 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 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 these neglected crops in 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, 2010). The assessment of genetic diversity of introductions (accessions) is now a pivotal strategy for their successful and efficient preservation, *in situ* as well as *ex situ* (Van Tienderen *et al.*, 2002). Simple sequence repeats (SSRs) are the most suitable markers for the genetic assessment of germplasms 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.

### **5.2.1 Microsatellites: Simple Sequence Repeats (SSRs)**

Microsatellites, or simple sequence repeats (SSRs), are tandem di- to tetra-nucleotides sequence motifs flanked by sequences and are present in most eukaryotes genomes (Robinson *et al.*, 2004). They are codominant molecular markers that distinguish homozygotic and heterozygotic individuals (Shehata *et al.*, 2009). They arise due to slippage-like events occurring randomly in stretches of repetitive sequences. This makes microsatellite a more powerful genetic marker (Gupta and Varshney, 2000; Reusch, 2001). Microsatellites are mostly useful in comparative and association studies, genetic diversity, marker-assisted selection, population and evolutionary studies (Nunome *et al.*, 2006; Shi *et al.*, 2011). Since SSRs have a high variability, they are especially good at distinguishing closely related individuals (Kumar *et al.*, 2009). A number of microsatellites are now available for a wide range of crops, such as groundnut (*Arachis hypogaea*) (Cuc *et al.*, 2008), pigeonpea (*Cajanus cajan*) (Saxena *et al.*, 2010), bambara groundnut (Basu *et al.*, 2007), chickpea (*Cicer arietinum*) (Sethy *et al.*, 2003) and common bean (*Phaseolus vulgaris*) (Blair *et al.*, 2011).

### **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 it is 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).

The second advantage of the SSR marker system is the polymorphic genetic information contents. The hyper-variable nature of SSRs produces very high allelic variations even among very closely related varieties (Wang *et al.*, 2004). The third advantage has to do with the co-dominant nature of SSR polymorphisms. Although homoplasious bands can be misleading in scoring SSR profiles, the SSR bands produced from the same set of primers are intuitively orthologous. Homoplasia is a phenomenon wherein different copies of a locus are identical in state despite not being identical by descent (Estoup *et al.*, 2002). In SSR analysis, homoplasia 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 non-repetitive DNA (Varshney *et al.*, 2005). Genomic sites of SSR markers, derived from genomic libraries, fall into either the transcribed region (genic SSRs) or the non-transcribed region (genomic SSRs). The SSRs, derived from expressed sequence tags (ESTs) or cDNAs, are mostly genic SSRs, which have the potential for application in such areas as gene function characterization (Ronning *et al.*, 2003), association analysis for gene tagging (Szalma *et al.*, 2005; Shin *et al.*, 2006; Crossa *et al.*, 2007) and quantitative trait loci (QTL) analysis (Breseghello *et al.*, 2006; Zeng *et al.*, 2009).

### **5.3 Materials and Methods**

#### **5.3.1 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 (Table 5.2). 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.

#### **5.3.2 DNA Isolation**

One (1) gramme of leaf tissue was harvested when seedlings developed the first two fully grown leaves (Appendix 8). The leaf tissue was placed in eppendorf self

standing tubes (Appendix 11) each containing two ceramic beads. The tubes with beads and leaf tissue were placed in the geno-grinder machine (Benchtop homogenizer, Fast prep\*–24) (Appendix 10) which was set to run for one minute at 4 up-down movements per second (4M/S). A modification of the cetyltrimethylammonium 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 Sodiumchloride (NaCl), 50mM Ethylenediaminetetraacetic acid (EDTA), 2% Polyvinylpyrrolidone (PVP) and 10µl of 2% β-mercaptoethanol) was added to the leaf tissue and ground to form a slurry. The slurry was incubated at 65<sup>0</sup>C for 15 minutes in a water bath with constant shaking. It was then centrifuged at 13000 rpm for five minutes.

Six hundred microliters (600µl) of the supernatant were transferred into a fresh eppendorf tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The eppendorf tubes were shaken well before separating the contents in a centrifuge at 13000 rpm for 5 minutes. Five hundred microliters (500µl) of the aqueous phase was transferred into a fresh tube and an equal volume of chloroform: isoamyl alcohol (24:1) added. The tubes were shaken well and then centrifuged for 5 minutes at 13000rpm. Four hundred microliters (400µl) 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 500µl of 70% ethanol and spun for one minute before they were air dried for one hour. The dried pellets were re-suspended in 50µl of sterile distilled water. RNA was removed by adding two



microliters (2 $\mu$ l) of pancreatic ribonuclease A (RNase A) (10mg/ml) and incubating the samples for one hour at 37°C. The samples were stored at minus 20°C.

### **5.3.3 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) in a 0.8% agarose gel using 1x TBE buffer 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 Nanodrop spectrophotometer (Thermo Scientific NanoDrop 2000C). The concentrations were used to guide the normalization of DNA of each sample at a concentration of 20 ng/  $\mu$ L. Additionally, the ratio of OD 260/280 was provided by the Nanodrop 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.

### **5.3.4 PCR optimization**

PCR optimization was carried out using four selected DNA samples obtained from accessions 11 (brown seed testa, collected from Lamu), 53 (black seed testa, collected from Eastern), 97 (white seed testa, collected from Makueni) and 100 (dotted brown-black that was collected from Makueni). A pre-mix containing dNTPs (dATPs, dCTPs, dGTPs and dTTPs), MgCl<sub>2</sub>, Tris-HCl (pH 9.0), KCl and TaqDNA was used. A master mix containing 2 $\mu$ L of sterile distilled water, 0.5 $\mu$ L of 10pmoles forward primer, 0.5 $\mu$ L of 10pmoles reverse primer, 5 $\mu$ L premix and 2 $\mu$ L of template DNA was prepared. Amplification was carried out in a Thermocycler machine (Techne-TC 412, Applied Biosystems 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<sup>0</sup>C for 1 minute; extension at 72<sup>0</sup>C for 1 minute; final extension at 72<sup>0</sup>C for 10 minutes and final hold at 4<sup>0</sup>C.

The diluted DNA samples were subjected to polymerase chain reaction (PCR) in a thermocycler machine (Techne TC 412, Applied Biosystems Veriti systems) amplification using simple sequence repeat (SSR) markers. A total of 34 SSR markers specific for Lablab developed by Kamau in 2014 (not published) 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).

### **5.3.5 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 1.0 $\mu$ L 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*.

### 5.3.6 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 Genetic Analysis in Excel (GenAIEx) version 6.2 software (Peakall, and Smouse, 2006).

**Table 5.1 List of ten SSR primer pairs (specific for *Lablab purpureus*)**

Primer name	Primer Sequence		EPS (bp)	Tm°C	Repeat motif
	Forward 5'-3'	Reverse 5'-3'			
Lab T1	ACCAGAATGGTTT-CTCAAGTTCCT	GGTGAACCTTCCT-ACACCATGACT	273	56.1	(TA)7
Lab T2	GTGCGCGTCACTT-ATTAGTTCTTA	CAATATCTTCACG-TAACCACGGTA	224	54.6	(TATATC)7
Lab T3	CAGATCGATTGGT-AGCTGGATTTTC	CCTCCTTACAGAA-AGGGTAGCCTAGT	194	57.8	(TG)7
Lab T6	TCAATCGTTGTTG-GAAGAGGGTAT	GTCTCCTTCAACT-GTGTCCACTGA	187	57.5	(TGG)6
Lab T7	CAGCAGTGTTGCC-TCATACAGAAC	TGTACTTAGCCAA-GATCAGGCACA	123	57.5	(ATG)6
Lab T14	GGCATGGTGAAG-ATTGAAGAAGAG	AGAAGCAGAGGA-CAGGTGAATTGT	255	57.8	(GA)8
Lab T24	GATCAGCTCCAG-ACTGCTGACG	TAACCCTCCATTC-ATTGTCCATTC	202	58.5	(TC)7
Lab T25	GGGTTGAAGCTC-ACACAAATTCTT	CCAATGATGGTTG-TATGAGTAGCAC	126	57.4	(TGGT)5
Lab T28	CTTTCTCCATGCA-GACCAAATTC	CCTGTAAATAACT-GTCCTGGGAAGC	204	57.9	(ATG)6
Lab T33	CTAACCATGGCCT-TGAGTGGTACT	AATGAGTGAATG-CAGCAGTAG	345	57.3	(CTTTTC)5

Key: EPS - Expected product size; Tm°C - Annealing temperatures

### 5.3.7 Grouping of *Lablab purpureus* into populations

The 96 Lablab accessions were grouped as indicated in Table 5.2. According to the grouping there were 15 populations of Lablab. The grouping was based on regions of collection of accessions. 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.

**Table 5.2 *Lablab purpureus* populations based on regions of collection**

Pop	Name of pop	Place of collection	Accessions	Seed coat colour
1	Embu	Mbeere- Siakago	29, 30	Brown (29,30,76) Black (77)
		Embu market	76, 77	
2	Genebank Coast	Coastal region (conserved at the genebank of Kenya)	65, 66, 67, 68, 69	Brown (65, 66, 67, 68), Black (69)
3	Genebank Eastern	Eastern Kenya region (conserved at the genebank of Kenya)	46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 60, 61, 62, 63, 64	Brown (46, 48, 50, 54, 55, 56, 57, 58, 59, 60, 63, 64), Whitish brown (49), Black (47, 51, 52, 53, 61, 62)
4	Lamu	Lamu-Mpeketoni	1, 2, 3, 5, 6	All Brown
		Lamu-Kilimani	7, 8, 9, 10	
		Lamu- market	12, 13	
5	Machakos	Machakos- Lalama	14, 15, 16, 17	Dark brown(14, 15, 16, 17, 18, 19, 20) Black (21)
		Machakos- Kathiani	18, 19, 20	
		Machakos- Yatta	21	
		Machakos market	81	
		KARI- Katumani	96	
6	Makueni	Kibwezi market	80	Black (80, 99) White (98) Dotted brown-black(100)
		Makueni- Mwema	98, 99	

Table 5.2 Continued

Pop	Name of pop	Place of collection	Accessions	Seed coat colour
7	Meru	Meru- Abothoguchi	31	All black
		Meru- Mihirigamieru	32	
		Meru market	78	
		Meru- Mikinduri	94, 95	
8	Murang'a	Maragwa- Makuyu	22	Black (22, 83, 91) Brown (92)
		Murang'a market	82, 83	
		Murang'a-Gathenda	91	
		Mrang'a- Kahuro	92	
9	Mwingi	Mwingi- Central	39, 40, 41	All brown
		Mwingi- Migwani	45	
10	Nairobi	Nairobi region (conserved at the genebank of Kenya)	70, 71, 72	Black (70, 71, 87, 88, 97) Whitish brown (72)
		Nairobi, Nyamakima	87, 88	
		Namanga market	97	
11	Nakuru	Nakuru-Lare	33, 34, 35	All black
		Nakuru-Bahati	36	
		Naivasha-Maragushu	37, 38	
		Njoro market	86	
12	Nyeri	Nyeri market	84	All black
		Karatina market	85	
		Kirinyaga- Mwema	89	
		Kirinyaga- Kagio	93	
13	Rift Valley	From genebank of Kenya	73, 74	Brown (73, 74) Black (75)
		Eldoret market	75	
14	Thika	Thika-Kakuzi	24, 25, 26, 27	All black
		Thika-Municipality	28	
		Thika market	79	
		Thika-Ithaga	90	
15	Western	Bungoma market	101, 102	All black
		Kisumu market	104, 105	

Key: Pop-Population

## **5.4 Results**

### **5.4.1 Simple Sequence Repeats (SSRs) profiles on agarose gel**

The ten SSR primer pairs revealed polymorphism in Lablab accessions (Appendix 11). The molecular sizes of SSR amplicons ranged from 185 to 500 base pairs (bp). Locus SSR LabT3 had the highest molecular size of 350 to 500bp while SSR LabT7 had the least molecular size of 185 to 195 bp (Appendix12). The other SSR loci had a molecular size that ranged from 186 to 196 with the exception of SSR LabT33 that had a molecular size of 300 to 400 bp. The smallest difference between the highest and lowest values of allele size was 4 bp at loci SSR LabT6 and SSR LabT24. The largest difference of 100bp was detected at loci LabT3 and LabT33. The allele sizes scored at the other loci had differences between 6 and 10 bp (Appendix12).

### **5.4.2 Markers' effectiveness in detecting allele availability and polymorphism**

A total of 43 alleles were detected and all were polymorphic (Table 5.3). 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 5.3 Characteristics of the 10 Lablab SSR markers indicating major allele frequency, number of alleles, expected heterozygosity and polymorphism information content (PIC).**

Marker	Major Allele Frquency	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

#### 5.4.2 Genetic distance between populations of *Lablab purpureus*

The level of relatedness between the 15 populations of Lablab was established through a genetic identity matrix (Table 5.4a) and a genetic distance matrix (Table 5.4b) derived from the proportion of shared (common) loci (Nei, 1983), using Genetic Analysis in Excel (GenAlEx) version 6.2 software (Peakall and 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 1.081 between Mwingi and Western populations (Table 5.4b). 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's genetic 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 that had 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.

Lablab accessions exhibited a high level of unbiased genetic identity (Table 5.4a). Pairwise comparison of Nei's unbiased genetic identity among the 15 populations ranged from 0.338 between Mwingi and Western populations to 0.912 between Embu and Meru populations. Populations collected from Murang'a and Nyeri also revealed a high genetic identity of 0.886 followed by genebank collection from coastal region and population from Nairobi with a genetic identity of 0.882. Nakuru and Western Lablab populations displayed a low genetic identity of 0.381 (Table 5.4a).



**Table5.4a *Lablab purpureus* pairwise population matrix of Nei genetic identity matrix**

E	GC	GE	LM	MC	MK	ME	MU	MW	NA	NK	NY	RV	TH	WE
1.000														EM
0.7001	1.000													GC
0.7060	0.661	1.000												GE
0.6460	0.608	0.7461	1.000											LM
0.5410	0.491	0.7620	0.728	1.000										MC
0.6470	0.711	0.8000	0.763	0.6911	1.000									MK
0.9120	0.710	0.7320	0.703	0.5780	0.7081	1.000								ME
0.6690	0.632	0.8710	0.710	0.7900	0.8190	0.6901	1.000							MU
0.5850	0.508	0.7810	0.742	0.5640	0.7040	0.5730	0.7181	1.000						MW
0.6890	0.852	0.7220	0.648	0.5620	0.8450	0.7560	0.7040	0.6031	1.000					NA
0.7060	0.594	0.6200	0.591	0.4220	0.5940	0.6160	0.6500	0.7280	0.6531	1.000				NK
0.6810	0.587	0.8240	0.680	0.6610	0.8240	0.7990	0.8860	0.6830	0.7750	0.6291	1.000			NY
0.7600	0.656	0.7090	0.795	0.5190	0.6740	0.7970	0.5980	0.7920	0.7450	0.7130	0.698	1.000		RV
0.5230	0.562	0.7790	0.683	0.6990	0.6400	0.5280	0.7430	0.5200	0.6260	0.5060	0.677	0.509	1.000	TH
0.6170	0.582	0.6400	0.609	0.5340	0.6990	0.7140	0.5750	0.3390	0.6640	0.3810	0.614	0.521	0.555	1.000WE

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

**Table 5.5b** *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	1.081	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

#### 5.4.3 Genetic differentiation of *Lablab purpureus* populations

The extent of genetic differentiation was estimated between and within populations using F statistics of Wright, (1951). In this study the  $F_{ST}$  was generally low ranging from 0.188 to 0.399 with a mean of 0.270 (Table 5.5), implying that the *Lablab* subpopulations used in this study did not have much differences in allele frequencies. The  $F_{IS}$  values indicate that SSR loci LabT1, LabT2, LabT14 and LabT33 were not

heterozygous while SSR loci LabT3, LabT6, LabT7, LabT24, LabT25 and LabT28 were heterozygous. The NM range was 0.377 to 1.081 with a mean of 0.733. This indicates that there is high gene flow among the Lablab populations.

**Table 5.6 F-statistics and estimates of differentiation of *Lablab purpureus* populations for each locus.**

Locus	$F_{IT}$	$F_{ST}$	$F_{IS}$	NM
LabT1	0.244	0.188	0.069	1.081
LabT2	0.401	0.399	0.004	0.377
LabT3	0.019	0.248	-0.305	0.757
LabT6	0.296	0.381	-0.136	0.407
LabT7	0.106	0.204	-0.123	0.978
LabT14	0.332	0.274	0.079	0.661
LabT24	0.139	0.215	-0.096	0.914
LabT25	0.257	0.309	-0.074	0.560
LabT28	0.142	0.213	-0.090	0.926
LabT33	0.274	0.273	0.002	0.667
Mean	0.221	0.270	-0.067	0.733
SE	0.037	0.023	0.036	0.076

Key:  $F_{IT}$  - estimates correlation of genes within individuals over all populations;  $F_{ST}$  - estimates correlation of genes of different individuals in the same population;  $F_{IS}$  - estimates correlation of genes within individuals within populations; NM- coefficient for gene flow between populations

#### **Analysis of molecular variance (AMOVA)**

Population diversity components were partitioned using analysis of molecular variance (AMOVA) (Table 5.6). 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 (Appendix 16). Estimated

variance among populations was 1.567 and within population was 8.65 out of 10.225. The extent of differentiation ( $\Phi_{PT}$  = 0.153) among the population was observed showing the low extent of differentiation. The estimation of the variance components among and within populations using analysis of molecular variance (AMOVA) was significant ( $P \leq 0.01$ ).

**Table 5.7 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 ( $\Phi_{PT}$ )
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 ( $\Phi_{PT}$ ) - 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.

#### **Mean allelic analysis across *Lablab purpureus* populations**

Among the Lablab bean accessions analyzed for genetic diversity, accessions collected from Nairobi and Eastern regions of Kenya but conserved at the genebank of Kenya showed the highest Shannon diversity index of  $I = 0.96$  and  $I = 0.90$ , respectively, while accessions collected from Western and Nakuru exhibited the lowest index of 0.464 and 0.576 respectively (Table 5.7). However an evaluation of diversity based on expected heterozygosity demonstrated that the highest population diversity existed among the Lablab bean accessions where Nairobi showed the highest followed by Genebank Eastern, Meru, Makueni, Nyeri, Murang'a, Thika, Riftvalley,

Embu, Lamu, Machakos, Genebank Coast, Mwingi, Nakuru and finally Western which had the least diversity (Table 5.7). The analysis of allelic patterns across the 15 Lablab bean populations revealed that accessions from Nairobi and Genebank Eastern had the largest number of different alleles ( $N_a$ ) of 2.9 with highest number (2.31) of effective alleles ( $N_e$ ) observed in Genebank Eastern (Table5.7, Appendix 13).

**Table5.8 Mean number of different loci ( $N_a$ ), number of effective loci ( $N_e$ ) Expected heterozygosity ( $H_e$ ), Shannon index ( $I$ ) across the 15 *Lablab purpureus* populations.**

	EM	GC	GE	LM	MC	MK	ME	MU	MW	NA	NK	NY	RV	TH	WE
$N_a$	2.30	2.30	2.90	2.60	2.60	2.50	2.60	2.70	2.30	2.90	1.90	2.20	2.40	2.80	1.90
$N_e$	2.05	1.83	2.31	1.97	1.92	2.17	2.26	2.16	1.71	2.51	1.64	2.10	2.01	2.15	1.51
$H_e$	0.48	0.44	0.56	0.47	0.46	0.51	0.53	0.51	0.40	0.58	0.36	0.51	0.48	0.51	0.30
$I$	0.73	0.67	0.90	0.75	0.74	0.81	0.85	0.83	0.63	0.96	0.58	0.75	0.75	0.84	0.46

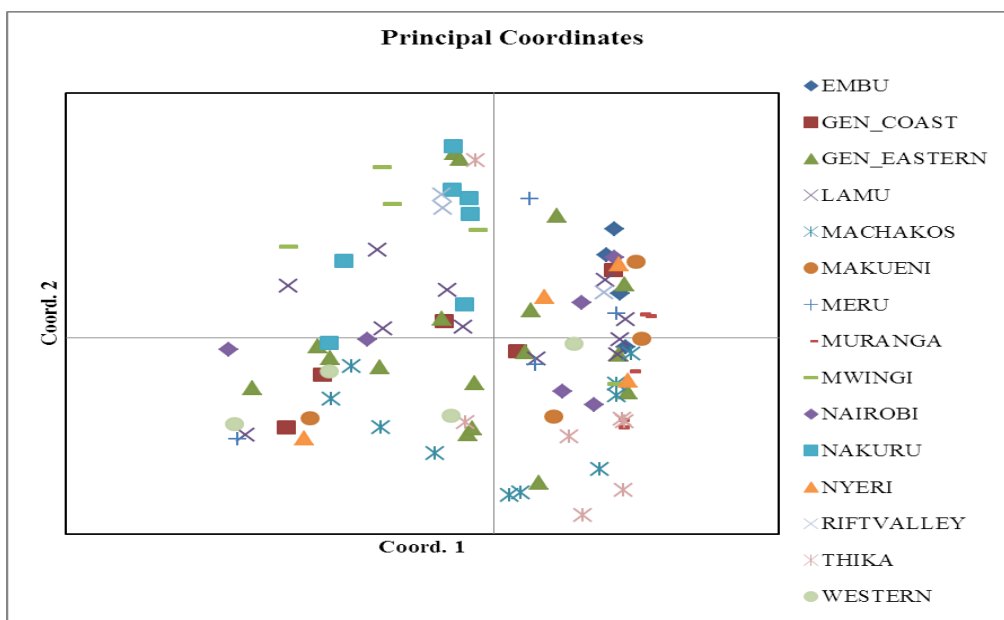
Key: EM - Embu; GC - Genebank Coast; GE - Genebank Eastern; MC - Machakos; MK - Makueni; ME - Meru; MU - Murang'a; MW - Mwingi; NA - Nairobi; NK - Nakuru; NY- Nyeri; RV - Rift Valley; TH - Thika; WE-Western.

#### 5.4.4 Principal coordinate analysis (PCoA)

The principal coordinate analysis was undertaken in order to confirm the clustering pattern obtained from unweighted pair group method with arithmetic averages (UPGMA) cluster analysis and exploit the resolving power of ordination. The 15 Lablab populations segregated with a high degree of overlap among them. However, Nairobi, Genebank Eastern, Machakos and Lamu populations depicted a higher degree of dispersion while Mwingi, Nakuru and Makueni had a low level of

dispersion. Evaluation of the 15 Lablab bean populations by principal coordinate analysis revealed 68.88% of the total variation existence, with the first principal component displaying 32.35% and the second displaying 54.64% variation (Appendix 17).

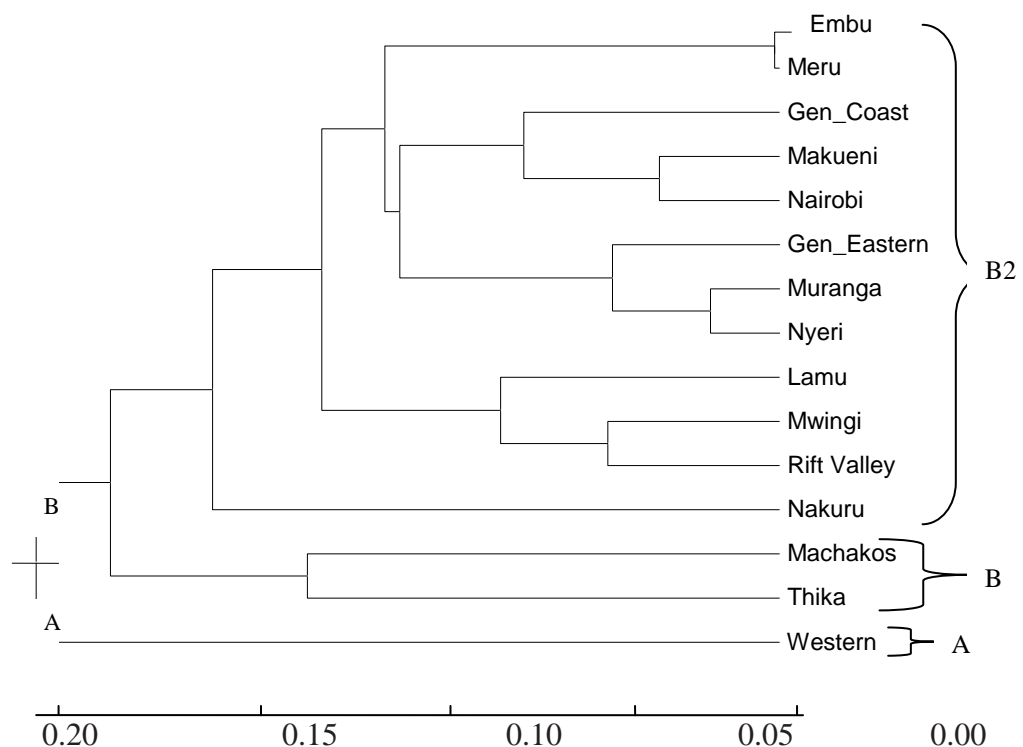
The PCoA for the 10 SSR markers clearly segregated the samples with no distinct sub-groups although the population diversity could be singled out between populations. Significant dispersion was observed especially for population from Nairobi, Lamu, Genebank Coast and Genebank Eastern while Nakuru and Mwingi populations clustered together with minimal dispersion (Fig.5.3).



**Fig.5. 1** Scatter plot showing the clustering pattern of 15 *Lablab purpureus* populations represented by different colours and symbols.

#### 5.4.4 Cluster Analysis

A dendrogram was constructed based on the similarity matrix data by applying unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the Genetic Analysis in Excel (GenAlEx) version 6.2 software (Peakall, and 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. 5.4). 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, Muranga, Nyeri, Gen\_Eastern, Nairobi, Makueni, Gen\_Coast, Meru and Embu populations (Fig.5.4).



**Fig.5. 2 Genetic relationship among 15 populations of *Lablab purpureus* using dendrogram based on Darwin's genetic identity distance.**

## 5.5 Discussion

### 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 and 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. Díaz *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. Cabral *et al.*, (2011), during characterization of common bean cultivars with SSR markers, found a PIC mean value of 0.50. In this study, a mean PIC value of 0.64 was obtained. The results showed that the SSR markers used were efficient in discriminating the Lablab populations.

### **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 1.081 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.

### **Genetic differentiation of *Lablab purpureus* populations**

The extent of genetic differentiation was estimated between and within populations using F statistics of Wright, (1951). Three F coefficients are generally used in genetic diversity studies. These are: ( $F_{IT}$ ) that estimates correlation of genes within individuals over all populations; ( $F_{ST}$ ) that estimates correlation of genes of different individuals in the same population and ( $F_{IS}$ ) that estimates correlation of genes within individuals within populations (Mohamad *et al.*, 2003).  $F_{ST}$  is a measure of genetic differentiation over subpopulations and is always positive.  $F_{ST}$  equals zero when subpopulations are identical in allele frequencies and one when they are fixed for different alleles.  $F_{ST}$  was generally high ranging from 0.188 to 0.399 with a mean of 0.270 implying that Lablab sub-populations used in this study had a lot of differences in allele frequencies. Kiambi *et al.*, (2005) suggested that an  $F_{ST}$  range of 0 - 0.05 indicates little differentiation, 0.05 – 0.15 moderate and 0.15 – 0.25 large while values above 0.25 indicate very large differentiation.

