CHARACTERIZATION (MORPHOLOGICAL AND MOLECULAR) AND SENSORY EVALUATION OF IMPROVED LABLAB GENOTYPES

BY

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DECLARATION

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This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To the almighty God for enabling me reach this far, it has surely taken your hand oh Lord.

To my family, friends and all those who helped me to lay the foundation for my education, thank you for your prayers and encouragement.

ABSTRACT

Lablab purpureus is a multipurpose legume mainly grown by subsistence farmers for pulse, forage and vegetable. Despite these diverse uses that can be combined successfully under various agronomic conditions, the bean has not been extensively exploited. Dolichos production in Kenya is constrained by low yielding varieties, pests, poor growing techniques and varieties with non-preferred taste and flavor. This study was initiated to access the diversity, cooking time and organoleptic traits of six Dolichos genotypes, (G2, B1, M5, LG1, W7 and G2), that had been bred at the University of Eldoret and two checks (Local Variety and DL1002) across three sites. The genotypes were characterized using morphological and molecular markers. Morphological characterization was done in three sites; University of Eldoret's farm, Njoro and in Mabanga FTC-Bungoma County. Morphological traits (qualitative and quantitative) were characterized using the descriptors of genus *Lablab*. Molecular characterization was done at the KEPHIS- Muguga laboratory, using ten SSR markers that are specific for Dolichos. Cooking time and organoleptic studies were carried out on-farm in Meru County, Ruiri sub location using an organized farmer group (Ruiri farmers group) that comprised of ten panelists (seven women and three men). Results from morphological characterization demonstrated a high variation for both qualitative and quantitative traits evaluated. Most of the quantitative traits were highly significant at 99.999% or ($P \leq 0.001$), except for number of racemes per plant and number of seeds per pod which were significant at 99.99% or ($P \leq 0.01$) and 99.95% or ($P \leq 0.05$) respectively. Microsatellite analysis produced six polymorphic primers mapping an average of 3.17 alleles per locus. There was a significant difference ($P \leq 0.001$) among the six improved genotypes and the two checks in terms of cooking time and sensory attributes evaluated. Cooking time ranged from 87-159 minutes, with genotype M5 taking the shortest time and Local variety taking the longest time to cook respectively. In terms of overall acceptability, genotypes G2, G1, M5 and B1 were highly rated because of their short cooking time and good organoleptic attributes. High variability among the genotypes evaluated could be exploited even further in breeding programs to produce genotypes that take even less time to cook and with even better organoleptic characters for easy adoption by farmers.

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CHAPTER ONE

INTRODUCTION

1.1 Overview of Lablab

Dolichos (*Lablab purpureus* (*L.*) Sweet) is a grain legume, indigenous to Asia and Africa (Pengelly *et al*, 2001). It is a multipurpose legume mainly grown by subsistence farmers for food, forage and vegetable purposes. Lablab is a legume well suited to most tropical environments as it is adaptable to a wide range of rainfall, temperatures and altitudes. It is reported to grow well under warm, humid conditions at temperatures ranging from 18^{o} C to 30^{o} C and is fairly tolerant to high temperatures (Cameron, 1988). It is a high grain – yielding, nitrogen fixing, dual purpose legume that improves soil fertility and some more genetically diverse varieties can survive a dry spell. It is more drought tolerant than common beans (*Phaseolus vulgaris*), soybean (*Glycine max*) or cowpea (*Vigna unguiculata*) and has grain yields that are higher than cowpeas (Adebisi *et al*, 2004), and therefore can virtually substitute these common legumes whose growth in drier areas is limited by high temperatures and low rainfall. The plant is also drought tolerant when established and will grow in areas where rainfall is less than 500mm, but loses leaves during prolonged drought periods (Gowda, 2009). This drought tolerance feature of *L. purpureus* is enhanced by its capability to extract soil water from at least 2m depth even in fine textured soil (Burkill, 1995). The plant is also tolerant to high temperatures but intolerant of shading from light. Lablab seeding rates range from 12-20kg/ha (Burkill, 1995). Its rows should be set 80-120cm apart with a space of 30-50cm between plants. Seeds ought to be sown to a depth of 3-10cm (Hendricksenn and Minson, 1985). Local

subsistence farmers in Africa traditionally grow it for human consumption, vegetable (flowers and immature pods), green manure, cover crop and concentrate feed. The pods and seeds contain high protein content (20-28%) and carbohydrates (55%) (Khan *et al*., 2005). Due to its potential for use as vegetable, pulse, weed control and soil improvement, lablab has been found to be of great significance (Murphy *et al.,* 1999). This plant offers solutions to the current problem of food insecurity following climatic inconsistencies particularly at harvest time (Burkill, 1995). Genetic improvement on yield and desease resistance has been successfully applied by researchers working on *L. purpureus* varieties from Indian ecogeographical zones, who have used genetic markers (Rai *et al.,* 2010; Innes, 2004). The crop improvement program in Kenya aims at employing these improved techniques.

Lablab grows like a vine and produces seeds of variety of colors and beautiful fragrant purple or white flowers which attract insect and bird pollinators. It is scientifically classified in the family: Fabaceae, the tribe: Phaseolae, Genus: *Dolichos* and Species: *L. purpureus* (Rai, 2010).

It is a crop with underutilized potential and is considered a neglected crop despite having potential to be used as a vegetable, pulse, and forage crop in tropical areas which have humid to semi-arid climates.

