ADDIS ABABAUNIVERSITY

INSTITUTE OF BIOTECHNOLOGY

Ethnobotanical Knowledge of Lablab (*Lablab purpureus* (L.) Sweet - Fabaceae) in Konso zone and genetic diversity of collections from Ethiopia using SSR markers

MSc. Thesis

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October, 2020

Addis Ababa, Ethiopia

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MSc. Thesis

Submitted to the Institute of Biotechnology for the partial fulfillment of the requirements for the degree of Master of Science in Biotechnology

By

Solomon Tamiru Workneh

October, 2020

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

INSTITUTE OF BIOTECHNOLOGY

MSc. THESIS APPROVAL SHEET

This is to certify that the thesis prepared by Solomon Tamiru, entitled **"Ethnobotanical Knowledge of Lablab (*Lablab purpureus* (*L.*) Sweet - Fabaceae) in Konso zone and genetic diversity of collections from Ethiopia using SSR markers"** submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology complies with the regulations of the University and meets the standard with respect to originality and quality.

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ABSTRACT

Ethnobotanical Knowledge of Lablab (*Lablab purpureus* (*L.*) Sweet - Fabaceae) in Konso zone and genetic diversity of collections from Ethiopia using SSR markers
Solomon Tamiru Workneh, MSc. Thesis
Addis Ababa University, 2020

Lablab is an important multipurpose legume crop used for human consumption, animal feed and soil conservation. In spite of these qualities, the potential value of this crop has not been fully utilized, and little research attention has been given to this crop. The main objective of the study was to build the knowledge base from farmers' perspectives and molecular genetic diversity analysis of Lablab collections using 15 SSR markers. The field study was conducted in December 2018 in six kebeles distributed in Konso zone by interviewing a total of 84 informants containing 72 randomly selected general informants and 12 purposively selected key informants (42 men and 42 women) of above 18 years age. The data were analyzed by entering the data into the excel spreadsheet version 2007 and summarized using descriptive statistics. A total of six Lablab farmers' varieties were identified and the majority of the farmers give names to their varieties based on seed color. The main cultivation practices in the study area were intercropping Lablab with sorghum, maize and finger millet, and sole crop in the margin of terracing and fence line. In Konso zone, Lablab is mainly used for human food in the form of boiled grain (NIFRO), animal feed and soil conservation purpose. The molecular genetic diversity study of 91 lablab collections from the entire country revealed a total of 225 alleles with an average of 14.80 alleles per locus. All markers across the entire populations were found to be highly polymorphic and informative with PIC values ranging from 0.92 to 0.78 with a mean value of 0.85. The average expected heterozygosity and gene diversity was 0.75 and 0.86 respectively, indicating a high level of genetic diversity. Analysis of molecular variance showed that 94% of the total genetic variation was attributed to within populations while only 6% was attributed to among populations. The smaller Fixation Index value (0.061) recorded indicates the presence of moderate population differentiation as a result of higher gene flow (Nm =3.820) among populations. Cluster, PCoA and Structure analysis revealed a weak association between geographical origin and genetic diversity confirming the presence of population admixtures due to seed exchange and sharing. The observed higher genetic diversity in Konso and West Wellega zones indicates hot spot area for genetic diversity and germplasm evaluation. Generally, ethnobotanical knowledge and genetic diversity obtained from this study provides inputs for Lablab conservation and improvement in Ethiopia.

Keywords/Phrases: Ethnobotany, Gene diversity, General informants, Heterozygosity, Key informants, Polymorphic

ACKNOWLEDGMENTS

On top of everything, I would like express my gratitude to the Almighty God for his endless opportunity and help that enabled me to complete my study successfully.

First of all I would like to express my particular gratitude and deepest appreciation to my supervisors, Dr. Tileye Feyisa, Prof. Zemede Asfaw and Dr. Tesfaye Disasa for their continuous support, guidance, valuable comments, suggestions and encouragements for the completion of the research.

I extend my thanks to the Ethiopian Institute of Agricultural Research (EIAR), National Agricultural Biotechnology Research Center (NABRC) and Addis Ababa University for the sponsorship and supporting the study program financially. I would like to thank the Ethiopian Biodiversity Institute (EBI) for providing me the study materials. I also would like to express my thanks to the McKnight Foundation for their financial, material and training support received through the Legume Diversity Project administered by the Department of Plant Biology and Biodiversity Management. I also thank Mr. Demeke Mekonen (GIS experts from EIAR) for his technical support in mapping the study area.

My Special appreciation goes to all staff members of plant biotechnology, particularly Kalkidan Tesfu, Messele Molla, Lidiya Ashenafi and Yetaseb Nigusu for their technical assistance during molecular laboratory work. My special appreciation also goes to Konso farmers for their kind spent of their time and for sharing their knowledge during sample collection. I also express my gratitude to Sisaye Aregaye for his support during field data collection and his genuine cooperation in every aspect of the research.

Finally, I would like to express my very profound gratitude to my dear family to Fasika Alebachew, Tamiru Workneh, my mother Qelemua Kifleand other close relatives for their continuous support, encouragement and patience throughout the period of my study. Last but not least, I would like to thank Mesfin Tadele for his strong contributions in the entire period of this work.

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LIST of ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
DArT	Diversity Array Technology
EBI	Ethiopian Biodiversity Institute
EIAR	Ethiopian Institute of Agricultural Research
GD	Genetic Distance
GPS	Geographic Positioning System
NABRC	National Agricultural Biotechnology Research Center
ODK	Open Data kit
UPGMA	Unweighted Pair-Group Methods Using Arithmetic Averages
PCoA	Principal Coordinates Analysis
PIC	Polymorphic Information Content
SNNPR	Southern Nations, Nationalities and Peoples Region
SSR	Simple Sequence Repeats

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Lablab (*Lablab purpureus* (L.) Sweet (Fabaceae) is one of the most important legume crops in the world and widely distributed throughout tropical and sub-tropical regions of Asia and Africa (Kimani *et al*., 2012).It is a monotypic genus in the family Fabaceae characterized by semi erect, bushy, perennial herb and cultivated as an annual (Kukade and Tidke, 2014). It is predominantly self-fertilizing crop with 2n = 22 chromosomes (Kukade and Tidke, 2014; She and Xiang, 2015).

Lablab is an important multipurpose legume crop used as food for human consumption, animal feed and soil conservation (Kimani et al., 2012; Robotham and Chapman, 2017). It is drought and salinity tolerant crop and thus can be grown in a wide range of environmental conditions and soil types (D'Souza and Devaraj, 2010). The crop is cultivated as a sole crop and intercropped with maize, finger millet, groundnut and sorghum and it can also be used as a cover crop since its dense green cover protects the soil against desiccation and minimize erosion by wind and rain. As a legume crop, it is also useful for biologically fixing atmospheric nitrogen into the soil (Robotham and Chapman, 2017).

In Ethiopia, Lablab is mostly grown in Konso special district of southern part of Ethiopia, and some parts of the Amhara and Benishangul Gumuz Regions (Tesfaye Awas, 2007). The crop is cultivated as hedge crop for its edible seeds and grows at altitudes ranging from 400 to 2350 m.a.s.l. Considerable agro-morphological diversity such as plant height, leaf size, flowering

days, seed color, number of seed per pod, seed size and shape has been reported (EBI, 2012). Robotham and Chapman (2017) reported the greatest genetic diversity of Lablab in Africa, making Ethiopia one of the probable centers for the domestication of the crop.

1.2. **Statement of the Problem**

Lablab is considered as a minor and neglected crop in most parts of Africa (Maass*et al*., 2010; Kimani *et al*., 2012). This has led to the threat of genetic erosion of naturally occurring wild species and cultivated Lablab varieties in Africa (Maass *et al*., 2010). Some of the reasons are limited research attention, and the decreasing cultivation area and demand in Africa due to the replacement crops of other superior economic importance (Maass *et al*., 2010).However, Lablab has the ability to simultaneously meet demands for human consumption, animal feed and exhibit high potential for soil conservation strategies (Kimani *et al*., 2012; Robotham and Chapman, 2017).As human food, it is consumed in the form of mature seeds, green pods or leaves; as animal fodder, used as feed or mixed with other feed as silage; and used as cover crops or intercrops for weed suppression and soil conservation (Maass *et al*., 2010).

Maass (2016) reported the existence of wild relatives and the availability of wild populations of Lablab in uncultivated fields proving Ethiopia as the center of origin and diversity. However, there is limited understanding regarding the available genetic diversity in cultivated farmers varieties; and hence considered as an orphan and underutilized crop in Ethiopia. In addition, there is no study on Lablab farmers' varieties, use and management along with its ethnobotanical knowledge. The potential value of this crop has not been fully utilized and little research attention has been given to this crop. The results obtained from this research will be of great importance in decision making on accession management, maximizing the sustainable use of accession resources as well as in developing breeding strategies of the crop for different uses.

1.3. **Research Questions, Hypotheses and Objectives**

1.3.1. **Research questions**

- Are there a difference in the indigenous knowledge of farmers on the use and management of Lablab in Konso zone of SNNPR region?
- Are there different farmers' varieties of Lablab in Konso zone? What are they, and which ones are more frequently cultivated by farmers?
- What cropping systems were used (sole cropping, intercropping) in the study area?
- What are the main production constraints of Lablab in Konso zone (insect pests, diseases, others)
- Is there molecular genetic diversity in Lablab populations?

1.3.2 Research hypotheses

- There is a difference in the indigenous knowledge of farmers on the use and management of Lablab in Konso zone of SNNPR region; (alternative hypothesis)
- There is no difference in the indigenous knowledge of farmers on the use and management of Lablab in Konso zone of SNNPR region(Null hypothesis)
- There are different farmers' varieties of Lablab in Konso zone(alternative hypothesis)
- The farmers of Konso grow the same variety of Lablab(Null hypothesis)
- Insects, disease, drought and others are the main production constraints of Lablab (alternative hypothesis)

- Insects, disease, drought and others are not main production constraints of Lablab (Null hypothesis)
- There is a molecular genetic diversity of Lablab populations (alternative hypothesis)
- There is no a molecular genetic diversity of Lablab populations(Null hypothesis)

1.3.3. **Research objectives**

1.3.3.1. **General objective**

To build the knowledge base from farmers' perspectives and assess molecular genetic diversity of Lablab collections using SSR markers

1.3.3.2. **Specific objectives**

- To document indigenous knowledge of farmers on the use and management of Lablab in Konso zone of SNNPR region;
- To identify Lablab farmers' varieties cultivated in Konso zone of SNNPR using farmers' criteria and nomenclature;
- To assess the molecular genetic diversity of Lablab populations using SSR marker; and
- To determine the pattern of population structure in Lablab collections

CHAPTER TWO

2 LITERATURE REVIEW

2.1. Origin and Distribution

Lablab is an ancient domesticated crop, widely distributed in many countries like China, Indonesia, Malaysia, Egypt, Philippines, Sudan, Papua New Guinea, East and West Africa, the Caribbean, Central and South America (Maass *et al*., 2005, 2016) where it has been used as a grain legume and vegetable for more than 3500 years (Maass *et al*., 2005). Lablab is now widely distributed throughout the tropics and sub-tropics (Kimani *et al*., 2012), where it has become naturalized in some areas (Tefera Tolera, 2006).

Eastern and southern Africa is suggested as the center of origin for *Lablab purpureus* (Maass *et al.,* 2005, 2016). Africa is the only continent where wild plants in greater variation have been recorded to occur naturally (Verdcourt 1970; Maass *et al*., 2005, Maass, 2016). Several reports support this suggestion; that the center of origin of *L. purpureus* is eastern and southern Africa (Verdcourt, 1970; Maass *et al*., 2005; Maass 2016; Robotham and Chapman, 2017). The existence of wild relatives and the availability of wild populations of Lablab in uncultivated fields prove Ethiopia as the center of origin and diversity (Maass, 2016). Molecular clusters resolved by Robotham and Chapman (2017) contain accessions from Ethiopia, a fact that supports this area to be considered a center of diversity and one of the most probable candidate areas of origin and domestication.

In Ethiopia, Lablab is mainly cultivated in Konso zone of southern Ethiopia. Gojjam, Gonder, Wollo, Gamo Gofa, Wellega, Harerge, Ilubabor, Kefa and Sidama are the growing regions in Ethiopia(Edwards, 1995). It is also reported to grow in Benishangul Gumuz Region (Tesfaye Awas, 2007). It is cultivated as a hedge crop for its edible seeds and grows at altitudes ranging from 400 to 2350 m.a.s.l. (Edwards, 1995). Considerable agro-morphological diversity has been reported in this species for plant height, leaf size, flower and seed color, number of seeds per pod, seed size and shape and seed yield (EBI, 2012).

2.2. Taxonomy of Lablab

Lablab (*L. purpureus* (L.) Sweet Fabaceae) is a species of bean that belongs to the family Fabaceae with 2n=22 chromosome (Verdcourt, 1970; Kukade and Tidke, 2014; She and Xiang, 2015) and commonly known as hyacinth bean, Egyptian bean,dolichos Lablab, field bean, amora guaya (Amharic) and Okala (Konso) (Edwards, 1995).Verdcourt (1970) recognized taxonomically three sub-species, *unicinatus*, *purpureus*, *bengalensis*. The first wild subspecies *unicinatus* that is found in East Africa representing an ancestral form and is widespread in Ethiopia (Verdcourt, 1970, 1971;Maass*et al.*, 2016). Although there were significant differences with respect to pod shape, it is presumed that ssp. *purpureus* and ssp. *bengalensis* are genetically very similar and most of the domesticated material in India belongs either to ssp. *purpureus* or ssp. *bengalensis*. Sub-species *uncinatus* was domesticated only in Ethiopia (Verdcourt, 1970, 1971; Maass*et al.,* 2016).

2.3. Botanical description of Lablab

Lablab is an herbaceous, climbing and warm-season annual or short-lived perennial with a vigorous taproot. It has a thick, herbaceous stem that can grow up to 91.44cm and the climbing

vines stretching up to 7.6 m from the plant (Valenzuela and Smith, 2002).It has trifoliate, long-stemmed and alternate leaves, Leaflets broad in the middle and 7.5 to 15 cm long (Duke *et al.,* 1981;Kukade and Tidke, 2014). The flowers grow in clusters on an unbranched inflorescence in the angle between the leaf and the main stem. It may have white, blue, or purple flowers depending on its variety. Pods are very variable in shape and color; they may be flat or inflated (Byregowda *et al*, 2015). Seed pod is 4 to 10 cm long and is smooth, flat, pointed, and contain 2 to 4 seeds (Cook *et al*., 2005). Dry Seeds color can be white, cream, pale brown, dark brown, red, black, or mottled depending on variety (Byregowda *et al.*, 2015).

2.4. Uses of Lablab

Lablab can be used in many different ways under a range of conditions due to its adaptability, which enhances its potential for use in the future. As a multipurpose legume, Lablab is used as a pulse crop for human consumption, as a fodder crop for livestock, as a rotational and cover crop to improve soil fertility and soil organic matter (Kimani *et al*., 2012). As human food, green pods, mature seeds and leaves are traditionally eaten as vegetables in Africa, south and south-east Asia (Tefera Tolera, 2006; Maass*et al*., 2010). Thereby, not only leaves but also flowers may be cooked and eaten like spinach (Tefera Tolera, 2006). Additionally, it is also used as herbal medicine and ornamental purposes (Maass*et al*., 2010).

For livestock production, Lablab is used as forage, hay, and silage. As forage, it is often sown with sorghum or finger millet (Maass *et al*., 2010). It is one of the most palatable legumes for animals (Valenzuela and Smith, 2002). The leaf has 21 to 38% crude protein whereas the seed contains 20 to 28% (Cook *et al*., 2005). Silage made from a mix of Lablab with sorghum raised

the protein content of sorghum around 11% with a 2:1 mixture of Lablab: sorghum (Sheahan, 2012).

It is also useful for biological nitrogen fixation of green manure to improve soil. It is also used as cover crop in mixed farming with crops like finger millet, groundnut, sorghum and maize as it reduces moisture loss, soil erosion and supplies nutrients by fixing atmospheric nitrogen into the soil (Maass*et al,* 2010). Its dense green cover can help to protect the soil against desiccation and decreases wind and water erosion when used as a cover crop. In addition, it is used as green manure and offers great potential for conservation strategies and stabilization of chemical and physical properties of soil (Kimani *et al*., 2012). *L. purpureus* has been used in the Philippines and China as a stimulant, to reduce fever, to reduce flatulence, to stimulate digestion, and as an antispasmodic in Namibia, the root has been used to treat heart conditions (Sheahan, 2012).

2.5. Environmental requirements of Lablab

Lablab is remarkably adaptable to wide areas under diverse climate, such as arid, semiarid, sub-tropical and humid regions at temperature range of 22–35 °C, lowlands and highlands. The crop is suitable for growing well as a rain-fed crop where the average annual rainfall is 600-800 mm (Vaijayanthi*et al.,* 2019). It requires adequate moisture during the early stages of growth, after which its deep roots enable it to exploit residual soil moisture which can reach up to 2 m below the soil surface (Cook *et al*., 2005).Lablab withstands frost for a limited period, although it is liable to leaf damage(Cook *et al*., 2005). The plant survives on a wide variety of soil types ranging from deep sands to heavy clays(Vaijayanthi*et al.,*2019). It is reported to do particularly well on sandy loams to clay in pH ranges of 4.5-7.5 (Cook *et al*., 2005). It does not grow well in

saline or poorly-drained soils, but grows better than other legumes under moisture stress conditions (Maass*et al.*, 2010; Vaijayanthiet *al.,* 2019).

2.6. Production constraints

The utilization and extent of other leguminous species such as common beans and cowpeas is far greater than Lablab generally, with this limitation being ascribed to Lablab's comparable poor cooking and eating qualities (Shivachi *et al*., 2012). Prolonged cooking times are listed as one of the major factors responsible for under-utilization of leguminous species in many diets as those lead to increased energy costs and, further have negative impacts on the nutritive value (Shivachi *et al*., 2012). The taste of some Lablab genotypes is further known to be accompanied by a bitter taste, a reduction thereof requires several water changes throughout the cooking, additionally resulting in loss of nutrients (Shivachi *et al*., 2012).