( $F_{IS}$ ) and ( $F_{IT}$ ) are measures of deviation from Hardy-Weinberg proportions within subpopulations and in the total population respectively, where positive values indicate a deficiency of heterozygotes and negative values indicate an excess of heterozygotes (Mohamad *et al.*, 2003). The  $F_{IS}$  values indicated that SSR loci LabT1, LabT2, LabT14 and LabT33 were not heterozygous. However, SSR loci LabT3, LabT6,

LabT7, LabT24, LabT25 and LabT28 were heterozygous. A high level of average heterozygosity correlates with high levels of genetic variation at loci. This has a critical importance for adaptive response to environmental changes.

The NM is a measure of gene flow (Ozie, 2012) and ranged from 0.377 to 1.081 with a mean of 0.733 implying that there was high gene flow among the Lablab populations. This could have come about due to movement of seeds from one place to another. A survey conducted in Lablab growing areas of Kenya indicated that a substantial percentage (20.4%) of Lablab growers obtained seed from neighbours and markets (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). Seed exchange for planting is common especially in crops that do not have structured seed certification programmes. For instance in Botswana (Brink *et al.*, 1996) reported that most farmers preferred to use the previous season's harvest as their seed stock and also exchanged bamba groundnut seeds with friends and family members. This movement of seeds across regions could have caused the high level of gene flow (NM = 0.733) observed in Lablab populations. Although Lablab is predominantly self fertilizing, there is some degree of cross pollination (Kukade and Tidke, 2014). Several other leguminous species like *Medicago trunculata* (Kamphius *et al.*, 2007), common bean (*Phaseolus vulgaris*) Tosti and Negri, (2005) and pigeonpea (*Cajanus cajan*) Songok *et al.*, (2010) are predominantly self pollinating but have also a low level of cross-pollination.

#### **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.* (2010) 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. Massawe *et al.*, (2003) found high levels (77.1%) of polymorphism among landraces and 28.7% within landraces using RAPDs markers. Wasike *et al.*, (2005) studied genetic diversity of 32 African and 9 Asian pigeonpea (*Cajanus cajan*) varieties, using AFLP and found that the analysis of molecular variance estimates between the two regions revealed a higher genetic variation of 92.16% within the populations while only 7.84% was among populations in the two regions. In cowpeas, Zannou *et al.*, (2008) also reported a higher percentage of variation within accessions (73%) as compared to among groups (26%). 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.

### **Mean allelic analysis across populations**

An evaluation of diversity based on expected heterozygosity demonstrated that the highest population diversity existed among the Lablab accessions from Nairobi followed by Genebank-Eastern, Meru, Makueni, Nyeri, Murang'a, Thika, Riftvalley, Embu, Lamu, Machakos, Genebank-Coast, Mwingi, Nakuru and finally Western in

that order. The analysis of allelic patterns across the 15 Lablab populations revealed that accessions from Nairobi and Genebank-Eastern had the largest number of different alleles ( $N_a$ ) of 2.9 with highest number (2.31) of effective alleles ( $N_e$ ) observed in Genebank-Eastern. The number of alleles is also referred to as allelic richness and is a measure of genetic diversity. A decrease in allelic richness could lead to a reduction in the population's potential to adapt to future environmental changes (Greenbaum *et al.*, 2015). In general Lablab exhibited a low allelic richness. Genetic variation within populations decreases as a result of selection for economically important traits. Genetic variation between and within populations is important as raw material for genetic improvement. Populations showing a great deal of variation would be able to adapt to changing environments whereas populations with less genetic variability would be less adaptable to sudden environmental changes.

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 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 and 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 and 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 and Prasanna, 2003). In previous studies, Kimani *et al.*, (2010) used amplified fragment length polymorphism (AFLP) markers to assay 50 Kenyan Lablab accessions and reported low diversity among them. Similarly, Shivash *et al.*, (2012) studied 13 Kenyan Lablab genotypes using SSRs specific for common bean and also reported low variability. Venkatesha *et al.*, (2007) used (AFLP) and randomly amplified polymorphic DNA (RAPD) markers to study the genetic diversity of Indian Lablab accessions and their results indicated that there was low genetic diversity among them.

Maass *et al.*, (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 subspecific taxonomic organization and also as cultivated and wild forms. The Kenyan Lablab revealed that the existing variations in cultivated forms had no geographic basis. Indeed, clustering of the accessions was not dependent on the geographical area of collection. Maass *et al.*, (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 Africa continents. Conversely, great diversity has also been reported for the wild forms from Africa (Maass *et al.*, 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 (Maass *et al.*, 2010), and these can also be included in the breeding programs to expand the genetic base of the Kenyan Lablab genotypes.

### **Principal coordinate analysis (PCA)**

The mathematic principal of PCA method lies in coordinate conversion and helps in exploring and visualizing similarities and dissimilarities of data. It is used to transform a set of original variables into a set of correlated variables (Zuur, 2007). The 15 Lablab populations generally segregated with a high degree of overlap among them. This indicated similarity among the populations. However, Nairobi, Genebank Eastern, Machakos and Lamu populations depicted a higher degree of dispersion while Mwingi, Nakuru and Makueni had a low level of dispersion. Evaluation of the 15 Lablab populations by principal coordinate analysis revealed 68.88% of the total variation existence, with the first principal component displaying 32.35% and the second displaying 54.64% variation. Somata *et al.*, 2009 evaluated the divergence between 39 lines of *Vigna unguiculata* using 48 pairs of SSR primers and observed that the first two principal coordinates accounted for 21.74% of the variation with the first principal coordinate accounting for 14.18% and the second accounting for 7.56%. Benchimol *et al.*, (2007) evaluated the divergence among 29 genotypes of dry beans using 87 SSR primer pairs and observed that the three first principal coordinates explained 45% of the total variation.



## 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 and Prasanna, 2003). Clustering pattern indicated a narrow genetic base of Lablab accessions. A dendrogram constructed on the basis of a genetic distance matrix and by unweighted paired group method with arithmetic averages (UPGMA), using GenAIEx version 6.2 software (Peakall, and 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 indicated 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 agro-morphological 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 throughout 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 populations 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.

## CHAPTER SIX

### GENERAL DISCUSSION

In Kenya, very few studies have focused on Lablab as a crop. A number of studies have concentrated on forage and soil improvement aspects of Lablab. Lack of information on adaptability and genetic diversity of Lablab has grossly hindered its improvement. To assess the adaptability potential and genetic diversity of Lablab with a view to identifying distinct genotypes to recommend to researchers and growers for further crop improvement, the following research objectives were formulated:

1. To assess the status of *Lablab purpureus* production in Kenya.
2. To evaluate the adaptability potential of *Lablab purpureus* to various agro-ecological environments in Kenya.
3. To assess the phenotypic diversity of *Lablab purpureus* grown in Kenya
4. To evaluate the genotypic diversity of *Lablab purpureus* grown in Kenya using simple sequence repeat (SSR) molecular markers.

#### **6.1 Assessment of the status of Lablab purpureus in Kenya**

To assess the status of Lablab production in Kenya, a reconnaissance baseline survey was conducted in Lablab growing areas of Kenya (Chapter2). Data was collected on different parameters such as cropping systems, cultural practices, yield, constraints to production and utilization of Lablab (Chapter 2). Results obtained from the survey revealed the status of Lablab growing in Kenya with reference to types of Lablab grown, farming practices, seed postharvest handling, utilization and source of seed by growers. Main challenges in Lablab production in Kenya were identified as; pests and diseases, unavailability of good quality seed, low yielding cultivars, cultivars that had

a long maturity period and lack of technical knowhow by farmers (Table 2.2; Table 2.3).

## **6.2 Evaluation of performance of Lablab under different agro-ecological environments**

In Kenya, there are no known Lablab genotypes for specific environments. Choice of Lablab genotypes to grow is based on colour preference and seed availability (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012) thus low yields are obtained. It is widely accepted that genotype (G), growing conditions (E) and their interaction (G x E) are key factors in optimization of phenotypic traits in agricultural crops. A genotype is considered to be more adaptive if it has high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments (Muhammad *et al.*, 2003). To evaluate the performance of Lablab genotypes under diverse environmental conditions, field trials of the forty five (45) accessions of Lablab collected during the survey (Chapter 1) were established in three locations with different agro-ecological environments (Chapter 3).

Significant differences and similarities among accessions were determined for seventeen quantitative traits related to vegetative, reproductive and yield and its components (Table 3.2). Generally, accessions collected from Lamu performed better in Bungoma than other accessions (Table 3.3). However, accession 36 also recorded a high seed yield in Bungoma. Accession 36 was the best performer across all the sites. Other accessions that recorded high seed yield per plant across the three sites were 22 and 23. Accession 36 was collected from Nakuru-Bahati while the two accessions 22 and 23 were sourced from Maragwa-Makuyu.

All accessions irrespective of places of collection recorded a shorter period of maturity in Bungoma while in Nakuru, all accessions took a long period to mature (Table 3.3). In Uasin-Gishu accessions were not significantly different from Nakuru in maturity period. In general, accessions collected from Lamu had the least days to maturity. Accessions 36 (collected from Nakuru-Bahati), 21 (collected from Machakos-Yatta) and accessions 39, 40, 41, 42, 43, 44 and 45 (collected from Mwingi) also recorded a short period to mature of about 120 days. Accessions 22 and 23 (collected from Murang'a-Makuyu), 24, 25, 26, 27 and 28 (collected from Thika) ranked third as regards maturity period which was about 147 days (Table 3.3).

Environmental effects were significant for all traits evaluated (Table 3.5). Accessions 22 and 23 (collected from Makuyu) and 36 (collected from Bahati) proved to have high yield potential and took a relatively short period to mature in Nakuru and Uasin-Gishu while accession 7 was the best performer in Bungoma (Table 3.3). The four accessions could therefore be adopted as suitable genotypes in the respective agro-ecological environments.

### **6.3 Assessment of Lablab phenotypic diversity**

There is much disagreements as to names and varieties of Lablab. It is not uncommon to find that morphologically similar cultivars do not bear the same name while cultivars bearing the same name may not be identical morphologically (Opong-Konadu *et al.*, 2003). This ambiguity in names necessitated the need to carry out a study on morphological characterization of Lablab. Forty five (45) accessions of Lablab collected during the survey (Chapter 1) were planted at Kenya Agricultural and Livestock Research Organization (KALRO), Nakuru-Njoro farm (Chapter 4).

Lablab accessions varied significantly in days to 50% flowering, duration to flowering and days to maturity (Table 4.4). For instance, accessions collected from Lamu (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13) were significantly different from all other accessions and similarly, accessions collected from Nakuru (33, 34, 35 and 36) and those collected from Mwingi (39, 40, 41, 42, 43, 44 and 45) were also significantly different from accessions collected from other regions. Lablab accessions were significantly different in plant height, leaf length and leaf width (Table 4.5).

Lablab accessions differed significantly in pod length. However, accessions collected from Murang'a, Thika and Meru were not significantly different. Similarly, accessions collected from Mbeere and Meru were not significantly different in pod length. Based on growth habit, pod colour, flower colour, seed testa colour and hypocotyl colour, Lablab accessions were not variable and could be grouped into three or four classes. In general, Lablab accessions had a wide diversity in quantitative traits and a narrow diversity in qualitative traits (Chapter 4).

#### **6.4 Evaluation of genotypic diversity of *lablab purpureus* using simple sequence repeat (SSR) molecular markers**

Information on genetic diversity on Kenyan Lablab is scanty. 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). DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships precisely than other markers (Singh *et al.*, 2012). Estimation of genetic diversity in a crop species is prerequisite for its improvement. Exchange of seed stock among farmers is a

common practice in Lablab production (Kamotho *et al.*, 2010; Kinyua and Kiplagat, 2012). However, the level of genetic relatedness or dissimilarity of Lablab genotypes used by growers in different regions has not been determined. In this study, ninety six (96) Lablab accessions comprising of the forty five accessions collected during the survey (Chapter 1) and additional forty one (41) collections from farmers and genebank of Kenya were characterized based on simple sequence repeat (SSR) molecular markers. Characterization was carried out in Biotechnology Institute, Kenya Agricultural and Livestock Research Organization (KALRO) molecular laboratory, Nairobi (Chapter 5).

Expected heterozygosity ranged from 0.23 to 0.46 and on average was 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 1.081 was found between Western and Mwingi populations while lowest genetic distance of 0.092 was found between Embu and Meru populations. Lablab populations exhibited a high level of relatedness as revealed by *Nei's* genetic identity and dendrogram based on unweighted pair group method with arithmetic averages (Chapter 5).

## CHAPTER SEVEN

### CONCLUSION AND RECOMMENDATIONS

#### 7.1 Conclusion

The most limiting constraints to Lablab production in Kenya include insect pests, diseases, poor seed quality, high cost of pesticides, poor soil fertility and lack of technical knowhow in crop husbandry by farmers. Based on seed coat colour two types of Lablab are commonly grown by farmers in Kenya. These are the black and brown seeded Lablab but each type exhibit colour shade variations among the accessions. However, other types also exist such as dotted and white colour seed coat but these are not popular.

Performance of Lablab accessions was affected by environment in which they were grown. Lablab accessions 36, 22 and 23 proved to have high performance potential and took a relatively short period to mature in site 1 and 2 while accession 7 was the best performer in site 3. The four accessions could therefore be adopted as useful resource for sustainable farming systems in the respective agro-ecological environments.

Morphological characterization revealed that Lablab genotypes grown in Kenya exhibit a high variability in such traits as days to 50% flowering, duration of flowering, days to maturity, number of racemes per plant, number of nodes per raceme, pod length, number of seeds per pod, days to germination, hypocotyl length, leaf length, leaf width, number of pods per raceme, raceme length, number of pods per plant, seed length, 100 seed weight, seed yield per plant and plant height.



However, genotypes were not variable in traits such as pod width, emerging cotyledon colour, number of seeds per pod, seed width, colour of flower keel, colour of flower standard, pod colour, main vein colour, seed testa colour and growth habit where accessions could be grouped into two or three classes. Principal component analysis indicated that traits that contributed highly to diversity were plant height, seed yield per plant, pods per plant and days to 90% mature pods.

On the basis of molecular characterization, Lablab genotypes grown in Kenya are of narrow genetic base and highly related. The low level of expected heterozygosity ( $H_e$ ), high Nei's genetic identity, high degree of dispersion as indicated by PCoA and clustering based on unweighted pair group method with arithmetic averages (UPGMA) revealed a high level of relatedness of Lablab grown in Kenya. The SSR analysis was successful in the estimation of genetic diversity among Lablab genotypes. The assayed marker loci had different capacities to discriminate assayed Lablab germplasm. The study found that Lablab 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 probably high heterosis. The results of this study are expected to benefit Lablab breeding efforts in Kenya as well as aid in conservation of Lablab germplasm.

## **7.2 Recommendations**

The following recommendations were made:

1. Lablab improvement programme in Kenya ought to focus on pest and disease resistance, high yielding cultivars, earliness in maturity, taste improvement and short-time to cook cultivars.

2. It is indispensable not only to embark on Lablab improvement but also to build capacity of growers by training them on crop husbandry practices such as cultivar choice, pest and disease control and seed storage practices that will maintain seed quality.
3. A greater number of sites (environments) and accessions need to be included in further studies so as to come up with distinct genotypes of Lablab for specific agro-ecological regions.
4. Accessions 22, 23 and 36 that performed highly could be recommended for adoption by farmers in the three environments studied while accessin 7 could be recommended for adoption in Site 3 (Bungoma).
5. A higher number of markers and a larger number of accessions collected from all parts of Kenya may be needed to design a genetic map for the Kenyan Lablab.
6. Genotypes from other countries in Africa, Asia, Europe and Australia could be introgressed to the Kenyan *Lablab purpureus* so as to create a wide genetic diversity.
7. The wild Lablab could be evaluated for diversity as it might offer some level of diversity to the cultivated type.
8. There is need to subject population number 15 (Western) to further agronomic and morphological characterization studies. Perhaps this population contains germplasm with unique alleles that determine desirable traits and thus could be used as a donor parent in breeding programmes.
9. Consequently, there is need for further selection in order to identify specific traits that could give added value to Lablab genotypes grown in Kenya. These

traits could be introgressed to desirable accessions by hybridization and backcross breeding method.

### **7.3 Suggestions for further studies**

Further studies need to focus on the following areas:

- Seed postharvest handling aspects.
- Seed quality aspects
- Seed maturity and harvest stages for maximum physiological maturity
- Agronomic, harvesting and postharvest practices that maximize quantity.
- Genetic diversity within accessions collected from same region, and
- Genetic diversity within accessions

## REFERENCES

- Agbogidi, O.M., and Egho, E.O. (2012). Evaluation of eight varieties of cowpea (*Vigna unguiculata* (L.) Walp) in Asaba Asfaw, agro-ecological environment, Delta State, Nigeria, *European Journal of Development*, (1) 2, 303-314.
- Addo-Quaye, A.A., Darkwa, A.A., and Ampiah, M.P. (2011). Performance of three cowpea (*vigna unguiculata* (L.) Walp) varieties in two agro-ecological zones of the central region of Ghana II: Grain yield and its components, *Journal of Agricultural and Biological Science*, Vol. 6, No. 2, February, 2011.
- Agbolade, J.O., Olakunle, T.P., Aina, D.A., Adeyemo, I.A., Aasa-Sadique, A. D., and Taiwo, J.O. (2013). Morpho-Genetic Vegetative Variabilities of Some Underutilized legumes. *International Journal of Engineering Science Invention* ISSN (Online): 2319-6734, ISSN (Print): 2319-6726 www.ijesi.org Volume 2 Issue 8, PP.71-79
- Ali, F., Sikadar, B., Roy, A.K., and Joarder, O.I. (2005). Correlation and genetic variation of twenty different genotypes of Lablab bean, *Lablab perupureus* (L.) Sweet. *Bangladesh J. Bot.*, 34 (2): 125-128.
- Ali, N., Javidfar, F., Elmira, J.Y., and Mirza, M.Y. (2003). Relationship among yield Components and selection criteria for yield improvement in winter rapeseed (*Brassic napus* L.). *Pakistan Journal of Botany*, v.35 (2), p.167-174.
- Amadou, H.I., Bebeli, P.J., and Kaltsikes, P.J. (2001), Genetic diversity in Bambara groundnut (*Vigna subterranea* L.) Germplasm revealed by RAPD markers, *Genome* 44: 995 -999.
- Asare, T.A., Gowda, S.B., Galyuon, A.I., Aboagye, L.L., Takrama, F.J., and Timko, P.M. (2010). Assessment of the genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm from Ghana using simple sequence repeat markers. *Plant Genetic Resources: Characterization and Utilization*, 8 (02):142-150.
- Asfaw, T., Assefa, B., Amsalu, K., Negash, F., Alemayehu, F., Gurum, Y., Rezene, C., Finenissa, M. and Chemed, D. (2008). Adaptation and Yield Stability of Small Red Bean Elite Lines in Ethiopia. *International Journal of Plant Breeding and Genetics*, 2: 51-63.
- Ascenso, J.C., Milheiro, A., Mota, M.I. and Cabral, M. (1981). Selecao preliminar da Mangueira. *Pesquisa Agropecuaria Brasileira*, 16:417-429.
- Atilla, D., Kamil, H., and Melek, E. (2010). Characterization of breeding lines of common bean as revealed by RAPD and relationship with morphological traits. *Pak. J. Bot.*, 42(6): 3839-3845, 2010.

- Azam-Ali, S.N., Aguilar-Manjarrez, J., and Bannayan-Avval, M. (2001). *A Global mapping system for Bambara groundnut production*, Food and Agriculture Organization of The United Nations, Rome.
- Balasubramaniun, P., Slinkard, A., Tyler, R., Vandenberg, A. (1999). Genotype and environment Effect on canning quality of dry bean in Saskatchewan. *Can J Plant Sci* 79:335-342.
- Basu, A.K., Samantha, S.K. and Sasmala, S.K. (2002). Genetic analysis for some seed parameters in Lablab bean. *Vegetables Science*. 29: 17-19.
- Basu, S., Roberts, J.A., Azam-Ali, S.N., and Mayes, S. (2007). Bambara groundnut. In: *Genomic mapping and molecular breeding in plants*, volume 3, Pulses, sugar and tuber crops (Eds. Kole C.), Springer, New York pp159 - 173.
- Bartel C., (2009). Enhancing food security in Africa through science, technology and innovation. *Summary of UNCTAD Technology and Innovation Report 2010. ATDF Journal* Volume 6, Issue 3 April 2009.
- Basavarajappa, P.S., and Byregowda, M. (2004). Assessment of field bean germ plasm of southern Karnataka and isolation of elite genotypes. *Mysore J. Agric. Sci.*, 38 (4): 474-479.
- Beaumont, M.A., Ibrahim, K.M.P., Boursot, and Bruford, M.W. (1998). *Measuring genetic distance* p.315 – 325. In A. Karp *et al.* (ed.).
- Beebe, S., Skroch, P., Tohme, J., Duque, M.C., Pedraza, F., and Nienhuis, J. (2000). Structure of Genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Science* 40: 264-273.
- Bechere, E., Belay, G., Mitiku, D., and Merker, A. (1996). Phenotypic diversity of tetraploid wheat landraces from northern and north-central regions of Ethiopia, *Hereditas* 124: 165-172.
- Benchimol, L.L., Campos, T., Carbonell, S.A.M., and Colombo, C. (2007). Structure of genetic diversity among common bean (*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. *Genet. Res. Crop Evol.* 54: 1747-1762.
- Bhadru, D., and Acharya, N.G. (2010). Studies on genetic parameters and interrelationships among yield and yield contributing traits in pigeonpea [(*Cajanus cajan* (L.) Mill sp)]. *Legume Res.*, 33 (1): 23 - 27.
- Biswas, M.D., Sanaullah, Z.M., Rahman, M.M. (2012). Assessment of genetic diversity in country bean (*Lablab purpureus* L.) using RAPD marker against photo-insensitivity. *J.Plant Develop.* 19 (2012): 65-71.

- Blair, W.M., Hurtado, N., Chavarro, C.M., Muñoz-Torres, C.M., Giraldo, M.E., Pedraza, F., Tomkims, J., and Wing, R. (2011). Gene-based SSR markers for common bean (*Phaseolus vulgaris* L.) derived from root and leaf tissues ESTs: an integration of theBMC series, *BMC Plant Biology* 11: 50.
- Boder, P., Deak, T., Bacso, R., Velich, I., Bisztray, G.D., Fascar, G., and Gyulai, P.(2006). Morphological and genetic investigation of medieval grape seeds. *Acta Horticulture (ISHS)* 713–718.
- Breseghele, F., and Sorrells, M.E. (2006). Association analysis as a strategy for improvement of quantitative traits in plants. *Crop Sci.*, 46, 1323–1330.
- Brink, M., Collinson, S.T., Andwigglesworth, D.J. (1996), Characteristics of bambara groundnut cultivation in Botswana, In: Proceedings of the International Bambara groundnut Symposium, 23 -25 July, University of Nottingham, UK pp. 133-142
- Cabral, P.D.S. and Lima, A.B.P. (2011). Genetic diversity in local and commercial Dry bean (*Phaseolus vulgaris*) accessions based on microsatellite markers *Genetics and MolecularResearch*, v. 10, pp. 140-149.
- Camacho Villa. T., Maxted N., Scholten M., and Ford-Lyod B., (2005). Defining and identifying crop landraces. *Plant genetic resources: Characterization and Utilization/* Volume 3/Issue 3, pp 373-384.
- Cameron, D. (1988). Tropical and subtropical pasture legumes. *Queensland Agricultural Journal*. March - April: 110-113.
- Cardoso, J., Silva, G., Rosa, J., Gazaffi, R., Marcal, J., Carbonell, S., Chiorato, A., Zucchi, M., Garcia, A., Benchimol, L. (2015).Developing a common bean core collection suitable for association mapping studies. *Genetics and molecular biology*, Vol. 38 No. 1ISSN 1415-4757.
- Casquero, P. A., Lema, M., Santalla, M., De Ron, A. M. (2006). Performance of common bean (*Phaseolus vulgaris* L.) landraces from Spain in the Atlantic and Mediterranean environments. *Genetic resources and Crop evolution*, August 2006, Volume 53, Issue 5, pp 1021-1032
- Chahal, G. S., and Gosal, S. S. (2002). *Principles and procedures of plant Breeding: Biotechnology and Conventional Approaches*, Narosa Publishing House, NewDelhi.604p
- Chemining'wa, G.N., Kitonyo, O. M., and Nderitu, J.H. (2014). Status, challenges and marketing opportunities for canning navy bean in Kenya.AJFAND Vol. 14. No. 5. August, 2014.
- Chiorato, A.F., Carbonell, S. A., Colombo, C.A., and Dias, L.A. (2005). Genetic diversity of common bean accessions in the germplasm bank of the

Instituto Agronômico-IAC. *Crop Breeding and Applied Biotechnology* 5: 1-9.

- Chipojola, F.M., Mwase, W.F., Kwapata, M.B., Bokosi, J.M., Njoloma, J.P. & Maliro, M.F. (2009). Morphological characterization of Cashew (*Anacardium occidentale* L.) in four populations in Malawi. *African Journal of Biotechnology*, 8:5173-5181.
- Chloupek, O. and Hrstkova, P. (2005). Adaptation of crops to environment. *Theor Appl Genet.* Nov; 11(7):1316-21.
- Cook, B.G., Pengelly, B.C., Brown S.D., Donnelly, J.L., Eagles D.A., Franco M.A., Hanson J., Mullen B.F., Patridge I.J., Peters M., and Schultze K. (2005). *Tropical forages: an interactive selection tool, Lablab purpureus*. CSIRO, DPI & F(Qld), CIAT, and ILRI, Brisbane, Australia.
- Crossa, J., Burgueno, J., Dreisigacker, S., Vargas, M., Herrera-Foessel, S.A., Lillemo, M., Singh, R.P., Trethowan, R., Warburton, M., and Franco J. (2007). Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics*, 177, 1889–1913.
- Cuc, M.L., Mace, E.S., Crouch, H.J., Quang, D.V., Long, D.T., and Varshney, K.R. (2008). Isolation and characterisation of novel microsatellites markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*), *BMC Plant Biology* 8: 55.
- Cui, Z., Cater, Jr. E.M., Burton, J. W., and Wells, R. (2001). Phenotypic diversity of modern Chinese and North American soybean cultivars, *Crop Science* 41: 1954-1967.
- Diaz, L. M. and Blair, M. W. (2006). Race structure within the Mesoamerican gene pool of common bean (*Phaseolus vulgaris* L.) as determined by microsatellite markers. *Theor. Appl. Genet.*, 114: 143-154.
- Diouf, D. and Hilu, K.W. (2005), Microsatellites and RAPDs markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal, *Genetic Resources and Crop Evolution* 52: 1057 – 1067.
- Ellis, J.R., and Burke, J.M. (2007). EST-SSRs as a resource for population genetic analyses, *Heredity* 99:125-132.
- Engels, J.M., and Visser, B. (2000). Strategies and methodologies in genetic diversity conservation. p. 26–31. In C. Almekinders and W. De Boef (ed.) *Encouraging diversity. The conservation and development of plant genetic resources*. Intermediate Technology Publ., London.