In Kenya, lablab is produced in over 7000 hectares (ha) (MOA, 2005) and it is predominantly grown by small scale farmers. The main lablab producing Counties are Eastern, Central and Coast where the legume is grown either as a pure stand or as an intercrop especially with maize. The grain yield of lablab on farmers' fields in Kenya is

low, (range between 800 to 900kg /ha) compared to the yield potential of 2700-3000 kg/ha. The low yield is attributed to use of unimproved varieties, influx of pests and low use of fertilizers (Kinyua *et al*., 2008). Despite the several uses of lablab, it's potential in improving agricultural systems of farmers and nutritional status of many households has not been fully exploited. It has also long cooking time and bitter taste compared to other grain legumes (Bressani, 2002). Lablab production in the country is constrained by low yielding varieties, pests, poor growing techniques and varieties with non-preferred taste and flavor, lack of certified seeds and limited information on production practices (Kinyua *et al.*, 2008). Therefore breeding programs have been developed to breed for the improvement of cooking time, taste and high yielding varieties.

1.2 Problem Statement

Lablab is one of the crops with potential to provide grain, vegetable and forage to farmers in addition to improving soil condition. Despite these diverse use that can be combined successfully under various agronomic conditions (Adebisi *et al.,* 2004), the bean has not been extensively exploited and has been classified by National Academy of Science (NAS) as a potential source of protein that has not yet been exploited (Osman, 2007). In Kenya, quality *L. purpureus* production is compromised by pests, diseases, low-yielding varieties, unpalatable grains, prolonged cooking time of grains, and insufficient information on best practices (Shivachi *et al.,* 2012; Kamau, 2009; Kinyua *et* Some of the most important characteristics considered in selecting dry bean varieties for production and consumption include good morphological characteristics and flavor quality (Shivachi *et al.,* 2012; Scott and Maiden, 1998), where flavor comprises of odor and taste. Breeding for improved cooking quality as well as improved yields has been done at the University of Eldoret, on six new genotypes. In view of this, it is therefore inevitable to carry out performance trials in various agro-ecological zones, diversity study (morphological and molecular characterization) as well as sensory evaluation of these new genotypes. Progress in genetic improvement is dependent on the extent of genetic diversity of existing germplasm and breeding stock, it is therefore essential to determine this diversity (Kimani *et al.,* 2012).

1.3 Justification

After development of a breeding program to improve on the cooking quality and yield of the Dolichos genotypes, a diversity study is inevitable. The new genotypes need to be characterized, morphologically to identify their morphological characteristics as well as their genetic diversities through molecular characterization.

Morphological characterization is important since it can be used to show the genetic distance of the new genotypes. The ultimate goal of a plant breeder is to be able to identify a superior genotype that can be released as a new cultivar to farmers for commercial production. To arrive at this goal, many experimental genotypes of high genetic potential are evaluated for their performance at various environmental conditions and at different locations (Aquash, 2007). This forms the basis of this research.

Cookability and organoleptic qualities are important attributes affecting performance, selection and acceptance of bean varieties developed by breeders (Shivachi *et al.*, 2012). According to Coelho *et al*, (2009), prolonged cooking has been listed as one of the major factors responsible for underutilization of beans in many diets. The improvement of locally adapted varieties is vital (Nene, 2006). This will minimize nutrient loss, reduce expenditure on fuel and shorten cooking time. The improvement is vital as it will help to fight food insecurity if successfully integrated in the farming system. Studies are being conducted to improve Dolichos production in Kenya with a primary aim of identifying and evaluating various genotypes to come up with stable and well adapted cultivars for release and possible commercialization.

1.4 Objectives

1.4.1 Main objective

To determine the diversity (morphological and molecular charactezation), cooking time and organoleptic traits of six improved dolichos genotypes.

1.4.2 Specific Objectives

i. To determine morphological and molecular diversity of six improved Dolichos genotypes.
ii. To develop descriptors for the improved genotypes.
iii. To evaluate the improved genotypes for cooking time and sensory attributes.

1.5 Hypothesis

i. Ho: The dolichos genotypes under investigation do not have similar morphological and molecular traits.
ii. Ho: The dolichos genotypes will not have the same descriptor traits.
iii. Ho: The dolichos genotypes under investigation do not have same cooking time and sensory qualities.

CHAPTER TWO

LITERATURE REVIEW

2.1 Geographical Distribution and Origin of *Lablab purpureus*

Lablab purpureus is considered to have originated in South East Asia, however there is also a possibility that it originated in Africa (Brigitte *et al.,* 2010; Deka and Sarkai, 1990). It has been known and used in Africa since the 8th Century. The legume has been found to occur wildly in Tropical Africa (Madagascar inclusive) and India co-existing with cultivated types (Mass *et al*, 2010; Wang *et al*, 2007). Man domesticated and dispersed it from the 8th Century and now *Lablab purpureus* has been cultivated throughout the African continent.