Ramesh and Byregowda (2016) reported anthracnose; Lablab yellow mosaic virus (LaYMV) diseases, pod borers (*Heliothis armigera* and *Adisura atkinsoni*) and bruchids (*Callosobruchus theobrome*) are major biotic production constraints in Lablab production. While pod borers cause damage in the field, bruchids cause damage both in the field and in storage. Breeding for resistance to these insect pests is currently limited to screening and identification of resistance sources in germplasm and breeding lines (Ramesh and Byregowda, 2016).

2.8. Farmers varieties (landrace) diversity

Farmer's varieties are populations of a cultivated plant with a historical origin, distinct identity, often genetically diverse and locally adapted associated with a set of farmers' practices of seed selection and field management as well as with farmers' knowledge base (Camacho-Villa *et al.*, 2005). They are heterogeneous local adaptations of domesticated species providing genetic

resources that meet current and new challenges for farming in stressful environments (Dwivedi *et al*., 2016). The main contributions of landraces to plant breeding have been traits for more efficient nutrient uptake and utilization, as well as useful genes. A systematic landrace evaluation may define patterns of diversity, which will facilitate identifying alleles for enhancing yield and abiotic stress adaptation, thus raising the productivity and stability of staple crops in vulnerable environments (Zeven, 1998). However, few agronomic and genetic data exist for such collections, and this scarcity has limited the use, management and conservation of this germplasm.

2.9. Genetic markers used in crop diversity study

Genetic diversity refers to variation in nucleotides, genes, chromosomes or whole genomes of organisms (Wang *et al.*, 2009*).*Genetic diversity can be assessed among different accessions /individuals within same species (intraspecific), among species (interspecific) and among genus and families by using different types of genetic markers(Mittal and Dubey, 2009). A genetic marker is any character that can be measured in an organism which provides information on the genotype of that organism. Determining genetic diversity can be based on morphological, biochemical, and molecular types of information (Goncalves *et al*., 2009).

2.9.1. Morphological markers

Morphological markers are usually visually characterized phenotypic characters appearance (Sumarani *et al*., 2004).Morphological traits were among the earliest markers used in genetic diversity assessment and they are the strongest determinants of the agronomic value of plants. Especially, if the traits are highly heritable, morphological markers are one of the choices for diversity studies because the inheritance of the marker can be monitored visually (Yoseph Beyene, 2005). Despite these advantages, morphological features have a number of limitations

including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Muthusamy *et al.,* 2008), which, in turn limits their utility for assessing real genetic diversity.

In their agro-morphological characterization of the species, Pengelly and Maass (2001) found far greater variation in wild forms from eastern and southern Africa than within cultivated landraces collected from Africa and Asia. They also found the wild and cultivated forms from the East African highlands, particularly Ethiopia, belonged exclusively to subsp. *uncinatus* and were distinct from the remainder of the collection studied. However, the expression of agro-morphological characters is often strongly influenced by environmental factors and diversity estimates based on such data may poorly reflect actual levels of genetic diversity.

2.9.2. Biochemical Markers

Isozymes are common enzymes expressed in the cells of plants. The enzymes are extracted, and run on denaturing electrophoresis gels. The denaturing component in the gels (usually SDS) unravels the secondary and tertiary structure of the enzymes and they are then separated on the basis of net charge and mass. Polymorphic differences occur on the amino acid level allowing single peptide polymorphism to be detected and utilized as a polymorphic biochemical marker. The technique is rapid and economical, and co-dominant nature of allozyme data makes it useful for the characterization of genetic variation in plant species (Weising *et al.,* 2005). Although protein markers circumvent environmental effects, the numbers of detectable isozymes are limited and they are typically tissue and developmental stage-specific (Park *et al*., 2009). For this reason, most researchers began to focus on the use of DNA marker systems for genetic and ecological analyses of plant populations.

2.9.3. DNA based markers

DNA based genetic markers are specific fragments of DNA that can be identified within the genome of the organism under study using a broad variety of techniques. Molecular markers are the most recent to be developed and have proven to be the powerful tools for genotype characterization and estimation of genetic variation both within and among plant populations by analyzing large numbers of loci distributed throughout the genome (Treuren *et al*., 2005). DNA based markers have many advantages over morphological and biochemical markers. The primary advantages include their availability in potentially unlimited number and the property that they generally are not affected by developmental differences or environmental influences. Nowadays, molecular marker technologies are increasingly being used to complement traditional methods because of their ability to measure diversity directly at the DNA level (Tadesse Abate, 2017).

The DNA based marker systems are generally classified as hybridization-based and PCR-based markers based on the PCR amplification of genomic DNA fragments (Weising *et al.,* 2005).The first reported non-PCR based molecular markers technique for assessment of DNA variation in selected organisms is restriction fragment length polymorphisms (RFLP).RFLP are co-dominant, reproducible, easily transferable between laboratories and relatively easy to score due to large size difference between fragments. RFLP is limited by the relatively large amount of high quality DNA required for restriction digestion, probes need to be developed, the technique is labor and time consuming (Botstein *et al.,* 1980).

PCR based polymorphisms result from DNA sequence variation at primer binding sites and from DNA length differences between primer binding sites. RAPD, AFLP, SSR and ISSR are among the major PCR based molecular markers (Staub *et al*., 1996).

Random Amplified Polymorphic DNAs (RAPDs) were the first PCR based molecular marker technique. The advantages of RAPDs are quick technique, easy to perform and comparatively cheap and little amount of DNA quantities are required to detect genetic variation. However, the results from RAPDs may not be reproduced in different laboratories and can only detect the dominant markers (Rabouam *et al.*, 1999).

Liu (1996) studied genetic variation of 40 Lablab accessions using random amplified polymorphic markers. The study revealed a high level of genetic variation in this species, but this was mainly limited to the difference between cultivated and wild forms.

Amplified Fragment Length Polymorphism (AFLP) technology is developed to overcome the limitation of reproducibility associated with RAPD. The method involves restriction digestion of genomic DNA with two different restriction enzymes and then ligating the fragments with specific adaptor sequences. PCR amplification will be carried out using pair of primers having complementary sequence with the adaptor sequence. AFLP markers are cost effective and there is no need of prior sequence information. Despite its attractiveness, the AFLP method requires clean and high molecular weight DNA for ensuring complete digestion by enzymes. Partial digestion of DNA results in non-reproducible variation in DNA profiles (Vos *et al*. 1995).

Kimani et al. (2012) studied the diversity on 50 Kenyan Lablab accessions using AFLP primers. The study showed the low genetic diversity in Lablab accessions. The study revealed that most of the genetic variation occurred within populations (99%) and only 1% variance was among the populations, while Principal Coordinate Analysis showed an overlap between accessions from different geographic origins. The overall mean expected heterozygosity (He) for the five

populations was 0.189. Maass et al. (2005) also used AFLP to determine sources of diversity in cultivated and wild L. purpureus related to provenance of germplasm. The study revealed landraces from Africa and Asia, belonging predominantly to subsp. purpureus, displayed moderate genetic diversity. The results support the suggested pathway of domestication and distribution of L. purpureus from Africa to Asia.

2.9.3.1. Simple sequence repeat

Simple sequence repeats (SSRs) also known as microsatellites are polymorphic loci present in DNA consisted of tandemly repeating units of 1-6 base pairs of DNA that are widely dispersed through genomes. Microsatellites can be amplified for identification by PCR using the unique sequences of flanking regions as primers (Park *et al.,* 2009). Therefore, specific primers are used to amplify microsatellites by PCR. The SSRs are the marker of choice in molecular diversity study as they are highly polymorphic resulting from high mutation rates that affect the number of repeating units, co-dominant, locus specific and has greater distribution and abundance in the genomes (Nadeem *et al*., 2018).The SSRs are mostly co-dominant markers, and are indeed excellent for studies of population genetics and mapping (Arif *et al.,* 2010). Microsatellites have been quite useful in various aspects of molecular genetic studies such as assessment of genetic diversity, measure population structure, marker-assisted selection and genetic linkage mapping (Arif *et al.,* 2010; Barcaccia, 2010).

Zhang *et al.*(2013) developed SSRs for Lablabs to investigate the genetic structure and diversity of different populations originating from China and Africa. A total of 459 Lablab ESTs from the National Center for Biotechnology Information (NCBI) database were downloaded and analyzed

to search for SSRs. Finally, 22 microsatellites were identified and SSR markers were subsequently screened on 24 Lablab accessions collected from both China and Africa. Among 22 SSRs, 11 markers showed polymorphism and revealed two to four alleles per locus. The polymorphic information content (PIC) values ranged from 0.0767 to 0.4864, with a mean of 0.286. The average observed and expected heterozygosity was 0.35 and 0.34, respectively.
Furthermore, both principal coordinate analysis (PCoA) and phylogenetic tree analysis indicated that all accessions were clustered into two main groups, and that all 19 Chinese accessions were clustered into the single group. These results suggest that there is a narrow genetic basis for Chinese Lablab accessions.

Robotham and Chapman (2017) investigated population genetic analysis of 91 Lablab accessions using five SSR markers designed from the Lablab transcriptome. This is the first study known to use microsatellites to look at genetic variation across a range of accessions from all over the world. They found that genetic variation was highest in eastern African accessions, and that cultivated lines from East Africa were more closely related to the wild subspecies, *L. purpureus* subsp. *uncinatus*Verdc., indicating an East African origin and sub-sequent dispersal. This study revealed PIC ranging from 0.209 to 0.741. The mean number of allele and heterozygosity showed a value of 7.4 and 0.205, respectively.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Ethnobotanical Field Survey

3. 1. 1. Description of the Study Area

The ethnobotanical field survey was carried out in Konso zone of SNNPR Regional state of Ethiopia (Fig. 1). The district is located about 600 km south West of Addis Ababa at5^{o}19’ – 5°35’ N latitude and 37°15’ – 37° 40’E longitude (Fig.1).The altitude of Konso zone varies from 550 to 2000 m.a.s.l. It has an annual rainfall that varies from 771 to 921 mm with highest precipitation being received from February to May and a short rainy season from September to November. The mean annual temperature of Konso zone ranges between 17.6 and27.50ºC (Kusse Haile *et al*., 2018).

The study area was selected purposively based on high production and economic importance of the crop. Six kebeles were purposively selected from Konso zone for this study (Fig. 1).

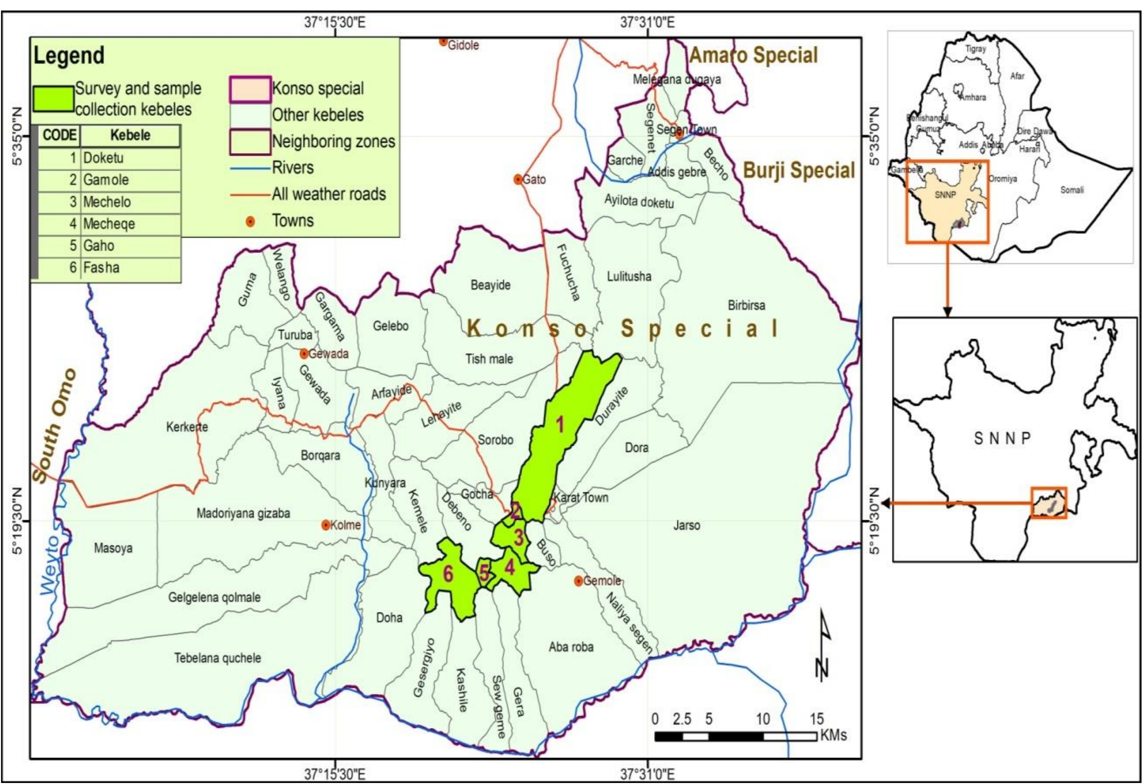

Figure 1 Map of Ethiopia showing SNNPR and the study zone

3.1.2. Materials used

The materials used to conduct this study were Global Positioning System (GPS) using android phone to collect longitude, latitude and elevation of study area; note book, digital camera to take pictures of the farmers' varieties; android phone to conduct structure interview using open data kit (ODK); pre- prepared hardcopy to conduct semi- structured interview and sample collection bag to collect different Lablab accessions.

3.1.3 Methods

3.1.3.1. Study site selection

The study sites were selected purposively to get areas that show greater diversity and production potential of the crop by referring literature sources.

The information of the production of the crop in the study area was obtained from wereda office expert/ agricultural development agents. In order to have valuable information on farmers' variety diversity, use and production systems, it was decided to collect data from those kebeles where Lablab is highly produced. Based on the information obtained from agricultural development agents for the high production kebeles of Lablab, six kebeles were selected purposively for this study.

3.1. 3.2. Informant selection for structured interview

Lists of farmers who produce Lablab in the study area along with their wealth category were obtained from Agricultural development agent/experts of the farmers. Twelve informants were selected from each kebele based on wealth status (6 low-income and 6 middle/high-income households) using stratified random sampling. Three women and three men were selected from middle/high income households and three women and three men were selected from low-income households.

A total of 72 general informants (12 informants x 6 kebeles = 72 general informants) having different age and sex categories were considered for the study.

3.1.3.3. Selection of key informants

The key informants were selected among the farmers who have already responded to the structured interview (by ODK), or based on the recommendations obtained from the informants during interviews and from local agricultural extension experts of each kebele. One man and one woman who are knowledgeable about the crop were selected from each kebele as key informant. A total of 12 key informants were selected from 6 kebeles.

3.1.3.4. Ethnobotanical Data Collection

Ethnobotanical data were collected in December 2018, following reconnaissance surveys. Ethnobotanical data were collected by interviewing the informants (farmers), observation and collection of data. Ethnobotanical data were collected in order to know the indigenous knowledge of farmers on production, use and managements of the crop. Different qualitative and quantitative ethnobotanical data collection methods were used to get ethnobotanical information or indigenous knowledge of the farmers on production, uses and management of the crop.

3.1.3.5. Structured and semi-structured interview methods

General informants were interviewed by using structured interview method using ODK software (open data kit) which is one of the formal interview methods in ethnobothany (Appendix 1) and the key informants were interviewed using semi-structured interview guide (Appendix 2). Semi-structured questions presented in Appendix 2 were used for discussion and interviewing the key informants who have better knowledge about the crop. The semi-structured interview contains open ended and closed questions. Both interview methods were used to collect the necessary

information about the local knowledge on plant parts used, management, cropping systems, seed supply, storage of the crop, local name of the landrace, time of cultivation and harvesting, production constraints and management taken by farmers to control the constraints. Interviews were done at the household level and in farmers' Lablab fields.

3.1.3.6. Field observation and guided field walk

The field observation was conducted with the help of local guides, language translators and participating informants to get the necessary information. The information was how the crop is cultivated, intercropped systems, management, cropping systems, seed supply, storage, local name of the landrace, time of planting and harvesting, production constraints and management taken by farmers to control the constraints and use of the crop.

3. 1.3.7. Seed collection method

The seed samples were collected from farmers' fields by using the seed collecting format of EBI protocol for genetic diversity study (Appendix 3).

3.2. Molecular Genetic Diversity Study

3. 2.1. Plant materials

A total of 91 accessions of Lablab were used for this experiment. Ten accessions were exotic materials which were obtained from Bako Agricultural Research Center. Twenty two accessions were obtained from Ethiopian Biodiversity Institute(EBI), and the remaining59 accessions were collected from Konso zone during ethnobotanical field survey, North Wollo, Gamo Gofa, West Wellega and West Gojjam (Appendix 4 and Fig. 3).The collected seeds were planted in pots at

National Agricultural Biotechnology Research Center (NABRC) greenhouse. Ten seeds from each of the accession were grown in a greenhouse and fresh leaves were collected from two-week-old plants for genomic DNA (gDNA) extraction.