- Estoup, A., Jarne, P., and Cornuet, J.M. (2002). Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Mol. Ecol.*, *11*, 1591–1604.
- Fan, X.M., Kang, H., Chen, Y., Zhang, J., and Xu, C. (2007). Yield stability of maize hybrids evaluated in multi-environment trials in Yunnan, China. *Agron. J.* *99*; 220-228.
- FAO. (2012). Grassland species index. *Lablabpurpureus*. <http://www.fao.org/ag/AGP/AGPC/doc/Gbase/DATA/Pf000047.HTM> (Accessed on 10<sup>th</sup> May, 2015).
- Garcia, A.A.F., Benchimol, L.L., Barbosa, M.M.A., Geraldi, O.I., Souza, Jr. L.C., and De Souza, A.P. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genetics and Molecular Biology* *27*: 579 – 588.
- Gemechu, K. Mussa, J. Tezera, W., Getnet, D. (2005). Extent a pattern of genetic diversity of morpho-agronomic traits in Ethiopian highland pulse landraces I: Field pea (*Pisum sativum* L.) *Genetic Resources and Crop Evolution* *52*, 539 – 549.
- Ghafoor, A., Ahmad, Z., Qureshi, A.S., and Bashir, M. (2001). Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE, *Euphytica* *123*: 367-378.
- Gepts, P. (2006). Plant genetic resources conservation and utilization: The accomplishments and future of a societal insurance policy. *Crop Science*, *46*, 2278–2292.
- Gibert, O., Dufour, D., Giraldo, A., Sánchez, T., Reynes, M., Pain, J.P., González, A., Fernández, A. and Diaz, A. (2009). Differentiation between cooking bananas and dessert bananas. 1. Morphological and compositional characterization of cultivated Colombian Musaceae (*Musa* sp.) in relation to consumer preferences. *Journal of Agricultural and Food Chemistry*, *57*:7857-7869.
- Gnanesh, B.N., Sekhar, R.M., RajaReddy, K. and Eswara, N.P. (2006). Genetic variability, character association and path analysis of pod yield and its component characters in field bean (*Lablab purpureus* (L) sweet). *Geobios* *33*, 163-168, 2006.
- Greenbaum, G., Templeton A., Zarmi Y., Bra-David, S. (2015). Allelic richness following population foundation events-A stochastic modeling framework incorporating gene flow and genetic drift. DOI:10.1371/journal.pone.0115203.
- González-Chavira, M.M., Torres-Pacheo, I., Villordo-Pineda, E., and Guevara-Gonzalez, G.R. (2006). DNA markers, *Advances in Agricultural and Food Biotechnology* *6*: 99 – 134.



- Gupta, P.K. and Varshney, R.K. (2000), The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat, *Euphytica* 113: 163-185.
- Gupta, P.K., Rustgi, S., Sharma, S., Singh, R., Kumar, N., and Balyan, H.S. (2003). Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Mol. Genet. Genomics*, 270, 315–323.
- Hair, J.R., Anderson, R.E., Tatham, R.L., and Black, W.C. (1995). *Multi-variate data analysis with readings*. 4th edition, Prentice-Hall, Englewood Cliffs, NJ.
- Hedrick, P.W. (2005). *Genetics of Population*, 3<sup>rd</sup> Ed.; Jones and Bartlett Pub. Co: Sudbury, MA, USA.
- Hoogendijk, M., and Williams, D. (2001). Characterizing the genetic diversity of home garden crops: Some examples from Americas. *2nd International Home gardens workshop*, 17-19 July 2001, Witzenhausen, Federal Republic of Germany. pp. 34-40.
- Islam, F., Das, S.S., and Fakir, M.S. (2009). Effect of stage of seed maturity on seed germination and seedling growth in Lignosus bean (*Dipogon lignosus*). *Abst. Int. Conf. Quality Seed and Food Security*, 17-19 February, 2009, Bangladesh Agric. Univ. Mymensingh. p. 66.
- Jaccourd, D., Peng, K., Feinstein, D., and Kilian, A. (2001). Diversity Arrays: a solid state technology for sequence information independent genotyping, *Nucleic Acids Research* 29: 1 - 7 Kabir, J. and Sen, S., 1987b, Studies on genetic variability and heritability in Lablab bean. *Ann. Agri. Res.*, 8 (1): 141-144.
- Jonah, P.M., Adeniji, O.T. and Wammanda, D.T. (2010). Variability and genetic correlations for yield and yield characters in some bambara groundnut (*Vigna subterranea*) cultivars, *International Journal of Agriculture and Biology* 12: 303 – 307.
- Kala, K.B., Soris, T.P., Mohan V.R., and Vadivel, V. (2010). Nutrient and chemical evaluation of raw seeds of five varieties of lablab purpureus (L.) Sweet. *Advances in Bioresearch*, Vol.1 (1) June 2010:44-53.
- Kamotho, G.N., Kinyua, M.G., Muasya, R.M., Orwa, D.O., and Kimani, E.N. (2010). Abaseline survey on production, Utilization and Marketing Constraints of Lablab bean: Impact on Lablab Bean Improvement in Kenya, *International Journal of Professional Practice*, Vol.1, pp. 21-29.
- Kamphius, L.G., Williams, H.A., D'souza, K.N., Pfaff, T., Singh, B.K., Oliver, R.P. and Lichtenzweig, J. (2007), The *Medicago truncatula* reference accession A17 has an aberrant chromosomal configuration, *New Phytologist* 174:

229-303.

- Kang, M.S. (2002). Genotype-environment interaction: Progress and prospects. In: M.S. Kang (Ed.), *Quantitative genetics, genomics and plant breeding*, CAB International: Wallingford: England, p.221-243.
- Katungi, E., Farrow, A., Mutuoki, T., Gebeyehu, S., Karanja, D., Alamayehu, F., Sperling, L., Beebe, S., Rubyogo, J. and Buruchara, R. (2010). Improving common bean productivity: An analysis of socio-economic factors in Ethiopia and Eastern Kenya. *Baseline Report Tropical legumes II*. Centro Internacional de Agricultura Tropical – CIAT. Cali, Colombia.
- Khourya, C.K., Bjorkman, A.D., Dempewolf, H., Ramirez-Villegasa, J., Guarino, L., Jarvis, A., Rieseberg, L.H. and Struik, P.C. (2014). A look at 10 orphan crops and their benefits. Increasing Homogeneity in Global Food Supplies and the Implications for Food Security. *Proceedings of the national Academy of Sciences*: January 2014.
- Kimani, E.N., Wachira, F.N., and Kinyua, M.G. (2012). Molecular diversity of Kenyan lablab bean (*Lablab purpureus*(L.) Sweet) accessions using amplified fragment length polymorphism markers. *Am J Plant Sci*.3:313-321.
- Kinyua, M.G., and Kiplagat, O.L. (2012). *Lablab (Lablab purpureus L. Sweet) bean Improvement using mutation and biotechnological techniques*. Dansten Agencies, Nairobi, Kenya.
- Koile, S. and Chemining'wa, G. (2014). Effect of inorganic and organic fertilizers on growth and yield of Lablab lablab (*lablab purpureus*). Project report ,University of Nairobi. [www./ plantscience.uonbi.ac.ke/Lablab%bean%20final520prposal-web](http://www.plantscience.uonbi.ac.ke/Lablab%bean%20final520prposal-web). (Accessed 17<sup>th</sup>, 2015).
- Konstantinos, T., Koutira, O., Papadopouios, I.I., Tokatlidis, I.S., Tamoutsidis, E. G., Vasiliki, P.M., and Sotirious, M.K. (2008). Genetic diversity in bean populations based on Random Amplified Polymorphic DNA markers. *Biotechnology* 7 (1): 109, 2008.
- Kukade, S.A. and Tidke, J A., (2014). Reproductive biology of Lablab lablab L. (Fabaceae). *Indian Journal of plant Sciences*, Vol.3 (2) April-June, pp 22-25.
- Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. and Pandey, B.K. (2009). Potential of molecular markers in plant biotechnology, *Plant Genomics Journal* 2: 141 – 162.
- Kumaresan, D. and Nadarajan, N. (2010). Genotype X environment interactions for seed yield and its components in sesame (*Sesamum indicum* L.). *Electronic journal of plant breeding*. 1(4): 1126-1132 (July 2010).
- Kushwaha, U.K., Ghimire, S.K., Yadav, N.K. and Ojha, B.R. (2013). genetic relatedness of lentil (*Lens culinaris* L.) germplasm by using SSR markers.

*International Journal of Applied Sciences and Biotechnology*, ISSN:2091-2609.

- Lazrek, F., Roussel, V., Ronfort, J., Cardinet, G., Chardon, F., Aouani, M.E. and Hugnet T. (2009). The use of the neutral and non- neutral SSRs to analyse the genetic structure of a Tunisian collection of *Medicago truncatula* lines and to reveal association with eco- environmental variables, *Genetica* 135: 391 – 402.
- Leon, A.J., Andrade, and Lee, F.H. (2003). Genetic analysis of seed-oil concentration across generations and environments in sunflower. *Crop Sci* 43:135-140.
- Li, G., Ra, W.H., Park, J.W., Kwon, S.W., Lee, J.H., Park, C.B., and Park, Y.J. (2011). Developing EST-SSR Markers to study molecular diversity in *Liriopeand Ophiopogon*. *Biochem Sys Eco*.39:241-252.
- Lohr, S. (1999). *Sampling: Design and analysis*. Pacific Grove, California: Duxbury Press.
- Ma, K.H., Kim, N.S., Lee, G.A., Lee, S.Y., Lee, J.K., Yi, J.Y., Park, Y.J., Kim, T.S., Gwag, J.G., Kwon, S.J. (2009). Development of SSR markers for studies of diversity in common buckwheat. *Theor. Appl. Genet.* 119, 1247–1254.
- Maass, B.L., Jannadass, R.H., Hanson, J., and Pengelly, B.C. (2005). Determining sources of diversity in cultivated and wild *Lablab purpureus* related to provenance of accession by using amplified fragment length polymorphism. *Genetic Resources and Crop Evolution* 52: 683-695.
- Maass, B. L. (2006). “Changes in seed morphology, dormancy and germination from wild to cultivated hyacinth bean germplasm (*Lablab purpureus*: Papilionoideae)”, *Genet. Res. Crop. Evol.* 53, pp. 1127-1135.
- Maass, B.L., and Usongo, M.F. (2007). Changes in seed characteristics during the domestication of the lablab bean (*Lablab purpureus* (L.) Sweet: Papilionoideae) *Australian Journal of Agricultural Research* 58:9–19.
- Maass, B.L., Knox, M.R., Venkatesha, S.C., Tefera, T.A., Ramme, S., and Pengelly, B.C. (2010). *Lablab purpureus*- A crop lost for Africa? *Tropical Plant Biology* 3 (3):123-135.
- Makanda, I., Tongoona, P., Madamba, R., Icishahayo, D., and Derera, J. (2009). Evaluation of bambara groundnut varieties for the production in Zimbabwe, *African Crop Science Journal* 16: 175-183.
- Malik, M.F.A., Ashraf, M., Qureshi, A.F., and Ghafoor, A. (2007), Assessment of genetic variability, correlation and path analyses for yield and its components in soybean, *Pakistan Journal of Botany* 39:405 – 413.
- Mauricio, R. B., Elisabetta, G. and Francesco, C. (2015). Conserving landraces and improving livelihoods: how to assess the success of on-farm conservation projects?, *International Journal of Agricultural Sustainability*, 13:2, 167-182,

DOI: 10.1080/14735903.2014.986363

- Massawe, F.J., Schenkel, W., Basu, S. and Temba, E.M. (2003). Artificial hybridization in bambara groundnut (*Vigna subterranea* (L.) Verdc.) In. Proceedings of the International bambara groundnut Symposium, Botswana College of Agriculture, Botswana, 8 -12 August 2003 pp. 193 - 209.
- Masi, P., Zeuli, S.L.P. and Donni, P. (2003). Development and analysis of multiplex microsatellite marker set in common bean (*Phaseolus vulgaris* L.), *Molecular Breeding* 11: 303 – 313.
- McDonald, L.M., Wright, P. and MacLeod, D.A. (2001). Nitrogen fixation by lablab (*Lablabpurpureus*) and Lucerne (*Medicago sativa*) rotation crops in an irrigated cotton farming system. *Australian Journal of Experimental Agriculture* 41:219-225.
- Mekbib F. (2003). Yield stability in common bean (*Phaseolus vulgaris* L.) genotypes. *Euphytica*. 30: 147-153.
- Ministry of Agriculture Annual Report, (2014). *Annual Crops Report for 2014*. Ministry of Agriculture, Kenya.
- Mogbo, C., Okeke, T. and Akunne C., (2014). Studies on the resistance of cowpea seeds (*Vigna unguiculata*) to weevil (*Callosobruchus maculatus*) infestations. *American Journal of Zoological Research* 2.2(2014):37-40.
- Moghaddam, M.J., and Pourdad, S.S. (2011). Genotype x environment interactions and simultaneous selection for high oil yield and stability in rainfed warm areas rapeseed (*Brassica napus* L.) from Iran. *Euphytica*, DOI: 10.1007/s10681-011-0371-8.
- Mohan, N., and Aghora, T.S. (2006) Collection and evaluation of Lablab bean (*Lablabpurpureus* [L.] Sweet) Germplasm in Tamil Nadu, India. Poster presented at *Int. Conf. on Indigenous Vegetables and Legumes: Prospects for Fighting Poverty, Hunger and Malnutrition*. 12–15 Dec. 2006, Patancheru, India. [Online 05.11.2007 from: [www.ivs2006.org/PosterSession.pdf](http://www.ivs2006.org/PosterSession.pdf)]
- Moore, J., Liu J., Zhou, K., and Yu, L. (2006). Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J Agric Food Chem* 54:5313-5322.
- Mondini, L., Noorani, A. and Pagnotta M.A. (2009). Assessing plant genetic diversity by molecular tools, *Diversity* 1: 19 -35.
- Mohammadi, A.S., and Prasanna, B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci.* 43: 1235-1248.

- Mrdja J., Crnobarac, J., Radi, C., Mikli, C. (2012). Sunflower seed quality and yield in relation to environmental conditions of production region, *Helia*, 35, Nr. 57 pp.123-134. DoI: 10.2298/HEL1257123M.
- Muhammad, A., Bakhsh, A., Haqqani, A.M., and Bashir, M. (2003). Genotype-environment interaction for grain yield in chickpea (*Cicer arietinum* L.). *Pakistan J Botn* 35(2):181-186.
- Murphy, A.M., and Colucci, C.E. (1999). A tropical forage solution to poor quality ruminant diets: A review of *Lablab purpureus* Livestock Research for Rural Development (11) 2: 1999. <http://www.cipav.org.co/lrrd/lrrd11/2/colu.htm>. (Accessed on 15th April, 2015).
- Nagy, S., Poczai, P., Cernak, I., Gorgi, A., Hegedus, G. and Taller, J. (2012). PICcalc: An online programme to calculate polymorphic information content for molecular genetic studies. *Bioche. Genet.* 50: 670-672. DOI 10.1007/s10528-012-9509-1.
- Navabi, A, Yang, R.C, Helm, J., and Spawer, D.M. (2006). Can spring wheat growing mega-environments in the Northern great plain be dissected for representative locations or Niche – Adapted Genotypes? *Crop Science* 46, 1107 – 1116.
- Nei, M., and Chesser, R.K. (1983). Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.* 47:253–259.
- Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Ngailo, J.A., Kaihura, F.B.S., Baijukya, F.P., Kiwambo, B.J. (2003). Changes in land use and its impact on agricultural biodiversity in Arumeru, Tanzania. In: Kaihura F, Stocking M, editors. *Agricultural biodiversity in smallholder farms of East Africa*. Tokyo: United Nations University Press; 2003. pp. 145–158.
- Nunome, T., Negoro, S., Miyatake, K., Yamaguchi, H., and Fukuoka H. (2006). A protocol for the construction of microsatellite enriched genomic library, *Plant Molecular Biology Reporter* 24: 305 – 312.
- Ntundu, W.H., Bach, I.C., Christiansen, J.L. and Andersen, S.B. (2004). Analysis of genetic diversity in bambara groundnut (*Vigna subterranea* L. Verdc) landraces using amplified fragment length polymorphism (AFLP) markers, *African Journal of Biotechnology* 3:220- 225.
- Oram, R. (1990). *Lablab - Macrotyloma*. In Register of Australian Herbage Plant Cultivars 3<sup>rd</sup> Edition. CSIRO - Australia pp. 173-174.
- Osman, E.M. A., Nada, B. H. and Yasin, M. I.D. (2015). Agronomic and molecular evaluation of six lablab bean (*Lablab purpureus* L) cultivars. *International Journal of Scientific Research in Agricultural Sciences*, 2(1), pp 0007 – 0015.

- Ouedraogo, M., Ouedraogo, J.T., Tignere, J.B., Balma, D., Dabire, C.B. and Konate, G. (2008). Characterization and evaluation of accessions of bambara groundnut (*Vigna subterranea* L. Verdcourt) from Burkina Faso, *Science and Nature* 5: 191 – 197.
- Ouborg, N.J., Vergeer, P., and Mix, C. (2006). The rough edges of the conservation genetics Paradigm for plants. *Ecology*, 94, 1233–1248.
- Ozie, O. M. (2012). Genetic diversity and population structure analysis of bambara groundnut (*Vigna subterranea* (L.) Verdc.) Landraces using morpho-agronomic characters and SSR Markers. Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy, March 2012, Loughborough, Leicestershire, LE12, 5RD, UK
- Parzies, H.K., Spoor, W. and Ennos, R.A. (2000). Genetic diversity of barley landrace accession (*Hordeum vulgare* ssp. *vulgare*) conserved for different length of time in ex situ gene banks, *Heredity* 84: 476 – 486.
- Pasquet, R.S., Schwede, S., and Gepts, P. (1999). Isozyme diversity in Bambara groundnut, *Crop Science* 39: 1228-1236.
- Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Glaser, A.I., Channing, I.C. Welham, S.J., Gilmour, A.R. Thompson, R. and Webster, R. (2009). *GensStat release 12 Reference Manual, Part1 Summary*. VSN International, Hemel Hempstead.
- Peakall R. and Smouse P.E. (2006). GENALEX 6.2: genetic analysis in Excel. Population genetic software for teaching and research [http://www. anu. edu. au/BoZo/GenALEX /](http://www.anu.edu.au/BoZo/GenALEX/). *Molecular Ecology Notes* 6:288-295.
- Pennacchio, M.L.V., and Havens, K. (2010). Use and abuses of plant derived – smoke: its ethnobotany as hallucinogen, perfume incense and medicine. Oxford Univ. Press Inc., New York.
- Pengelly, B.C., and Lisson, S.N. (2003). Strategies for using improved forages to enhance production in Bali cattle. In: Entwistle K. and Lindsay D.R. (eds), Strategies to improve Bail cattle in eastern Indonesia. *Proceedings of a workshop 4–7 February 2002, Bali, Indonesia*. ACIAR Proceedings No. 110. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia. pp. 29–33.
- Purseglove, J.W. (1974). *Lablab niger*, In "Tropical Crops : Dicotyledons." London : Longman pp.273-276.
- Pierrier, X., and Jacquemoud, J.P. (2006). Darwinsoftware. [http://www. darwin. Cirad.fr/](http://www.darwin.Cirad.fr/)  
Darwin. (Accessed 15<sup>th</sup> April, 2015).
- Rai, N., Kumar, A., Singh, P.K., Singh, M., Datta, D., and Rai, M. (2010). Genetic relationship among Hyacinth bean (*Lablab purpureus*) genotypes cultivars

- from different races based on quantitative traits and random amplified polymorphic DNA marker *African Journal of Biotechnology* Vol. 9 (2), pp. 137-144.
- Rai, N., Singh, P.K., Rai, A.C., Rai, V.P. and Singh, M. (2011). Genetic diversity in Indian bean (*Lablab purpureus*) germplasm based on morphological traits and RAPD markers. *Indian Journal of Agricultural Sciences* 81 (9): 801–6,
- Reusch, B.T. (2001). New markers- old questions: population genetics of sea grasses, *Marine Ecology Progress Series* 211: 261-274.
- Reza, M, Armon, M, Shabani, A., and Daryaci, A. (2007). Identification of stability and adaptability in advanced durum genotypes using AMMI analysis. *Asian Journal of Plant Science* 6 (8), 1261 – 1268.
- Robinson, A.J., Love C.G., Batley, J., Barker, G., Edwards, D., (2004). Simple sequence repeat marker loci discovery using SSR primer bioinformatics ,20 (9): 1475-6.*Epub* 2004 Feb 12.
- Ronning, C.M., Stegalkina, S.S., Ascenzi, R.A., Bougri, O., Hart, A.L., Utterbach, T.R., Vanaken, S.E., Riedmuller, S.B., White, J.A., and Cho, J. (2003). Comparative analyses of potato expressed sequence tag libraries. *Plant Physiol.*, 131, 419–429.
- Savitha, B.N. (2008). *Characterization of avare (Lablab purpureus (L.) sweet) local collections for genetic variability*. Thesis submitted to the University of Agricultural Science Dharwad in partial fulfillment of the requirement for the degree of Master of Science (Agriculture) in genetics and plant breeding.
- Saxena, R.K., Prathima, C., Saxena, K.B., Singh, N.K., and Varshney, R.K. (2010). Novel SSR markers for polymorphism detection in pigeonpea (*Cajanus spp.*), *Plant Breeding* 129: 142 – 148.
- Schippers, R.R. (2000). *African indigenous vegetables: An overview of the cultivated species*, p 95. Chatham, UK: Central Avenue, Chatham Maritime.
- Schnable, P.S. and Springer N. M. (2013). Progress toward understanding heterosis in crop plants. *Annual Review of plant biology*, Vol.64:71-88. [www.doi.org/10.1146/annurev-arplant-042110-103827](http://www.doi.org/10.1146/annurev-arplant-042110-103827).
- Seehalak, W.P., Sommanas, W., and Srinives, P. (2009). Microsatellites markers for mungbean developed from sequence database, *Molecular Ecology Resources* 9: 862 – 864.
- Semagn, K., Bjornstad, and Ndjiondjop, M.N. (2006). An overview of molecular marker methods for plants. *Afr. J. Biotechnol.*, 5, 2540–2568.
- Sethy, K.N., Shokeen, B., and Bhatia, S. (2003). Isolation and characterisation of sequence- microsatellite site markers in chickpea (*Cicer arietinum*L.), *Molecular Ecology Notes* 3:428 – 430.

- Shean, C.M. (2012). Plant guide for lablab (*Lablab purpureus*). USDA-Natural Resources Conservation Service, Cape May Plant materials Center. Cape May, NJ. 08210. <http://plants.USDA.gov/> (Accessed on 15<sup>th</sup> June, 2015).
- Shehata, A.I., Al-Ghethar, H. A. and Al-Homaidan, A.A. (2009). Application of simple sequence repeats (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. *Saudi journal of Biological Sciences*. Volume 16, Issue 2, October 2009, Pages 57-62.
- Shibairo, S., Mutitu, E., Obukosia, S., Ngugi, E., Magomere, T. (2015). The population structure of wild sorghum species in agro-ecological zones of Western Kenya. [Erepository.uonbi.ac.ke/handle/11295/86434](http://erepository.uonbi.ac.ke/handle/11295/86434). (Accessed on 24<sup>th</sup> June, 2015).
- Shi, C., Navabi, A., and Yu, K. (2011). Association mapping of common bacterial blight resistance QTL in Ontario bean breeding populations, *BMC Plant Biology* 11: 52.
- Shivachi, A., Kiplagat, K.O., and Kinyua, G.M. (2012). Microsatellite analysis of selected *Lablabpurpureus* genotypes in Kenya. *Rwanda Journal*, ISSN 2305-2678, Volume 28, Series E, 2012: Agricultural Sciences, DOI: [http:// dx. doi. org/10 .4314/rj.v 28i1.3](http://dx.doi.org/10.4314/rj.v28i1.3) (Accessed 25th July, 2015).
- Sidlauskas, G., Bernotas, S. (2003). Some factors affecting seed yield of spring rapeseed (*Brassica napus* L.). *Agronomy Research*, v.1, p.229-243.
- Singh, S.P. (2001). Broadening the genetic base of common bean cultivars: a review. *CropScience* 41:1659-1675.
- Singh, S.K., Lavanyan, R.G., Bhat, K.V., Babu, G.S., Arya, L., Verma, M., Hussain, Z., Roy, S., Rathi, R.S., and Misra, A.K. (2012). Microsatellite markers revealed genetic diversity in mungbean mutant lines. *Indian Journal of hill Farming*, 25(1): 38-43.
- Shin, J.H., Kwon, S.J., Lee, J.K., Min, H.K., and Kim, N.S. (2006). Genetic diversity of maize Kernel starch synthesis genes with SNAPs. *Genome*, 49, 1287–1296.
- Skerman, P.J, Cameron, D.G., and Riveros, F. (1991). Leguminosas forrajeras tropicales. colección, *FAO: Producción y Protección Vegetal*, No. 2. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma.
- Spooner, D., Van Treuren, R., deVicente, C. (2005). Molecular markers for gene bank management. *IPGRI Technical Bulletin* No.10. Rome, Italy: International Plant Genetic Resources Institute.
- Somata, P., Sommanas, W., and Srinives, P. (2009). Molecular diversity assessment of AVRDC the World vegetable center elite-parental mungbeans. *Breed. Sci.* 59: 149-157.