2.2 Uses of *Lablab purpureus*

The young green pods of *L. purpureus* are eaten boiled. In Kenya and Nigeria, the dried seeds are eaten as a pulse after prolonged cooking with several changes of water (Amole *et al.*, 2013). In Kenya, Madagascar, and Malawi *L. purpureus* is grown on a small scale for the green seeds and also the dried pulse (Burkill, 1995). The Indian community in East Africa mostly appreciates the ripe seeds because it is a popular pulse in India. It is grown on a large scale in Ethiopia for the pulse. It has also been grown as a fodder, either green or silage (Innes, 2004). Large scale farmers of *L. purpureus* in Kenya and Zimbabwe it has been used as an annual or short-lived fodder crop to supplement poor quality hay and stover (Burkill, 1995). This is because their stems are stronger and more fibrous than the cowpea whose purpose is similar. In East Africa, its leaves are crushed and sniffed as a

cure for headache, they also help to accelerate child birth and treat stomach discomfort (Innes, 2004). Green leaves crushed in vinegar have been used successfully to treat snake bites (Cameron, 2008). Various strains of this plant are easily adaptable to different climatic conditions as per the geographical distribution (Innes, 2004; Pengelly & Maass, 2001). *Lablab purpureus* varieties such as Highworth, Rongai and White have been used successfully as cover crops to suppress weed growth and retard soil erosion. *Lablab purpureus* beans are good sources of amino acids lysine and contain 20-28% crude protein (Urga *et al*, 2009; Bressani, 2008). They can therefore be substituted with other beans and be used complementarily with maize which has generally very low lysine. Its green pods are also good sources of protein and fiber (Innes, 2004). In Kenya dolichos has been used to improve soil fertility and reduce striga weed infestation in Nyanza (Onyango *et al.,* 2012). Dolichos has been extensively used as a cover crop in Machackos County (Karuma *et al.,* 2011).

2.3 Adaptability of Dolichos

Lablab purpureus combines a great number of qualities that can be used successfully under various conditions. One of its advantages is its adaptability, not only is it drought resistant, it is able to grow in a diverse range of environmental conditions worldwide (Macharia *et al.,* 2011). Staying green during the dry season, it has been known to provide up to six tons of dry matter/ha (Amole *et al.,* 2013; Ewanish *et al.,* 2006; Murphy *et al.,* 1999). *Lablab* is remarkably adaptable to wide areas under diverse climatic conditions such as arid, semi-arid, sub-tropical and humid regions where temperatures vary between 22°C–35°C, low lands and uplands and many types of soils and the pH varying from 4.4 to 7.8. Being a

legume, it can fix atmospheric nitrogen to the extent of 170 kg/ha (Rajagopalan and Raju, 2008) besides leaving enough crop residues to enrich the soils with organic matter. It is a drought tolerant crop and grows well in dry lands with limited rainfall. The crop prefers relatively cool seasons (temperature ranging from 14-28^{0}C).

Lablab purpureus with its ability to out-yield conventional crops, especially during the dry season, and its enhanced nutritive value, is a fodder crop of great significance for the Tropics. Lablab can be used advantageously as a cover crop (Karuma *et al.,* 2011). Its dense green cover during the dry season protects the soil against the action of the sun's rays and decreases erosion by wind or rain. As green manure it provides organic matter, minerals and fixes nitrogen into the soil thereby improving crop yields in an economic and environmentally friendly manner (Murphy *et al*., 1999).

The ultimate goal of any plant breeder is to be able to identify superior genotypes that can be released as varieties in certain target areas. To arrive at this goal, many experiments involving genotypes of high genetic potential are evaluated for performance capabilities under various environmental conditions in order to select a cultivar suitable for a given area. Most of the existing varieties of Dolichos have a number of short comings and farmers face various problems while growing the crop. This include: low yields, poor cooking and flavor qualities, susceptibility to pests and diseases, poor agronomic characteristics among others all lowering the economic value of the crop (Shivachi *et al.,*2012). In the past, little had been done as far as improving this crop with most researchers concentrating on maize and common bean (Kinyua *et al.,* 2007). This has seen a decline in production of the crop and yet it is one of the staple foods in some parts of the

country. Its market value also reduced with consumers citing its poor cooking qualities, lack of taste and non-appealing appearance when compared to common bean. Studies are being conducted to improve Dolichos production in Kenya with a primary aim of identifying and evaluating various genotypes to come up with stable and well adapted cultivars for release in each of the study regions (Kinyua *et al.,* 2008).

This will be done by introducing new varieties which are not only high yielding but also stable and locally adaptable to various agro-ecological zones as well as farmer preferences. Trials are designed to forecast the performance of the genotypes in a given environment. This makes adaptability trials an important concept of any breeding program (Aquash, 2007), put into considerations before variety release. During trials, the cultivars are routinely evaluated on regional basis and the data generated is used as a platform for recommending a suitable cultivar for that region (Yan *et al.,* 2002). Farmer trials provide a valid data for effective cultivar evaluation. The farmer managed trials have been found to be widely accepted as opposed to trials done at research institutions (Aquash, 2007) as research conditions may not necessarily represent intended environment for growing the cultivar finally.

Farmers need to be involved during adaptability trials since it equips them with the relevant expertise for handling the yet to be released cultivars in terms of the technology and production method to be employed. Furthermore, farmer involvement in the trials hastens the adoption rate as they play an important role in awareness creation and information dissemination regarding the new crop (Tuaeli and Ennis, 2003; Douwe, 1994). Douwe (1994) further adds that the role played by farmers in adaptability trials should be

emphasized since it allows them to give their suggestions regarding the yet to be released cultivars. (Olubayo, 2006) suggests that the incorporation of farmers in variety assessment enhances greater adoption and farmer managed trials are more convincing to other farmers than well managed demonstrations done at the research centers. This is so because the cultivars are subjected to the effect of natural selection in a given environmental setup and only the well adapted genotypes are favored and grown to maturity giving substantial yields while the poor adapted genotypes are characterized by poor or low yields.