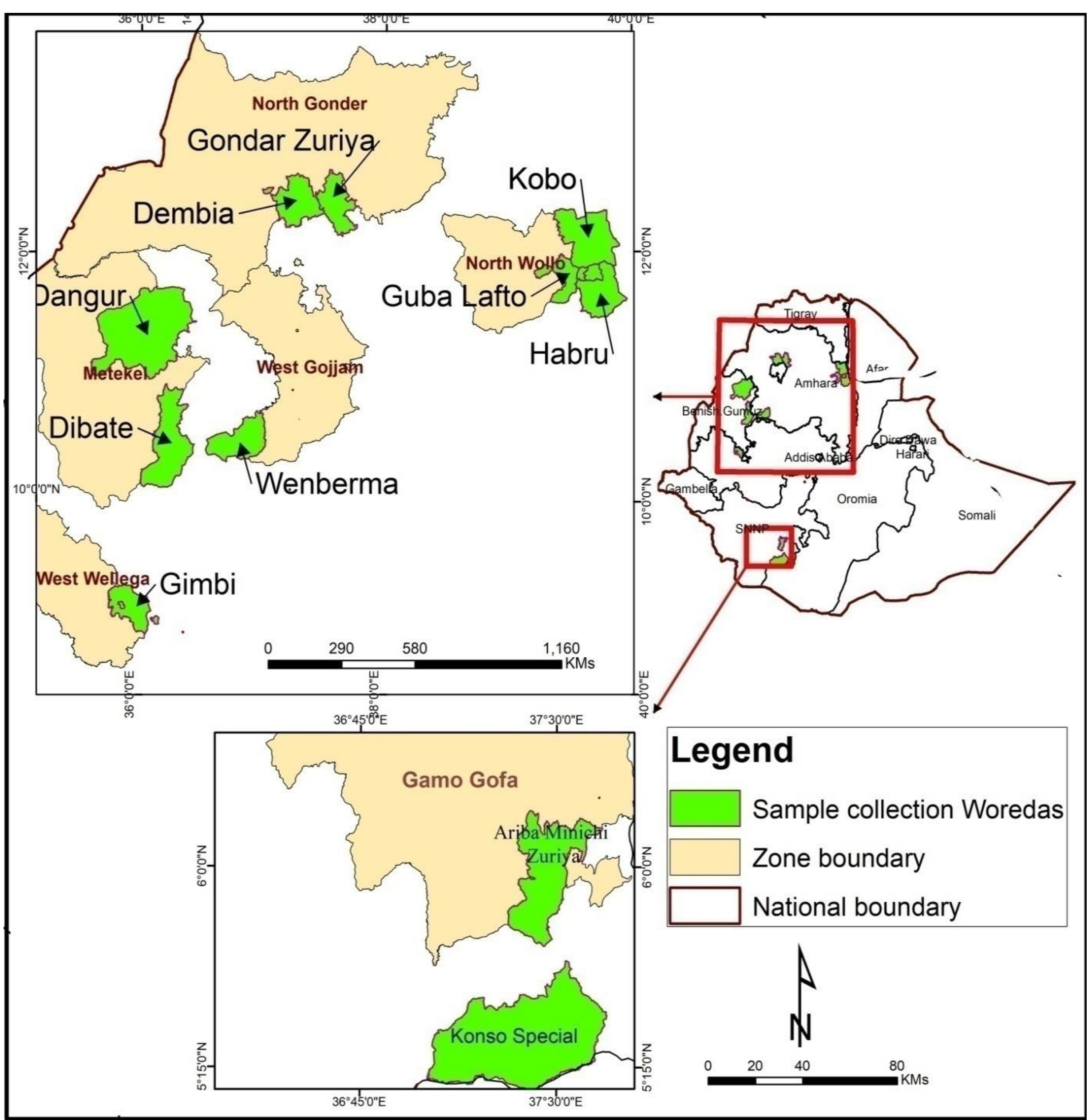

Figure 2 Map of Ethiopia showing sample collection areas of Lablab accessions

3.2.2. DNA Extraction

Two weeks after planting, equal amount of bulk leaf samples were collected from five plants of each accession as suggested by Gilbert *et al.* (1999). About 100 mg of fresh leaves were placed in 2 ml autoclaved and labeled Eppendorf tubes and freeze dried for 24 hours at −80°C. After 24 hours the leaves were further dried in liquid nitrogen and then grounded using Geno Grinder (MM-200, Retsch) for 3 min. Genomic DNA was extracted using plant DNA extraction protocol based on the method of Diversity Array Technology (DArT) with some minor modification (Appendix 5). Then the DNA pellet was air dried and dissolved in 100 µl of nuclease free water and kept at room temperature until the DNA pellet were dissolved.

The concentration of DNA was quantified using nano drop spectrophotometer (ND-8000, Thermo scientific). The level of DNA purity was determined by the 260/280 absorbance ratio. The quality of DNA was further assessed using 1% agarose gel in 1xTAE buffer using a standard lambda DNA, Biolabs, New England. For gel preparation, 1% agarose powder was dissolved in 1x TAE buffer. The mixture was boiled in microwave oven at 100°C. After agarose was completely dissolved and cooled to 50-60°C, it was casted on gel tray with comb. After solidifying, the gel was placed in a gel tank containing 1x TAE buffer. Five micro liters DNA from each sample was taken and mixed with 2µl loading dye which contains gel red and loaded in the well. The Gel was run at constant voltage of 100 volts for 40 min. The gel was visualized under UV light and subsequently photographed using a BioDoc-It^{TM}imaging System (Cambridge, UK).

Samples with high band intensity, lesser smear, purity with 1.8 to 2 at 260/280 nm were selected for further PCR analysis. Purified and working concentration of DNA was stored in the refrigerator (-20ºC) till the next use.

3.2.3. Primer selection and optimization

A total of 20 SSR primers were used for PCR amplification. PCR optimization and testing of SSR primers was done using twelve representative Lablab accessions. Out of the 20 tested primers, 15 SSR primers were selected for final analysis on the basis of reliability, polymorphism and their specificity to target region (Table1).

Table 1List of SSR markers used in this study for genotyping Lablab accessions

S.No.	Markers	Forward primers (5' to 3')	Reverse primers (5' to 3')	Annealing Temp
1	c17963_g1_i1	TGATGAGGAGGAGTGTGATAG	GATCTAGAGATGCAGAGGAGAG	50.8
2	c21512_g2_i1	GCCAAGTTTCTACGACCTC	GAGATCGACCTGGAAATACTC	56.7
3	c13353_g1_i1	GAAGCTTCACAAGTGAAAGAA	GTTCTCGTTCTGAACAATCAT	50.2
4	Lpxu-009	GCCCAGCTAAGATTGAG	GTTCTGATCCTATGACCG	58
5	Lpxu-013	CTCTACTATCATCCGTCTC	TCGGTCCATACTCTTC	55.4
6	Lpxu-002	TTCCGCAAAGACAAGTT	CGTCAGCGAGAAGGGTA	53.8
7	Lpxu-010	AGCCTGACATTTCACCTG	TGCCACTTCAATCTCCC	58.8
8	KTD241	GTTAAGCCTTGAGATCTGACAC	CTTCACCTCACTCACAACATT	58
9	KTD195	TGGTTGAATGAGAGAGTAAAGG	GTTTCTTCAAGGTACATGTCTCAC	51.9
10	KTD255	GAACTGAAAGAGAGGGATGAT	GGGCAGAGAGACAGTAATAATAAG	50.7
11	KTD138	GATGAAGAAGGTTGTAGAGTTGTG	CTATCTCACACTTTCCTTACACCT	50.7
12	KTD272	AATCTTAACAGGGTCAGAAGC	CTCTCCCTCCCATAACTAACTT	50.7
13	KTD245	AAGGAGAGAGTTAAGGTTGTAGAG	AAAAGTGCCACATTCTCTCTC	50.7
14	KTD249	ACTACCCTATAGTCTCTCTGTGCT	AGAAGATGATCTCAGATTCCAC	58
15	KTD199	TTCTTCTCTTCAACTTCACTCC	ACGAAGACAAGGAAGAGAAATC	58

3.2.4. PCR and gel electrophoresis

Lyophilized primers for the target genes were reconstituted using nuclease free water to obtain 100 µM stock solutions. All primers were stored at -20°C and then finally diluted to working concentration of 10 µM. PCR reaction was carried out with a thermal cycler (GeneAmp®PCR System 9700) in a total volume of 12.5 µl reaction containing 6.25 µl one Taq 2x Master Mix (M04821) Biolabs England, with standard buffer (which contain all PCR reaction components, $MgCl_2$, PCR buffer, dNTPs and Taq DNA polymerase), 0.5 µl forward primer, 0.5 µl reverse primer, 0.25 µl DMSO, 3 µl nuclease-free water and 2 µl genomic DNA. The PCR was programmed at initial denaturation (preheating) step of 3 min at 94°C followed by 35 cycles of a denaturation at 94°C for 1 min, annealing at 50.2 - 58°C depending of the primers for 2 min and elongation at 72°C for 1 min, with a final elongation at72°C for 10min followed by a holding step at 4°C.PCR amplification of each primer was optimized using "gradient" methodology.

PCR products were loaded on 3% agarose gel (w/v) with gel red containing 6x loading dye. Electrophoresis was performed in1× TAE buffer at 100 constant volts for 3 h and 30 min. The gel was stained with gel red and visualized under UV light using a BioDoc-it™ imaging system (Cambridge, UK). DNA fragment sizes were estimated by comparing the DNA bands with a 100 and 50 base pair DNA ladder as molecular ruler (Appendix 7).

3.3. Data Analysis

3.3.1 Ethnobotanical data analysis

Ethnobotanical data were analyzed by entering the data into the excel spreadsheet version 2007 and summarized using descriptive statistics to identify the most common widely used Lablab farmers variety in the study areas. To determine proportions of different farmers' varieties, importance as fodder, food, and soil conservation; source of seed, planting date, harvesting time, Gender and age role in cropping of Lablab and intercropping of Lablab with other crops. Then the results were presented with graphs and tables.

Beta Diversity: Whittaker (1960) divided the diversity into various components. The best known are diversity in one spot that the author called alpha diversity, and the diversity along gradients that the author called beta diversity. The basic diversity indices are indices of alpha diversity. Beta diversity should be studied with respect to gradients (Whittaker, 1960), it is a measure of general heterogeneity (Tuomisto, 2010): how many more species/variety have in a collection sites compared to an average site. Beta diversity calculated as gamma divided by alfa. This indicates the degree to which farmers within the same ethnic group or region share the same landrace.

Gamma diversity: the total number of landraces within a region or among farmers of a certain ethnic group.

Alpha diversity: the average number of landraces listed by each farmer.

3.3.2. Molecular genetic diversity data scoring and analysis

The amplified products were scored based on fragment band size using PyElph 1.4 software package (Pavel and Vasile, 2012). Clearly resolved and unambiguous bands were scored for each primer and samples. Bands with the same fragment size were treated as identical fragments.

Different statistical software packages were performed to compute the standard indices of genetic diversity. Major allele frequency (MAF), the number of allele (Na), and gene diversity; PIC and heterozygosity were computed using Power marker ver. 3.25 software (Liu and Muse, 2005).

Genetic diversity parameters such as; number of effective alleles per locus (Ne), Shannon information index (I), fixation index (F) (Nei‟s, 1978), gene flow (Nm) and percent polymorphism (% P),allelic frequency, observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F) and estimate of the deviation from Hardy-Weinberg Equilibrium (HWE) over the entire populations were computed with GenAlEx ver. 6.502 software (White and Peakall, 2015). Furthermore, Analysis of molecular variance (AMOVA) was done to partition the total genetic variation within and among genotypes and estimate of its variance components using the same software. AMOVA uses the estimated F-statistics such as genetic differentiation (F_{ST}), fixation index or inbreeding coefficient (F_{IS}) and overall fixation index (F_{IT}) to compare the genetic differentiation among and within populations.

Rarified allelic richness (Ar) and private rarified allelic richness (Arp) were computed using HP-Rare 1.1 software (Kalinowski, 2005).

To examine the genetic relationship between the different accessions, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based Neighbor-Joining tree and hieratical clustering

(dendrogram) were generated using DARwin var. 6.0.13 (Perrier and Jacquemoud-Collet, 2006).A dendrogram was generated based on the dissimilarity matrix as input data in order to visualize pattern of cluster within and among the accessions. To examine the pattern of variation among samples and resolving power of coordination, principal coordinate analysis (PCoA) was carried out using GenAlex ver.6.502 software.

The population structure and admixture patterns of the 91 accessions were determined by the Bayesian model-based clustering method of Pritchard *et al*. (2000) using the Structure ver. 2.3.1 software. To estimate the true number of population cluster (K), a burn-in period of 100,000 was used in each run, and data were collected over 200,000 Markov Chain Monte Carlo (MCMC) replications for K = 1 to K = 10 using 20 iterations for each K. The structure output results were zipped into one zip archive, and the zipped file was uploaded into the web-based program STRUCTURE HARVESTER ver. 0.6.92(Dent and Bridgett, 2012).

The most likely K value was determined using the ΔK method of Evanno *et al.* (2005) using the web-based STRUCTURE HARVESTER ver. 0.6.92 (Dent and Bridgett, 2012). Bar plot for the optimum K was determined using Clumpak beta version (Kopelman *et al.*, 2015).

CHAPTER FOUR

4 RESULTS

4.1 Ethnobotanical study

4.1.1. Legume crops grown in the study area

The result of this study indicated that the farmers grow different legume crops. All of the farmers (100%) interviewed in Konso cultivated Lablab, which they call Okala in Konso local language and other leguminous crops including pigeon pea (*Cajanus cajan*, 100%), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) by varying proportions of farmers. The latter legume crop in particular was seen under cultivation in Konso by very few farmers. The proportions of Konso farmers growing the different legume crops are shown in Fig.3.

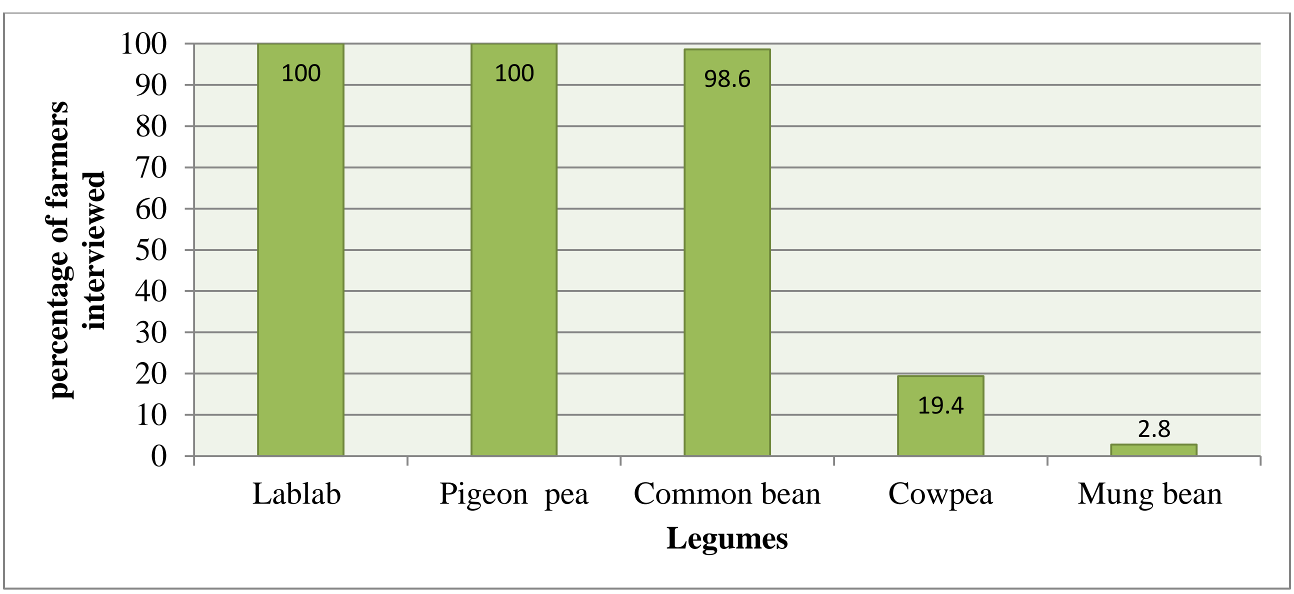

Figure 3 Legume crops grown in the study area, Konso zone (n=72)

4.1.2 Diversity of Lablab farmers' varieties cultivated in Konso zone

A total of six Lablab farmers' varieties were collected from 6 different surveyed kebeles of Konso zone. The collected six farmers' varieties locally called tima, ata, abora, budeyata, tima burburisata and abora burburisata (Konso language).The result of the study indicated that the farmers grow different varieties of Lablab in the study area (Fig.4). More than 90% of the farmers grow tima variety while few farmers (6%) grow budhayata and abora burburisata varieties.

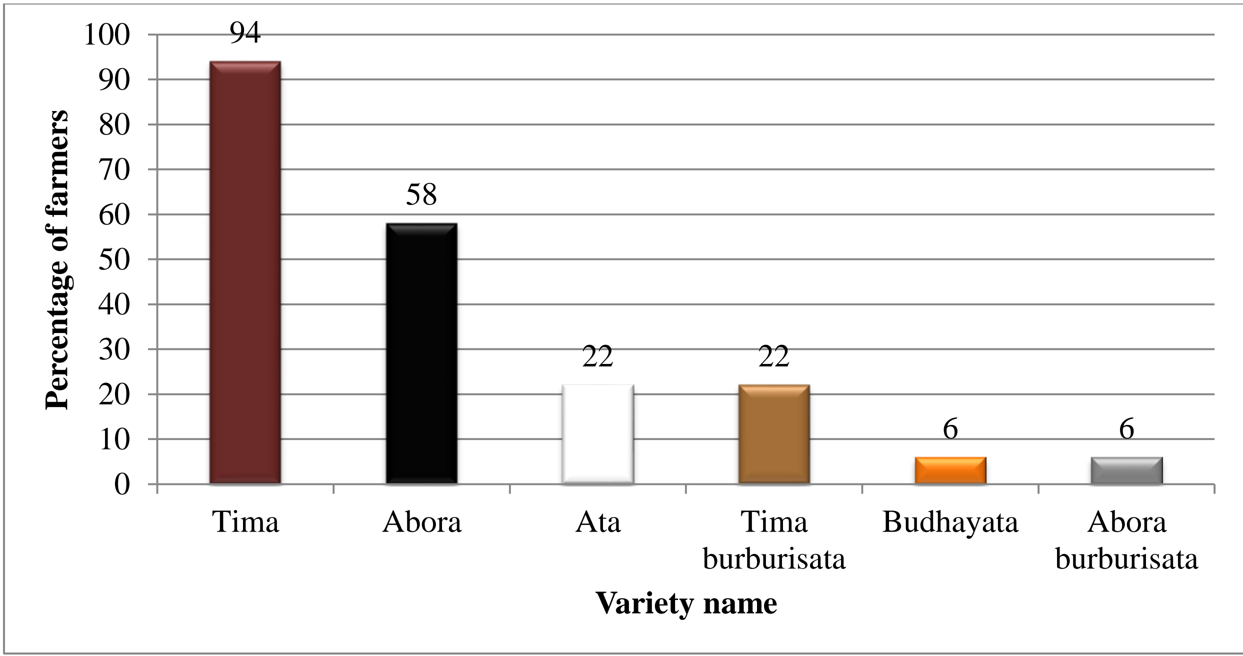

Figure 4 Distribution of farmers' varieties of Lablab in the study area (n=72)

Table 2 Farmers' varieties of Lablab collected from Konso zone and their meanings

Local name of variety in Konso language	Translation	Picture of Lablab farmers' varieties
Okala tima	Red- seeded Lablab	
Okala ata	White- seeded Lablab	
Okala abora	Black- seeded Lablab	
Okala budhayata	Yellow- seeded Lablab	
Okala tima burburisata	Red-spotted Lablab	
Okala abora burburisata	Black-spotted Lablab	

4.1.3. Landraces diversity and richness

The diversity of farmers' varieties is different among the households. The total number of varieties (gamma diversity) is the highest in Mecheke and Mechelo kebeles (5) followed by Gamole, Doketu, Gaho and Fasha kebeles (4). Thehighest average number of varieties per household (alpha diversity) was found in Fasha (2.7) followed by Mechelo (2.4) whereas the lowest in Doketu (1.4). The highest Beta diversity was recorded in Doketu (2.86) and the lowest was obtained from Fasha kebele (1.5) (Table 3).