- Songok, S., Ferguson, M., Muigai, A.W. and Silim, S. (2010). Genetic diversity in pigeonpea (*Cajanus cajan* (L.) Mills.) Landraces as revealed by simple sequencerepeat markers, *African Journal of Biotechnology* 9: 3231 – 3241
- Stevens, J.M. (2012). Bean, hyacinth - *Dolichos lablab* (L.) or *Lablab purpureus* (L.) Sweet. Institute of Food and Agricultural Sciences (IFAS) Univ. of Florida Extension. <http://edis.ifas.ufl.edu/mu019>. (Accessed on 10th August, 2015).
- Stuart, G. (2011). Stuartxchange - Philippine alternative medicine. [http://www.stuartxchange.org/Alt Med. html](http://www.stuartxchange.org/Alt%20Med.html), (Accessed 10<sup>th</sup> August, 2015).
- Sultana, N. Ozaki, Y. Okubo, H. (2001). Morphological and physiological variation in lablab bean (*Lablab purpureus* (L.)Sweet). *Journal of the Faculty of Agriculture, Kyushu University* 45.(2):465-472.
- Szalma, S.J., Buckler, E.S., Snook, M.E., and McMullen, M.D. (2005). Association analysis of candidate genes for maysin and chlorogenic acid accumulation in maize silks. *Theor. Appl. Genet.*, 110, 1324–1333.
- Tariqul, I. (2010). Morpho-agronomic diversity of hyacinth bean (*Lablab purpureus* (L.) Sweet) accessions from Bangladesh. *PGR Newsletter, FAO Bioversity*. Issue No.156, pp 73-78.
- Tosti, N. and Negri, V. (2005). On-going on-farm microevolutionary process in neighbouring Cowpea landraces revealed by molecular markers, *Theoretical and Applied Genetics* 110: 1275 – 1283.
- Valenzuela, H., and Smith J. (2002). Sustainable agriculture green manure crops. SA-GM-7. Cooperative Extension Service, College of Tropical Agric. And Human Resources, Univ. of Hawaii at Manoa. [http://www.ctahr.hawaii.edu/oc/freepubs/pdf/ Green Manure Crops /lablab.pdf](http://www.ctahr.hawaii.edu/oc/freepubs/pdf/Green%20Manure%20Crops/lablab.pdf) (accessed 14 July, 2015).
- Van Zonneveld, M., Dawson, I., Thomas, E., Scheldeman, X., van Etten, J., Loo, J., & Hormaza, J. I.(2014).Application of molecular markers in spatial analysis to optimize in situ conservation of plant genetic resources. In R. Tuberosa, A. Graner, and E. Frison (Eds.), *Genomics of plant genetic resources* (pp.67 – 91). Dordrecht: Springer.
- Van Tienderen, P.H., De Haan, A.A., Van der Linden, C.G., and Vosman, B. (2002). Biodiversity assessment using markers for ecologically important traits. *Trends Ecol. Evol*, 17, 577–582.
- Varshney, R.K., Close, T.J., Singh, N.K., Hoisington, D.A., and Cook, D.R. (2009). Orphan legume crops enter the genomics era. *Plant Biol.* 2009 Apr, 12 (2): 202 - 10.
- Varshney, R.K., Graner, A., and Sorrells, M.E. (2005). Genic microsatellite markers in plants: Features and applications. *Trends Biotechnol.* 23, 48–55.

- Vargas, M. Crossa, J, Van Eeuwijk, F., Sayre, K.D. and Reynolds, M.P. (2001). Interpreting treatment x environment interaction in agronomy trials. *Agronomy Journal* 93, 949 – 960.
- Venkatesha, S.C., Byre Gowda, M., Mahadevu, P., Mohan Rao, A., Kim, D. J., Ellis, T. H. N., and Knox, M. R.( 2007). “Genetic diversity within *Lablabpurpureus* and the application of gene specific markers from a range of legume species”. *Plant Genetic Resour.*5 (3), pp. 154 -171.
- Vigouroux, Y., Barnaud, A., Scarcelli, N., and Thuillet, A. C. (2011a). Biodiversity, evolution and adaptation of cultivated crops. *Comptes Rendus Biologies*, 334, 450–457.
- Virk, P.S., Newbury, H. J., Jackson, M. T. and Ford-Lloyd, B. V. (2000). Are mapped or anonymous markers more useful for assessing genetic diversity? *Theoretical and Applied Genetics*. 100: 607–613.
- Visscher, P.M., Hill, W G and Wray, N. R. (2008). Heritability in the genomics era—concepts and misconceptions. *Nature Reviews Genetics* 9, 255-266 (2008) doi:10.1038/nrg.2322.
- Wang, X., Rinehart, T.A., Wadl, P.A., Spiers, J.M., Hadziabdic, D., Windham, M.T., and Trigiano, R.N. (2009). A new electrophoresis technique to separate microsatellite alleles, *African Journal of Biotechnology* 8: 2432 – 2436.
- Wang, M.L., Gillaspie, A.G., Newman, M.L., Dean, R.E., Pittman, R.N., Morris, J.B., Pederson, G.A. (2004). Transfer of simple sequence repeat (SSR) markers across the legume family for germplasm characterization and evaluation. *Plant Genet Resour* ; 2(2):107–119. doi: 10.1079/PGR200441.
- Wasike, S., Okori, P. and Rubaihayo, P.R. (2005). Genetic variability and relatedness of the Asian and African pigeonpea as revealed by AFLP, *African Journal of Biotechnology* 4: 1228 – 1233.
- Wren, J.D., Forgacs, E., Fondon, J.W., Pertsemlidis, A., Cheng, S.Y., Gallardo, T., Williams, R.S., Shohet, R.V., Minna, J.D., and Garner, H.R. (2000). Repeat polymorphisms within regions: Phenotypic and evolutionary implications. *Am. J. Hum. Genet.* 67, 345–356.
- Wright, S. (1951). The genetical structure of populations. *Ann. Eu- gen.*15:323–354.
- [www.tropicalforages.info/key/Forages/Media/.../Lablab\\_purpureus.htm](http://www.tropicalforages.info/key/Forages/Media/.../Lablab_purpureus.htm). Factsheet—Lablab purpureus -Tropical forages. (Accessed on 15th April, 2015).
- Yaming, G., Guwen, Z., Shengchun, X., Weihua, M., and Qizan, H. (2013). Development of EST- SSR markers to study genetic diversity hyacinth bean (*Lablab purpureus* L.) *POJ* 6(4):295-301 (2013) ISSN: 1836-3644.
- Yan, W., and Hunt, L.A. (2001). Interpretation of genotype x environment interaction for winter wheat yield in Ontario. *Crop Science* 41, 656 – 663.

- Zane, L., Bargelloni, L. and Patarnello, T. (2002). Strategies for microsatellites isolation: a review, *Molecular Ecology* 11: 1 – 16.
- Zannou, A., Kossou, D.K. Ahnchédé, A., Zoundjihékpon, J., Agbicodo, E., Struik, P.C  
and Sannin, A. (2008) Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA, *African Journal of Biotechnology* 7: 4407 - 4417
- Zeng, Y., Yang, S., Cui, H., Yang, X., Xu, L., Du, J., Pu, X., Li, Z., Cheng, Z., and Huang, X.(2009).QTLs of cold-related traits at the booting stage for NIL-RILs in rice revealed by SSR. *Genes Genom.* , 31, 143–145.
- Zuur, A.F. (2007). Principal coordinate analysis and non-metric analysis. <http://www.link.springer.com/.../10.1007%2F978> - - Springer Science + Businee Media.

## APPENDICES

### APPENDIX I: Interview schedule guide

District.....  
 Division.....  
 Location.....  
 Village.....  
 Farmer Name.....  
 Date of interview.....

#### A. Background Information

1. Age of the farmer.....years
2. Sex            ( a) Male            (b) Female
3. Education level of farmer:  
                   (a) None            (b) Non formal (c) Primary    (d) Secondary (e) Tertiary
4. Total land under cultivation for all the crops.....acres  
                   (a) Owned.....acres            (b) Rented .....acres

#### B. Crops Production Trend

Crop Production Statistics

Dolikos Beans

	2002	2003	2004	2005	2006
Variety					
Acreage					
Yield					

Common Beans

	2002	2003	2004	2005	2006
Variety					
Acreage					
Yield					

#### C. Utilization /Consumption

How do you utilize dolikos Beans? For,

- (a) Livestock feed    (b) Human food    (c) Commercial    (d) Soil conservation

If used as human food, what part of the crop do you consume?

- (a) Leaves    (b) Dry beans            (c) Green pods

How do you prepare the leaves / dry grains?

How do you consider the following about beans?

Market (demand) ....price.....Taste / flavor.....Cook ability....Variety.....

**D. Source of the seed**

Source of seed

- (a) Own Save (b) From neighbours (c) Purchased from market

i) If purchased from the market,  
 Why do you buy seed?  
 Are you satisfied with the quality of seed bought? Yes / No  
 If No, what would be the better alternative?

ii) If farmer uses own saved seed,  
 Do you treat the seed before planting? Yes / No  
 If yes, indicate the type of chemical used.....rate.....  
 Are you satisfied with the quality? Yes /No  
 If No, indicate the reason .....

**E. Seed Production Details**

How do you grow the crop?

1. single stand crop
2. Mixed cropping

If mixed cropping, what crop do you plant with?.....

If single crop, what spacing do you use for the crop?.....

How many times do you weed the crop before harvesting?,.....  
 Which weeds are important in the area? List them in order of importance

- 1.
- 2.
- 3.
- 4.

Which are (is) the most problematic insect pest(s)

- 1.
- 2.
- 3.
- 4.

Ho do you control it/them?.....

Which are/is the most prevalent disease(s) of this crop in the area?

- 1.
- 2.
- 3.
- 4.

How do you control it /them.....

What are you indicators for maturity?

Pod Colour	Leaf Colour	Seed Clour	Others(Specify)

How do you dry your seed?

- (a) On the plant (b) As pods (c) a grain (d) others  
 (specify)

Do you harvest seed separate from grain? Yes /No

Do you sort the seed to be stored for future planning? Yes /No

If Yes, what is your criterion?

- (a) Size (b) colour (c) Shape (d) Pest (e) Diseases  
(f) Others (specify)

How do farmers store the Lablab seed?

- (a) In Pots (b) In plastic containers (c) in gunny bags (d) Other (specify)

Which method of storage do you prefer and why?.....

For how long do you store the seed?.....

Have you ever experienced problems with germination of the stored seed? Yes / No

If Yes, after storage of how long?.....

In which type of container?.....

How do you avoid infestation by storage pest?

- (a) Sanitation (b) Chemical spray (c) Traditional practices (d) Others (specify)

How do you control storage pests?

- (a) Cleaning (b) Sun drying (c) Traditional practices (d) other (specify)

In case of technology development, which aspects would you want addressed?

.....

**APPENDIX II: Map of Kenya showing localities where Lablab bean was collected**



Key: Red squares indicate areas covered by the field survey. (Source: Kamotho *et al.* 2010)

**APPENDIX III: Lablab bean accessions collected during the survey**

Region	County	Specific Area	Accession
Coast	Lamu	Lamu -Mpeketoni 1	1
		Lamu –Mpeketoni 2	2
		Lamu – Mpeketoni 3	3
		Lamu – Mpeketoni 4	4
		Lamu – Mpeketoni 5	5
		Lamu – Mpeketoni 6	6
		Lamu – Kilimani 1	7
		Lamu – Kilimani 2	8
		Lamu – Kilimani 3	9
		Lamu – Kilimani 4	10
		Lamu – Market 1	11
		Lamu – Market 2	12
		Lamu – Market 3	13
Eastern	MACHAKOS	Kalama 1	14
		Kalama 2	15
		Kalama 3	16
		Kalama 4	17
		Kathiani 1	18
		Kathiani 2	19
		Kathiani 3	20
		Yata	21
Central	MURANG'A	Makuyu 1	22
		Makuyu 2	23
	THIKA	Kakuzi 1	24
		Kakuzi 2	25
		Kakuzi 3	26
		Kakuzi 4	27
		Thika municipality	28
Eastern	MBEERE	Siakago 1	29
		Siakago 2	30
	MERU	Abothoguchi	31
		Mihiriga Mieru	32
Rift Valley	NAKURU	Lare Mugumoini	33
		Lare Mt. Clare	34
		Lare Keriri	35
		Bahati Solai	36
		Naivasha Maragushu 1	37
		Naivasha Maragushu 2	38
Esatern	MWINGI	Central 1	39
		Central 2	40
		Central 3	41
		Migwani 1	42
		Migwani 2	43
		Migwani 3	44
		Migwani 4	45



**APPENDIX IV:** Photos on Lablab intercropped with maize, pure stand and grown on terraces.



**Plate1:** Photograph showing a *Lablab purpureus* intercropped with maize in Lamu-Mpeketoni. (Source:Author, 2006)



**Plate 2:** Photograph showing a pure stand of *Lablab purpureus* in Mwingi county. (Source:Author, 2006)



**Plate 1:** Photograph showing *Lablab purpureus* planted on top of a terrace as a soil conservation measure in Mwingi county. Source:Author, 2006)

**APPENDIX V: Plates showing various activities carried out in the molecular laboratory at KALRO, Biotechnology Institute, Nairobi (source: Author, 2014)**



Leaf tissue for DNA



Leaf tissue and buffer in eppendorf self standing tubes



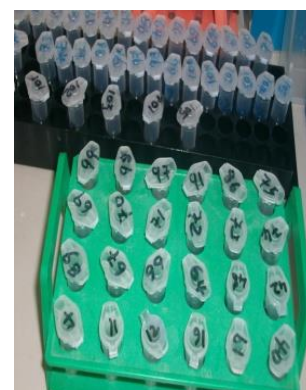
Leaf tissue slurry in waterbath at 65°C



Decanting the supernatant



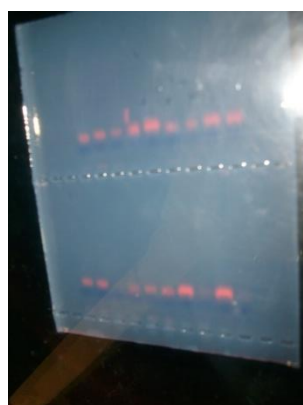
Vortexing the samples for thorough mixing



Labeled samples



Samples in centrifuge



Gel showing presence of DNA



Samples in thymocycler machine

**APPENDIX VI: Fischers protected test for means of *Lablab purpureus* site x accession interaction for days to mature pods, days to flowering, duration flowering and pods per raceme**

Days to mature pods			Days to flowering			Duration flowering			Pods per raceme		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
1	14	193a	1	15	121.67a	2	37	44.67a	1	36	12.1a
1	15	193a	1	19	121.67a	1	37	44.67a	1	24	10.9b
1	16	193a	1	20	121.67a	1	38	44.67a	1	27	10.9b
1	19	193a	2	15	121.67a	2	38	44.67a	1	25	10.9b
2	16	193a	2	17	121.67a	3	38	40.67b	1	26	10.9b
2	17	193a	2	20	121.67a	3	37	40.33b	1	28	10.9b
2	18	193a	1	16	121.67a	1	15	35.67c	1	32	10.8c
2	19	193a	2	14	121.67a	2	14	35.67c	2	36	10.8c
2	20	193a	2	18	121.67a	2	16	35.67c	1	31	10.7c
2	14	193a	1	37	121.33a	2	17	35.67c	1	35	10.2d
2	15	193a	2	37	121.33a	1	18	35.67c	1	34	10.2d
1	17	192a	1	14	121.33a	1	20	35.67c	1	33	10.1d
1	18	192a	1	17	121.33a	1	14	35.33c	1	22	9.7e
1	20	192a	1	18	121.33a	1	17	35.33c	1	23	9.7e
2	38	188b	1	38	121.33a	1	19	35.33c	2	32	9.6ef
1	37	187c	2	16	121.33a	2	15	35.33c	2	27	9.6fg
2	37	187c	2	19	121.33a	2	20	35.33c	2	26	9.6fg
1	38	187c	2	38	121.33a	1	16	35.33c	2	25	9.6fg
3	14	177d	1	33	113.67b	2	18	35.33c	2	28	9.6fg
3	15	177d	1	35	113.67b	2	19	35.33c	2	24	9.5fg
3	17	177d	2	33	113.67b	2	33	31.67d	2	31	9.5fg
3	20	177d	2	34	113.67b	2	35	31.67d	1	29	9.5g
3	16	176d	3	37	113.67b	3	16	31.67d	1	30	9.5g
3	18	176d	1	34	113.67b	3	20	31.67d	1	43	9.3h
3	19	176d	2	35	113.33c	1	33	31.33d	2	35	9.2hi
3	37	175e	3	38	113.33c	1	34	31.33d	1	39	9.2hi
3	38	175e	3	15	112.67d	1	35	31.33d	1	40	9.2hi
1	34	173f	3	17	112.67d	2	34	31.33d	1	41	9.2hi
1	35	173g	3	19	112.67d	3	14	31.33d	1	44	9.2hi
2	33	173g	3	20	112.67d	3	15	31.33d	2	33	9.2hi
1	33	173g	3	14	112.33d	3	17	31.33d	2	34	9.2hi
2	34	173g	3	16	112.33d	3	19	31.33d	1	45	9.1i
2	35	172g	3	18	112.33	3	18	31.33d	1	42	9.1i
2	30	159h	1	42	103.67e	1	41	27.67e	1	2	8.8j
1	30	159h	1	43	103.67e	2	40	27.67e	1	7	8.8j
1	29	158i	3	34	103.67e	2	45	27.67e	1	9	8.8j

Days to mature pods			Days to flowering			Duration flowering			Pods per raceme		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
2	29	158hi	3	35	103.67e	1	39	27.67e	3	3	8.8jk
2	31	158hi	1	40	103.33f	1	40	27.67e	3	6	8.8jk
2	32	158i	2	42	103.33f	1	42	27.67e	3	8	8.8jk
1	31	158hi	2	43	103.33f	1	43	27.67e	3	10	8.8jk
1	32	158i	1	39	103.33f	1	44	27.67e	3	13	8.8jk
3	33	158ij	1	41	103.33f	1	45	27.67e	1	1	8.8jk
3	34	157j	1	44	103.33f	2	42	27.67e	1	8	8.8jk
3	35	157j	1	45	103.33f	2	43	27.67e	1	4	8.7jk
1	26	154k	2	45	103.33f	2	39	27.33ef	1	11	8.7jk
1	28	154k	3	33	103.33f	2	41	27.33ef	3	1	8.7jk
2	22	153l	1	30	102.67g	2	44	27.33ef	3	2	8.7jk
2	23	153l	2	30	102.67g	3	34	26.67fg	3	4	8.7jk
2	24	153l	2	39	102.67g	3	35	26.33gh	3	5	8.7jk
2	25	153l	2	41	102.67g	3	33	26ghi	3	7	8.7jk
2	26	153l	2	44	102.67g	1	22	25.67hij	3	9	8.7jk
2	27	153l	1	29	102.33g	1	23	25.67hij	3	11	8.7jk
2	28	153l	1	31	102.33g	1	26	25.67 hij	3	12	8.7jk
1	22	153l	1	32	102.33g	1	29	25.67 hij	1	3	8.7jk
1	24	153l	2	29	102.33g	1	30	25.67 hij	1	5	8.7jk
1	25	153l	2	31	102.33g	2	25	25.67 hij	1	6	8.7jk
1	27	153l	2	32	102.33g	2	26	25.67 hij	1	10	8.7jk
1	23	153l	2	40	102.33g	2	27	25.67 hij	1	12	8.7jk
1	21	146n	1	22	98.67h	2	28	25.67 hij	1	13	8.7jk
2	21	145n	1	23	98.67h	2	29	25.67 hij	2	23	8.7kl
3	29	142o	1	25	98.67h	2	30	25.67 hij	2	22	8.6l
3	30	142o	1	27	98.67h	2	31	25.67 hij	2	29	8.4m
3	31	141o	1	28	98.67h	2	32	25.67 hij	2	30	8.4m
3	32	141o	2	22	98.67h	2	22	25.33ijk	2	39	8.4m
3	22	137p	2	23	98.67h	2	23	25.33 ijk	2	40	8.4m
3	24	137p	2	26	98.67h	2	24	25.33 ijk	2	41	8.4m
3	25	137p	2	27	98.67h	1	24	25.33 ijk	2	43	8.4m
3	27	137p	1	26	98.33h	1	25	25.33 ijk	2	44	8.4m
3	28	137p	2	25	98.33h	1	27	25.33 ijk	2	1	8.4m
3	23	136p	2	28	98.33h	1	28	25.33 ijk	2	42	8.4m
3	26	136p	1	24	98.33h	1	31	25.33 ijk	2	9	8.3n
2	43	127q	2	24	98.33h	1	32	25.33 ijk	2	2	8.3n
1	42	127q	1	21	93.67i	3	41	24.67kl	2	3	8.3n
2	39	127q	2	21	93.33i	3	45	24.67l	2	6	8.3n
2	40	127q	2	36	91.67j	3	39	24.33l	2	7	8.3n

Days to mature pods			Days to flowering			Duration flowering			Pods per raceme		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
2	41	127q	3	29	91.67j	3	40	24.33l	2	10	8.3n
1	43	126r	3	31	91.67j	3	42	24.33l	2	11	8.3n
1	45	126r	3	32	91.67j	3	43	24.33l	2	12	8.3n
2	42	126r	1	36	91.33k	3	44	24.33l	2	45	8.3n
2	44	126r	3	30	91.33k	3	22	21.67m	2	4	8.3n
2	45	126r	3	40	90.67k	3	23	21.67m	2	8	8.3n
1	39	126r	3	42	90.67k	3	24	21.67m	2	13	8.3n
1	41	126r	3	43	90.67k	3	26	21.67m	3	31	8.3n
2	36	126s	3	39	90.67k	3	30	21.67m	3	36	8.3n
1	40	126s	3	41	90.67k	3	25	21.33m	2	5	8.2n
1	44	126s	3	44	90.67k	3	27	21.33m	3	32	8.2n
3	21	125s	3	45	90.67k	3	28	21.33m	3	33	7.8o
1	36	125s	2	5	89.00l	3	31	21.33m	3	34	7.8o
2	4	124t	1	1	88.67l	3	32	21.33m	3	35	7.7p
1	3	124t	1	2	88.67l	3	29	21.33m	3	45	7.7q
1	7	124t	1	3	88.67l	1	21	17.67n	3	39	7.7q
1	10	124t	1	4	88.67l	2	21	17.67n	3	40	opq
1	1	123t	1	5	88.67l	1	36	17.33no	3	43	7.7opq
1	2	123t	1	6	88.67l	2	36	17.33no	3	44	7.7opq
1	4	123t	1	7	88.67l	1	6	16.67op	3	41	7.6pqr
1	5	123t	1	8	88.67l	1	11	16.67op	3	42	7.6pqr
1	6	123t	1	9	88.67l	2	7	16.67op	3	22	7.6qr
1	8	123t	1	10	88.67l	2	9	16.67op	3	23	7.6qr
1	9	123t	1	11	88.67l	1	4	16.33p	3	25	7.6qr
1	11	123t	1	12	88.67l	1	5	16.33p	3	26	7.6qr
1	12	123t	1	13	88.67l	1	8	16.33p	3	28	7.6qr
1	13	123t	2	1	88.67l	1	10	16.33p	3	24	7.5r
2	1	123t	2	2	88.67l	1	13	16.33p	3	27	7.5r
2	2	123t	2	3	88.67l	2	1	16.33p	3	29	7.3s
2	3	123t	2	4	88.67l	2	2	16.33p	3	30	7.3s
2	5	123t	2	6	88.67l	2	3	16.33p	1	21	6.6t
2	6	123t	2	8	88.67l	2	12	16.33p	2	21	5.8u
2	7	123t	2	9	88.67l	1	1	16.33p	1	16	5.7v
2	8	123t	2	10	88.67l	1	2	16.33p	1	20	5.7vw
2	9	123t	2	11	88.67l	1	3	16.33p	1	15	5.6vwx
2	10	123t	2	12	88.67l	1	7	16.33p	1	14	5.6wx
2	11	123t	2	13	88.67l	1	9	16.33p	1	17	5.6wx
2	12	123t	3	22	88.67l	1	12	16.33p	1	19	5.5x
2	13	123t	3	23	88.67l	2	4	16.33p	1	18	5.4y

Days to mature pods			Days to flowering			Duration flowering			Pods per raceme		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
3	36	112u	3	25	88.67l	2	5	16.33p	2	16	5.3yz
3	45	109v	3	26	88.67l	2	6	16.33p	2	18	5.3yz
3	39	109v	3	27	88.67l	2	8	16.33p	2	20	5.3yz
3	41	109v	3	28	88.67l	2	10	16.33p	2	17	5.2z
3	40	108v	3	24	88.67l	2	11	16.33p	3	21	5.2z
3	42	108v	2	7	88.33l	2	13	16.33p	2	15	5.2z
3	43	108v	3	21	83.33l	3	21	14.67q	2	19	5.2z
3	44	108v	3	36	83.33l	3	36	14.67q	2	14	5.2z
3	1	107w	3	1	79.67m	3	7	13.67r	1	37	4.9A
3	3	107w	3	2	79.67m	3	8	13.67r	1	38	4.8AB
3	4	107w	3	3	79.67m	3	10	13.67r	3	14	4.8ABC
3	6	107w	3	5	79.67m	3	2	13.67r	3	16	4.8ABC
3	7	107w	3	6	79.67m	3	3	13.67r	3	17	4.7BCD
3	8	107w	3	8	79.67m	3	4	13.33r	3	15	4.7BCD
3	9	107w	3	9	79.67m	3	5	13.33r	2	38	4.7CDE
3	10	107w	3	11	79.67m	3	6	13.33r	2	37	4.6DEF
3	11	107w	3	12	79.67m	3	11	13.33r	3	19	4.6DEF
3	12	107w	3	13	79.67m	3	13	13.33r	3	20	4.6EF
3	2	106w	3	7	79.67m	3	9	13.33r	3	18	4.5F
3	5	106w	3	4	79.33m	3	1	13.67r	3	37	4.3G
3	13	106w	3	10	79.33m	3	12	13.33r	3	38	4.3G

**APPENDIX VII:** Fischers protected test for means of *Lablab purpureus* site x accession on for number of seeds per pod, 100 seed weight, seed yield per plant and number of racemes per plant