In essence, (Douwe, 1994) argues that working with farmers in adaptability evaluation studies is challenging at times with several bottle necks to be overcome. However understanding farmers' needs and the proper designs in the experimental setup can be used to overcome some of these challenges.

2.4 Cultivation of *Lablab purpureus*

Certain varieties of *Lablab purpureus* can grow in an array of climatic conditions and soil types ranging from an altitude of sea level to 2000m in tropical regions. It is drought tolerant but grows best in areas ranging from 200-2500mm annual rainfall. Their soil preference is sandy or clayish well drained at a pH ranging from 4.4-7.8. It has done well both in nutrient poor and nutrient rich soils. For use as a pulse, it should be allowed to mature 150-200 days after planting (Innes, 2004; Burkill, 1995). It grows well in warm climate with an average temperature of 18^{o}C. Many cultivars are fairly tolerant to high temperatures and some are able to stand even frost for a limited period. Cold weather generally does not suit this crop as it affects pollination and seed set (Gowda, 2013).

2.5 Genetic Resources of *Lablab purpureus*

The National Gene Bank of Kenya-Muguga holds over 340 accessions of *Lablab purpureus* (Adebisi and Bosch, 2004). More than 250 lines both indigenous and exotic are maintained in India in the University of Agricultural Science, Bangalore. The International Institute of Tropical Agriculture, Ibadan, Nigeria maintains a germplasm collection at its legume unit. Genetic diversity using genetic markers shows that variation in cultivated *L. purpureus* is low but cultivars from India are the most divergent (Burkill, 1995). Diversity based on morphological characteristics in accessions from the collection of CSIRO (Australia) and ILRI (Ethiopia) showed that the cultivated *L. purpureus* in Ethiopia are very similar to wild African plants and different from cultivated *L. purpureus* elsewhere (Burkill, 1995). Research is now geared towards exploring *L. purpureus*'s genetic diversity as a starting point of its reassessment before crop improvement. Molecular, morphological and physiological approaches are being used. Morpho-agronomic studies have arrived at a core collection from the Australian CSIRO gene bank and the ILRI gene banks in Ethiopia (Pengelly & Maass, 2001).

2.6 Breeding of *Lablab purpureus*

Increasing genetic erosion and habitat destruction has heightened the necessity of *L. purpureus* plant germplasm collection (Rai *et al.,* 2010). The cultivars "Rongai" and "Highworth" are selections from landrace materials (Maass *et al.,* 2005). They have been grown widely in the tropics. "Rongai" was selected from a late flowering landrace in Rongai, Kenya close to the equator. It was then exported to South Africa to large commercial farms which were interested in it as a fodder crop. The small scale farmers

then took it up as a vegetable and a pulse crop. These small scale farmers made further selections based on seed color. A white seeded cultivar "Koala" has also been released in Australia. Recently, another cultivar was released in Australia and is called "Endurance", which is a result of crosses between "Rongai" and a perennial wild parent (Bruce and Maass, 2001). There is need to breed for vegetable *Lablab purpureus* while concentrating on palatability with regards to seed coat tenderness (Rai *et al.*, 2010). As a pulse and forage crop, other legumes such as cowpeas enjoy more popularity than *L. purpureus* but its relatively much higher yield than cowpeas' and spectrum of adaptability to differing ecological zones compared to any other legume make it a promising solution to the current food security question; http://www.fao.org/DOCREP/004/AC145E/AC145E09.htm. Most of the improvement work of the climbing vegetable types and bush pulse types of *L. purpureus* is done in India where several cultivars including Co.9 and Co. 12 have been released (Gowda, 2008). The qualities to look out for in improved cultivars are short-duration to maturity, day length neutrality, uniform maturity, and disease resistance. The cultivar "Highworth" is a popular pulse and forage cultivar whose origin is Queensland, Australia. It matures quickly and its high yield is not compromised under widely differing environmental conditions. Its pods are easy to harvest because they grow above the foliage at the top of the stems and they mature at the same time yet are non-shattering (Burkill, 1995).

2.7 Contribution of Genetics to *Lablab purpureus* Improvement

Agriculture, the source of the world's food faces the challenge of increasing demand for food, water deficit, soil salinity and unpredictable weather and in the next 50 years there

will be need for a doubled agricultural production. *Lablab purpureus* has comfortably adaptable varieties whose yield is not affected by climatic changes (Innes, 2004). Molecular genetics will work complimentarily with plant breeding to supply seed that meet the current demands of *L. purpureus* (Rai *et al.,* 2010).

2.8 Morphological Characterization

Traditionally, genetic diversity is evaluated in crop species based on differences in morphological characters and qualitative traits (Schut *et al*., 1997). Morphological characterization is the first step in genetic relationship studies in most breeding programs (Van Beuningen and Busch, 1997; Cox and Murphy, 1990). Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field. Most important agronomic characteristics are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions.

Plant morphological traits are grouped as either polygenic (variable) or monogenic (constant). Polygenic characteristics are associated with large genotype by environment interactions. Monogenic characteristics are salient, thus identifying the species or genotype, for example, petiole color, root skin and pulp color, and stem color (Elias *et al*., 2001). Morphological characterization of dolichos uses characters which include; Leaf color, leaf anthocyanin, growth habit, leaf persistence, branch orientation, number of flower buds/ raceme, number of raceme/ plant, raceme position, days to 50% flowering, pod color, number of pods/ plant, number of seeds/ pod, days to maturity, plant height (cm), seed color, 1000 seed weight among others (Gowda, 2006). Phenotypic markers still

play an important role in classical plant breeding and complement the use of molecular tools in the identification of specific markers linked to traits and QTL (Kizito *et al.,* 2007; Okogbenin *et al.,* 2007).