Table 3 Diversity of the farmers' varieties within each kebele

Kebeles	Alpha diversity	Gamma diversity	Beta diversity
Gamole	1.6	4	2.5
Doketu	1.4	4	2.86
Mecheke	2.3	5	2.17
Gaho	2.2	4	1.82
Fasha	2.7	4	1.50
Mechelo	2.4	5	2.10

4.1.4. Intercropping of Lablab with other crops

The major crops grown with Lablab in the study area were sorghum, common bean, maize, finger millet and cowpea in their order of importance. Traditional cropping systems reported by farmers showed that, Lablab is grown as field crop, mostly intercropped with cereal crops such as sorghum and maize (Fig.5). Thus, the study result indicated that almost all (99%) of the farmers intercropped Lablab with sorghum followed by maize (89%) whereas 15% of the farmers intercropped Lablab with cowpea (Fig.5). Farmers also planted this crop in their home gardens, field margins and terracing areas as a sole crop.

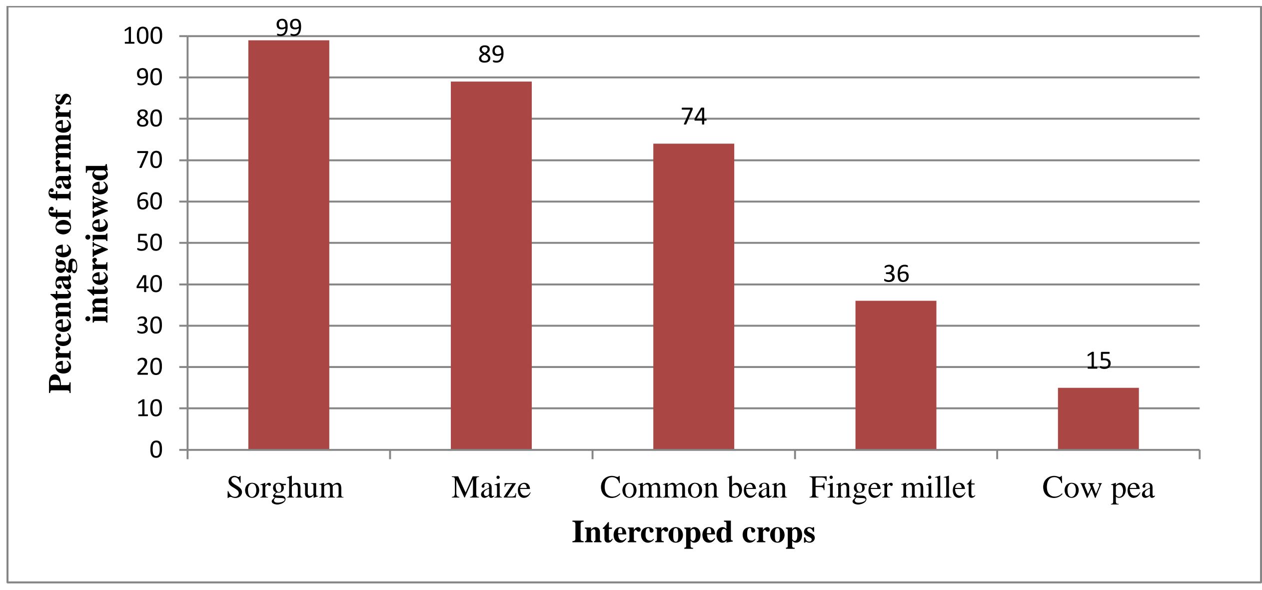

Figure 5 Intercropping of Lablab with other crops (n=72)

4.1.5. Lablab seed source

Farmers obtain the seeds of Lablab varieties from different sources (Fig.6). The study result showed more than 70% of the farmers obtained the seed of the Lablab varieties from their family members (grandfather, father, uncles and brothers) for the first time and 21% from market. Only

2% of respondents obtained Lablab seed from formal agent (agricultural office) (Fig.6).The majority of farmers (70%) used their home saved seed (relied more on own sources) for the next planting season.

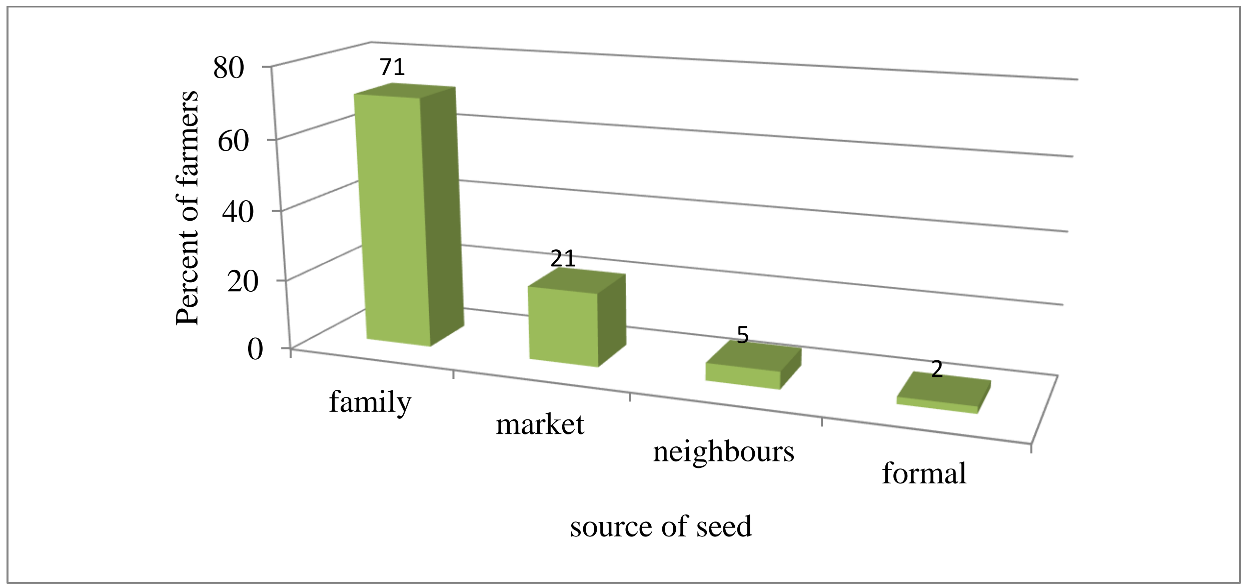

Figure 6 Seed source for Lablab farmers' varieties

4.1.5. Gender and age role in cropping of Lablab in Konso zone

Most of the management /agronomic activities such as land preparation; hoeing, planting, weeding, harvesting, threshing and storage of Lablab were the responsibility of both female and male adults. Men and women often work together in the field, but the agronomic activities such as weeding, hoeing, fertilizer application, harvesting, storage, and food and animal feed preparation were mostly carried out by women informants in the study area (Fig.7).

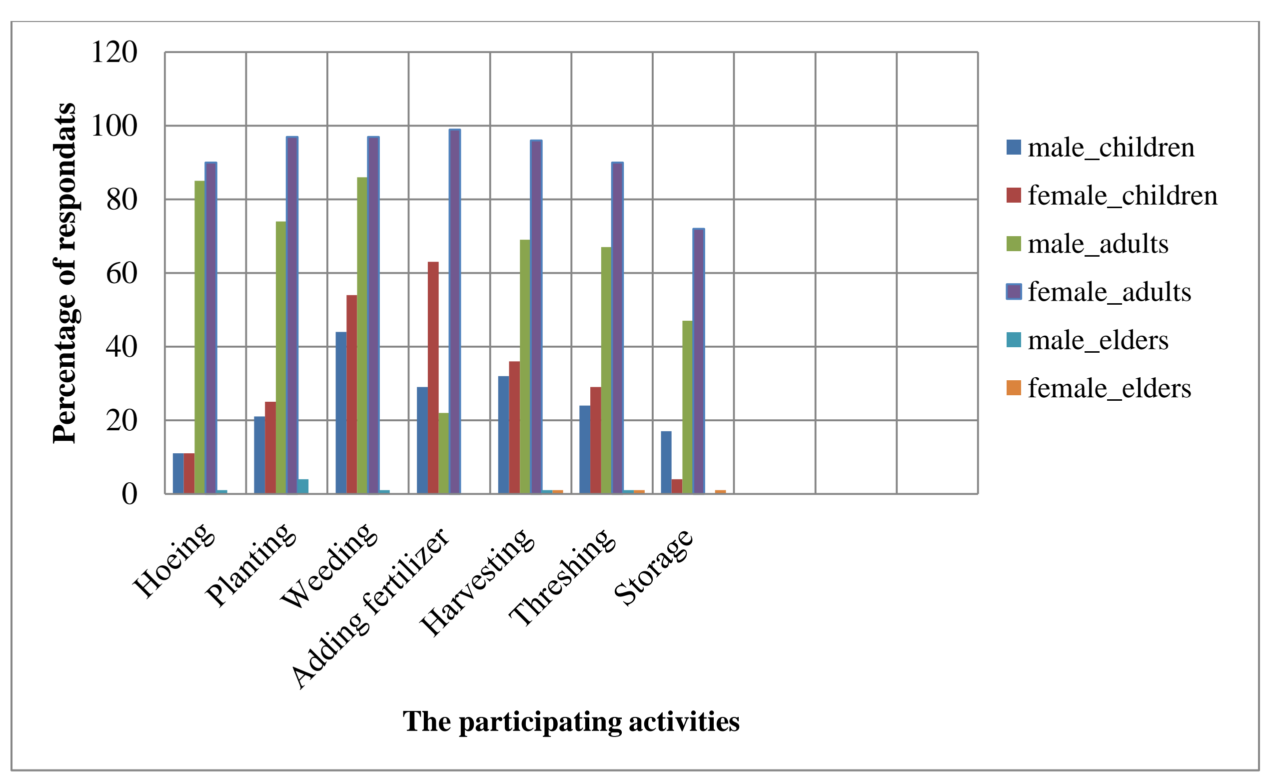

Figure 7 Role of gender and Age in participating of Lablab cropping (Konso, n=72)

4.1.6. Planting and harvesting time of the crop

The planting time of Lablab was done between mid-February to late March (Fig.8). The majority (80%) of the farmers in the study area plant the crop in early March. Thus the most appropriate planting months for all varieties is early March.

The harvesting time is between mid-November to mid-January for the main cropping season. Majority of the farmers (80%) in the study area harvest the crop in late November to late December (Fig. 9).

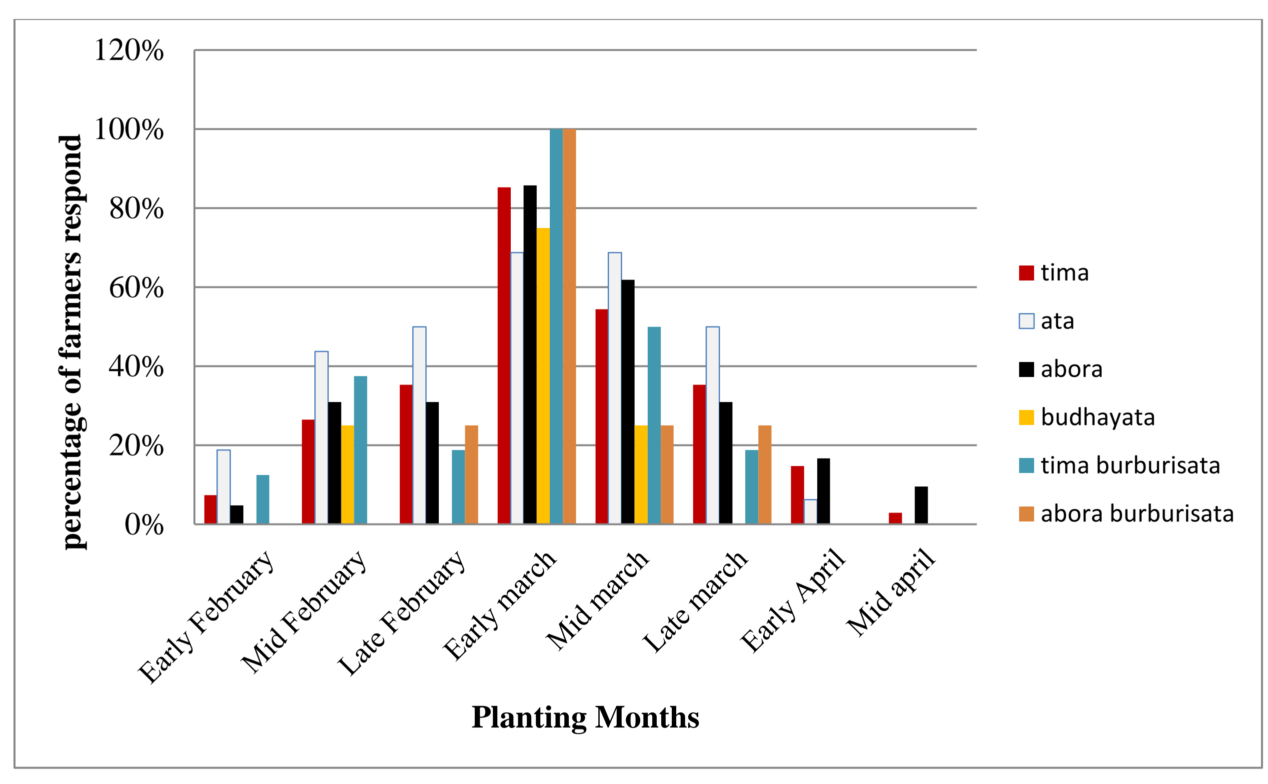

Figure 8 planting time of Lablab in Konso zone

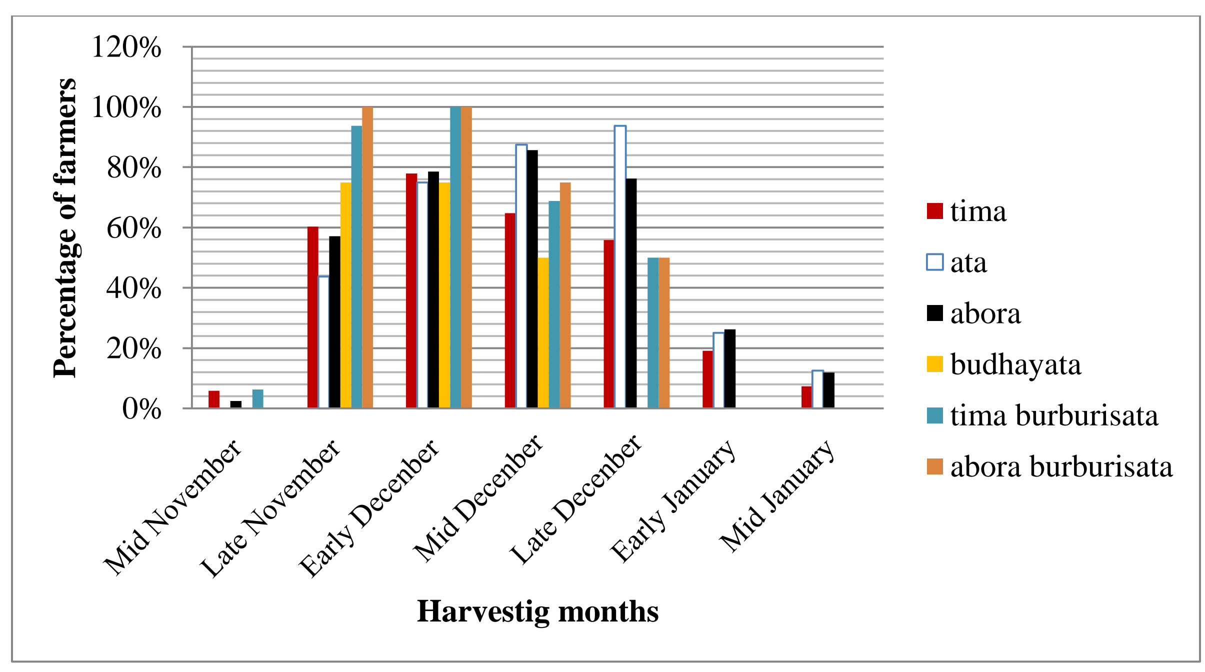

Figure 9 Harvesting time of Lablab in Konso

4.1.7. Uses of Lablab in the study area

In the study area, Lablab is mainly used for human food in the form of boiled grain (*nifro*), animal feed and soil conservation (Fig. 10). In Konso zone, the dry seeds are eaten as boiled grain (*nifro*) after prolonged cooking with several changes of water. During the survey time it was observed that whole plant is used as a fodder for cattle, either green or as hay. Furthermore, Lablab also plays an important role in improving soil fertility and soil conservation for cereal crops (such as sorghum and maize) when grown via intercropping. The key informants were asked about the use of the crop and they confirmed that it has tremendous uses as food, forage, and improving soil fertility.