Number of seeds per pod			100 seed weight (g)			Seed yield per plant (g)			Number of racemes per plant		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
1	32	4.2a	3	22	32.6a	1	36	203.5a	1	22	13.3a
1	25	4.2a	3	23	32.6a	1	22	188.1b	1	25	13.2a
1	27	4.2a	1	23	32.6a	1	23	188.1b	1	26	13.2a
1	28	4.2a	1	22	32.6a	1	32	181.5c	1	32	13.2a
1	22	4.1ab	2	22	31.3b	1	31	181.4c	1	36	13.2a
1	24	4.1ab	2	23	31.3b	1	25	181.4c	1	23	13.1a
1	26	4.1ab	3	36	29.9c	1	28	181.4c	1	24	13.1a
1	31	4.1ab	1	36	29.9c	1	26	181.4c	1	27	13.1a
1	36	4.1bc	2	36	29.6d	1	24	181.4c	1	28	13.1a
1	23	4.0bc	3	31	29.4e	1	27	181.4c	1	31	13.1a
1	16	4.0cd	3	32	29.4e	1	29	173.8d	1	29	12.6b
1	14	3.9de	3	35	29.4e	1	30	173.8d	1	30	12.6b
1	17	3.9de	3	33	29.4e	2	36	157.1e	1	33	12.6b
1	29	3.9de	3	34	29.4e	1	34	153.7f	1	34	12.6b
1	30	3.9de	3	25	29.4	1	35	153.7f	1	35	12.4b
1	15	3.8e	3	27	29.4e	1	33	153.7f	2	23	11.6c
1	21	3.8e	3	26	29.4e	1	6	138.5g	2	25	11.6c
1	33	3.8ef	3	28	29.4e	1	7	138.3gh	2	26	11.6c
1	35	3.8ef	3	24	29.4e	1	8	138.1hi	2	28	11.6c
1	1	3.7fg	1	24	29.4e	1	9	138.1hi	1	39	11.6c
1	7	3.7fg	1	25	29.4e	1	3	138.1hi	1	43	11.6c
1	34	3.7fg	1	32	29.4e	1	1	138.1hi	1	45	11.6c
1	10	3.6g	1	26	29.4	1	12	138.1i	1	21	11.6c
1	12	3.6g	1	27	29.4e	1	5	138.1i	1	42	11.6c
1	42	3.6g	1	28	29.4e	1	2	138.0i	2	22	11.4d
1	43	3.6g	1	33	29.4e	1	11	138.0i	2	24	11.4d
1	45	3.6g	1	34	29.4e	1	13	138.0i	2	36	11.4d
1	2	3.6g	1	35	29.4e	1	4	138.0i	1	40	11.4d
1	4	3.6g	1	31	29.4e	1	10	137.9i	2	27	11.3e
1	5	3.6g	2	26	28.9f	2	23	124.2j	1	1	11.2e
1	13	3.6g	2	28	28.9f	2	22	124.2j	1	2	11.2e
1	39	3.6g	2	24	28.9f	2	25	121.3k	1	3	11.2e
1	41	3.6g	2	27	28.9f	2	28	121.3k	1	4	11.2e
1	3	3.6gh	2	31	28.9f	2	24	121.3k	1	5	11.2e
1	6	3.6gh	2	35	28.9f	2	26	121.3k	1	8	11.2e
1	8	3.6gh	2	32	28.9f	2	27	121.3k	1	9	11.2e
1	9	3.6gh	2	33	28.9f	2	31	121.0l	1	10	11.2e
1	11	3.6gh	2	34	28.9f	2	32	121.0l	1	11	11.2e
1	40	3.6gh	2	25	28.9f	1	44	119.2m	1	12	11.2e
1	44	3.6gh	3	29	28.8f	1	42	119.2m	1	44	11.2e
1	18	3.5hi	3	30	28.8f	1	40	119.2m	2	31	11.2e
1	20	3.4ij	1	29	28.7f	1	41	119.2m	1	41	11.2e
1	19	3.4ij	1	30	28.7f	1	45	119.2m	2	32	11.1e
2	23	3.4ijk	2	30	28.2g	1	39	119.2m	1	6	11.1e
2	24	3.4ijk	2	29	28.2g	1	43	119.2m	1	7	11.1e
2	27	3.4ijk	3	39	27.9h	2	30	113.8n	1	13	11.1e

Number of seeds per pod			100 seed weight (g)			Seed yield per plant (g)			Number of racemes per plant		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
2	22	3.3jkl	3	40	27.9h	2	29	113.8n	2	29	10.6f
2	25	3.3jkl	3	44	27.9h	2	1	112.3o	2	30	10.6f
2	26	3.3jkl	3	41	27.8h	2	4	112.3o	2	33	10.6f
2	28	3.3jkl	3	43	27.8h	2	2	112.3o	2	34	10.6f
2	36	3.3jkl	3	45	27.8h	2	13	112.3o	2	35	10.6f
2	30	3.3klm	3	42	27.8h	2	6	112.3o	2	45	10.2g
2	40	3.3klm	1	39	27.8h	2	7	112.3o	2	39	10.2g
2	41	3.3klm	1	41	27.8h	2	9	112.3o	2	40	10.2g
2	44	3.3klm	1	43	27.8h	2	10	112.3o	2	41	10.2g
2	45	3.3klm	1	45	27.8h	2	11	112.3o	2	42	10.2g
3	3	3.3klm	1	40	27.8h	2	8	112.3o	2	43	10.2g
3	4	3.3klm	1	42	27.8h	2	12	112.3o	3	1	10.2g
3	5	3.3klm	1	44	27.8h	2	3	112.3o	3	2	10.2g
3	8	3.3klm	2	45	27.2i	2	5	112.3o	3	4	10.2g
3	11	3.3klm	2	39	27.2i	2	33	105.8p	3	6	10.2g
3	13	3.3klm	2	41	27.2i	2	35	105.8p	3	7	10.2g
2	43	3.3klm	2	43	27.2i	2	34	105.8p	3	9	10.2g
2	2	3.2lmn	2	40	27.2i	2	39	96.4q	3	10	10.2g
2	3	3.2lmn	2	42	27.2i	2	40	96.1r	3	11	10.2g
2	6	3.2lmn	2	44	27.2i	2	41	96.1r	3	13	10.2g
2	8	3.2lmn	3	1	24.6j	2	42	96.1r	2	44	10.1h
2	29	3.2lmn	3	6	24.6j	2	43	96.1r	3	3	10.1h
2	39	3.2lmn	1	2	24.6j	2	44	96.0r	3	5	10.1h
3	1	3.2lmn	1	6	24.6j	2	45	96.0r	3	8	10.1h
3	2	3.2lmn	1	12	24.6j	3	7	95.1s	3	12	10.1h
3	6	3.2lmn	3	3	24.6j	3	2	95.1s	3	32	9.9h
3	7	3.2lmn	3	4	24.6j	3	1	95.1s	1	17	9.8i
3	9	3.2lmn	3	9	24.6j	3	10	95.1s	3	31	9.8i
3	10	3.2lmn	3	11	24.6j	3	5	95.0s	1	14	9.8i
3	12	3.2lmn	3	12	24.6j	3	12	95.0s	1	15	9.8i
2	42	3.2lmn	1	1	24.6j	3	9	95.0s	1	16	9.8i
2	1	3.2mn	1	3	24.6j	3	6	95.0s	2	1	9.6ij
2	4	3.2mn	1	5	24.6j	3	13	95.0s	2	4	9.6ij
2	5	3.2mno	1	8	24.6j	3	11	95.0s	2	5	9.6ij
2	7	3.2mno	1	9	24.6j	3	3	95.0s	2	6	9.6ij
2	10	3.2 mno	1	10	24.6j	3	4	95.0s	2	9	9.6ij
2	12	3.2 mno	1	13	24.6j	3	8	95.0s	2	10	9.6ij
2	13	3.2 mno	3	2	24.6j	3	36	92.9t	2	11	9.6ij
2	21	3.2 mno	3	5	24.6j	3	32	85.7u	2	13	9.6ij
2	9	3.1 nop	3	7	24.6j	3	31	85.7u	3	36	9.6ij
2	11	3.1 nop	3	8	24.6j	3	22	85.3v	2	2	9.4k
2	14	3.1 nop	3	10	24.6j	3	23	85.3v	2	3	9.4k
2	15	3.1 nop	3	13	24.6j	3	24	78.0w	2	7	9.4k
2	17	3.1 nop	1	7	24.6j	3	25	77.9w	2	8	9.4k
2	18	3.1 nop	1	11	24.6j	3	27	77.9w	2	12	9.4k
2	19	3.1 nop	1	4	24.6j	3	28	77.9w	1	18	9.2l
2	20	3.1 nop	2	2	24.1k	3	26	77.9w	1	20	9.2l
2	31	3.1 nop	2	4	24.1k	1	21	75.2x	3	22	9.2l
2	32	3.1 nop	2	6	24.1k	3	35	73.7y	3	24	9.2l
2	33	3.1 nop	2	7	24.1k	3	34	73.7y	3	25	9.2l
2	34	3.1 nop	2	9	24.1k	3	33	73.7y	3	26	9.2l



Number of seeds per pod			100 seed weight (g)			Seed yield per plant (g)			Number of racemes per plant		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
2	35	3.1 nop	2	10	24.1k	3	30	68.4z	3	28	9.2
3	36	3.1 nop	2	11	24.1k	3	29	68.4z	3	23	9.11
3	23	3.1opq	2	12	24.1k	3	39	64.8A	3	27	9.11
3	29	3.1 opq	2	13	24.1k	3	45	64.7A	1	19	9.0m
3	30	3.1 opq	2	1	24.1k	3	43	64.5B	3	33	8.9n
3	31	3.1 opq	2	3	24.1k	3	44	64.5B	3	34	8.9n
3	25	3.1 opq	2	5	24.1k	3	41	64.5B	3	35	8.9n
3	27	3.1 opq	2	8	24.1k	3	42	64.5B	3	44	8.2o
3	24	3.0pqr	3	19	23.1l	3	40	64.5B	2	21	8.8n
3	26	3.0 pqr	3	20	23.1l	2	21	63.5C	3	45	8.3o
3	32	3.0 pqr	3	18	23.1lm	1	14	63.2D	2	14	8.2o
2	16	3.0 pqr	1	20	23.1lmn	1	15	63.2D	2	15	8.2o
3	28	3.0 pqr	1	18	23.1lmno	1	17	63.1D	2	17	8.2o
3	34	3.0qrs	2	14	23.0lmno	1	16	63.1D	3	29	8.2o
3	40	3.0 qrs	3	14	23.0lmno	1	20	56.0E	3	30	8.2o
3	44	3.0 qrs	3	15	23.0mno	1	18	56.0E	3	41	8.2o
3	33	3.0 qrs	3	17	23.0mno	1	19	56.0E	3	42	8.2o
3	42	3.0 qrs	3	16	23.0no	2	14	51.6F	2	16	8.1o
3	22	2.9rst	1	17	23.0no	2	17	51.6F	3	39	8.1o
3	35	2.9 rst	1	15	23.0no	2	15	51.6F	3	40	8.1o
3	39	2.9 rst	1	16	23.0o	2	16	51.4F	3	43	8.1o
3	41	2.9 rst	1	14	23.0o	3	21	41.4G	2	19	7.6p
3	43	2.9 rst	1	19	22.8p	2	19	40.9H	2	20	7.6p
3	45	2.9stu	2	19	22.6q	2	18	40.9H	2	18	7.4p
1	38	2.9 stu	2	18	22.6q	2	20	40.9H	3	21	7.2p
1	37	2.8tu	2	20	22.6q	3	14	35.1I	3	18	6.2q
3	21	2.8u	2	17	22.4q	3	15	35.0I	3	20	6.2q
3	14	2.7v	2	15	22.4q	3	17	35.0I	3	16	6.2q
3	16	2.7v	2	16	22.4q	3	16	35.0I	3	17	6.2q
3	18	2.7v	1	21	19.9r	3	19	30.2J	3	19	6.2q
3	20	2.7v	3	21	19.9r	3	18	30.0JK	3	14	6.1q
3	15	2.6v	3	37	19.6s	3	20	30.0K	3	15	6.1q
3	17	2.6v	3	38	19.6s	1	38	23.1L	1	37	5.4r
3	19	2.6v	1	37	19.6s	1	37	23.0L	1	38	5.4r
2	37	2.6v	1	38	19.6s	2	37	19.2M	2	37	5.2r
2	38	2.6v	2	37	19.4t	2	38	19.2M	2	38	5.2r
3	38	2.5w	2	38	19.4t	3	38	17.7N	3	37	4.6s
3	37	2.4w	2	21	19.3t	3	37	17.7N	3	38	4.6s

**APPENDIX VIII: Fischers protected test for means of *Lablab purpureus* site x accession interaction for days to germination, plant height, leaf length and raceme length**

Days to germination			Plant height			Leaf length			Raceme length		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
1	1417.7a		1	38	374.7a	1	14	13.1a	1	17	44.0a
1	3717.8a		1	37	374.3a	1	17	13.1a	1	14	44.1a
1	3817.7a		3	37	354.4b	1	39	13.1a	1	18	43.7ab
1	1617.6a		3	38	353.7bc	1	33	13.0ab	1	19	43.5ab
1	1817.7a		1	14	351.4bcd	1	35	13.0ab	1	20	43.2bc
1	2017.8a		1	15	350.7cde	1	42	13.0ab	1	16	42.7cd
1	1717.3ab		1	20	349.8de	1	43	12.9abc	1	15	42.3d
1	1917.2ab		1	17	349.6de	1	44	12.9abc	1	35	37.3e
1	1517.0ab		1	16	348.8def	1	19	12.9abc	1	34	37.0e
2	3816.3bc		1	19	348.3ef	1	41	12.9abc	1	33	36.7e
2	1415.7cd		1	18	346.0f	1	40	12.9abcd	1	21	34.0f
2	1915.6cd		1	35	337.9g	1	15	12.8bcd	1	26	33.6fg
2	1515.3de		1	33	336.6g	1	45	12.8bcd	1	9	33.4fgh
2	1615.1de		1	34	335.9g	1	34	12.7bcde	1	8	33.3fgh
2	1715.3de		3	15	330.2h	1	18	12.7bcde	1	2	33.4fgh
2	1815.2de		3	17	330.1	1	20	12.7cde	1	13	33.3ghi
2	2015.3de		3	19	330.0h	1	16	12.7cde	1	23	33.2ghi
2	3715.2de		3	18	329.8h	1	31	12.7cde	1	5	33.2ghi
1	614.7def		3	20	329.5h	1	32	12.6de	1	6	33.1ghi
1	814.6 def		3	14	329.4h	1	29	12.5ef	1	4	33.2ghi
1	3014.7 def		3	16	328.5h	1	37	12.3fg	1	12	33.2ghi
1	314.8 def		2	37	328.1h	1	38	12.3fg	1	27	33.2ghi
1	1014.7 def		2	38	328.0h	1	30	12.3g	1	7	33.1ghi
1	1314.6def		3	33	316.2i	3	17	12.3g	1	10	33.1ghi
1	114.3efg		3	35	316.0i	3	14	12.2g	1	25	33.0ghi
1	214.2 efg		3	34	315.8i	3	19	12.2g	1	3	33.1ghi
1	414.3 efg		2	16	298.2j	3	20	12.2g	1	11	32.9hi
1	514.1 efg		2	20	298.0j	3	15	12.2g	1	22	32.8hi
1	714.3 efg		2	14	298.0j	3	16	12.2g	1	24	32.9hi
1	914.1 efg		2	17	297.9j	3	18	12.2g	1	1	32.7hi
1	1114.3 efg		2	19	294.7k	1	25	11.9h	1	31	32.9hi
1	1214.0 efg		2	18	294.6k	1	24	11.8hi	1	28	32.7ij
1	2114.2 efg		2	15	291.3l	1	23	11.8hij	1	32	32.6ijk
1	2914.3 efg		2	34	283.2m	1	27	11.8hij	1	36	32.2jk
1	4014.2 efg		2	35	283.2m	1	28	11.8hij	3	17	32.1jk
1	4314.1 efg		2	33	283.1m	1	26	11.7hjkl	3	14	32.2jk
1	4414.3 efg		1	30	273.2n	1	22	11.6hjkl	3	20	32.1jk
1	3914.0fgh		1	31	273.0n	1	36	11.6hjkl	3	15	32.2jk
1	4513.7ghi		1	29	272.7n	2	18	11.6ijklm	3	16	32.1jk

Days to germination			Plant height		Leaf length		Raceme length				
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean			
1	4113.3	hij	1	32	271.4n	2	14	11.5jklm			
1	4213.3	hij	3	31	259.0o	2	16	11.5jklm			
1	3513.0	ijk	3	32	258.9o	2	17	11.5klm			
1	2312.7	jkl	3	30	258.1o	2	19	11.5klm			
1	2612.6	jkl	3	29	257.9o	2	20	11.5klm			
1	3112.7	jkl	2	32	234.3p	15	11.5klm	1	43	30.1l	
1	2212.2	lm	2	31	233.9p	3	29	11.4lm	1	45	30.0lm
1	2712.3	lm	2	30	232.1p	3	30	11.4lm	1	39	29.8lmn
1	3212.1	lm	2	29	231.9p	3	33	11.4lm	1	42	29.8lmno
1	3312.3	lm	1	22	225.7q	3	34	11.4lm	3	7	29.4mnop
1	3412.2	lm	1	25	224.9qr	3	39	11.4lm	3	3	29.3nop
1	2412.1	lm	1	28	224.8qr	3	40	11.4lm	3	1	29.3 nop
1	2512.2	lm	1	27	224.5qr	3	41	11.4lm	3	8	29.3 nop
2	2412.1	lm	1	26	222.3r	3	42	11.4lm	3	33	29.3 nop
2	2812.0	lm	1	23	222.0r	3	44	11.4lm	3	4	29.2 nopq
2	3212.1	lm	1	24	222.0r	3	31	11.4lm	3	35	29.2 nopq
2	3612.2	lm	3	23	214.3s	3	32	11.4lm	3	13	29.1opq
1	3611.8	lm	3	28	214.2s	3	35	11.4mn	3	9	29.1pq
2	111.7	lm	3	26	214.0s	3	43	11.4mn	3	11	29.1pq
2	611.6	lm	3	24	213.9s	3	45	11.4mn	3	2	29.1pq
2	711.5	lm	3	27	213.9s	3	36	11.1n	3	10	29.0pqr
2	811.7	lm	3	25	213.8s	3	25	10.8o	3	12	29.0pqrs
2	911.8	lm	3	22	213.7s	3	27	10.8o	3	34	29.0pqrs
2	1111.6	lm	1	39	206.5t	3	37	10.8o	3	6	28.9pqrs
2	1211.7	lm	1	41	202.1u	3	38	10.8o	1	29	28.8qrst
2	2111.6	lm	1	44	201.6uv	3	23	10.8o	3	5	28.8qrst
2	2511.7	lm	1	43	201.0uv	3	24	10.8o	1	30	28.6qrstu
2	2911.8	lm	1	42	200.7uv	3	22	10.8o	2	16	28.4rstu
2	3011.7	lm	1	45	200.2uv	3	26	10.8o	2	14	28.4rstu
2	3311.6	lm	1	40	198.6v	3	28	10.8o	2	18	28.3stu
2	4411.7	lm	1	21	193.2w	2	33	10.7o	2	20	28.3stu
2	4511.8	lm	2	27	187.2x	2	34	10.7o	2	15	28.3tu
2	211.7	lm	2	23	187.2x	2	35	10.7o	2	19	28.1u
2	311.6	lm	2	25	187.1x	2	40	10.4p	2	17	28.1u
2	411.7	lm	2	22	187.1x	2	42	10.4p	3	39	27.3v
2	1311.8	lm	2	26	187.0x	1	21	10.4p	3	27	27.3v
2	4011.7	lm	2	28	187.0x	2	39	10.3p	3	25	27.2v
2	4111.6	lm	2	24	186.9x	2	43	10.3p	3	41	27.2v
2	4311.7	lm	3	41	177.2y	2	44	10.3p	3	22	27.1v
1	2811.3	m	3	39	177.1y	2	31	10.3p	3	43	27.2v
2	511.1	m	3	44	176.9y	2	32	10.3p	3	45	27.1v
2	1011.3	m	3	42	176.9y	2	45	10.3p	3	26	27.0v
2	2211.2	m	3	45	176.8y	2	41	10.3p	3	42	27.1v

Days to germination			Plant height			Leaf length			Raceme length		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
2	2311.3m		3	40	176.8y	3	21	10.0q	3	28	27.0v
2	2611.1m		3	43	176.5y	2	36	9.8qr	3	44	26.9vw
2	2711.3m		3	21	174.6y	2	30	9.8r	3	23	26.9vw
2	3111.2m		1	5	161.2z	2	29	9.7r	3	40	26.9vw
2	3411.1m		1	11	160.4zA	2	37	9.5s	3	24	26.7vw
2	3511.3m		1	8	160.2 zA	2	38	9.4st	3	21	26.3wx
2	3911.2m		1	2	160.0 zA	2	23	9.4st	3	32	25.9xy
2	4211.3m		1	12	160.0 zA	2	28	9.3st	3	31	25.9xy
3	159.7n		1	13	159.4 zAB	1	12	9.3stu	1	37	25.3yz
3	179.8n		1	10	159.4 zAB	2	25	9.3stu	2	3	25.2z
3	379.7n		1	4	159.3 zAB	2	26	9.3stu	2	4	25.1z
3	389.3n		2	21	159.1 zAB	1	5	9.3stuv	1	38	25.2z
3	149.2n		1	9	158.5ABC	1	8	9.2stuvw	2		25.1z
3	169.1n		2	39	158.3 ABC	1	9	9.2stuvw	2	10	25.1z
3	189.3n		2	44	158.4 ABC	1	11	9.2stuvw	2	6	25.0z
3	199.2n		2	43	158.1 ABC	2	22	9.2stuvw	2	8	25.0z
3	209.3n		2	42	158.1BCD	1	2	9.2stuvw	2	7	25.0zA
3	27.6o		2	41	158.0 BCD	1	1	9.2tuvwx	2	2	24.8zAB
3	47.7o		2	40	158.1 BCD	1	3	9.2tuvwx	2	13	24.9 zAB
3	67.6o		2	45	157.9 BCD	1	6	9.2 tuvwx	2	1	24.8zAB
3	87.7o		1	6	156.6 BCD	1	4	9.2 tuvwx	2	12	24.8zAB
3	107.5o		1	7	156.0CD	2	27	9.2 tuvwx	2	9	24.7zABC
3	257.7o		1	3	155.3DE	1	7	9.2 tuvwx	2	5	24.7zABC
3	267.5o		1	1	152.9E	1	11	9.2 tuvwx	3	36	24.7zABC
3	307.6o		3	11	137.3F	2	24	9.2 tuvwx	2	44	24.3ABCD
3	327.7o		3	12	137.3 F	1	13	9.2 tuvwx	2	39	24.2BCD
3	347.8o		3	4	136.9 F	3	12	9.1uvwx	2	23	24.1CD
3	357.7o		3	9	136.7 F	3	3	9.0vwxy	2	25	24.1CD
3	407.6o		3	7	136.9 F	3	1	9.0 vwxy	2	27	24.1CD
3	437.7o		3	10	136.8 F	3	8	9.0 vwxy	2	41	24.1CD
3	17.2o		3	8	136.8 F	3	9	9.0 vwxy	2	40	23.9D
3	37.3o		3	2	136.7 F	3	2	9.0 vwxy	2	43	24.0D
3	57.1o		3	5	136.7 F	3	13	9.0 vwxy	2	45	23.90D
3	77.3o		3	6	136.8 F	3	5	9.0 vwxy	3	29	24.0D
3	97.2o		3	1	136.6 F	3	6	9.0 vwxy	2	28	23.9D
3	117.3o		3	13	136.5 F	3	10	9.0 vwxy	3	30	24.0D
3	127.0o		3	3	136.2 F	2	21	8.8 xyz	2	42	23.9D
3	137.3o		2	2	124.2G	3	7	8.7A	2	22	23.8DE
3	227.2o		2	6	124.3 G	3	4	8.7A	2	24	23.7DE
3	237.3o		2	10	124.2 G	3	11	8.7A	2	26	23.8DE
3	247.1o		2	3	124.1 G	2	10	8.2B	2	35	23.1EF
3	277.3o		2	7	124.2 G	2	12	8.1BC	2	33	23.1F
3	287.0o		2	11	124.3 G	2	5	8.0BC	2	34	23.0F

Days to germination			Plant height			Leaf length			Raceme length		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
3	317.3o		2	13	124.2 G	2	7	8.1BC	2	31	23.2F
3	337.1o		2	4	124.1 G	2	8	8.1BC	2	32	23.0F
3	367.3o		2	8	124.2 G	2	9	8.0BC	2	21	22.7F
3	417.2o		2	12	124.3 G	2	6	8.0BC	3	37	22.1G
3	447.1o		2	1	123.6 G	2	13	7.9C	3	38	22.0G
3	217.2o		2	5	123.7 G	2	11	7.9C	2	36	21.6GH
3	397.3o		2	9	123.6 G	2	1	7.9C	2	29	21.1H
3	427.2o		1	36	122.8 G	2	2	7.8C	2	30	21.2H
3	457.1o		3	36	119.8 G	2	3	7.8C	2	38	19.2I
3	297.0o		2	36	104.1 G	2	4	7.5D	2	37	19.1I

**APPENDIX IX:** Means of *Lablab purpureus* site x accession interaction for pod length, pod width, seed length and seed width

Pod length			Pod width			Seed length			Seed width		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
3	36	5.6a	3	36	2.2a	1	14	1.1a	1	17	0.9a
1	32	5.6a	1	32	2.2a	1	17	1.1a	1	14	0.9a
1	29	5.6a	1	29	2.2a	1	39	1.1a	1	18	0.9a
1	34	5.6a	1	34	2.2a	1	33	1.1a	1	19	0.9a
3	22	5.6a	3	22	2.2a	1	35	1.1a	1	20	0.9a
3	23	5.6a	3	23	2.1a	1	42	1.0b	1	16	0.9a
1	31	5.5a	1	31	2.2a	1	43	1.0b	1	15	0.9a
1	22	5.5a	1	22	2.3ab	1	44	1.0b	1	35	0.9a
1	23	5.5a	1	23	2.3ab	1	19	1.1a	1	34	0.9a
1	30	5.5a	1	30	2.1a	1	41	1.0b	1	33	0.9a
1	33	5.5a	1	33	2.1a	1	40	1.0b	1	21	0.9a
1	35	5.5a	1	35	2.2a	1	15	1.1a	1	26	0.9a
3	31	5.5a	3	31	2.2a	1	45	1.0b	1	9	0.9a
3	29	5.5a	3	29	2.2a	1	34	1.1a	1	8	0.9a
3	30	5.5a	3	30	2.1a	1	18	1.1a	1	2	0.9a
3	33	5.5a	3	33	2.2a	1	20	1.1a	1	13	0.9a
3	34	5.5a	3	34	2.2a	1	16	1.1a	1	23	0.9b
3	32	5.4ab	3	32	2.1a	1	31	1.1a	1	5	0.9a
3	35	5.4ab	3	35	2.2a	1	32	1.1a	1	6	0.9a
1	12	5.4ab	1	12	2.1a	1	29	1.1a	1	4	0.9a
3	2	5.4ab	3	2	2.1a	1	37	0.8c	1	12	0.9a
3	4	5.4ab	3	4	2.2a	1	38	0.8c	1	27	0.9a
3	9	5.4ab	3	9	2.2a	1	30	1.1a	1	7	0.9a
3	13	5.4ab	3	13	2.1a	3	17	1.1a	1	10	0.9a
1	5	5.4ab	1	5	2.2a	3	14	1.1a	1	25	0.9a
1	11	5.4ab	1	11	2.2a	3	19	1.1a	1	3	0.9a
1	36	5.4ab	1	36	2.1a	3	20	1.1a	1	11	0.9a
3	12	5.4ab	3	12	2.2a	3	15	1.1a	1	22	0.9a
1	1	5.3abc	1	1	2.2a	3	16	1.1a	1	24	0.9a
1	8	5.3abc	1	8	2.2a	3	18	1.1a	1	1	0.9a
1	9	5.3abc	1	9	2.2a	1	25	1.1a	1	31	0.9a
1	10	5.3abc	1	10	2.2a	1	24	1.1a	1	28	0.9a
3	1	5.3abc	3	1	2.1a	1	23	1.1a	1	32	0.9a
3	3	5.3abc	3	3	2.2a	1	27	1.1a	1	36	0.9a
3	5	5.3abc	3	5	2.2a	1	28	1.1a	3	17	0.9a
3	6	5.3abc	3	6	2.1a	1	26	1.1a	3	14	0.9a
3	7	5.3abc	3	7	2.2a	1	22	1.1a	3	20	0.9a
3	8	5.3abc	3	8	2.2a	1	36	1.1a	3	15	0.9a
3	10	5.3abc	3	10	2.1a	2	18	1.0b	3	16	0.9a
3	11	5.3abc	3	11	2.1a	2	14	1.0b	3	18	0.9a