2.9 Genetic Diversity

Genetic variability and genetic diversity of a taxon is of great importance for plant geneticists, breeders and taxonomists (Rai *et al.,* 2010; Prince *et al.,* 2004). Frankel *et al.,* (1995) defined genetic diversity as the product of interplay of biotic factors, physical environment, artificial and plant characters. The knowledge of genetic diversity and relationship among sets of germplasm as well as the potential merit would be beneficial to all phases of crop improvement (Geleta, 2003; Lee, 1995). Evaluation of genetic diversity among adapted or elite germplasm provides the estimation of genetic variation among segregating progeny for pure line development (Manjarrez-Sandoval *et al.,* 1997) and the degree of heterosis in the progeny of certain parental combinations (Geleta, 2003; Barbosa-Neto *et al.,* 1997; Cox and Murphy, 1990). The information about genetic diversity in available germplasm is important for the optimal design of a breeding program (Geleta, 2003) and the notion of genetic relationships among lines, population or species has been an important tool for effective management of genetic diversity in a given gene pool (Manjarrez Sandoval *et al.,* 1997). The study of genetic diversity has been of interest to plant breeders and germplasm curators. It is a process where variation among individuals or groups of individuals is analyzed using specific methods of combination (Mohammadi *et al,* 2003).

In plant species, it can assist in the evolution of germplasm as possible sources of genes that can improve the performance of cultivars (Geleta, 2003; Yang *et al.,* 1996).

2.10 Molecular Characterization

Knowledge about genetic diversity in the germplasm collection is very useful for plant breeders. Assessment of genetic diversity using DNA markers is one of the key tools for germplasm conservation and crop improvement (Somta *et al.,* 2009). Due to the advancement of molecular techniques, large numbers of highly informative DNA markers have been developed for identification of genetic polymorphism (Fevzi, 2001). Molecular markers based on DNA sequences are independent of environmental conditions and show a higher level of polymorphism (Rai *et al.,* 2010). This necessitates assessment of genetic diversity in selected dolichos genotypes using the modern molecular approaches. Molecular markers combine many desirable marker properties that include; high level of polymorphism and information content, an ambiguous designation of alleles, even dispersal, selective neutrality high reproducibility, co-dominance and simple genotyping assays (Grisi *et al.,* 2007; Chen-Dao *et al.,* 2001). Assessment of genetic variation in available germplasm collections forms an integral part of any crop improvement program (Somta *et al.,* 2009; Gnanash *et al.,* 2006).

2.11 Organoleptic Traits

There has been a worldwide interest in searching for new and potential uses of unconventional legumes. Pengelly and Maass, (2001) concluded that because of its already well-established uses as a pulse, vegetable and forage, lablab is a priority genus in

developing multi-purpose legumes in both commercial and small holder farming systems in the tropics. Like other legumes, Lablab seeds contain anti-nutritional factors; trypsin and chymotrypsin inhibitors, tannins, phytohemagglutinins (lectins), lathyrogens, cyanogenic glycosides and goiterogenic factors, saponins and alkaloids (Wanjekeche *et al.,* 2003; Vijayakumari *et al.,* 1995). These anti-nutritional factors limit the usage of the legume, unless they are eliminated through processing e.g. by pre-soaking and subsequent discarding of the liquid and/or by heat treatment at relatively elevated temperatures (Wanjekeche *et al.,* 2003). Prolonged cooking has a negative impact on beans; reducing their nutritive value especially vitamins and certain amino acids (Urga *et al.,* 2009; Bressani, 2008).

Sensory factors are a major determinant of the consumers' subsequent purchasing behavior (Mkanda *et al.,* 2007; Watts *et al*., 1989). Some of the most important characteristics considered in selecting dry bean varieties for production and consumption are fast food cooking and good flavor quality traits (Shivachi *et al.,* 2012; Scott and Maiden, 1998). In India for example, lablab is valued for its nutritional and sensory attributes (Venkatachalam and Sathe, 2007).

Cookability and organoleptic qualities of beans are important attributes affecting preference, selection and acceptance of bean varieties developed by breeders. Previous studies have shown that cooking time is an important trait in breeding of common beans especially where 96% of the beans consumed are prepared at household level (Shivachi *et al.,* 2012; Susana *et al*., 2003).

Apart from cooking time, sensory characteristics such as appearance, texture and taste contribute to consumers' choice of a particular bean variety (Mkanda, 2007; Sanzi and Attienza, 1999).

Descriptive sensory evaluation identifies, describes and quantifies sensory attributes of a food material or product using human subjects (Mkanda *et al*., 2007). Sensory attributes that influence acceptance of cooked beans are visual appearance, texture and flavour-taste and aroma (parker, 2002) as they contribute to consumers' like or dislike of certain bean varieties.

Consumer sensory evaluation is a process of evaluating personal opinion of a particular product in terms of specific sensory attributes.

Appearance; It is most important to consumers since they have certain expectations on how food should look like (Parker, 2002). It is divided into color and geometric (shape and size) attributes.

Texture; this is a quality felt with fingers, tongue and teeth (Parker, 2002). According to Mkanda (2007), fast cooking beans have soft texture that is preferred by most consumers.