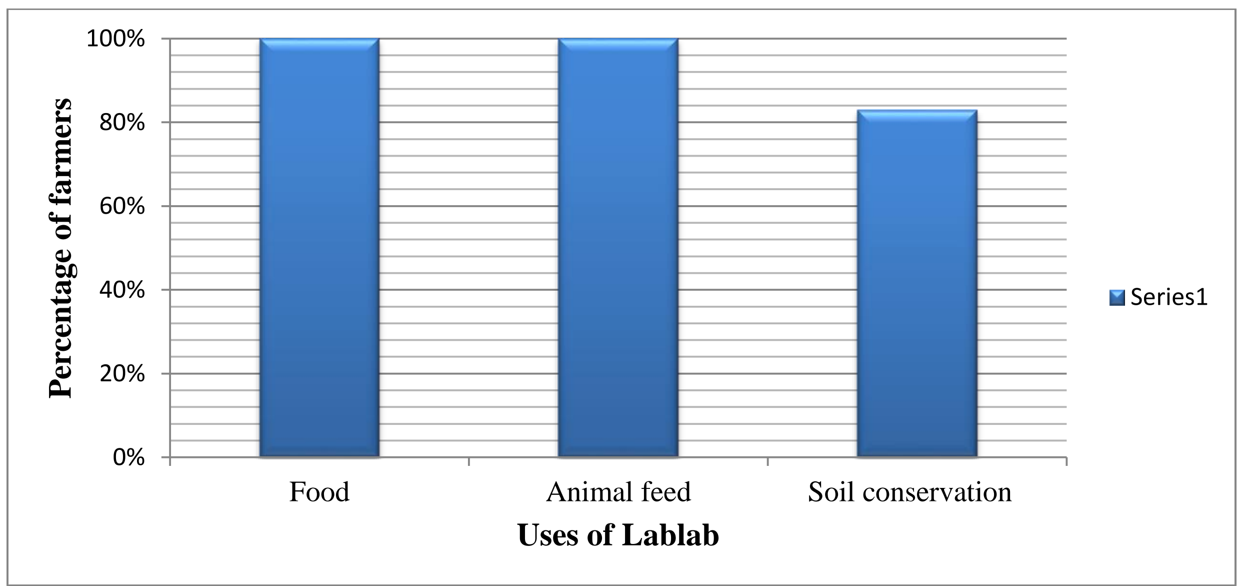

Figure 10 Uses of Lablab in Konso zone

4.1.8. Production constraints of the crop in Konso zone

In the study area, the farmers are facing different constraints of Lablab production. As the farmers mentioned, drought, shortage of land, insects and diseases are the most important constraints for Lablab production. Even though they could not identify the names of the insects and diseases properly, the generated information indicated occurrence of some major insect pest such as bruchid beetles (storage pest), powdery mildew, aphids and pod borers are the most important problems for farmers. The most serious insect pest contributed to significant loss of stored Lablab in the study area is bruchid beetles, which also attack the crop in the field. They used ash for repel of these bruchid beetles.

4.2. Molecular genetic diversity

All the 15SSR markers were polymorphic and produced a total of 1764 bands with an average of 117.6 bands per locus (Table 4). The highest number (180) of bands per locus was recorded from the marker KTD255, out of which 171 (95%) were polymorphic. KTD299 resulted in the highest percentage of polymorphic bands (97.8%), whereas c13353_g1_i1produced the smallest percentage (91.11) of polymorphic bands (Table 4).

Table 4 Number of polymorphic bands, monomorphic bands and percentage of polymorphic bands of each primer

Primers	**Total no of bands**	**No. of Monomorphic bands**	**No of polymorphic bands**	**Percentage of polymorphic bands**
c17963_g1_i1	99	7	92	92.93
c21512_g2_i1	134	5	129	96.2
c13353_g1_i1	90	8	82	91.11
Lpxu-009	89	3	86	96.6
Lpxu-013	160	6	154	96.25
Lpxu-002	178	5	173	97.19
Lpxu-010	141	6	135	95.74
KTD241	91	6	85	93.4
KTD195	91	3	88	96.7
KTD255	180	9	171	95.00
KTD138	91	4	87	95.6
KTD272	147	7	140	95.23
KTD245	91	4	87	95.6
KTD249	91	3	88	96.7
KTD199	91	2	89	97.8
Total	1764	78	1686	95.58

The results resolved 225 alleles across all the accessions with an average of 14.80 alleles per locus for the 15 SSR markers. The allele frequency distribution reflects that 47.11 % of the alleles were rare (0.01 to 0.05), 28 % range from 0.05 to 0.10, and 24.89 % were higher than 0.10 (Table 5).

Table 5 Summary of the number of alleles with their respective frequencies

Markers	**Number of alleles with their frequency**			
	Rare alleles(0.01 - 0.05)	**Common alleles (0.05 - 0.1)**	**Abundant alleles (0.1 or higher)**	**Total**
c17963_g1_i1	9	3	4	16
c21512_g2_i1	9	3	3	15
c13353_g1_i1	8	4	4	16
Lpxu-009	3	3	3	9
Lpxu-013	9	8	2	19
Lpxu-002	6	7	3	16
Lpxu-010	10	3	4	17
KTD241	7	2	6	15
KTD195	4	2	4	10
KTD255	12	5	7	24
KTD138	4	5	3	12
KTD272	13	6	2	21
KTD245	3	3	5	11
KTD249	6	4	3	13
KTD199	3	5	3	11
Total	106	63	56	225
Percentage	47.11	28	24.89	

The results showed that the major allele frequency (MAF) per locus ranged from 0.32 (c21512_g2_i1marker)to 0.13 (Lpxu-002marker) with an average of 0.22 per marker (Table 6).The highest gene diversity (0.92), allelic richness (9.25), polymorphic information content (0.92), number of alleles (21), effective number of alleles (6.87) and Shannon's Information Index (1.99) were obtained for the SSR marker KTD272 (Table 6).

The lowest gene diversity (0.80), allelic richness (4.5), polymorphic information content (0.78), number of alleles (9.00), effective number of alleles (3.53), and Shannon's information index (1.32) were recorded for SSR marker Lpxu-009 (Table 6). The study also revealed that all the markers were highly informative with the PIC ranging from 0.78 (Lpxu-009 marker) to

0.92(KTD272 marker) with an average 0.85 (Table 6). The observed heterozygosity (Ho) varied from 0.00 to 0.98, with a mean of 0.27 and expected heterozygosity (He) ranged from 0.69 (Lpxu-009 and KTD 241) to 0.84(Lpxu-013), with a mean of 0.75(Table 6). The 15 markers showed highly significant ($p<0.0001$) deviation from HWE (Table 6).

Table 6 Summary of different diversity parameters of 91 Lablab accessions across 15 SSR markers

Marker	MAF	GD	Ar	Arp	PIC	Na	Ne	I	Fst	Nm	Ho	He	PHWE[a]	F
c17963_g1_i1	0.26	0.84	5.75	0.88	0.83	16.00	4.05	1.48	0.15	1.37	0.17	0.71	0.000***	0.73
c21512_g2_i1	0.32	0.80	6.12	0.75	0.78	15.00	4.06	1.49	0.10	2.03	0.42	0.71	0.037*	0.39
c13353_g1_i1	0.24	0.87	5.50	1.00	0.86	16.00	4.19	1.53	0.14	1.44	0.00	0.75	0.000***	1.00
Lpxu-009	0.27	0.80	4.50	0.38	0.78	9.00	3.53	1.32	0.12	1.79	0.00	0.69	0.000***	1.00
Lpxu-013	0.18	0.90	8.75	0.75	0.89	19.00	6.74	1.99	0.08	2.87	0.77	0.84	0.000***	0.08
Lpxu-002	0.13	0.90	8.63	0.50	0.90	16.00	6.40	1.93	0.09	2.50	0.95	0.82	0.001***	-0.15
Lpxu-010	0.19	0.87	6.63	1.00	0.87	18.00	4.31	1.61	0.12	1.67	0.37	0.75	0.000***	0.51
KTD241	0.25	0.85	5.25	0.75	0.84	14.00	3.59	1.39	0.20	0.98	0.00	0.69	0.000***	1.00
KTD195	0.23	0.84	5.00	0.38	0.82	10.00	4.01	1.43	0.13	1.59	0.00	0.72	0.000***	1.00
KTD255	0.14	0.91	8.88	1.00	0.90	21.00	6.51	1.89	0.10	2.22	0.98	0.81	0.004**	-0.24
KTD138	0.25	0.86	5.88	0.50	0.85	12.00	5.02	1.67	0.08	2.71	0.00	0.79	0.000***	1.00
KTD272	0.15	0.92	9.25	0.50	0.92	21.00	6.87	1.99	0.09	2.46	0.44	0.83	0.001***	0.47
KTD245	0.20	0.86	4.75	0.38	0.85	11.00	3.72	1.36	0.19	1.05	0.00	0.70	0.000***	1.00
KTD249	0.21	0.86	5.25	0.38	0.85	13.00	3.98	1.43	0.16	1.24	0.00	0.71	0.000***	1.00
KTD199	0.32	0.82	4.75	0.25	0.81	11.00	3.76	1.38	0.14	1.53	0.00	0.70	0.000***	1.00
Mean	0.22	0.86	6.33	0.64	0.85	14.80	4.72	1.60	0.12	1.83	0.27	0.75		0.65

Where MAF = Major allele frequency, NA= Number of alleles, Ne = Effective number of alleles, GD= Gene diversity, Ar = Allelic richness, Arp = Private allelic richness, Ho = Observed heterozygosity, He = Expected heterozygosity, Fst = Inbreeding coefficient within subpopulations relative to total (genetic differentiation among subpopulations), Nm = Gene flow estimated from Fst= 0.25(1-Fst)/Fst, PIC = Polymorphic information content, I = Shannon's Information Index, F = Fixation Index, PHWE [a] = *P*-value for deviation from Hardy Weinberg Equilibrium, ns = not significant, * = $P < 0.0001$ and hence highly significant.

4.2.2. Genetic relationship within and among populations

The number of observed alleles (Na) was higher for accessions collected from Konso (9.53) followed by north Wollo (7.67) and West Wellega (7.40). Similarly, accessions collected from Konso showed the highest value for number of effective alleles (6.40), Shannon's information index (1.96), private allele richens (1.67) and expected heterozygosity (0.83). The inbreeding coefficient (fixation index) value ranged from 0.59 for Metekel accessions to 0.70 for GamoGofa and West Wellega accessions. All Lablab populations showed similar value in percentage of polymorphism (100) (Table 7).

Table 7 Summary of genetic diversity indices for eight Lablab populations grouped over 15 markers.

Population	**Genetic parameters**								
	N	**Na**	**Ne**	**Arp**	**I**	**Ho**	**He**	**PPL**	**F**
Konso	24	9.53	6.40	1.67	1.96	0.31	0.83	100	0.64
North Wollo	22	7.67	5.08	0.60	1.74	0.26	0.78	100	0.69
Gamo Gofa	9	6.47	5.18	0.53	1.68	0.25	0.78	100	0.70
West Wellega	10	7.40	5.77	0.73	1.83	0.26	0.80	100	0.70
Metekel	6	4.40	3.46	0.33	1.28	0.27	0.67	100	0.59
North Gonder	5	4.33	3.82	0.07	1.34	0.27	0.70	100	0.67
West Gojjam	5	4.27	3.59	0.20	1.33	0.27	0.70	100	0.63
Exotic	10	6.53	4.50	0.87	1.63	0.30	0.76	100	0.61
Mean	11.38	6.33	4.72	0.63	1.60	0.27	0.75	100	0.65

N= Number of genotypes, Na= Number of different alleles, Ne = Effective number of alleles, Arp = Private allele, I = Shannon's Information Index, Ho = Observed heterozygosity, He = Expected heterozygosity, PPL= Percentage of polymorphic loci, F = Fixation Index.

4.2.3. Analysis of molecular variance

AMOVA showed highly significant differences (P<0.001) among the populations (AP) and within populations (WP). A low level of variation was observed among populations (6%), whereas high level of variation was revealed within populations (94%) (Table8).Genetic differentiation among the populations was moderate (F_{ST} = 0.061). The analysis also confirmed the presence of considerable (3.820) gene flow (Nm) among the studied populations (Table 8).

Table 8 AMOVA based on standard permutation across the full data set of Lablab genotypes collected from different geographic origin

Source of variation	df	SS	MS	Variance		F-statistics	P value
				Estimated	%		
Among Populations (AP)	7	143.356	20.49	0.40	6	0.061	0.001
Among individuals (within population)	83	997.479	12.08	6.00	94	1.000	0.001
Total	90	1180.35		6.40	100		Nm=3.820

Where, df = degree of freedom, SS=sum of squares, MS=mean squares and Nm=gene flow

4.2.4. Magnitude of genetic distance between populations

The magnitude of genetic distances between populations originated from different geographical origins showed more differentiations between West Gojjam and the rest collections (GD range from 0.121 to 0.951). The highest value of GD (0.951) was observed between West Gojjam and Metekel populations followed by West Gojjam and Exotic materials (0.908).The smallest genetic distance (GD=0.121) was observed between populations from West Gojjam and North Wollo (Table 9).

Table9 Pair wise population matrix of Nei's genetic distance between eight Lablab populations

	Konso	**North Wollo**	**Gamo Gofa**	**West Wellega**	**Metekel**	**North Gonder**	**West Gojjam**	**Exotic materials**
Konso	0.000							
North wollo	0.332	0.000						
Gamo Gofa	0.189	0.427	0.000					
West Wellega	0.422	0.425	0.312	0.000				
Metekel	0.718	0.772	0.379	0.449	0.000			
North Gonder	0.441	0.164	0.457	0.519	0.814	0.000		
West Gojjam	0.474	0.121	0.680	0.570	0.951	0.436	0.000	
Exotic materials	0.501	0.632	0.669	0.499	0.508	0.430	0.908	0.000

4.2.5 Genetic interrelationships between Lablab accessions

The dendogram of cluster analysis based on neighbor-joining algorithm using the un-weighted pair group method (UWPGM) categorized the 91 Lablab accessions into three major clusters consisting of 48%, 33% and19 % of the accessions in cluster I, II, and III, respectively forming different sub-clusters (Fig. 11).Eighty percent of Exotic materials were grouped together in cluster one but in separate sub-clusters. The first cluster mostly composed of accessions from all populations. The second cluster contained accessions mainly from West Gojjam, North Wollo and North Gondar. It was observed that accessions from geographically nearest populations of North Wollo, West Gojjam and North Gonder constitute more than 80% of the total accessions in the group, and in the third cluster comprised almost entirely Konso accessions (88%).

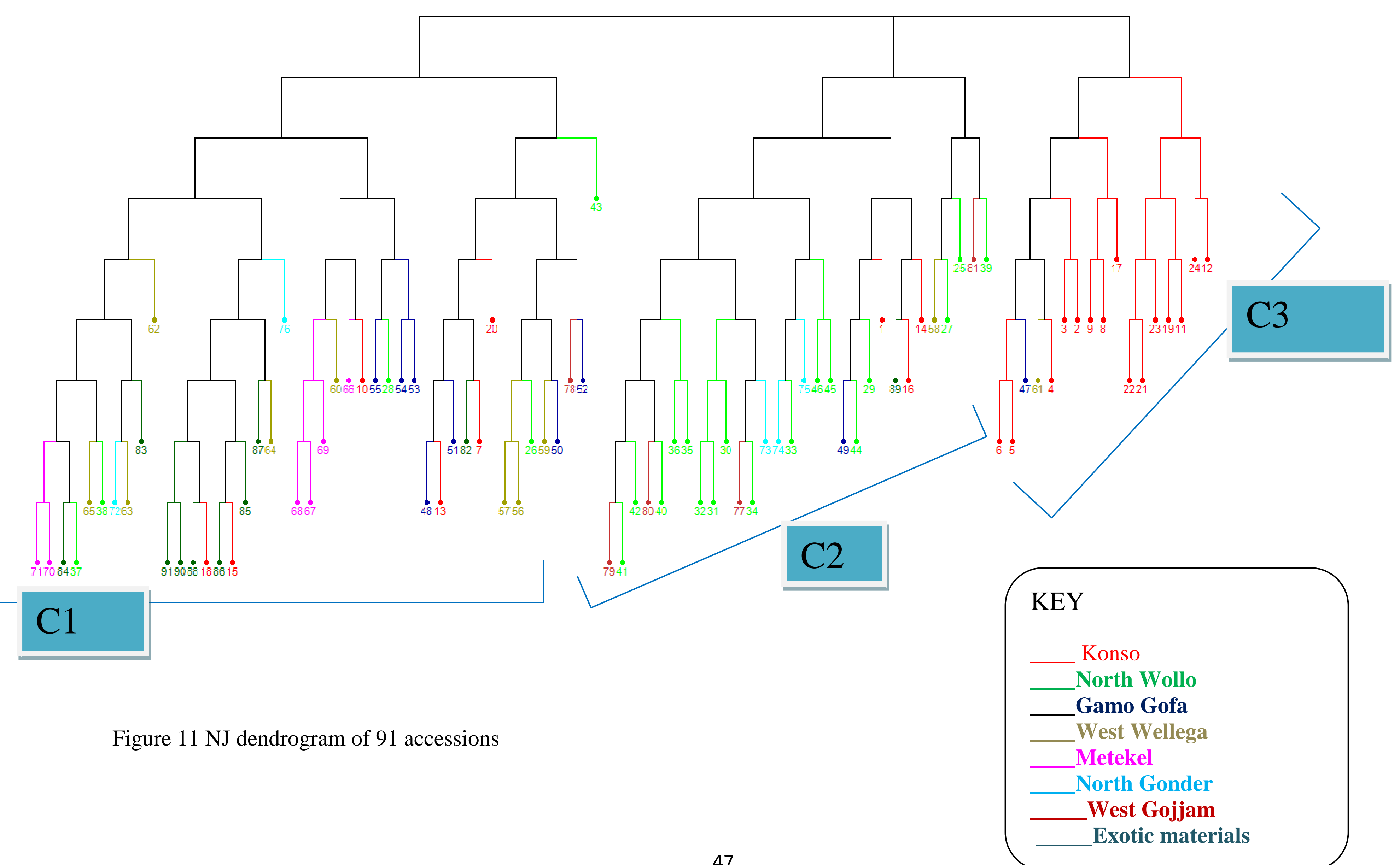

Figure 11 NJ dendrogram of 91 accessions

4.2.6. Principal coordinate analysis (PCOA)

PCoA revealed that the first three coordinates explain 22.55 % of the genetic variation. The first, second, and third principal coordinates, respectively explained about 9.04 %, 6.78 % and 6.36 % of the gross variation, respectively (Table 10).

The PCoA analysis displayed in (Fig. 12) showed that accessions from different collection sites were clustered together confirming the result of NJ cluster analysis.