Pod length			Pod width			Seed length			Seed width		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
1	2	5.3abc	1	2	2.2a	2	16	1.0b	3	19	0.9a
1	3	5.3abc	1	3	2.2a	2	17	1.0b	1	41	0.9a
1	6	5.3abc	1	6	2.1a	2	19	1.0b	1	40	0.9a
1	7	5.3abc	1	7	2.2a	2	20	1.0b	1	44	0.9a
1	4	5.3abc	1	4	2.1a	2	15	1.0b	1	43	0.9a
3	24	5.3abc	3	24	2.2a	3	29	1.1a	1	45	0.9a
3	26	5.3abc	3	26	2.2a	3	30	1.1a	1	39	0.9a
3	27	5.3abc	3	27	2.1a	3	33	1.1a	1	42	0.9a
3	28	5.3abc	3	28	2.2a	3	34	1.1a	3	7	0.9a
1	13	5.2abc	1	13	2.2a	3	39	1.1a	3	3	0.9a
3	25	5.2abc	3	25	2.2a	3	40	1.1a	3	1	0.9a
1	21	5.2abc	1	21	2.1a	3	41	1.1a	3	8	0.9a
1	25	5.2abc	1	25	2.2a	3	42	1.1a	3	33	0.9a
1	26	5.2abc	1	26	2.2a	3	44	1.1a	3	4	0.9a
3	21	5.2abc	3	21	2.2a	3	31	1.1a	3	35	0.9a
1	24	5.1bcd	1	24	2.2a	3	32	1.1a	3	13	0.9a
1	27	5.1bcd	1	27	2.1a	3	35	1.1a	3	9	0.9a
2	22	5.1bcd	2	22	2.1a	3	43	1.1a	3	11	0.9a
2	23	5.1bcd	2	23	2.1a	3	45	1.1a	3	2	0.9a
2	7	5.1bcd	2	7	2.1a	3	36	1.1a	3	10	0.9a
1	28	5.1bcd	1	28	2.2a	3	25	1.1a	3	12	0.9a
2	1	5.1bcd	2	1	2.0a	3	27	1.1a	3	34	0.9a
2	2	5.1bcd	2	2	2.1a	3	37	0.8c	3	6	0.9a
2	4	5.1bcd	2	4	2.1a	3	38	0.8c	1	29	0.9a
2	5	5.1bcd	2	5	2.0a	3	23	1.2d	3	5	0.9a
2	6	5.1bcd	2	6	2.0a	3	24	1.1a	1	30	0.9a
2	9	5.1bcd	2	9	2.1a	3	22	1.2d	2	16	0.8b
2	10	5.1bcd	2	10	2.0abc	3	26	1.1a	2	14	0.8b
2	11	5.1bcd	2	11	2.1a	3	28	1.1a	2	18	0.8b
2	12	5.1bcd	2	12	2.1a	2	33	1.0b	2	20	0.8b
2	3	5.0cde	2	3	2.0abc	2	34	1.0b	2	15	0.8b
2	8	5.0cde	2	8	2.0abc	2	35	1.0b	2	19	0.8b
2	13	5.0cde	2	13	2.1a	2	40	1.0b	2	17	0.8b
3	15	5.0cde	3	15	2.2a	2	42	1.0b	3	39	0.9a
3	17	5.0cde	3	17	2.2a	1	21	1.1a	3	27	0.9a
3	20	5.0cde	3	20	2.1a	2	39	1.0b	3	25	0.9a
3	14	4.9def	3	14	2.2	2	43	1.0b	3	41	0.9a
3	16	4.9def	3	16	2.2a	2	44	1.0b	3	22	0.9a
3	18	4.9def	3	18	2.1a	2	31	1.0b	3	43	0.9a
3	19	4.9def	3	19	2.2a	2	32	1.0b	3	45	0.9a
1	41	4.9def	1	41	2.1a	2	45	1.0b	3	26	0.9a
3	45	4.9 def	3	45	2.2a	2	41	1.0b	3	42	0.9a
1	15	4.9 def	1	15	2.2a	3	21	1.1a	3	28	0.9a
1	16	4.9 def	1	16	2.1a	2	36	1.0b	3	44	0.9a

Pod length			Pod width			Seed length			Seed width		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
1	17	4.9 def	1	17	2.2a	2	30	1.0b	3	23	0.9a
1	19	4.9 def	1	19	2.2a	2	29	1.0b	3	40	0.9a
1	20	4.9 def	1	20	2.2a	2	37	0.7d	3	24	0.9a
1	39	4.9 def	1	39	2.2a	2	38	0.7d	3	21	0.9a
1	40	4.9 def	1	40	2.2a	2	23	1.0b	3	32	0.9a
1	43	4.9 def	1	43	2.1a	2	28	1.0b	3	31	0.9a
1	44	4.9 def	1	44	2.2a	1	12	1.1a	1	37	0.9a
1	45	4.9 def	1	45	2.2a	2	25	1.0b	2	3	0.8b
3	39	4.9 def	3	39	2.1a	2	26	1.0b	2	4	0.8b
3	41	4.9 def	3	41	2.2a	1	5	1.1a	1	38	0.9a
3	43	4.9 def	3	43	2.1a	1	8	1.1a	2		0.8b
1	14	4.8efg	1	14	2.2a	1	9	1.1a	2	10	0.8b
1	18	4.8efg	1	18	2.1a	1	11	1.1a	2	6	0.8b
3	40	4.8efg	3	40	2.1a	2	22	1.0b	2	8	0.8b
3	42	4.8efg	3	42	2.1a	1	2	1.1a	2	7	0.8b
3	44	4.8efg	3	44	2.1a	1	1	1.1a	2	2	0.8b
1	42	4.8efg	1	42	2.1a	1	3	1.1a	2	13	0.8b
2	24	4.8efg	2	24	2.2a	1	6	1.1a	2	1	0.8b
2	25	4.8efg	2	25	2.2a	1	4	1.1a	2	12	0.8b
2	26	4.8efg	2	26	2.1a	2	27	1.0b	2	9	0.8b
2	27	4.8efg	2	27	2.2a	1	7	1.1a	2	5	0.8b
2	28	4.8efg	2	28	2.1a	1	11	1.1a	3	36	0.9a
2	31	4.7fgh	2	31	2.1a	2	24	1.0b	2	44	0.8b
2	32	4.7fgh	2	32	2.2a	1	13	1.1a	2	39	0.8b
2	33	4.6fgh	2	33	2.0abc	3	12	1.1a	2	23	0.8b
2	34	4.6fgh	2	34	2.1a	3	3	1.1a	2	25	0.8b
2	35	4.6fgh	2	35	2.1a	3	1	1.1a	2	27	0.8b
2	30	4.6fgh	2	30	2.0abc	3	8	1.1a	2	41	0.8b
2	39	4.6fgh	2	39	2.1a	3	9	1.1a	2	40	0.8b
2	41	4.6fgh	2	41	2.1a	3	2	1.1a	2	43	0.8b
2	42	4.6fgh	2	42	2.0abc	3	13	1.1a	2	45	0.8b
2	45	4.6fgh	2	45	2.1a	3	5	1.1a	3	29	0.8b
2	14	4.5ghi	2	14	2.1a	3	6	1.1a	2	28	0.8b
2	15	4.5ghi	2	15	2.0abc	3	10	1.1a	3	30	0.8b
2	16	4.5ghi	2	16	2.1a	2	21	1.0b	2	42	0.8b
2	17	4.5ghi	2	17	2.1a	3	7	1.1a	2	22	23.8DE
2	18	4.5ghi	2	18	2.1a	3	4	1.1a	2	24	23.7DE
2	19	4.5ghi	2	19	2.0abc	3	11	1.1a	2	26	23.8DE
2	20	4.5ghi	2	20	2.1a	2	10	1.0b	2	35	23.1EF
2	29	4.5ghi	2	29	2.0abc	2	12	1.0b	2	33	23.1F
2	40	4.5ghi	2	40	2.1a	2	5	1.0b	2	34	23.0F
2	43	4.5ghi	2	43	2.1a	2	7	1.0b	2	31	23.2F
2	44	4.5hij	2	44	2.0abc	2	8	1.0b	2	32	23.0F
2	36	4.5hij	2	36	2.2a	2	9	1.0b	2	21	22.7F



Pod length			Pod width			Seed length			Seed width		
Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean	Site	Ac.	Mean
3	38	4.5 hij	3	38	1.8cd	2	6	1.0b	3	37	22.1G
3	37	4.4 hij	3	37	1.9cd	2	13	1.0b	3	38	22.0G
1	37	4.4 hij	1	37	1.9cd	2	11	1.0b	2	36	21.6GH
1	38	4.4 hij	1	38	1.8d	2	1	1.0b	2	29	21.1H
2	21	4.3ijk	2	21	2.0abc	2	2	1.0b	2	30	21.2H
2	37	4.11	2	37	1.8cd	2	3	1.0b	2	38	19.2I
2	38	4.11	2	38	1.8cd	2	4	1.0b	2	37	19.1I

## APPENDIX X: Absorbance curve obtained by Nanodrop spectrophotometer

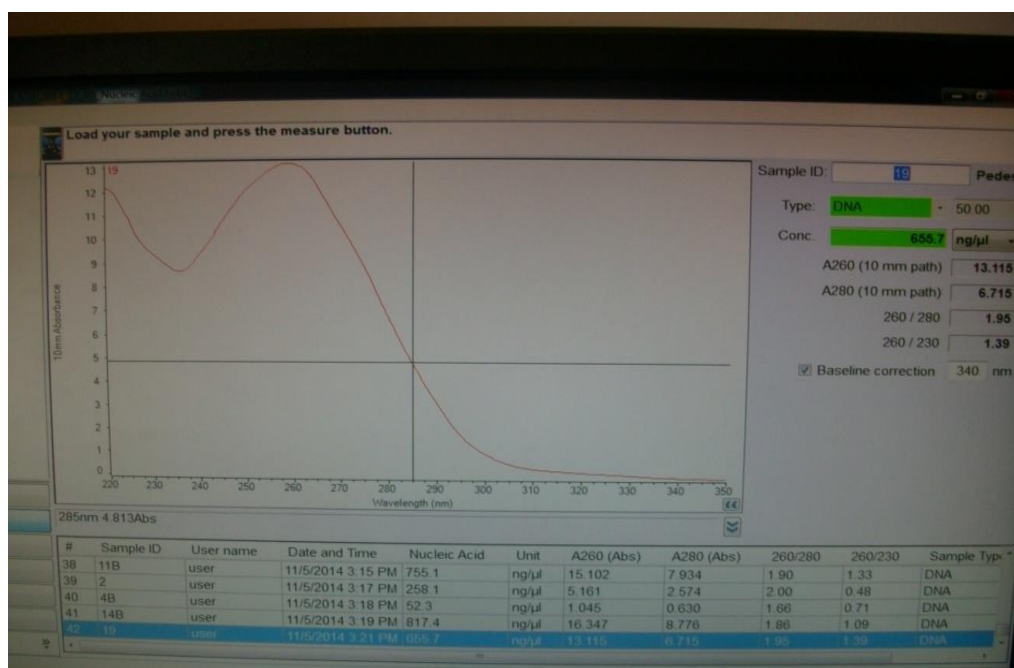


Fig. 2 Absorbance curve showing high concentration and purity of isolated DNA of *Lablab*.

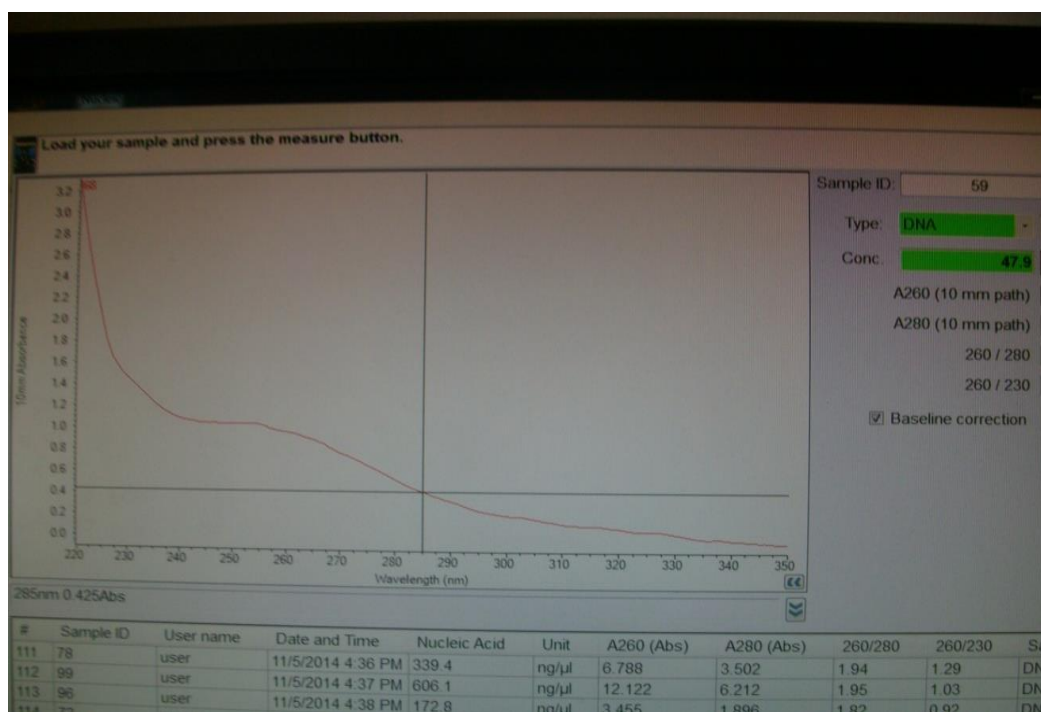


Fig.3 Absorbance curve showing low concentration and purity of isolated DNA of *Lablab purpureus*. DNA samples that had low concentration and purity were discarded and more leaves harvested for fresh DNA extraction.

**APPENDIX XI: Agarose gel profiles of SSR polymerase chain reaction (PCR) products**

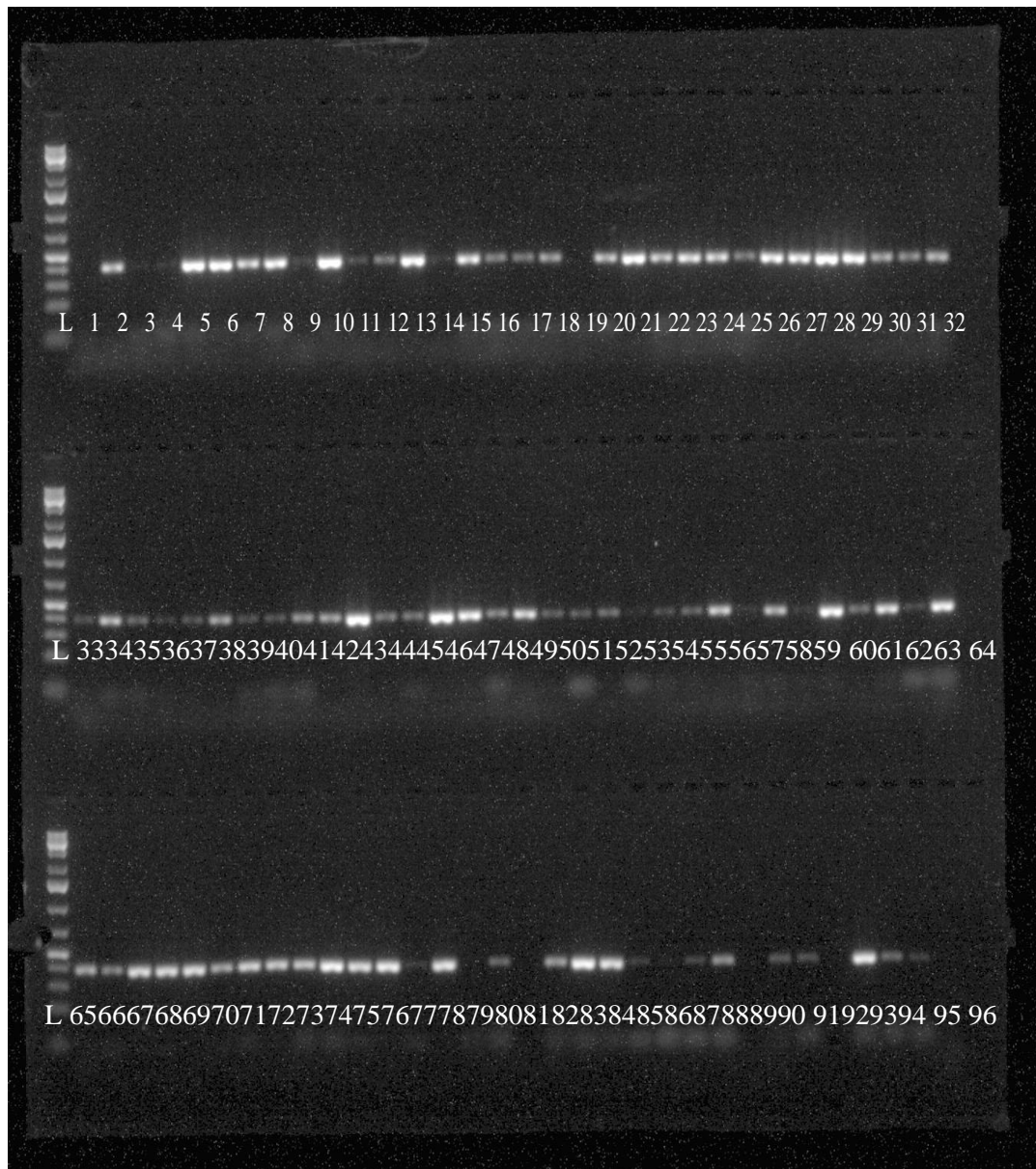


Plate 4: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT3.

Key:L is DNA ladder; Numbers 1 to 95 are are DNA samples from *Lablab purpureus* accessions; Number 96 is sterile double distilled water which replaced one of DNA templates used during screening of primers.

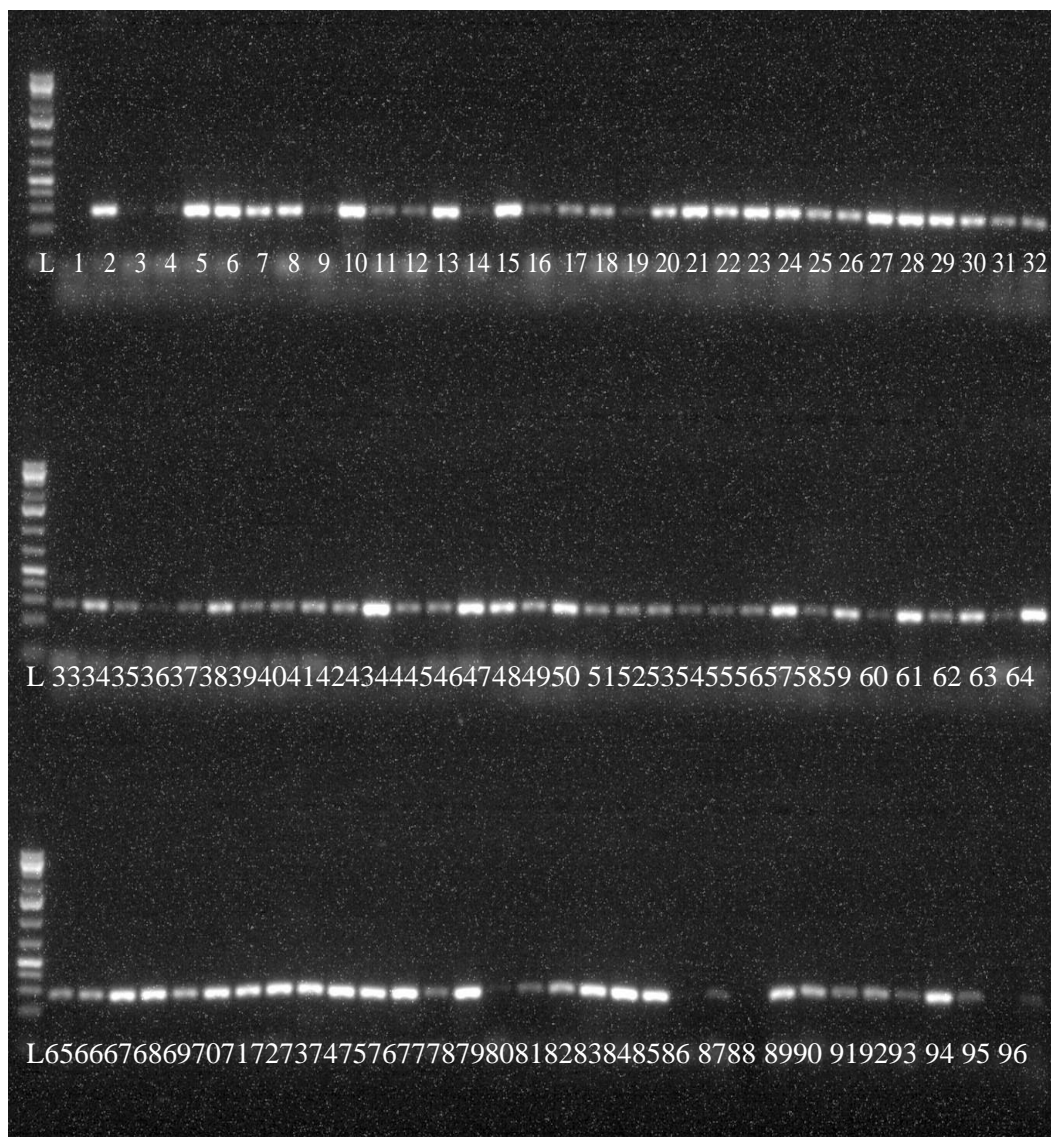


Plate 5: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT1.

Key: L is DNA ladder; numbers 1 to 96 are DNA samples from *Lablab purpureus* accessions.

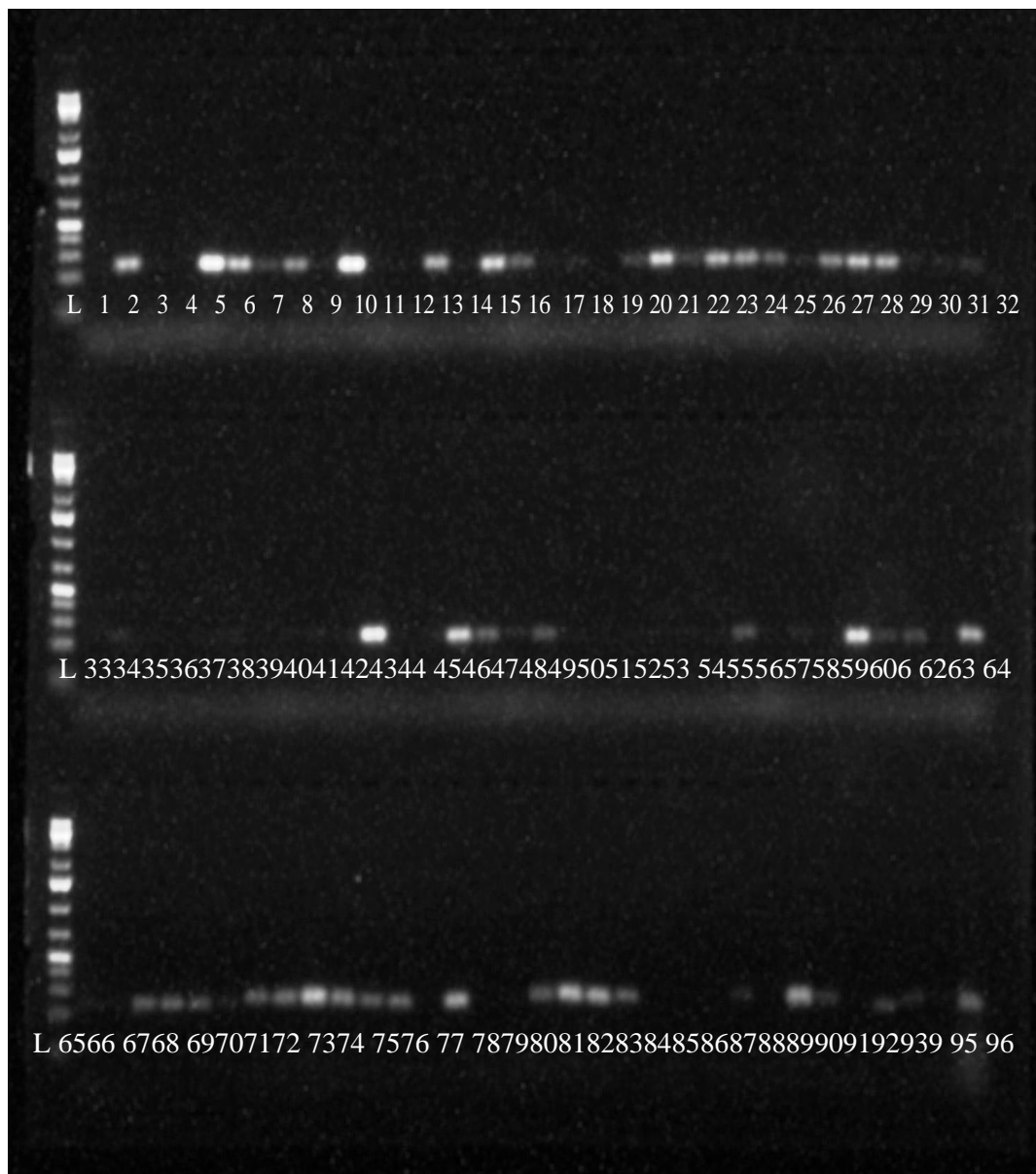


Plate 6: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT2.

Key: L is DNA ladder ; Numbers 1 to 96 are DNA samples from *Lablab purpureus* accessions.