Flavour; It comprises of odor and taste. It is defined as a perceived attribute resulting from integrated responses to a complex mixture of stimuli on several senses i.i. smell, taste, touch sigh and even hearing (Lawless and Lee, 1993). Flavour, like appearance and texture, is a quality factor that influences the decision to purchase and consume a food product.

Over the years, farmers in Kenya preferred other legumes over lablab bean because of the bitter taste (Wanjekeche *et al*., 2000). Prolonged cooking time also increases the cost of

utilizing the bean due to increase in amount of fuel needed (Shivachi *et al.*, 2012). Odor of the lablab was also reported to affect acceptance (Kim and Chung, 2008). Also a study on common bean reported that bitter taste contributes to consumers' dislike of some bean varieties (Mkanda *et al*., 2007). Therefore there is a need to carry out cooking time and organoleptic studies on improved lablab lines by the breeder, with the aid of the consumers/farmers, to ascertain whether he or she has achieved this objective.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Organoleptic Traits

3.1.1 Source of Genotypes

The genotypes comprised of 6 lines that had been bred at University of Eldoret (W7, M5, B1, G1, G2 and LG1) and two checks (DL1002 and a local land race (Local variety) collected from farmers' field). The genotypes were selected based on adaptability, yield and ability to withstand pests and other diseases. The genotypes are as described in table 3.1 below.

Table 3.1: Description of the Genotypes Used in the Study

Entry	Genotype code	Seed color
1	LG1	Black
2	G2	Black
3	W7	Black
4	M5	Brown
5	G1	Black
6	B1	Dotted (Brown with black dots)
7	Local variety	Black
8	DL1002	Black

3.1.2 Study Site

Cooking time and organoleptic studies were carried out on-farm in Meru County, Ruiri sub location using an organized farmers group (Ruiri farmers group). This study site was selected because of the popularity of the crop in the region.

3.1.3 Methods

3.1.3.1 Cooking Time

Cooking of the 8 genotypes was done to ascertain the cooking time of each genotype at a farm in Ruiri-Meru County. Saucepans 'sufurias' used in the experiment were made of stainless steel with tight fitting lids of same size. Heating system used was charcoal since it was the most convenient in the study site. A quarter (¼) Kgs of each genotype was weighed, cleaned and cooked in accordance to Gisslen (2007) protocol with few modifications in terms of the quantity of water, source of heat and grains used. All the eight genotypes were coded differently to avoid bias when scoring.

After the "jiko's" lit, one and a half liters of water was put in each saucepan and let to boil. Water from all the source pans was let to boil before the seeds were put in to take care of errors that may have arisen due to the different intensities of the source of heat. Once the water in all the source pans was boiled, each ¼ kg seed genotype was poured into the separate saucepans simultaneously and then covered with tight fitting lids of the same size and then timing started. During the cooking process, the samples remained covered with water and it was added intermittently as its level dropped until the grains were fully cooked to acceptable tenderness. Tenderness was determined using the method of Njoku and

Ofuya (1989), by subjectively pressing the beans in between fingers until no hard material was found-as traditionally done. One person was allowed to determine the tenderness of all the genotypes; this was to take care of errors that could have arisen due to various people having different textures on their fingertips as well as different strengths when pressing the beans. Samples were allowed to cook for the first sixty minutes. For the next thirty minutes sampling was done at an interval of ten minutes and at intervals of five minutes for the rest of the cooking time. The cooking time was recorded for the genotypes that had cooked to the required tenderness. This was calculated from the initiation of cooking until 80% of the grains were cooked. Three sample replicates of each genotype was cooked separately and cooking time recorded.

3.1.3.2 Organoleptic Tests

After cooking the seeds to the acceptable tenderness, organoleptic tests were done. The taste panel consisted of 7 women and 3 men from the Ruiri farmers group in Meru. The panelists were trained on what they were expected to do and how they were to carry out the scoring. Females formed the majority of the panelists' since they are usually involved in preparation of meals therefore are likely to be more sensitive to taste than men (Shivachi *et al.*, 2012; Kigel, 1999). The attributes evaluated included; appearance, texture, taste, and overall acceptability as described in section2.11. Appearance (size and shape), was rated by sight, texture by rubbing gently between the thumb and index fingers of the hand and also in the mouth and taste in the mouth. Evaluations were done through quantitative descriptive analysis. The panelist indicated the intensity of the specified characteristic (Appearance, Taste and Texture), by checking an appropriate category and ordering them

using five descriptive terms (1= Very bad, 2= Bad, 3= Fair, 4= Good and 5= Very good) (Appendix 1). The cooked samples used for tasting were code blinded from the panelist and served on ten plates then given to the taste panel for evaluation. One sample was evaluated at a time by all panelists. They rated each sample depending on the intensity of the sensation perceived. After testing and scoring one sample, the panelists were given water for rinsing the plate and their mouths before proceeding to the next sample.

3.1.4 Data Analysis

The data obtained from both studies were subjected to statistical analysis using genstat discovery (10^{th} edition, 2007) version. The means were separated by the Duncan's Multiple Range Test (DMRT).

3.2 Morphological Characterization of Dolichos Genotypes

3.2.1 Experimental Sites

The study was carried out in three locations representing different agro-climatic zones with different climatic and soil conditions (Table 3.2).