Table 10 Percentage of variation explained by the first three principal components using 15 SSR markers across 91 genotypes

Axis	**1**	**2**	**3**
Individual %	9.04	6.78	6.36
Cumulative %	9.40	16.18	22.55

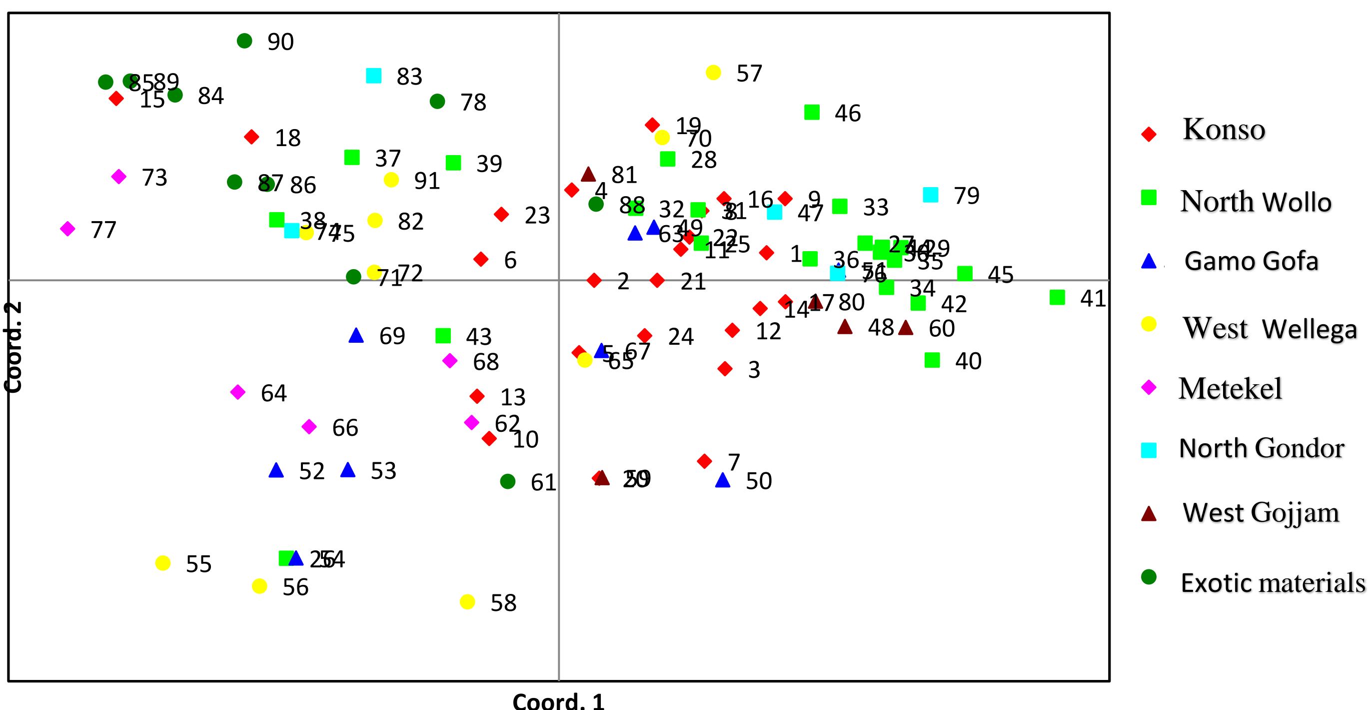

Figure 12 PCoA of 91 accessions and eight populations of Lablab using 15 SSR markers

4.2.7. Population structure

The STRUCTURE analysis divided the Lablab accessions into four genetic clusters. The four groups were determined by Bayesian algorithm implemented in the software STRUCTURE. The algorithm allows estimating the true number of clusters (K) in populations of the study. Based on the true number of cluster (K*)* suggested by Evanno *et al.* (2005), the real structure showing a clear peak of the populations was set at $K = 4$ (Fig. 13). It means that K = 4 found to be the most likely number of clusters to divide the 91 accessions in to four (Fig. 14). Based on this value, Clumpak result (bar plot) showed wide admixtures and hence there was no clear geographic origin-based structuring of populations (Fig.14).

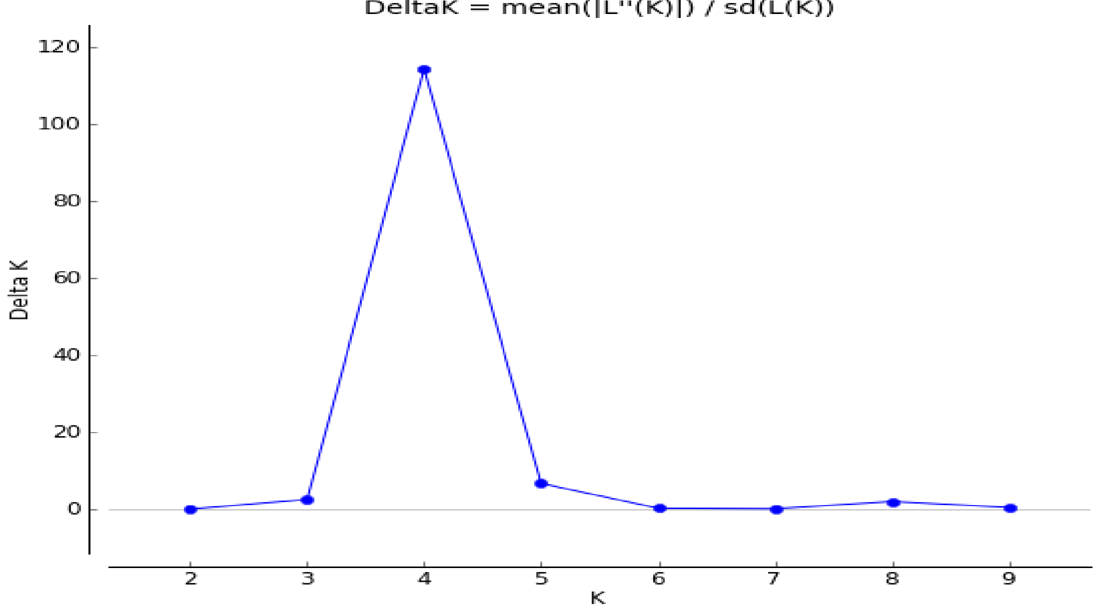

Figure 13 Results of the STRUCTURE analysis of 91 Lablab accessions: The highest peak at K=4

Table 11The Evanno output table

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-6249.630000	0.991596	—	—	—
2	20	-5863.860000	44.184808	385.770000	6.700000	0.151636
3	20	-5484.790000	31.307959	379.070000	81.985000	2.618663
4	20	-5187.705000	1.230950	297.085000	140.490000	114.131402
5	20	-5031.110000	4.262431	156.595000	28.885000	6.776650
6	20	-4903.400000	80.100102	127.710000	29.215000	0.364731
7	20	-4804.905000	56.571412	98.495000	13.395000	0.236780
8	20	-4693.015000	15.791413	111.890000	32.785000	2.076128
9	20	-4613.910000	7.784053	79.105000	4.470000	0.574251
10	20	-4539.275000	11.208590	74.635000	—	—

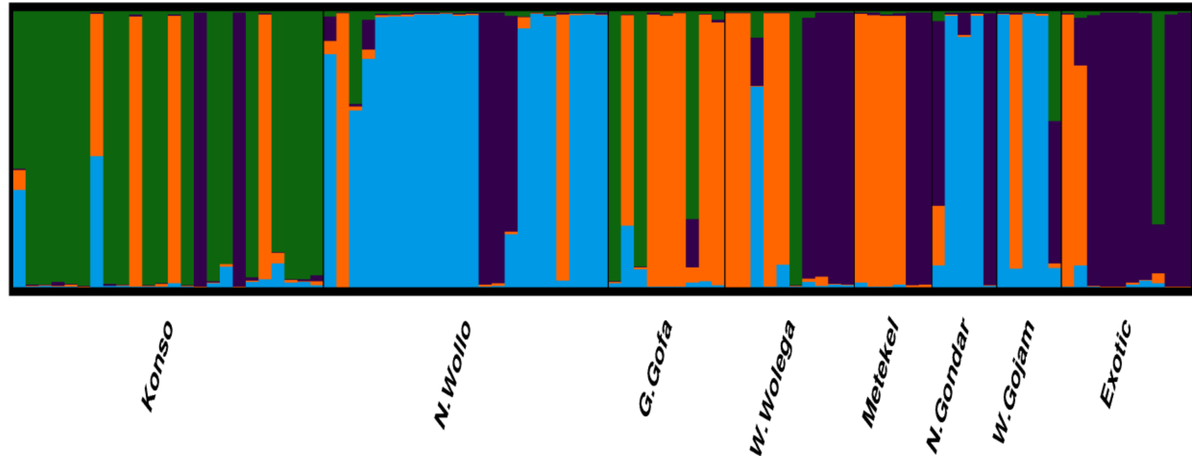

Figure 14 Estimated population structure of 91Lablab accessions as revealed by 15 polymorphic SSR markers for (K=4)

CHAPTER FIVE

5 DISCUSSION CONCLUSION AND RECOMMENDATIONS

5.1. DISCUSSION

5.1.1. Diversity of farmers' varieties of Lablab in Konso zone

Landraces/ farmers' variety are populations of a cultivated crop with a historical origin, distinct identity, often genetically diverse and locally adapted, and associated with a set of farmers' practices of seed selection and field management as well as with a farmers' knowledge base (Camacho-Villa *et al.*, 2005).Farmers who cultivate local varieties of Lablab in the study zone use morphological traits of seeds for identification and selection of varieties. For example, the varieties called Okala tima, Okala ata and Okala abora were named based on their seed color meaning red seeded, white seeded and black seeded, respectively in Konsogna languge (Konso language). Similar results were reported by Tura Bareke*et al*. (2018) in Common bean in Southern and Eastern Ethiopia use morphological traits of seeds for identification and selection of farmers' varieties. Based on names and descriptions given by farmers, six Lablab local varieties were identified in the study area.

5.1.2. Cropping systems and management practice

In the study area, all Lablab varieties were grown as an intercrop cropping with sorghum, maize and finger millet and grown as a sole crop in the margin of terracing and fence line. Konso farming system utilizes a multiple cropping system which incorporates a combination of different crops. This is probably because of Konso people are well known for their traditional land management and conservation practices, intercropping of the crop with other cereal crops can protect soil erosion and improve soil fertility. In this study, sorghum is the major

intercropped cereal (99%) and maize is the second most intercropped crop (89%) cereal with Lablab in all surveyed areas of Konso. Likewise, Menfese Tadesse, (2010) in Konso people of southern Ethiopia have been known for their indigenous intensive agricultural land management and soil conservation practices.

5.1.3. Use of Lablab in the study area

Farmers used Lablab as food in the form of boiled grains (*nifro*). As the respondent said that, the dry seeds are eaten as boiled grain (*nifro*) after prolonged cooking with several changes of water. *Nifro* is the major food that the farmers used from seed of Lablab. Tefera Tolera (2006) and Maass*et al*. (2010) reported that green pods, mature seeds and leaves are traditionally eaten as vegetables in Africa, South and South-east Asia. The use of Lablab for food in this study seems to vary considerably with other countries, as some uses reported from other countries were not recorded in the study area.

The farmers of the study area also mentioned that the fodder of Lablab is very important for their cattle production. This is because of Lablabis one of the most palatable legumes for animals (Valenzuela and Smith, 2002). The leaf has crude protein of 21 to 38% and the seed contains 20 to 28% crude protein (Cook *et al*., 2005). Additionally, Lablab also plays a great role in improving soil fertility and making it suitable for cereal crops such as sorghum, maize and finger millet when grown as intercropping/mixed cropping. Similar report was mentioned by Kimani *et al*. (2012), Lablab is a multipurpose legume crop used as a pulse crop for human consumption, as a fodder crop for livestock, as a rotational and cover crop to improve soil fertility and soil organic matter.

5.1.4. Gender and age roles in production and management of Lablab in the study area

In the study area, most of the management practices/agronomic practices in the cropping of Lablab were mostly done by both men and women adults. Both of them participate in hoeing, planting, weeding, adding organic fertilizers, harvesting, threshing, and storage. Men and women often work together in the field, but most activities are carried out by women. Male and female children also participate in planting, weeding, harvesting, threshing and storage activities. This indicates that the crop is well managed by the male and female adults within the study area. The reason for this phenomenon is that ploughing and crop cultivation in Konso is done mainly by hand hoeing; thereby resulting in a higher demand for household labor than in a farming system using the ox plough method. Similar study conducted by Menfese Tadesse (2010) showed men have relatively low work burdens than women with increasing work load during the peak seasons. Women are engaged throughout the year in addition to their regular household responsibilities. Similarly, studies conducted by Abayneh Feyso *et al*. (2019) in Konso on gender roles in crop production have indicated that women play a great role than men on farm work activities.

5.1.5. Planting month of Lablab in the study area

As both the key and general informants mentioned, planting of Lablab was done following the start of rain from February to April. Based on the survey result, planting time of Lablab was mainly from mid February to late March. The extension of planting time could be variability of rainfall in Konso. According to key informants' information, March was the main planting season of the crop in Konso zone. Abayneh Feyso *et al*. (2019) have reported planting of crop in

Konso zone starts as soon as the main rains begin in February. This is because of the production of cycle crop is entirely dependent on rainfall.

5.1.6. Production constraint of Lablab

Farmers are facing different constraints of Lablab production. As the farmers mentioned, drought, shortage of land, insects and diseases are the most important constraints for Lablab production. Even though they could not identify the names of the insects and diseases, the descriptions they provided showed that bruchid beetles (storage pest), powdery mildew, aphids and pod borers are the most important problems for farmers. The most serious pest causing losses to stored Lablab is bruchid beetles, which also attack the crop in the field. Similar results were reported by Ramesh and Byregowda (2016), biotic stresses, such as anthracnose, Lablab yellow mosaic virus (LaYMV) diseases, pod borers (*Heliothis armigera* ,*Adisura atkinsoni*) and bruchids (*Callosobruchus theobrome*), are major biotic production constraints in Lablab.

5.1.7. Genetic diversity study using SSR markers

Molecular characterization of plant genetic diversity and relationships using microsatellite markers is promising because of their co-dominance and ability to reveal a high number of alleles per polymorphic locus.

The 15 SSR loci used in this study were highly polymorphic, resulting in 95.59% polymorphisms and highly effective in differentiation of 91Lablab accessions. The results also revealed 225 alleles across all the accessions. The number of alleles per locus varied from nine for Lpxu-009 to 21 for KTD255 and KTD272. The values recorded in the present study are different from previous studies using SSRs markers on Lablab (Zhang *et al.,* 2013; Robotham and Chapman, 2017; Keerthi *et al.,* 2018). This study recorded higher total number of allele (225) as compared

to report of 133 alleles by Keerthi*et al*. (2018). Similarly, the mean number of alleles (14.80) detected in this study was higher than the mean number of alleles reported by Zhang *et al.* *(*2013), Robotham and Chapman (2017) and Keerthi *et al.*(2018).According to their report, the mean number of alleles ranged from 2.65 to 7.4. The differences in the mean number of alleles and total number of alleles between the present study and the previous studies could be attributed to the genetic materials and the number of genotypes used for this study was different and the genotypic difference of the tested genotypes may cause the difference. Likewise, the mean number of effective alleles (4.75) and Shannon information index (1.60) were higher than the previous study reported by Keerthi *et al.* (2018).

PIC value of the present study indicates the markers used in the study were highly informative and availability of high allelic variation in the marker loci and their distribution within the studied Lablab genotypes. The result also indicates the usefulness of the markers for further genetic analysis such as genetic diversity, genetic linkage map construction and QTL mapping of Lablab genotypes in the future. The 15 SSR markers used in this study were selected from Zhang *et al. (*2013), Robotham and Chapman (2017) and Keerthi *et al.* (2018) studies based on their high PIC values. The mean value of PIC was higher than that reported by Zhang *et al.* (2013), Robotham and Chapman (2017) and Keerthi *et al.* (2018). The reason for this result may be attributed to the differences in the genetic diversity of the tested Lablab accessions, the number (sample size) of accessions used for this study. The major allele's frequency across the accessions ranged from 0.13 to 0.32. This result indicated that the SSR markers used in this study are very informative, and can be used in genetic diversity studies and in marker assisted breeding in Lablab.

Heterozygosity can be considered as a measure of the amount of genetic variation within genotypes. This parameter indicates how much the variation exists in the population and how the variation distributed across the alleles of analyzed markers. The observed heterozygosity (H_o) is the proportion of heterozygous individual in population samples and expected heterozygosity (H_e) is the probability of an individual being heterozygous in any locus (Hirpara and Gajera, 2018). The observed heterozygosity revealed low values in relation to expected hetrozygosity at all markers, which showed high amount of homozygosity.The observed lower heterozygosity in Lablab genotypes reflects a low level of diversity within Lablab accessions or low levels of out crossing. Robotham and Chapman (2017) also reported very low observed heterozygosity (0.205) suggesting the inbreeding index characteristic of a minimum out crossing species. All of the markers (100%) exhibited significant deviations from Hardy-Weinberg equilibrium between observed and expected heterozygosity in which all of them showed excess heterozygosity that led to a significant deviation from HWE across all populations. Such excess heterozygosity is expected in sexually reproducing organisms that can maintain their heterozygosity through sexual recombination. The average genetic diversity (0.86) and expected heterozygosity (0.75) detected among the 91 Lablab accessions showed high levels of variation within the studied Lablab populations.

5.1.8. Genetic relationship among populations

The high genetic variation observed within the tested Konso and West Wellega accessions could be used as a potential source of important traits in the future Lablab breeding programs as the private alleles provide a unique genetic variability in certain loci (Kalinowski, 2005).

5.1.9. Genetic distance between populations of Lablab

Pairwise comparison of Nei's unbiased genetic distance among the eight populations ranged from 0.121 between West Gojjam and North Wollo populations to 0.951 between West Gojjam and Metekel populations. Metekel populations have larger genetic distance between the population of North Gondar, West Gojjam and North Wollo. This could be because of low genetic material exchange between the populations. Low genetic distances were also observed between populations of North Gondar and North Wollo (0.164) and Konso and Gamo Gofa (0.189). The low value of genetic distance indicates high frequency of identical alleles among accessions, thus leads genetic homogeneity. This could be attributed to the proximity of these geographical regions; hence there could be higher chances of exchanging and sharing of seed through agricultural systems and farmers

5.10. Analysis of molecular variance

A low level of variations was observed among populations (6%), while high levels of variation were revealed within populations (94%). This low genetic differentiation among population may be due to gene flow as a result of exchange of germplasm by farmers across populations which are geographically close to each other. This leads to an increase in the distribution of gene among different populations. Similarly, Kimani *et al*. (2012) reported 99% variation within and 1% variation among Lablab populations using amplified fragment length polymorphism (AFLP) markers. Kamotho *et al.* (2016)reported 85 % of genetic variation within populations and 15% among populations for the tested Kenyan Lablab accessions using SSR markers. The low level of genetic variability among Lablab populations may be as a result of gene flow (introduction and migration of alleles or genotypes) from one population to another through seed exchange.