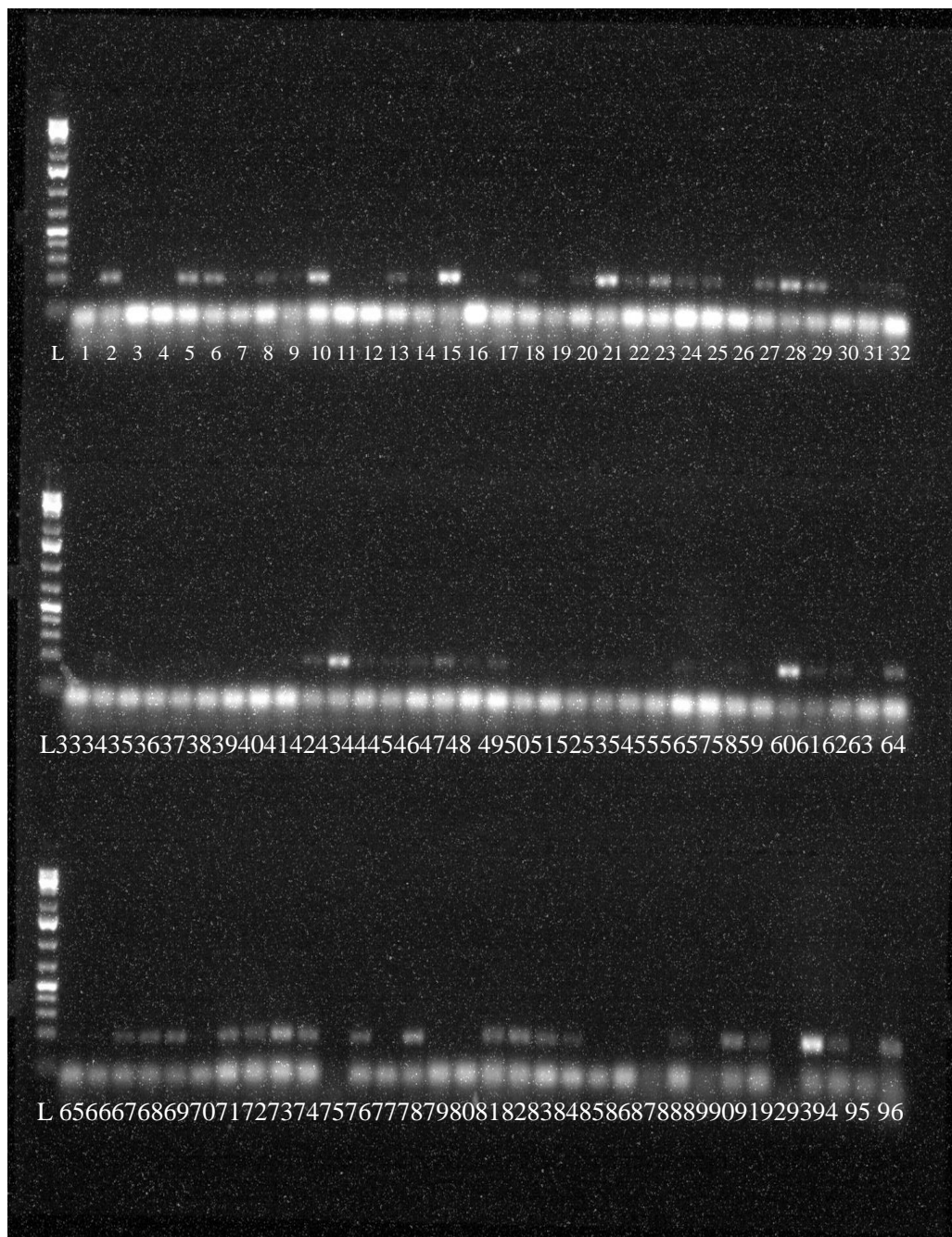


Plate 7: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT6.

Key:L is DNA ladder; Numbers 1 to 96 are DNA samples from *Lablab purpureus* accessions.

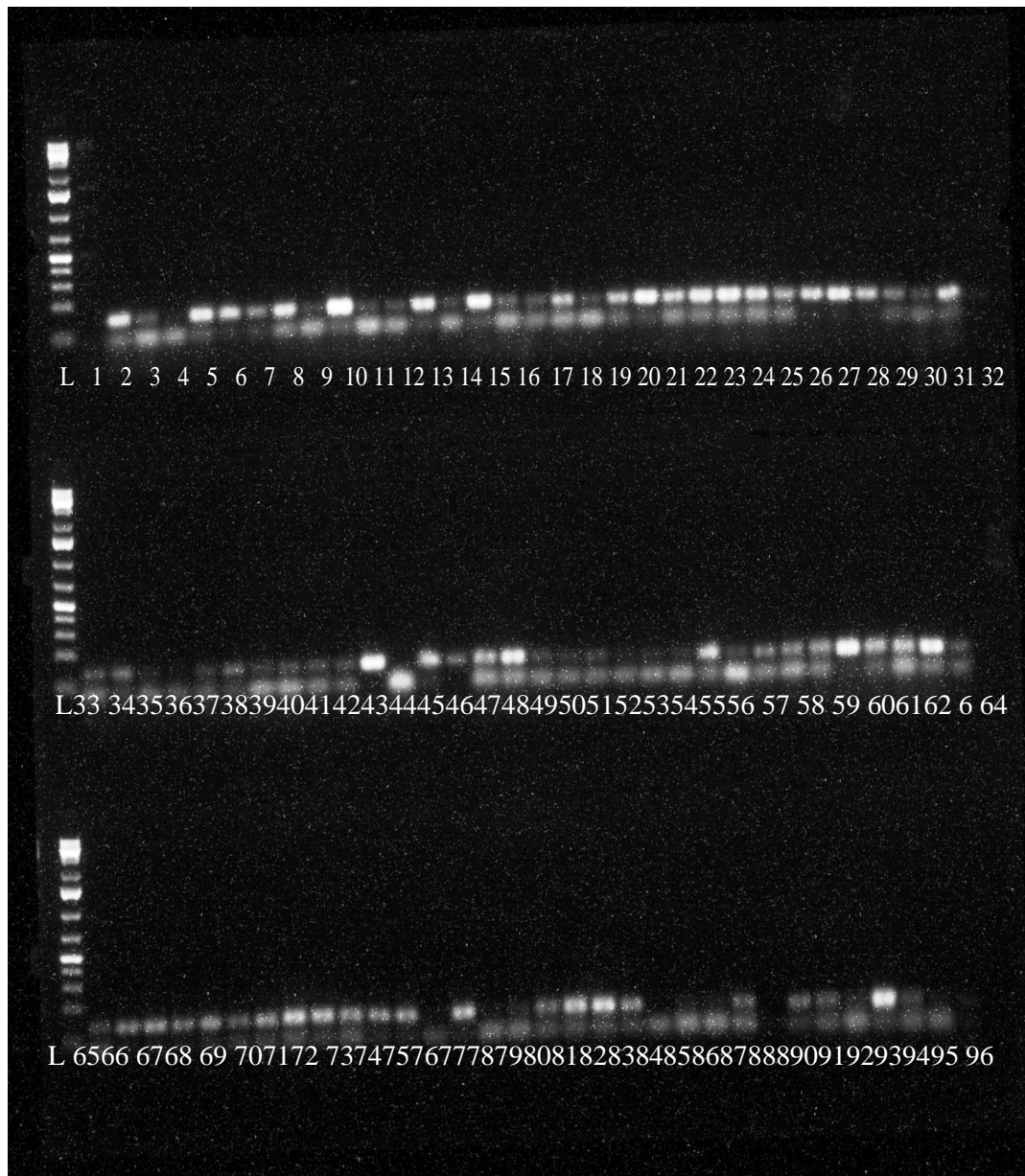


Plate 8: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT7.

Key:L is DNA ladder; Numbers 1 to 95 are DNA samples from *Lablab purpureus* accessions; Number 96 is sterile double distilled water which replaced one of the DNA templates used during screening of primers.

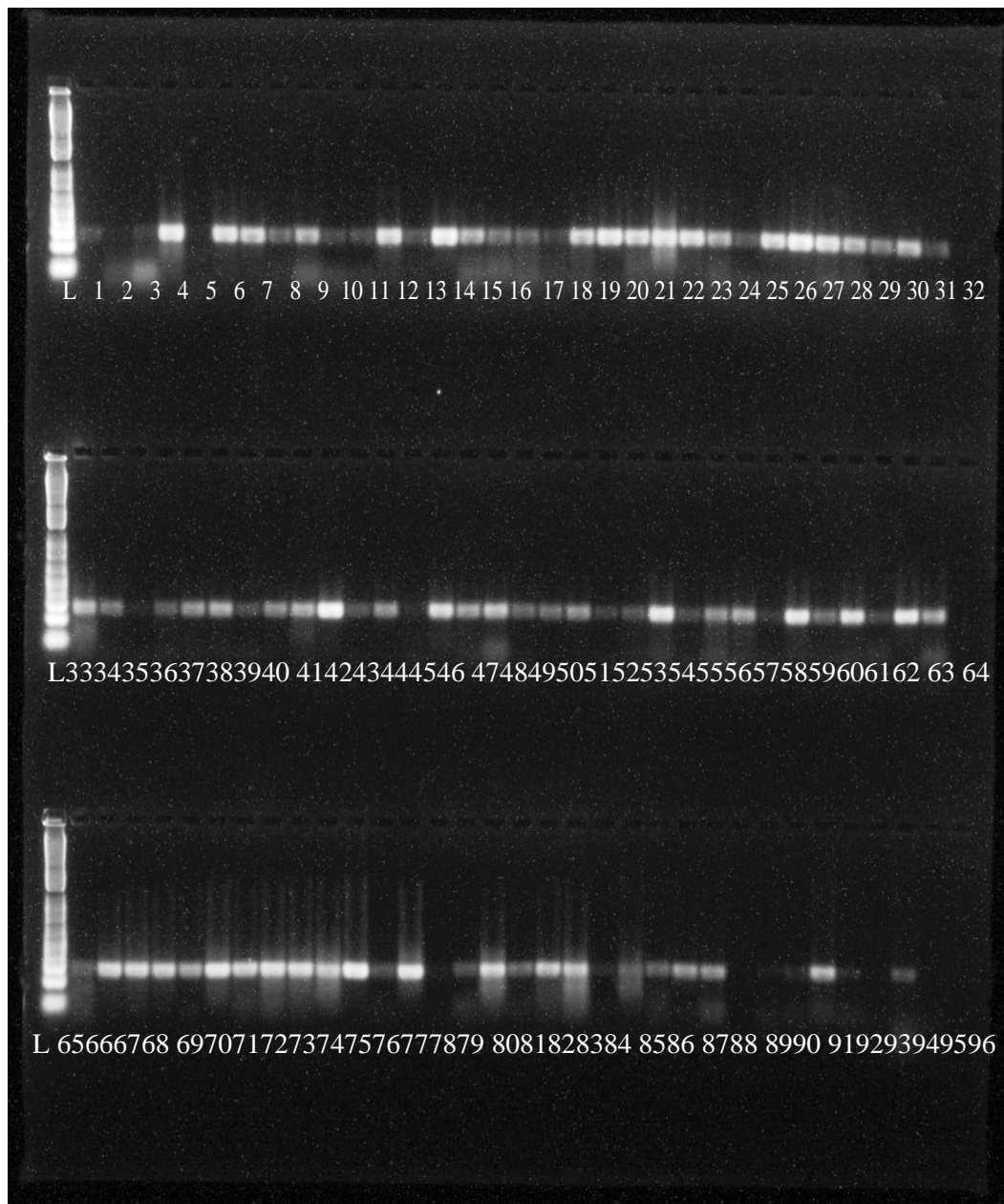


Plate 9: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT14

Key: L is DNA ladder; Numbers 1 to 95 are DNA samples from *Lablab purpureus* accessions; Number 96 is sterile double distilled water which replaced one of the DNA templates used during screening of primers.



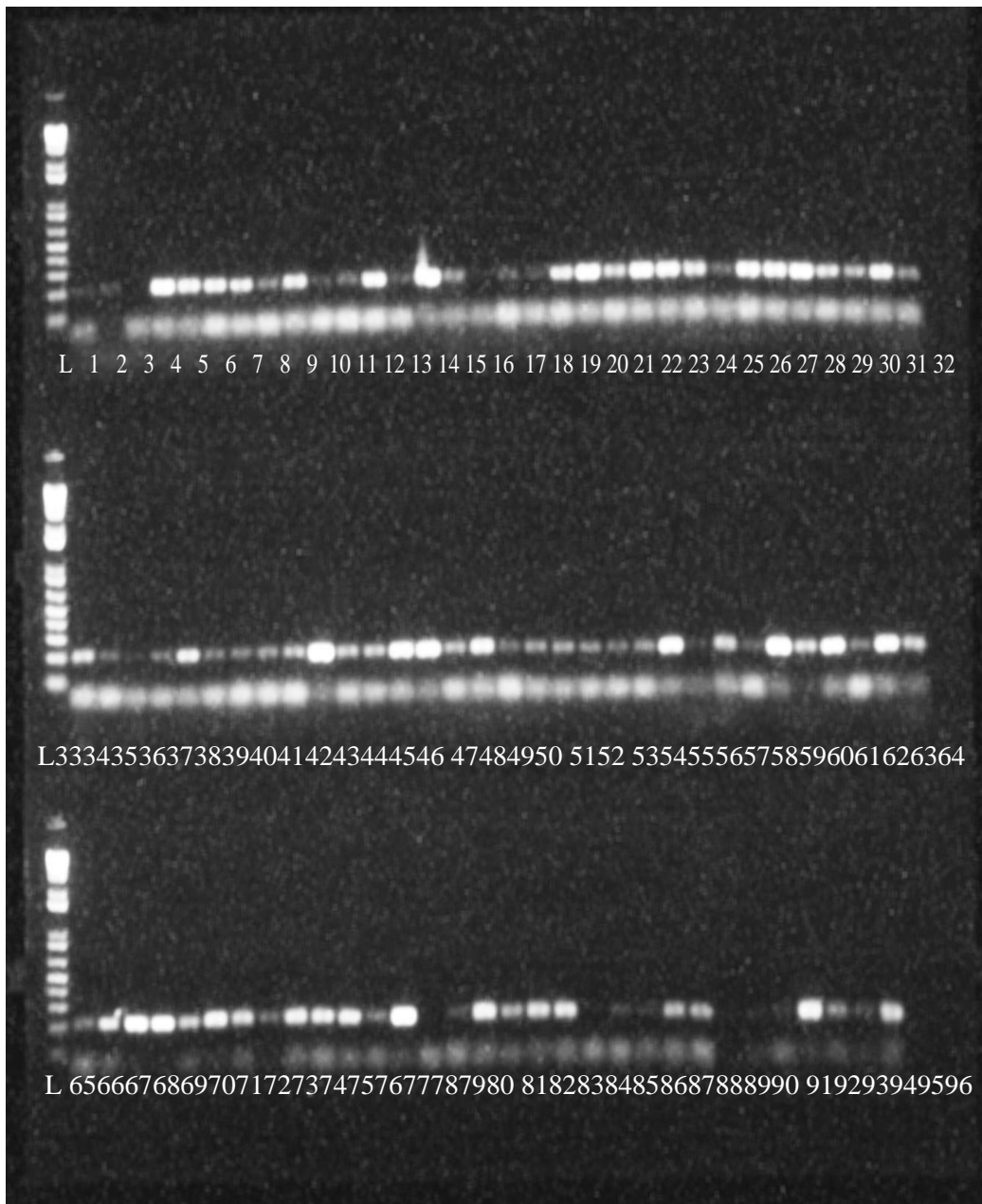


Plate 10: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT24.

Key:L is DNA ladder; Numbers 1 to 95 are DNA samples from *Lablab purpureus* accessions; Number 96 is sterile double distilled water which replaced one of the DNA templates used during screening of primers.

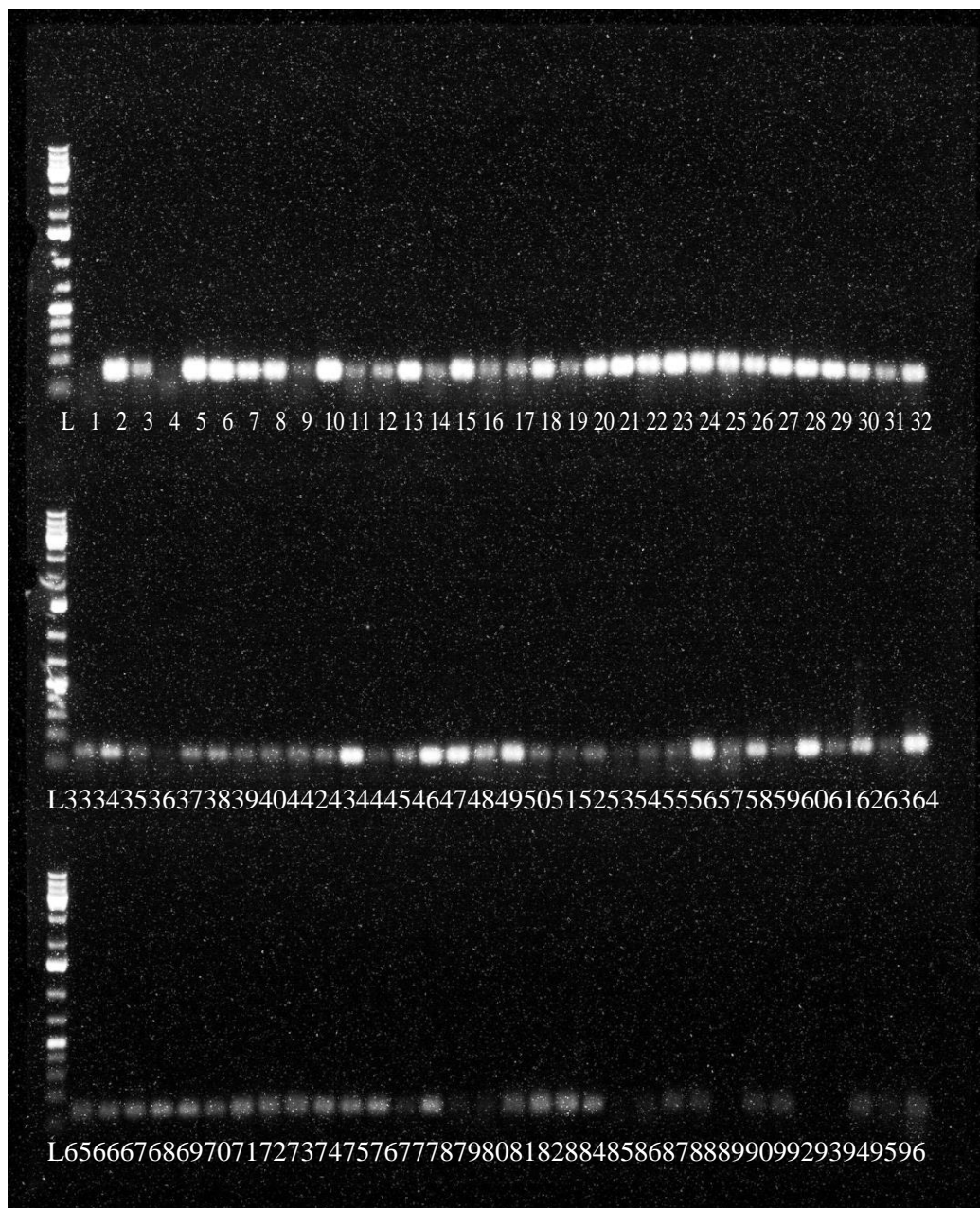


Plate 11: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT25.

Key:L is DNA ladder; numbers 1 to 96 are are DNA samples from *Lablab purpureus* accessions.

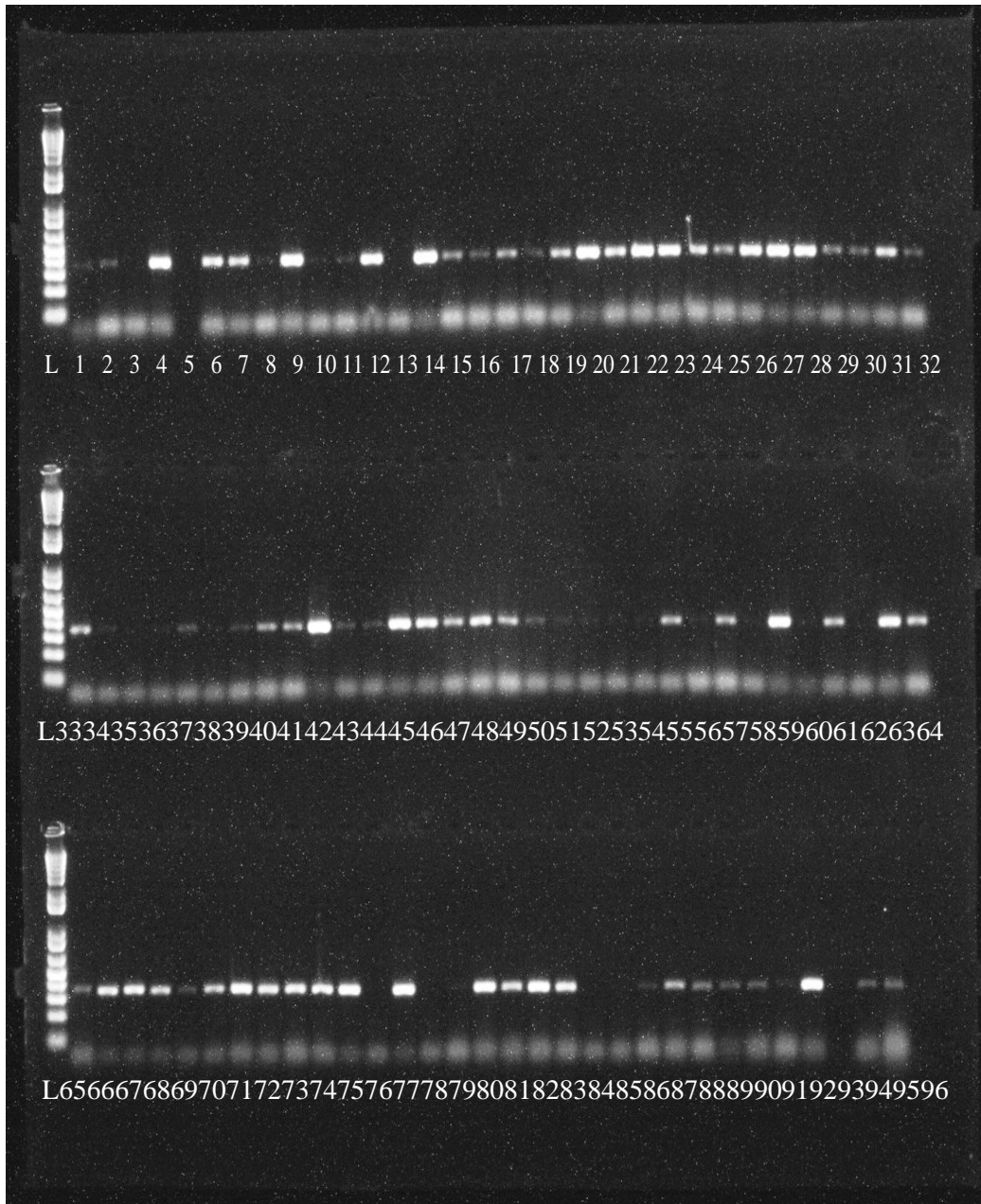


Plate 12: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT33.

Key: L is DNA ladder; Numbers 1 to 95 are are DNA samples from *Lablab purpureus* accessions; Number 96 is sterile double distilled water which replaced one of the DNA templates used during screening of primers.

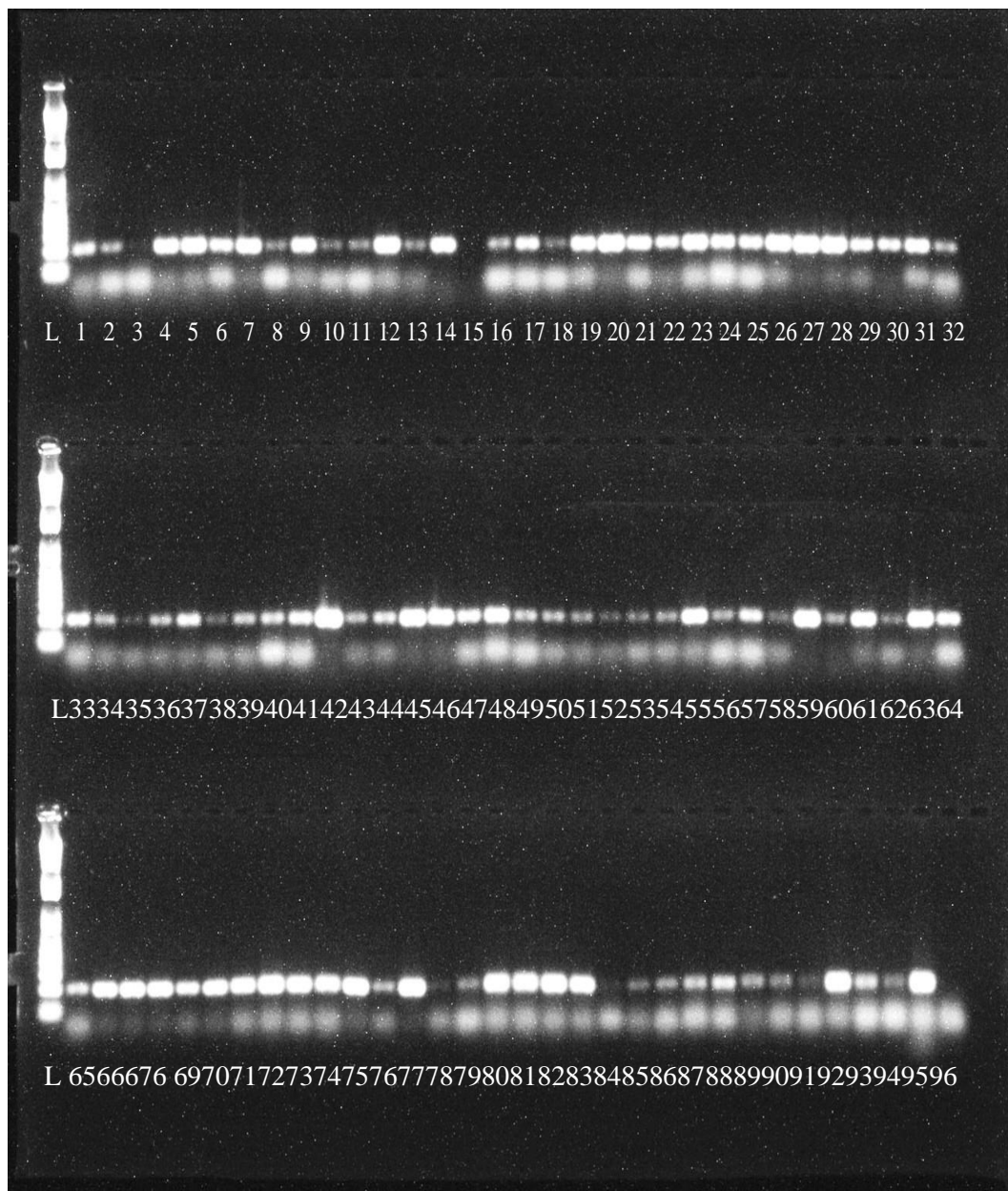


Plate 13: PCR amplification of *Lablab purpureus* accessions using SSR primer LabT28.