Table 3.2: Description of experimental sites used in the study

Location	County	Coordinates	Elevation (masl)	Soil type	Mean Annual Temperature (^{o}c)	Mean Annual Rainfall (mm)
University of Eldoret	Uasin-Gishu	0^{o} 34'N 35^{o} 17'E	2134	Rhodic ferralsols	16.6^{o}c	1124
Njoro-Nakuru	Nakuru	0^{o}20'N 35^{o}56'E	1920	Nitisols	22^{o}c	800
Mabanga FTC	Bungoma	0^{o}35N 34^{o}37'E	1440	Acrisols and Nitisols	26^{o}c	700

3.2.2 Experimental Design

The experimental design for the trials was Randomized Complete Block Design (RCBD) with three replications. Uniformity in management was ensured particularly within the blocks.

3.2.3 Planting in the Field Plots

Each experimental site measured 32 m by 13.25 m. There were 3 blocks (replicates) and each block had 8 experimental units representing each of the 8 genotypes, each plot measured 4 m by 3.75 m with 6 rows of 4m each. Each unit had 48 seeds sown. The seeds were sown 1½ inches deep with plant spacing of 0.5 m between plants by 0.75 m between

rows. Management of each site was as per the recommended standards for each study region.

3.2.4 Morphological Characters Scored

During morphological characterization, qualitative and quantitative traits mainly yield related components, were measured at different growth stages on all the plants. Morphological evaluation was carried out on each site. Morphological traits evaluated included both qualitative and quantitative traits. The variables were scored using Gowda (2008), descriptor list for the genus *lablab*. All agronomic practices were carried out including weeding.

The qualitative traits that were measured included;

Main stem pigmentation; no pigmentation, localized at the node, extensive or almost solid, leaf anthocyanin; present or absent.

Leaf color; pale green, green, dark green, purple or dark purple, leaf hairiness on inner surface; glabrous, low pubescent, moderately pubescent or highly pubescent, leaf shape; round, ovate, ovate lanceolate or linear lanceolate, growth habit; determinate, semi determinate or indeterminate, branch orientation; short and erect lateral branches or branches tending to be perpendicular to main stem, flower color; white, cream, light yellow, pink or purple, pod curvature; straight, slightly curved or curved, pod beak; short beak, medium length, long beak or thick beak, pod color of mature pods, white, cream, green, green with purple stature, dark purple or red, unripe pod attachment; erect, intermediate, or pendant, seed color at maturity, white, green, cream, purple brown or black, seed shape when dry round oval or flat.

The quantitative traits included following;

Days to 50% flowering; days from emergence to 50% of the plants producing flowers, days to maturity; from emergence to stage when 50% of the plants have mature pods, flower buds/raceme; average number of buds from 10 randomly chosen racemes, racemes/ plant; average number of racemes from 10 randomly chosen plants, pods/plant; average number of pods from 10 randomly chosen plants, seeds/pod; average number of seeds from 10 randomly chosen ripe pods, plant height (cm); measured on 10 random matured plants from the cotyledon scar to tip of the plant, 1000 seed weight (g); average weight of 1000 seeds.

Data was taken from four inner rows since one outer row from both sides of each plot was removed to take care of border effect. Values used for analysis were measured from 10 plants chosen at random in each plot.

3.2.5 Data Analysis

The quantitative data obtained was subjected to analysis of variance using genstat 13th edition and the means of the genotypes separated using least significant difference (LSD) at 5% level of probability of the same software. This was to check on any difference among genotypes. Coefficient of variation (CV) was used to measure statistically the dispersion of data around the mean. The qualitative parameters scored were used to develop descriptors for the genotypes.

3.2.5.1 Cluster Analysis

The morphological data were scored as binary data and were used to construct a dendogram. A dendogram was constructed for the quantitative data by the Unweighted Pair-Group method (UPGMA) (Sokal and Michener, 1958) clustering using the sequential agglomerative hierarchical nested (SAHN) program and tree plot of Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1.

3.3 Genetic Diversity of the Dolichos Genotypes using SSR Markers.

3.3.1 Plant Materials

The plant materials used in this study was the 6 genotypes bred at Eldoret University and the two checks (section 3.1.1 and table 1). Ten plant samples of each genotype were planted in a greenhouse at KEPHIS Muguga. After 10 days of emergence, young fresh leaf samples were obtained for DNA extraction.

3.3.2 DNA Extraction

Young fresh leaves of each genotype were harvested and ground into fine powder under liquid nitrogen using a motor and pestle. The extracts were transferred to labeled eppendorf tubes each containing 500 µl of 2 x CTAB mecaptoethanol extraction buffer and the samples placed on ice. The contents were subsequently incubated in a water bath at 65^{o} c for 1 hour with invasions after every fifteen minutes. This was followed by addition of 500 µl of chloroform-isoamyl alcohol (24:1) and inversion for 5 minutes at room temperature to mix. The mixture was then centrifuged at 14000 rpm for 10 minutes. Without disturbing the bottom layer, 400 µl of the top clear layer was pipetted into fresh eppendorf tubes and

250 µl of isopropanol added. The contents were then gently mixed by a few inversions and then incubated at room temperature for 10 minutes.

The mixture was then centrifuged at 14000 rpm for 10 minutes to pellet the DNA. The supernatant was then gently discarded using the yellow tips and 320 µl of 1 x TE was added. The samples were then placed on ice. A further 40 µl of Magnesium chloride was added and the contents incubated on ice for 10 minutes followed by a centrifugation to14000 rpm for 10 minutes and the supernatant discarded. The pellet was then vacuum dried for 5 minutes before adding 5 µl of R-nae enzyme and placed in a water bath (37^{o}c) for two hours. 40 µl of sodium acetate was added followed by 250 µl of isopropanol and the contents incubated for 15 minutes at room temperature. This was followed by a 10 minutes centrifuge of 14000 rpm so as to re-pellet the DNA and the supernatant discarded.