The study also showed the presence of moderate genetic differentiation among Lablab populations (F_{ST}= 0.061). The magnitude among and within population differentiation was quantified using F-statistics (F_{it}, F_{is} and F_{st}) also known as fixation indices (Wright, 1951). Fixation index (Fst) is a measure of population differentiation due to genetic structure. According to Wright (1951), Fst value ranges from 0 to 0.05 is considered as low, 0.05 to 0.15 moderate, and 0.15 to 0.25 large and greater than 0.25 indicate very large genetic differentiations. A low level of genetic differentiation among populations could be indicative of a high gene flow (Nm = 3.280).According to Slatkin (1985) and Waples (1987), Nm values grouped into three categories: Nm > 1.00 high, 0.25-0.99 intermediate and 0.000 – 0.249 low. Therefore, the high Nm value of 3.280 observed in this study indicates high gene flow among populations which will agree with the AMOVA result showing low variation among the populations.

5.11. Cluster analysis and relationship among accessions

The dendogram of cluster analysis based on neighbor-joining algorithm using UWPGM categorized the 91 Lablab accessions into three main clusters (I, II, and II) by forming different sub groups. Zhang*et al*. (2013)clustered 24 Lablab accessions in to two clusters using 11 EST-SSR markers. The differences might be due to the number and type of accessions used for the study. The clustering model showed the existence of weak relationships between the pattern of genetic diversity and geographical origins of collections. Accessions collected from different populations clustered together, which may be attributed to the existence of gene flow among neighboring populations. Most of exotic materials were grouped with geographically distant accessions in Cluster I. Accessions collected from Konso were also grouped in all clusters with other geographically distant populations. This indicates accessions in one cluster might be

evolved from different lines of ancestry or independent events of evolutionary forces such as genetic drift, mutation, migration, selection and germplasm exchange might be separated them into related but different gene pools (Gemechu Keneni *et al.,* 2012).

Moreover, this result is clearly reflected on the model based population genetic structure analysis finding showing high potential of admixtures of gene across populations. It revealed the existence of weak sub-structuring (K=4) of the eight populations of Lablab. This finding may indicate germplasm exchange between farmers, leading to gene flow across adjoining populations.

The overall grouping pattern of PCoA corresponds with the clustering dendrogram which explains about conformity of results obtained from UPGMA analysis. The reason for these inter mixing could be explained by gene flow from one area to the other.

5.2. CONCLUSION

The production and utilization of Lablab in different forms around Konso area implies that the crop has tremendous potential to significantly contribute to both food and nutritional security of the country. The indigenous knowledge to use intercropping type of cultivation practice in the area may also contribute to nutritional diversity. The different biotic and abiotic constraints affecting Lablab productivity are good indicators for the underutilization of the crop which demands strong attention towards its genetic improvement and conservation in the future. Successful molecular characterization of the crop helped to identify distinct grouping between different Lablab accessions collected from different parts of Ethiopia. The abundance of different alleles observed among the collections provides more evidence for novel alleles that can be efficiently exploited through future breeding program for the trait of interest. The existence of relatively higher genetic diversity in Konso and West Wellega collections also reveals that these areas could be considered as hot spot for genetic diversity as well as sources of desirable genes for genetic improvement and conservation. The results create an insight to exploit this underutilized genetic resource through integration of modern tools to the existing indigenous knowledge. Indigenous knowledge on Lablab production and management has tremendous contribution for the genetic improvement and better use of the crop.

5.3. RECOMMENDATIONS

- The limited collection of farmers' varieties of Lablab at the Ethiopian Biodiversity Institute signals the need to mount more collection of germplasm and conservation work.
- Further ethnobotanical studies with seed collection of farmers' varieties should be conducted particularly in areas not covered by this study in order to have a full documentation of Lablab farmers' varieties and indigenous knowledge held by farmers in Ethiopia.
- Further analysis should be done on the genetic diversity and population structure of Lablab accessions by including additional collections from all over the country using high resolution markers.
- Germplasm from Konso and West Wellega showed relatively high level of genetic diversity and should be the main target area for future collection and population improvement.

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APPENDICES

Appendix 1 Structured interview and informed oral consent

"Hello, first I will introduce myself; my name is Solomon Tamiru and I am a student at Addis Ababa University in the Institute of Biotechnology and this is my colleague who is assisting me as a local guide and translator. We are conducting a study of the crop Lablab as part of my education at the university." The purpose of this study is to identify different types of Lablab and to understand their uses and management by farmers. With your permission, I would like to ask you questions about Lablab. Of course, your participation is entirely voluntary. These interviews require less than one hour. I do not anticipate that the interview will pose any risks to you. Because I am a student, I cannot pay you.

I hope that my research will benefit farmers in Ethiopia by promoting the diversity of Lablab. If you agree to an interview, you do not have to answer all of my questions, and you can tell me at any time if you would like to stop. I would record your answers to my questions using my cell phone and my notebook. I would include this information in my thesis, and it would be shared with other researchers who are interested in Lablab. Do you have any questions? In case you have any questions in the future, here is my contact information. Solomon Tamiru phone number +251910991535. Do you agree to participate in this study of the crop Lablab? If yes, proceed with the interview. If no, thank the farmer and continue to the next randomly-selected household.

Section 1: **Basic information**

Date of interview:	***Start time of interview:***
Name of interviewer:	
Region:	Zone:
Woreda/District:	Kebele/Sub-District:
Latitude (in decimal degrees):	*Longitude (in decimal degrees):*
Altitude (in meters):	
Agro ecological classification	
Random number of household from farmers' association list:	
Relative wealth on farmers' association list: ☐ Low income ☐ Middle/High income	

Section 2: General information about informant

Name of informant:
Language spoken by informant during interview: ☐ Amharic ☐ Other (specify): ___________
Age (observed): ☐ 18 to 30 ☐ 30 to 45 ☐ 45 to 60 ☐ 60+
Gender (observed): ☐ Male ☐ Female
Is the informant the household head? ☐ Yes ☐ No
If no, household-head gender: ☐ Female-headed ☐ Male-headed

Section 3: Market access

How far is the closest market from your home? In minutes on foot
Do you go to any other larger market? ☐ Yes ☐ No
If yes, how far is this larger market from your home? In minutes (on foot)
How far is the nearest road with vehicle transport from your home? In minutes (on foot)

Section 4: Interspecific diversity of legume crops

Are you growing any legumes this year? ☐ Yes ☐ No If *yes, how many hectares.*
Which legumes did you plant in 2010 E.C (Meskerem to Pagume)?
For each of the legumes you listed, how many hectares of land did you plant in 2010 E.C (Meskerem to Pagume) including any areas under intercropping? *Convert any local units to hectares* (e.g. 1 timad or qurt = 0.25 hectare).

Section 5: Use of Lablab (*Lablab purpureus)*

How do you use Lablab (*Lablab purpureus)*? ☐ Food ☐ Spice ☐ Medicine ☐ Fodder/Forage ☐ Fuel ☐ Market ☐ Bee forage ☐ Other (specify): ________

Section 6: Cropping practices

Do you rotate Lablab with other crops? ☐ Yes ☐ No
If yes, with which crops do you rotate Lablab*?*
If yes, how often do you plant Lablab within the crop sequence?
Do you intercrop Lablab with other crops? ☐ Yes ☐ No
If yes, with which crops do you plant Lablab in the same field?

Section 7: Intraspecific diversity of Lablab (*Lablab purpureus)*

Note: Be sure to include all varieties, including landraces and released/improved types.

What varieties of Lablab have you grown in the past three years (for the 2009, 2010, and 2011 harvests)?
Are there any other varieties that you have grown in the past, prior to 2009?
Are there any other varieties of Lablab grown by other farmers in your community, but not by yourself?
Are there any other varieties of Lablab that you remember from a long time ago, or have heard about from Elders, that are no longer grown by your community?
If you run out of a particular variety, whom would you ask to replenish your seeds? (*Record name as a potential key informant*).

Section 8: Key attributes of varieties

Instructions: At the top of each column, write the names of all varieties harvested in 2009, 2010, and 2011. Use additional sheets if necessary

Question	**Variety 1**	**Variety 2**	**Variety 3**	**Variety 4**
Local vernacular name of variety				
Is this a **traditional** or a **new** variety?				
Where did you first obtain this variety? (e.g. family, neighbors*, DA, market, food aid, research center)				
For how many years have you planted this variety?				
Where do you plant this variety? (e.g. main fields, field margins, home gardens, fence lines, other places)				
Are the places where you grow this variety rain fed, irrigated, or both?				

Do you plant this variety on soils with low, moderate, and/or high fertility?				
How many times per year do you sow this variety?				
When do you usually sow this variety?				
When do you usually harvest this variety?				
Is this variety grown mainly for home use or mainly for the market?				
How many hectares of these varieties did you harvest in 2009 E.C. and 2010 E.C., including area under intercropping? Make sure you convert any local units to hectares (e.g. 1 timad or qert = 0.25 hectare).	2010: 2009:	2010: 2009:	2010: 2009:	2010: 2009:
How much of this variety did you harvest in 2008 and 2007? Record with local units to be converted later. Be sure to include the name of the units. Later, go to a local market to determine the conversion factor from local units to kilograms.	2010: 2009:	2010: 2009:	2010: 2009:	2010: 2009:
Based on your experience, was the yield for this variety in 2010 and 2011 a very high yield, a high yield, a medium yield, a low yield, or a very low yield?	2010: 2009:	2010: 2009:	2010: 2009:	2010: 2009:
What is the current price of this variety at your local market? (use farmer units)				

* If a particular variety came from friends or family, ask for the name of the individual who provided it as a potential key informant. Record that person's name in your notebook.

Section 9: Rating of attributes for varieties of Lablab (*Lablab purpureus)*

Ask the informant to rate the varieties of Lablab (*Lablab purpureus)* planted for the 2009, 2010, and 2011 harvests. This is **NOT** a ranking activity, so multiple varieties may receive the same score.

Rating criteria and scale	**Var. 1**	**Var. 2**	**Var. 3**	**Var. 4**	**Var. 5**
Local name (copied from previous pages)					
Best yield this variety has ever given you (5=very high, 4=high, 3=medium, 2=low, 1=very low)					
Yield under drought conditions (5=very high, 4=high, 3=average, 2=low, 1=very low)					
Yield when excessive rain causing water logging (5=very high, 4=high, 3=average, 2=low, 1=very low)					
Yield when rainy season begins late (5=very high, 4=high, 3=average, 2=low, 1=very low)					
Yield when rainy season ends early (5=very high, 4=high, 3=average, 2=low, 1=very low)					
Resistance to frost (5=never affected by frost, 4=rarely affected by frost, 3=sometimes affected by frost, 2=often affected by frost, 1=always affected by frost)					
Resistance to common diseases (5=never affected by diseases, 4=rarely affected, 3=sometimes					

affected, 2=often affected, 1=always affected by diseases)					
Tolerance to common insect pests (5=never affected by insect pests, 4=rarely affected, 3=sometimes affected, 2=often affected, 1=always affected)					
Tendency for seeds to detach/shatter (5=seeds never detach/shatter 4=rarely detach/shatter, 3=detach/shatter about half the time, 2=often detach/shatter, 1=always detach/shatter)					
Importance as food for the household (5=extremely important, 4=very important, 3=somewhat important, 2=not so important, 1=not at all important)					
Importance as a source of income (5=extremely important, 4=very important, 3=somewhat important, 2=not so important, 1=not at all important)					
Importance as fodder for livestock (5=extremely important, 4=very important, 3=somewhat important, 2=not so important, 1=not at all important)					
Effect on soil fertility (5=very positive effect on					

soil fertility, 4=some positive effect, 3=no effect, 2=some negative effect, 1=very negative effect on soil fertility)					
Ease of harvesting (5=Very easy to harvest, 4=easy, 3=neither easy nor difficult, 2=somewhat difficult, 1=very difficult to harvest)					
Ease of preparing as food (5=very easy to harvest, 4=easy, 3=neither easy nor difficult, 2=somewhat difficult, 1=very difficult to harvest)					
Taste (5=very good taste, 4=good taste, 3=neither good nor bad taste, 2=bad taste, 1=very bad taste)					

Section 10: Gender roles in production and management of Lablab (*Lablab purpureus*)

Which gender and age groups live in your household (including the respondent)?	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No

When it comes to Lablab (*Lablab purpureus*), who within the family usually participates in the following activities? Check any that apply.

Activity	Gender and age groups*			Is this true for all varieties?*
Ploughing with animals (mares)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Preparing the soil by hand with a hoe (mekofer)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Leveling soil and removing uprooted weeds (gulgualo)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Sowing (zer mezerat)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Weeding (marem) and cultivation (kutkuato)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Fertilizer application (madaberia, fig, kompost…)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Harvesting (mached ena	☐ M-children	☐ M-adults	☐ M-Elders	☐ Yes

mesebseb)	☐ F-children	☐ F-adults	☐ F-Elders	☐ No
Threshing (mewkat)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Storage (makemachet)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Marketing (meshet)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Seed selection (zer memret)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Food preparation (megib mazegajet)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Collection of fodder for animals (meno mesebsebe)	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐ Yes ☐ No
Other activity (specify):	☐ M-children ☐ F-children	☐ M-adults ☐ F-adults	☐ M-Elders ☐ F-Elders	☐Yes ☐ No

* Note: For this classification only, **children** are individuals 14 and younger, **adults** are ages 15 to 59, and **Elders** are 60 or older.

** Take detailed notes of any exceptions for particular varieties.

Section 11: Closing and follow-up questions

We are looking for both men and women who have a lot of knowledge about different Lablab. Is there anyone from your community who you recommend? (*Ask this if I haven't recorded any names of potential key informants in my notebook)*	
Thank you very much for answering my questions. Do you have any comments and/or questions you would like to raise at this time? (Record farmers' questions in my notebook, if relevant.)	
At any point during the interview, did the informant indicate that s/he had gained some new knowledge? ☐ Yes ☐ No	
If yes, what knowledge did he/she report to have gained as a result of the interview (Record farmers' statements in your notebook)	
Time interview was completed:	*Duration of interview (minutes):*

Appendix 2 Semi-structured interview with key informants

Note: Additional notes were taken in field notebooks. Informants raised their own points during the interview – not necessarily as responses to questions - and these were recorded. Second, interviewer was ready to dig deeper by asking additional questions whenever interesting information or ideas are sensed. This approach generates a richer, more complete understanding.

Section 1: Basic information about Key Informant

Date of interview:	Start time of interview:
Name of interviewer:	
Region:	Zone:
Woreda/District:	Kebele/Sub-District:
Language spoken during interview: ☐ Amharic ☐ Other (specify): ____________________	
Name of informant:	
Age (observed): ☐ 18 to 30 ☐ 30 to 45 ☐ 45 to 60 ☐ 60+	
Gender (observed): ☐ Male ☐ Female	

Section 2: Knowledge of varieties listed within the same kebeles

Compile a list of all of the varieties that have been mentioned by the general informants within the same kebele. For each variety, ask the following questions of the key informant. Remember to note if the key informant says that two or more local names refer to the same variety.

Are there any other varieties that you know that we can add to our list? You may include any that you remember or have heard about from a long time ago, but are no longer planted in the kebele.

Names of varieties (from ODK survey)	Have you heard of this variety?	Is this variety planted in this kebele?	Have you planted this variety yourself?
1.	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
2.	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
3.	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
4.	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

Section 3: Attributes/characteristics of varieties

Instructions: Ask the key informants the following questions about each of the varieties with which s/he is familiar. Record the information in your notebook. The following table is intended to keep track of the questions you have asked.

Question	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
What is the meaning of the local name? (e.g. color, shape, nutritional quality, origin, etc.)										
Are there alternative names for this variety?										
How do you recognize this variety? How does it differ from similar varieties? (By appearance or other features?)										
What are the ideal growing conditions for this variety (e.g. amount and timing of rainfall, sunlight, temperature)?										
Characterize the soil types on which the variety is planted.										
What fraction of the farmers in your community plants this variety? (e.g. almost all, half, one out of ten)										
Over the past 5 years, has this variety become more commonly planted or less commonly planted in your community?										
If applicable…What is the main reason that this variety is becoming more or less common?										

Section 4: Use of Lablab *(Lablab purpureus)* as food

What foods do you prepare using Lablab?

Note: Add a note if foods are of special cultural value. You may include foods that the key informant does not prepare her/himself.

Name of food	Which parts are used?	Which variety is preferred?	Why is this variety preferred?

*For example, color, taste, ease of preparation, etc

Section 5: Nutritional value of foods prepared with Lablab (*Lablab purpureus)*

Do any of the foods you prepare with Lablab *have* any special health benefits?

Name of food	Health benefit	Specific group who use it or for whom it is recommended*?	Which variety is preferred?	Why is this variety preferred?

*For example: children, elderly, pregnant women, lactating women, sick people, people with broken bones, people who have lost a lot of blood.

Section 6: Use of f Lablab (*Lablab purpureus)* as medicine

Do you use Lablab ***as*** medicine?

Name of ailment treated with Lablab	Which parts are used?	Which variety is preferred?	Why is this variety preferred?

Section 7: Use of Lablab (*Lablab purpureus)***as fodder**

Do your animals consume Lablab?

Which animals consume Lablab?	Which part(s) do they consume?	When (in which season) do they consume Lablab?

Section 8: Other specific uses of Lablab (*Lablab purpureus*)

Do you use Lablab for any other purpose?

Description of use	Which parts do you use?	Which variety do you prefer?	Why do you prefer this variety?

Section 9: Crop Management

1. Do the people in your community apply fertilizers to Lablab? Which fertilizers are used (including manure, compost, crop residues)?
2. Do people in your community use pesticides or herbicides on Lablab?
3. What else do people in your community do to prevent and control pests, weeds, and diseases affecting Lablab?
4. Are there any varieties of Lablab that produce more residues (stems, leaves, etc.) than others?
5. What do people in your community do with the residues from Lablab? After the harvest?
6. Does anyone in your community ever use inoculants for Lablab?
7. How do people in your community store Lablab?
8. What are the major production constraints for Lablab in your community (e.g. weeds, pests, diseases, drought, low fertility etc?)