Key:L is DNA ladder; numbers 1 to 96 are are DNA samples from *Lablab purpureus* accessions.

**APPENDIX XII: Allele frequencies by population for codominant data**

Allele Frequencies by Populations																
Lo	AI	EM	GC	GE	LM	MC	MK	ME	MU	MW	NB	NK	NY	RV	TH	WE
1	292	0.50	0.10	0.29	0.06	0.07	0.13	0.38	0.3	0.70	0.20	0.79	0.38	0.67	0.21	0.17
	294	0.50	0.20	0.41	0.38	0.21	0.38	0.38	0.5	0.20	0.30	0.21	0.38	0.17	0.57	0.17
	296	0.00	0.70	0.29	0.56	0.71	0.50	0.25	0.2	0.10	0.50	0.00	0.25	0.17	0.21	0.67
2	236	0.50	0.00	0.00	0.20	0.10	0.00	0.33	0.0	0.00	0.13	0.00	0.00	0.00	0.00	1.00
	242	0.13	0.25	0.17	0.50	0.30	0.67	0.50	0.3	0.50	0.38	0.00	0.67	0.50	0.00	0.00
	248	0.38	0.75	0.50	0.30	0.60	0.33	0.17	0.5	0.50	0.25	0.00	0.00	0.50	0.10	0.00
	254	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.2	0.00	0.25	0.00	0.33	0.00	0.90	0.00
3	350	0.38	0.33	0.59	0.00	0.00	0.00	0.50	0.2	0.70	0.30	0.17	0.38	0.50	0.00	0.17
	400	0.50	0.67	0.41	0.25	0.25	0.83	0.50	0.6	0.20	0.70	0.67	0.63	0.33	0.42	0.83
	450	0.13	0.00	0.00	0.69	0.75	0.17	0.00	0.2	0.10	0.00	0.17	0.00	0.17	0.58	0.00
	500	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	206	1.00	0.75	0.13	0.10	0.10	0.33	0.75	0.3	0.00	0.50	0.00	0.50	0.50	0.10	0.25
	209	0.00	0.25	0.63	0.90	0.50	0.33	0.25	0.6	0.50	0.25	0.00	0.50	0.50	0.80	0.75
	212	0.00	0.00	0.25	0.00	0.40	0.33	0.00	0.1	0.50	0.25	0.00	0.00	0.00	0.10	0.00
7	185	0.50	0.40	0.27	0.50	0.06	0.33	0.25	0.2	0.75	0.40	1.00	0.33	0.83	0.14	0.25
	188	0.50	0.60	0.42	0.50	0.61	0.50	0.50	0.5	0.13	0.40	0.00	0.33	0.17	0.50	0.75
	191	0.00	0.00	0.31	0.00	0.33	0.17	0.00	0.3	0.13	0.20	0.00	0.33	0.00	0.36	0.00
	195	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	253	0.50	0.50	0.03	0.06	0.00	0.17	0.33	0.0	0.00	0.50	0.50	0.00	0.17	0.14	0.00
	255	0.50	0.50	0.44	0.72	0.17	0.17	0.67	0.1	0.38	0.25	0.29	0.38	0.83	0.43	0.50
	257	0.00	0.00	0.53	0.22	0.83	0.67	0.00	0.9	0.63	0.25	0.21	0.63	0.00	0.43	0.50
24	200	0.25	0.38	0.12	0.65	0.11	0.33	0.25	0.0	0.80	0.40	0.36	0.00	0.67	0.14	0.00
	202	0.50	0.50	0.71	0.35	0.33	0.67	0.38	0.4	0.20	0.50	0.36	0.38	0.33	0.36	0.88
	204	0.25	0.13	0.18	0.00	0.56	0.00	0.38	0.6	0.00	0.10	0.29	0.63	0.00	0.50	0.13
25	186	0.75	0.00	0.34	0.50	0.50	0.33	0.83	0.3	0.13	0.10	0.21	0.33	0.50	0.14	1.00
	190	0.25	0.13	0.63	0.50	0.40	0.67	0.17	0.5	0.88	0.30	0.36	0.67	0.33	0.50	0.00
	194	0.00	0.88	0.03	0.00	0.10	0.00	0.00	0.2	0.00	0.60	0.43	0.00	0.17	0.36	0.00
28	201	0.38	0.10	0.26	0.65	0.28	0.38	0.25	0.5	0.80	0.33	0.57	0.38	0.67	0.21	0.00
	204	0.50	0.30	0.50	0.30	0.72	0.25	0.63	0.5	0.20	0.42	0.43	0.63	0.33	0.14	0.25
	207	0.13	0.60	0.24	0.05	0.00	0.38	0.13	0.0	0.00	0.25	0.00	0.00	0.00	0.64	0.75
33	300	0.00	0.25	0.04	0.14	0.00	0.50	0.33	0.1	0.00	0.75	0.00	0.50	0.50	0.07	0.75
	350	0.63	0.75	0.54	0.57	0.19	0.50	0.50	0.8	0.75	0.25	0.80	0.50	0.17	0.36	0.25
	400	0.38	0.00	0.42	0.29	0.81	0.00	0.17	0.1	0.25	0.00	0.20	0.00	0.33	0.57	0.00

**KEY:**

Lo – Locus; AI – alleles base pairs; EM – Embu; GC - Genebank coast; GE - Genebank Eastern; LM - Lamu; MC - Machkos; Mk – Makueni; ME - Meru; MU - Murang'a; Mw – Mwingi; NB –Nairobi; NK – Nakuru; NY – Nyeri; RV - Rift Valley; TH – Thika; WE –Western; 1, 2, 3, 6, 7, 14, 24, 25, 28 and 33 are SSR primers-LabT1, LabT2, LabT3, LabT6, LabT7, LabT14, LabT24, LabT25, LabT28, LabT33.

**APPENDIX XIII: Sample size, number of alleles, number of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index**

<b>Pop</b>	<b>Locus</b>	<b>N</b>	<b>Na</b>	<b>Ne</b>	<b>I</b>	<b>Ho</b>	<b>He</b>	<b>UHe</b>	<b>F</b>
<b>EM</b>	<b>LabT1</b>	4	2.0	2.0	0.7	1.0	0.5	0.6	-1.0
	<b>LabT2</b>	4	3.0	2.5	1.0	0.3	0.6	0.7	0.6
	<b>LabT3</b>	4	3.0	2.5	1.0	1.0	0.6	0.7	-0.7
	<b>LabT6</b>	4	1.0	1.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT7</b>	4	2.0	2.0	0.7	1.0	0.5	0.6	-1.0
	<b>LabT14</b>	4	2.0	2.0	0.7	1.0	0.5	0.6	-1.0
	<b>LabT24</b>	4	3.0	2.7	1.0	1.0	0.6	0.7	-0.6
	<b>LabT25</b>	4	2.0	1.6	0.6	0.5	0.4	0.4	-0.3
	<b>LabT28</b>	4	3.0	2.5	1.0	1.0	0.6	0.7	-0.7
	<b>LabT33</b>	4	2.0	1.9	0.7	0.8	0.5	0.5	-0.6
<b>GC</b>	<b>LabT1</b>	5	3.0	1.9	0.8	0.4	0.5	0.5	0.1
	<b>LabT2</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
	<b>LabT3</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT6</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
	<b>LabT7</b>	5	2.0	1.9	0.7	0.8	0.5	0.5	-0.7
	<b>LabT14</b>	5	2.0	2.0	0.7	0.2	0.5	0.6	0.6
	<b>LabT24</b>	4	3.0	2.5	1.0	0.5	0.6	0.7	0.2
	<b>LabT25</b>	4	2.0	1.3	0.4	0.3	0.2	0.3	-0.1
	<b>LabT28</b>	5	3.0	2.2	0.9	0.2	0.5	0.6	0.6
	<b>LabT33</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
<b>GE</b>	<b>LabT1</b>	17	3.0	2.9	1.1	0.4	0.7	0.7	0.5
	<b>LabT2</b>	6	3.0	2.6	1.0	0.3	0.6	0.7	0.5
	<b>LabT3</b>	16	2.0	1.9	0.7	0.4	0.5	0.5	0.1
	<b>LabT6</b>	4	3.0	2.1	0.9	0.3	0.5	0.6	0.5
	<b>LabT7</b>	13	3.0	2.9	1.1	0.4	0.7	0.7	0.4
	<b>LabT14</b>	16	3.0	2.1	0.8	0.1	0.5	0.5	0.8
	<b>LabT24</b>	17	3.0	1.8	0.8	0.2	0.5	0.5	0.5
	<b>LabT25</b>	16	3.0	2.0	0.8	0.4	0.5	0.5	0.2
	<b>LabT28</b>	17	3.0	2.7	1.0	0.3	0.6	0.6	0.5
	<b>LabT33</b>	13	3.0	2.1	0.8	0.3	0.5	0.6	0.4
<b>LM</b>	<b>LabT1</b>	8	3.0	2.2	0.9	0.8	0.5	0.6	-0.4
	<b>LabT2</b>	5	3.0	2.6	1.0	0.6	0.6	0.7	0.0
	<b>LabT3</b>	8	3.0	1.9	0.8	0.6	0.5	0.5	-0.4
	<b>LabT6</b>	5	2.0	1.2	0.3	0.2	0.2	0.2	-0.1
	<b>LabT7</b>	9	2.0	2.0	0.7	0.6	0.5	0.5	-0.1

## APPENDIX XIII: Continued

Pop	Locus	N	Na	Ne	I	Ho	He	UHe	F
	<b>LabT14</b>	9	3.0	1.7	0.7	0.3	0.4	0.5	0.2
	<b>LabT24</b>	10	2.0	1.8	0.6	0.5	0.5	0.5	-0.1
	<b>LabT25</b>	10	2.0	2.0	0.7	0.6	0.5	0.5	-0.2
	<b>LabT28</b>	10	3.0	1.9	0.8	0.6	0.5	0.5	-0.2
	<b>LabT33</b>	7	3.0	2.3	1.0	0.9	0.6	0.6	-0.5
<b>MC</b>	<b>LabT1</b>	7	3.0	1.8	0.8	0.4	0.4	0.5	0.0
	<b>LabT2</b>	5	3.0	2.2	0.9	0.6	0.5	0.6	-0.1
	<b>LabT3</b>	8	2.0	1.6	0.6	0.3	0.4	0.4	0.3
	<b>LabT6</b>	5	3.0	2.4	0.9	0.6	0.6	0.6	0.0
	<b>LabT7</b>	9	3.0	2.1	0.8	0.3	0.5	0.5	0.3
	<b>LabT14</b>	9	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT24</b>	9	3.0	2.3	0.9	0.4	0.6	0.6	0.2
	<b>LabT25</b>	10	3.0	2.4	0.9	0.4	0.6	0.6	0.3
	<b>LabT28</b>	9	2.0	1.7	0.6	0.6	0.4	0.4	-0.4
	<b>LabT33</b>	8	2.0	1.4	0.5	0.4	0.3	0.3	-0.2
<b>MK</b>	<b>LabT1</b>	4	3.0	2.5	1.0	0.3	0.6	0.7	0.6
	<b>LabT2</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT3</b>	3	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT6</b>	3	3.0	3.0	1.1	0.7	0.7	0.8	0.0
	<b>LabT7</b>	3	3.0	2.6	1.0	1.0	0.6	0.7	-0.6
	<b>LabT14</b>	3	3.0	2.0	0.9	0.3	0.5	0.6	0.3
	<b>LabT24</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT25</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT28</b>	4	3.0	2.9	1.1	0.5	0.7	0.8	0.2
	<b>LabT33</b>	4	2.0	2.0	0.7	0.0	0.5	0.6	1.0
<b>ME</b>	<b>LabT1</b>	4	3.0	2.9	1.1	0.3	0.7	0.8	0.6
	<b>LabT2</b>	3	3.0	2.6	1.0	0.3	0.6	0.7	0.5
	<b>LabT3</b>	2	2.0	2.0	0.7	1.0	0.5	0.7	-1.0
	<b>LabT6</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
	<b>LabT7</b>	2	3.0	2.7	1.0	1.0	0.6	0.8	-0.6
	<b>LabT14</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT24</b>	4	3.0	2.9	1.1	0.8	0.7	0.8	-0.1
	<b>LabT25</b>	3	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT28</b>	4	3.0	2.1	0.9	0.8	0.5	0.6	-0.4
	<b>LabT33</b>	3	3.0	2.6	1.0	0.3	0.6	0.7	0.5
<b>MU</b>	<b>LabT1</b>	5	3.0	2.6	1.0	1.0	0.6	0.7	-0.6
	<b>LabT2</b>	5	3.0	2.6	1.0	1.0	0.6	0.7	-0.6
	<b>LabT3</b>	5	3.0	2.3	1.0	0.8	0.6	0.6	-0.4
	<b>LabT6</b>	5	3.0	2.2	0.9	0.8	0.5	0.6	-0.5
	<b>LabT7</b>	5	3.0	2.6	1.0	1.0	0.6	0.7	-0.6
	<b>LabT14</b>	5	2.0	1.2	0.3	0.2	0.2	0.2	-0.1

## APPENDIX XIII: Continued

Pop	Locus	N	Na	Ne	I	Ho	He	UHe	F
	<b>LabT24</b>	5	2.0	1.9	0.7	0.4	0.5	0.5	0.2
	<b>LabT25</b>	5	3.0	2.6	1.0	1.0	0.6	0.7	-0.6
	<b>LabT28</b>	5	2.0	2.0	0.7	1.0	0.5	0.6	-1.0
	<b>LabT33</b>	5	3.0	1.5	0.6	0.4	0.3	0.4	-0.2
<b>MW</b>	<b>LabT1</b>	5	3.0	1.9	0.8	0.4	0.5	0.5	0.1
	<b>LabT2</b>	1	2.0	2.0	0.7	1.0	0.5	1.0	-1.0
	<b>LabT3</b>	5	3.0	1.9	0.8	0.4	0.5	0.5	0.1
	<b>LabT6</b>	1	2.0	2.0	0.7	1.0	0.5	1.0	-1.0
	<b>LabT7</b>	4	3.0	1.7	0.7	0.3	0.4	0.5	0.4
	<b>LabT14</b>	4	2.0	1.9	0.7	0.3	0.5	0.5	0.5
	<b>LabT24</b>	5	2.0	1.5	0.5	0.4	0.3	0.4	-0.3
	<b>LabT25</b>	4	2.0	1.3	0.4	0.3	0.2	0.3	-0.1
	<b>LabT28</b>	5	2.0	1.5	0.5	0.4	0.3	0.4	-0.3
	<b>LabT33</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
<b>NB</b>	<b>LabT1</b>	5	3.0	2.6	1.0	0.6	0.6	0.7	0.0
	<b>LabT2</b>	4	4.0	3.6	1.3	0.3	0.7	0.8	0.7
	<b>LabT3</b>	5	2.0	1.7	0.6	0.6	0.4	0.5	-0.4
	<b>LabT6</b>	4	3.0	2.7	1.0	0.5	0.6	0.7	0.2
	<b>LabT7</b>	5	3.0	2.8	1.1	0.8	0.6	0.7	-0.3
	<b>LabT14</b>	4	3.0	2.7	1.0	0.5	0.6	0.7	0.2
	<b>LabT24</b>	5	3.0	2.4	0.9	0.6	0.6	0.6	0.0
	<b>LabT25</b>	5	3.0	2.2	0.9	0.2	0.5	0.6	0.6
	<b>LabT28</b>	6	3.0	2.9	1.1	0.5	0.7	0.7	0.2
	<b>LabT33</b>	4	2.0	1.6	0.6	0.5	0.4	0.4	-0.3
<b>NK</b>	<b>LabT1</b>	7	2.0	1.5	0.5	0.1	0.3	0.4	0.6
	<b>LabT2</b>	0	0.0	0.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT3</b>	6	3.0	2.0	0.9	0.7	0.5	0.5	-0.3
	<b>LabT6</b>	0	0.0	0.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT7</b>	5	1.0	1.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT14</b>	7	3.0	2.6	1.0	0.3	0.6	0.7	0.5
	<b>LabT24</b>	7	3.0	3.0	1.1	0.4	0.7	0.7	0.4
	<b>LabT25</b>	7	3.0	2.8	1.1	0.4	0.6	0.7	0.3
	<b>LabT28</b>	7	2.0	2.0	0.7	0.3	0.5	0.5	0.4
	<b>LabT33</b>	5	2.0	1.5	0.5	0.0	0.3	0.4	1.0
<b>NY</b>	<b>LabT1</b>	4	3.0	2.9	1.1	0.8	0.7	0.8	-0.1
	<b>LabT2</b>	3	2.0	1.8	0.6	0.0	0.4	0.5	1.0
	<b>LabT3</b>	4	2.0	1.9	0.7	0.8	0.5	0.5	-0.6
	<b>LabT6</b>	2	2.0	2.0	0.7	0.0	0.5	0.7	1.0
	<b>LabT7</b>	3	3.0	3.0	1.1	0.0	0.7	0.8	1.0
	<b>LabT14</b>	4	2.0	1.9	0.7	0.3	0.5	0.5	0.5
	<b>LabT24</b>	4	2.0	1.9	0.7	0.8	0.5	0.5	-0.6



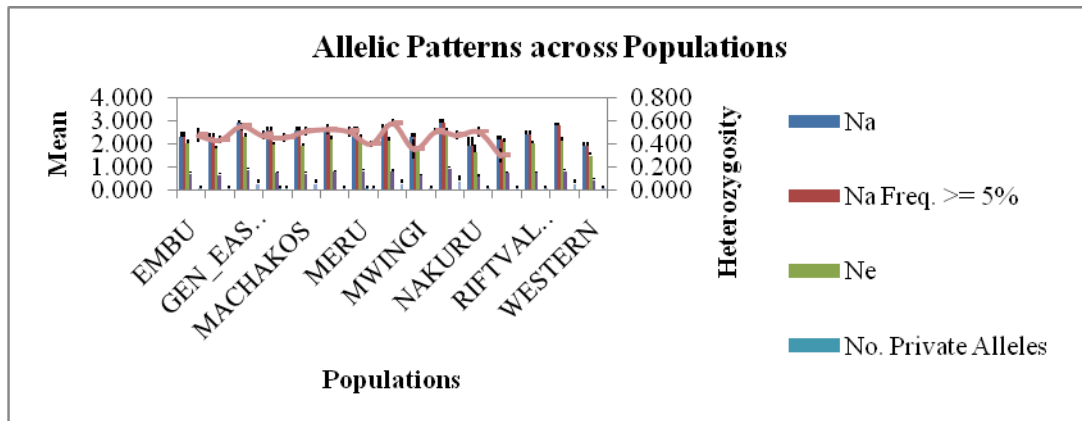
## APPENDIX XIII: Continued

Pop	Locus	N	Na	Ne	I	Ho	He	UHe	F
	<b>LabT25</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT28</b>	4	2.0	1.9	0.7	0.8	0.5	0.5	-0.6
	<b>LabT33</b>	3	2.0	2.0	0.7	1.0	0.5	0.6	-1.0
<b>RV</b>	<b>LabT1</b>	3	3.0	2.0	0.9	0.3	0.5	0.6	0.3
	<b>LabT2</b>	1	2.0	2.0	0.7	1.0	0.5	1.0	-1.0
	<b>LabT3</b>	3	3.0	2.6	1.0	0.7	0.6	0.7	-0.1
	<b>LabT6</b>	1	2.0	2.0	0.7	1.0	0.5	1.0	-1.0
	<b>LabT7</b>	3	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT14</b>	3	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT24</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT25</b>	3	3.0	2.6	1.0	0.7	0.6	0.7	-0.1
	<b>LabT28</b>	3	2.0	1.8	0.6	0.7	0.4	0.5	-0.5
	<b>LabT33</b>	3	3.0	2.6	1.0	0.3	0.6	0.7	0.5
<b>TH</b>	<b>LabT1</b>	7	3.0	2.4	1.0	0.6	0.6	0.6	0.0
	<b>LabT2</b>	5	2.0	1.2	0.3	0.2	0.2	0.2	-0.1
	<b>LabT3</b>	6	2.0	1.9	0.7	0.5	0.5	0.5	0.0
	<b>LabT6</b>	5	3.0	1.5	0.6	0.4	0.3	0.4	-0.2
	<b>LabT7</b>	7	3.0	2.5	1.0	0.4	0.6	0.6	0.3
	<b>LabT14</b>	7	3.0	2.6	1.0	0.6	0.6	0.7	0.1
	<b>LabT24</b>	7	3.0	2.5	1.0	0.7	0.6	0.6	-0.2
	<b>LabT25</b>	7	3.0	2.5	1.0	0.7	0.6	0.6	-0.2
	<b>LabT28</b>	7	3.0	2.1	0.9	0.3	0.5	0.6	0.5
	<b>LabT33</b>	7	3.0	2.2	0.9	0.4	0.5	0.6	0.2
<b>WE</b>	<b>LabT1</b>	3	3.0	2.0	0.9	0.3	0.5	0.6	0.3
	<b>LabT2</b>	1	1.0	1.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT3</b>	3	2.0	1.4	0.5	0.3	0.3	0.3	-0.2
	<b>LabT6</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
	<b>LabT7</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3
	<b>LabT14</b>	1	2.0	2.0	0.7	1.0	0.5	1.0	-1.0
	<b>LabT24</b>	4	2.0	1.3	0.4	0.3	0.2	0.3	-0.1
	<b>LabT25</b>	1	1.0	1.0	0.0	0.0	0.0	0.0	#N/A
	<b>LabT28</b>	4	2.0	1.6	0.6	0.5	0.4	0.4	-0.3
	<b>LabT33</b>	2	2.0	1.6	0.6	0.5	0.4	0.5	-0.3

**KEY:**

EM= Embu, GC=Genebank coast, GE=Genebank Eastern, LM=Lamu, MC=Machkos, Mk=Makueni, ME=Meru, MU=Murang'a, Mw=Mwingi, NB=Nairobi, NK=Nakuru, NY=Nyeri, RV=Rift Valley, TH=Thika, WE=Western, Na=Number of different alleles, Ne=Number of effective alleles, I=Shannon's Information Index, Ho=Observed heterozygosity, He=Expected heterozygosity, UHe=Unbiased expected heterozygosity, F=fixation Index  $(He - Ho) / He = 1 - (Ho / He)$

**APPENDIX XIV: Chart on allelic patterns for co-dominant data across populations**



**KEY:**

Na = No. of Different Alleles

Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%

Ne = No. of Effective Alleles =  $1 / (\text{Sum } \pi^2)$

I = Shannon's Information Index =  $-1 * \text{Sum } (\pi * \text{Ln } (\pi))$

No. Private Alleles = No. of Alleles Unique to a Single Population

No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations

No. LComm Alleles (<=50%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations

He = Expected Heterozygosity =  $1 - \text{Sum } \pi^2$

UHe = Unbiased Expected Heterozygosity =  $(2N / (2N-1)) * He$

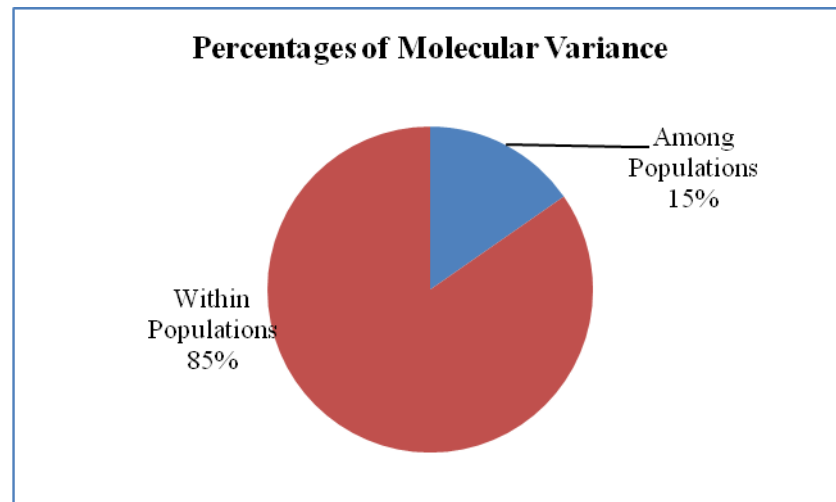
**APPENDIX XV: Allelic co-dominant data across populations**

Population	EM	GC	GE	Lm	MC	MK	ME	MU	MW	NB	NK	NY	RV	TH	WE
Na	2.3	2.3	2.9	2.6	2.6	2.5	2.6	2.7	2.3	2.9	1.9	2.2	2.4	2.8	1.9
Na Freq. $\geq$ 5%	2.3	2.3	2.6	2.6	2.6	2.5	2.6	2.7	2.3	2.9	1.9	2.2	2.4	2.8	1.9
Ne	2.1	1.8	2.3	1.9	1.9	2.2	2.3	2.2	1.7	2.5	1.6	2.1	2.0	2.2	1.5
I	0.7	0.7	0.9	0.8	0.7	0.8	0.9	0.8	0.6	0.9	0.6	0.8	0.8	0.8	0.5
No. LComm Alleles ( $\leq$ 50%)	0.1	0.1	0.3	0.1	0.3	0.1	0.1	0.3	0.1	0.4	0.1	0.1	0.1	0.3	0.1
He	0.5	0.4	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.6	0.4	0.5	0.5	0.5	0.3
UHe	0.5	0.5	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.7	0.4	0.6	0.7	0.6	0.4

**KEY:**

EM= Embu, GC=Genebank coast, GE=Genebank Eastern, LM=Lamu, MC=Machkos, Mk=Makueni, ME=Meru, MU=Murang'a, Mw=Mwingi, NB=Nairobi, NK=Nakuru, NY=Nyeri, RV=Rift Valley, TH=Thika, WE=Western, Na=Number of different alleles, Ne=Number of effective alleles, I=Shannon's Information Index, Ho=Observed heterozygosity, He=Expected heterozygosity, UHe=Unbiased expected heterozygosity, F=fixation Index  $(He - Ho) / He = 1 - (Ho / He)$

**APPENDIX XVI:** Percentages of analysis of molecular variance (AMOVA) for among and within *Lablab purpureus* populations



**APPENDIX XVII: Principal Coordinates Analysis (PCA)**

Principal Coordinates Analysis (PCA)			
PCA via Covariance matrix with data standardization			
No. Samples	96	No. Pops.	15
Percentage of variation explained by the first 3 axes			
PC Axis	1	2	3
% variation	32.35	22.29	14.24
Cum %	32.35	54.64	68.88