Section 10: Closing and follow-up questions

<table>
<tr><td colspan="2">I have asked all of the questions I had for you. Thank you very much for spending this time with me and sharing your knowledge. Do you have any comments and/or questions you would like to raise at this time? (Record farmers' questions below)</td></tr>
<tr><td colspan="2">(During the interview, did the informant indicate s/he had gained some new knowledge or some kind of appreciation of what you are doing? ☐ Yes ☐ No)</td></tr>
<tr><td colspan="2">If yes, what knowledge did he/she report to have gained as a result of the interview?</td></tr>
<tr><td>Time interview was completed:</td><td>Duration of interview (in minutes):</td></tr>
</table>

Additional notes:

Appendix 3 Lablab (*Lablab purpureus*) Seed Passport Descriptors

Ethiopian Biodiversity Institute   Accession No ___________

Collection Record Sheet

Collection No ____________   Crop___________________

Date__________________________   Genus__________________

Country_______________________   Species________________________

Region _______________________   Local/Vernacular Name__________

Zone__________________________   Ethnic Group__________________

Woreda________________________   Language______________________

Keble___________________

Village /Site_____________

Farmer's Name_________________

Latitude_________________Longitude _________ Altitude________________ (M)

Topography Genetic Status

1. Swampy
2. Flood plain
3. Plain level
4. Undulated
5. Hilly
6. Hilly dissected
7. Steeply dissected
8. Mountaineer
9. Other (specify)

1. Wild
2. Weed
3. Primitive cultivar/Landrace
4. Breeding line
5. Advanced cultivars

Source of Collection

1. Field
2. Backyard
3. Farm store/Threshing place
4. Agricultural Institute
5. Natural vegetation
6. Other (specify)

Sample

1. Single line
2. Pure line/clone
3. Population/mixture
4. Other (specify

Site : 1. Level
2. Sloppy
3. Summit
4. Depression

Soil Texture

1. Sand
2. Sandy loam
3. Loam
4. Clay loam
5. Clay
6. Silt
7. Highly organic
8. Other (specify)

Nature of Samples

1. Seed
2. Spikes
3. Pods
4. Cherry
5. Tuber
6. Rhizomes

Herbarium Specimen Yes,☐ No☐

Photographs Yes, ☐ No ☐

Soil Color

1. Black
2. Brown
3. Orange
4. Red
5. Yellow
6. Other (specify)

Sowing Month:

1 2 3 4 5 6 7 8 9 10 11 12

Early/Mid/Late

Harvesting Month

1 2 3 4 5 6 7 8 9 10 11 12

Early/Mid/Late

Stoniness

1. None
2. Low
3. Medium
4. Rocky

Origin of Seed

1. Local
2. Elsewhere

Usage (specify) ________________________________

Drainage

1. Poor
2. Moderate
3. Well drained

Disease and Pests_______________________________

Note (Associated wild weedy species, crop, local flora, disturbance factor) _________________

Shade Yes No

Remark____

Collectors Name (s):____

Appendix 4 List of the tested accessions and their geographical origins

code	Accession	Region	Zone	Woreda/Districts	Latitude	Longitude	Altitude
1	001	SNNPR	Konso	Konso special district	5° 20' 10.1142"	37° 24' 24.5232"	1638.2
2	002	SNNPR	Konso	Konso special district	5° 19' 49.8174"	37° 24' 23.9502"	1643.3
3	003	SNNPR	Konso	Konso special district	5° 20' 1.8342"	37° 25' 16.2582"	1583.1
4	004	SNNPR	Konso	Konso special district	5° 17' 28.4208"	37° 23' 46.7874"	1744.5
5	005	SNNPR	Konso	Konso special district	5° 20' 1.7226"	37° 25' 15.4914"	1586.6
6	006	SNNPR	Konso	Konso special district	5° 20' 3.2742"	37° 25' 13.278"	1619.8
7	007	SNNPR	Konso	Konso special district	5° 20' 3.1842"	37° 25' 14.9592"	1568.5
8	008	SNNPR	Konso	Konso special district	5° 17' 20.7132"	37° 21' 32.4828"	1842.8
9	009	SNNPR	Konso	Konso special district	5° 17' 19.518"	37° 21' 33.8826"	1864.5
10	010	SNNPR	Konso	Konso special district	5° 18' 51.2202"	37° 24' 30.7548"	1719.5
11	011	SNNPR	Konso	Konso special district	5° 17' 32.19"	37° 22' 39.684"	1855
12	012	SNNPR	Konso	Konso special district	5° 20' 7.7892"	37° 24' 31.5822"	1576
13	013	SNNPR	Konso	Konso special district	5° 17' 30.732"	37° 22' 39.8382"	1757
14	014	SNNPR	Konso	Konso special district	5° 17' 27.1392"	37° 23' 48.7788"	1735.4
15	015	SNNPR	Konso	Konso special district	5° 17' 28.6008"	37° 23' 46.0674"	1715
16	016	SNNPR	Konso	Konso special district	5° 17' 27.1062"	37° 23' 46.5534"	1488.3
17	017	SNNPR	Konso	Konso special district	5° 19' 49.944"	37° 24' 23.5332"	1644.3

18	018	SNNPR	Konso	Konso special district	5° 17' 29.0868"	37° 21' 32.58"	1901
19	019	SNNPR	Konso	Konso special district	5° 17' 20.9754"	37° 21' 32.0652"	1848
20	020	SNNPR	Konso	Konso special district	5° 20' 10.6362"	37° 24' 25.416"	1578
21	021	SNNPR	Konso	Konso special district	5° 18' 49.2474"	37° 24' 33.3714"	1614.9
22	022	SNNPR	Konso	Konso special district	5° 18' 51.2202"	37° 24' 30.7548"	1719.5
23	023	SNNPR	Konso	Konso special district	5° 18' 44.2548"	37° 24' 38.9874"	1533.9
24	024	SNNPR	Konso	Konso special district	5° 18' 51.1734"	37° 24' 31.1112"	1913.1
25	031	Amahara	North Wollo	Guba Lafto	11° 55' 27’’	39° 29’ 47’’	2025.4
26	032	Amahara	North Wollo	Guba Lafto	11° 53’ 27 ‘‘	39° 29’ 47’’	2025.4m
27	033	Amahara	NorthWollo	Kobo	12° 0’ 39’’	39° 37’ 25’’	1613.8
28	034	Amahara	NorthWollo	Guba Lafto	11° 53' 27"	39° 29’ 47’’	2071m
29	035	Amahara	NorthWollo	Guba Lafto	11° 53’ 33’’	39° 29’ 46’’	1858.3m
30	036	Amahara	NorthWollo	Guba Lafto	11° 53’ 30’’	39° 29’ 37’’	2097.4m
31	037	Amahara	NorthWollo	Kobo	12° 10’ 00’’	39° 38’ 00’’	1630m
32	038	Amahara	NorthWollo	Guba lafto	11 43 00	39 35 00	1880
33	039	Amahara	NorthWollo	Habru	12° 0’ 39’’	39° 37’ 25’’	1613.8
34	040	Amahara	NorthWollo	Habru	11° 45 1	39° 36’ 50’’	1833.5m
35	041	Amahara	NorthWollo	Kobo	12° 8' 49"	39° 37’ 56’’	1474m
36	042	Amahara	NorthWollo	Habru	11° 39 43	39° 39’ 30’’	1610.3m
37	043	Amahara	NorthWollo	Guba lafto	11°49' 30"	39° 35’ 13’’	1870.7m
38	044	Amahara	NorthWollo	gubalafto	11 50 00	39 44 00	1875
39	045	Amahara	NorthWollo	Guba lafto	11° 53 42	39° 27’ 31’’	2214.1m
40	046	Amahara	NorthWollo	Guba lafto	11° 53' 33"	39° 29’ 46’’	1858.3m
41	047	Amahara	NorthWollo	Guba lafto	11° 53 42	39° 27’ 31’’	2242.9m
42	048	Amahara	NorthWollo	woldia	11 49 55	39 36 9	1919.8

43	049	Amahara	NorthWollo	kobo	12° 0 ' 36"	39° 37’ 31’’	1604.8m
44	050	Amahara	NorthWollo	kobo	12° 0' 41"	39° 37’ 27’’	1622.5 m
45	051	Amahara	NorthWollo	Habru	11° 40’ 26’’	39° 39 23	1617.2m
46	052	Amahara	NorthWollo	kobo	12° 0’ 41’’	39° 37’ 27’’	1622.5 m
47	060	SNNPR	Gamogofa	Arebaminch zuriya	6 2 19.7	37 33 25.2	1225
48	061	SNNPR	Gamogofa	Arebaminch zuriya	5º 51’ 57 ‘’	37 º 28’ 41’’	1116.5
49	062	SNNPR	Gamogofa	Arebaminch zuriya	5 º 51’ 58’’	37 º 28’ 42’’	1119
50	063	SNNPR	Gamogofa	Arebaminch zuriya	6º 2’ 5’’	37º 34’ 34’’	1196.3
51	064	SNNPR	Gamogofa	Arebaminch zuriya			
52	065	SNNPR	Gamogofa	Arebaminch zuriya			
53	211427	SNNPR	Gamogofa		05° -25-00-N	37° -24-00-E	1440.00
54	212974	SNNP	Gamogofa		05° -14-00-N	37° -01-00-E	1580
55	211476	SNNP	Gamogofa				
56	211413	Benishangul and Gumuz	Metekel	Mandura			
57	240517	Benishangul and Gumuz	Metekel	Debate			
58	211414	Benishangul and Gumuz	Metekel	Dangure			
59	211556	Benishangul and Gumuz	Metekel	Mandura			
60	240516	Benishangul and Gumuz	Metekel	Debate			
61	240518	Benishangul and Gumuz	Metekel	Bulen			
62	211415	Orimiaya	West Wellega				
63	211419	Orimiaya	West Wellega				
64	211420	Orimiaya	West Wellega				
65	211421	Orimiaya	West Wellega				
66	211425	Orimiaya	West Wellega				

67	211426	Orimiaya	West Wellega				
68	070	Orimiaya	West Wellega	Gimbi	9^{0}11’40’’	35^{0}57’26’’	1868
69	071	Orimiaya	West Wellega	Gimbi	9^{0}11’44’’	35^{0}57’34’’	1873.2
70	072	Orimiaya	West Wellega	Gimbi	35^{0}57’28’’	35^{0}57’28’’	1871.1
71	073	Orimiaya	West Wellega	Gimbi	9^{0}11’30’’	E35^{0}57’22’’	1822.7
72	211406	Amahara	North Gondor	GONDAR ZURIA	12-37-00-N	37-28-00-E	NA
73	211407	Amahara	North Gondor	DEMBIA	12-25-00-N	37-19-00-E	1830.00
74	211408	Amahara	North Gondor	DEMBIA	12-25-00-N	37-19-00-E	1830.00
75	211422	Amahara	North Gondor	DEMBIA	12-25-00-N	37-19-00-E	1830.00
76	211423	Amahara	North Gondor	DEMBIA	12-25-00-N	37-19-00-E	1830.00
77	211409	Amahara	West Gojjam	BURE	10-42-00-N	37-04-00-E	2120.00
78	211410	Amahara	west Gojjam	BURE	10-42-00-N	37-04-00-E	2120.00
79	080	Amahara	West Gojjam	BURE	10 42 36	37 4 12	2102
80	081	Amahara	West Gojjam	BURE	10 42 36	37 4 12	2102
81	082	Amahara	West Gojjam	BURE	10 42 36	37 4 12	2102
82	10984	USA					
83	6529	Mali					
84	147	Australia					
85	7379	Keneya					
86	11614	Mali					
87	14417	Keneya					
88	14429	Keneya					
89	14455	Keneya					
90	14461	Kenaya					
91	14487	Keneya					

Appendix 5 Plant DNA Extraction Protocol for Diversity Array Technology (DArT)

BUFFER STOCK SOLUTIONS

EXTRACTION BUFFER STOCK

To make 500 ml:

- ✓ 0.35 M sorbitol 31.9 g sorbitol
- ✓ 0.1M TrisHCl pH 8.0 50 ml 1M TrisHCl pH 8.0 h
- ✓ 5 mM EDTA pH 8.0 5 ml 0.5 M EDTA pH 8.0

Fill up to 500 ml nuclease free water

LYSIS BUFFER STOCKTo make 500 ml:

- ✓ 0.2 M Tris HCl pH 8.0 — 100 ml 1M Tri HCl pH 8.0
- ✓ 0.05 M EDTA pH 8.0 — 50 ml 0.5 M EDTA pH 8.0
- ✓ 2M NaCl — 200 ml 5 M NaCl
- ✓ 2% CTAB 10 g CTAB

Fill up to 500 ml with nuclease free water

SARCOSYL STOCK 5% (w/v)

FRESH BUFFER WORKING SOLUTION:

- ✓ 0.5 % (w/v) sodium di-sulfite (= sodium meta-bisulfite)
- ✓ 2 % (w/v) PVP-40 (K29-32) Sigma
- ✓ Dissolve in required volume of extraction buffer stock;
- ✓ Add same volume of lysis buffer stock and 0.4 volume of extraction (=lysis) buffer stock of sarcosyl stock

For example to make 120 ml of working solution:

Add 0.6 g sodium di-sulfite (= sodium metabisulfite) and 2.4 g PVP-40 (K29-32) to 50 ml extraction buffer stock and dissolve.

Add 50 ml lysis buffer stock and 20ml Sarcosyl stock

For example to make 30 ml:

Add 0.15 g sodiumdisulfite (= sodium metabisulfite) and 0.6 g PVP-40 (K29-32) to 12.5 ml extraction buffer stock and dissolve; add 12.5 ml lysis buffer stock and 5 ml sarcosyl stock

*This buffer may settle into two layers on standing. Heat to 65ºC and shake immediately before adding to extraction tubes.

PROTOCOL

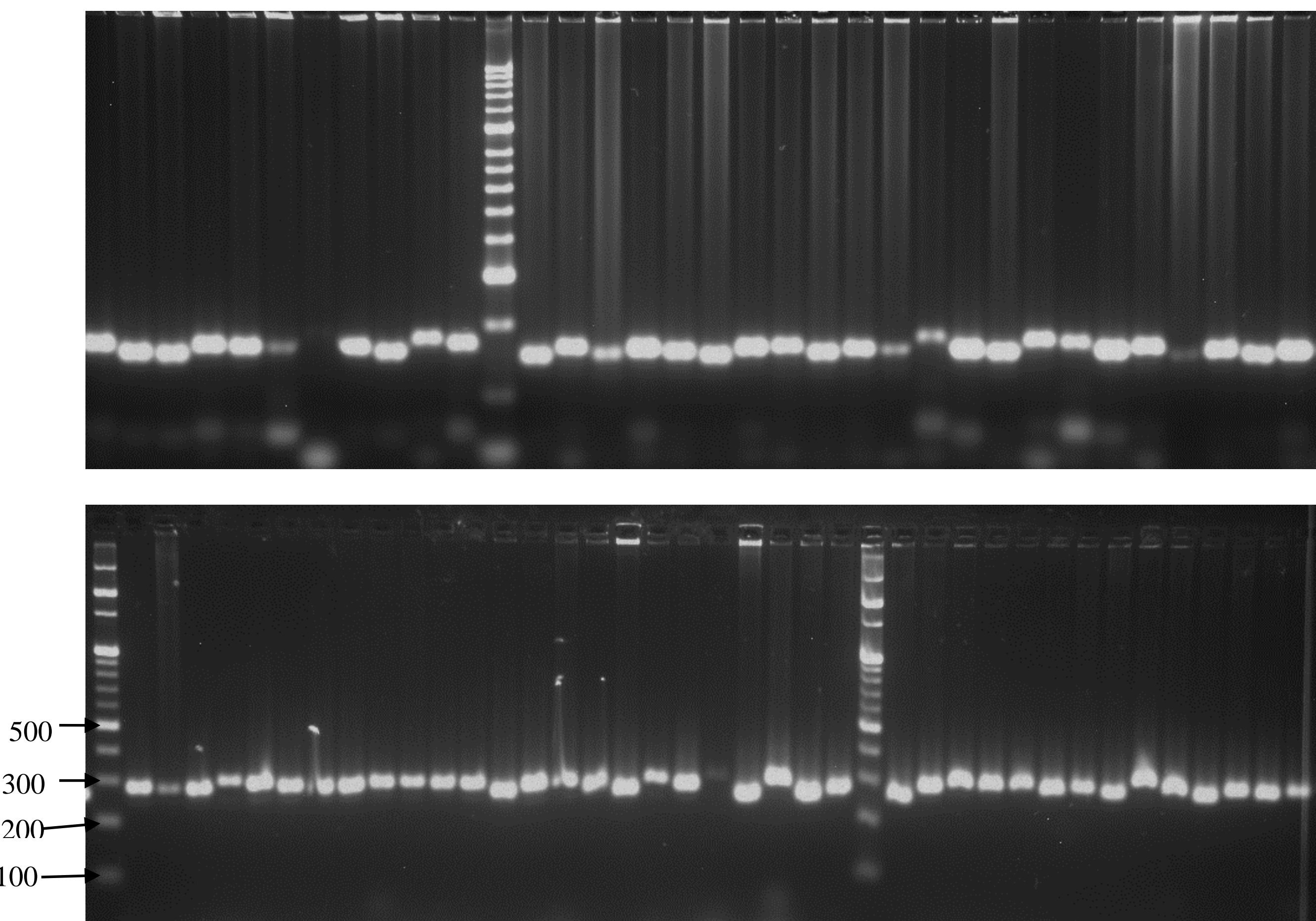
For 2 ml Eppendorf tubes:

- ✓ Aliquot 1 ml of freshly prepared preheated to 65ºC well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3,4dayes old “fresh buffer solution” works fine)
- ✓ Grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,
- ✓ Suspend powder in 1 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary)
- ✓ Incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking
- ✓ Cool down for 5 min and add 1 ml of chloroform : isoamyl alcohol (24 : 1) mixture
- ✓ mix well for 30 min
- ✓ Spin 20 min for 10,000 g, RT

- ✓ Transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible
- ✓ Spin 30 min at 10,000 x g, RT,
- ✓ Discard supernatant and wash pellet with 1 ml 70 % EtOH
- ✓ Discard EtOH, dry pellet and dissolve in 100 µl of nuclease free water
- ✓ Check DNA quality and quantity on 1% agarose gel

Appendix 6 Quality and quantity of Lablab genomic DNA, using Nano Drop Spectrophotometry

Appendix 7Samples of gel images showing the different sizes of PCR products of SSR markers generated from lablab accessions



A, Intercropping of lablab with Sorghum in the study area, Konso zone

B, Interview with key informants (photo by Solomon, December, 2018)

Lablab seedlings grown in green house