A 1ml aliquot of 70% ethanol was then added to the pellet followed by another centrifuge of 14000 rpm for 5 minutes. The supernatant was discarded followed by a quick spin of 2 minutes. The supernatant was then gently discarded and any liquid from the tube drained off using a clean tissue paper. The pellet was then vacuum dried for 3 minutes to remove any remaining liquid and the DNA pellet re suspended in 50 µl of 1 x TE. It was then left to stand for 10 minutes at room temperature before storing at 4^{o}c.

3.3.3 DNA Quantification

The quality and quantity of the DNA was verified by electrophoresis on a 0.8% (w/v) agarose gel, for 30 minutes at 80 volts. Lambda (λ) phage DNA was used as the standard. After electrophoresis, the gel was stained in ethidium bromide (10mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet trans

illuminator. The concentrations of the samples were determined by comparing band sizes and intensities of the test DNA with those of standard λ DNA. Between 0.5µg and 1µg of high quality DNA was obtained and was diluted to 0.01µg/µl with deionized distilled water for the PCR amplification.

3.3.4 PCR Amplification

Subsets of 10 SSR primer pairs specific to dolichos were selected from 25 primers developed by Kirkhouse foundation. The PCR reactions were performed in a Master cycler (Eppendorf®) using in a final volume of 20µl Bioneer AccuPower® containing 4µl pre-mix (1U Top DNA, 250µM each dNTP, 10mM Tris-HCl pH 9.0, 30mM KCl, 1.5mM MgCl, stabilizer and tracking dye), 0.0025ng/µl of each forward and reverse primer, 0.5ng of template DNA, and 6µl of double distilled water (ddH2O). The PCR cycles consisted of: 92°C for 3 minutes for initial denaturation, 92^{o}C for 3 minutes for actual denaturation, annealing at 56^{o}Cfor 30 seconds, Extension at 72^{o}C for 1 minute, followed by 34 cycles of 30 seconds at 92°C, 1 minute at 56°C, 1 minute at 72°C, and a final extension step of 5 minutes at 72°C (Appendix II).

The DNA fragments were separated on 4% agarose gel run at 100 volts (V) for 2 hours (h) using 0.5M TBE buffer. The DNA fragments in gel was visualized by staining in 0.5ug/mg ethidium bromide for 30 minutes and rinsed in distilled water for 20 minutes, visualized and photographed on ultraviolet (UV) trans-illuminator at 312nm. Allele sizes were scored using a 1000 base pair (bp) molecular size ladder.

3.3.5 Polymerase Chain Reaction (PCR) and Polyacrylamide Gel Electrophoresis

Polymerase chain reaction on the isolated genomic DNA obtained was carried out and the SSR primers used were as follows:

Ten primer pairs were selected from a list of 25 primers that were developed by Kirkhouse trust (Table 3.3).

Table 3.3: Description of the SSR Primers used in the Study

Primer name	Forward primer	Reverse primer	Annealing temperature	Approx product size
LABRRT 1	TGGATTCTACAGTTTCGATGACGA	GTCAGACGGTGGTTTCTGCCTTAT	56	100
LABRRT2	GCCATGTTCTGAAAGATGTAACAGTG	GGCAAGCAGTCATATCCAGAAACT	56	170
LABRRT8	TCAGAACTCTACTTTCTGAGCTTGA	ATCATACAGTCCGTGTTGTTCG	56	190
LABRRT23	GGGAGTGTGAAATAGAGAATCAGTT	CAGCACTATCCACACCTGCAATAC	56	170
LABRRT49	CATGCTCTCAAGCTGTTCATCAAT	GAGTCCAACGTTGTTAGCGAGAGT	56	220
LABRRT50	TCACAGAGCCAAAGACAAACTCA	GATGAGGAGCCTCGTTGAATTG	56	100
LABRRT52	CAGGTTTGTGATTCGCATGAGTC	TGGTGACTATTCATGATGGGAATG	56	90
LABRRT53	GGAAGAACTAAGGTCATCATGC	GATCGCAATGATCTTCCAAAGG	56	190
LABRRT77	AGTTTAGCACACCGATCAAATGGT	CACAAACCTCCATTACTCTCAGTCA	56	180
LABRRT83	ATAAGAAGATCGCTTGTCGCCTTC	TCTGAGTTTTGGGTCGTTTAATCC	56	100

3.3.6 Statistical Analysis

SSR primers that showed distinct and scorable DNA bands were considered for analysis. The generated DNA bands were scored based on their band marker sizes. The software package Power Marker version 3.25 (Liu and Muse, 2005) was used for the following statistics analysis: sample size, major allele frequencies, number of alleles per polymorphic locus, and Polymorphism Information Content (PIC).

The similarity matrix was subjected to UPGMA by selecting the SAHN program and tree plot of NTSYS.

3.4 Comparing the Genetic Distance of Dolichos Based on Morphological and SSR Markers.

3.4.1 Genetic Similarities.

Similarity matrices for morphological, SSR and combined marker types were subjected to UPGMA (Sokal and Michener, 1958). A dendogram was constructed from both the quantitative and molecular data by the Unweighted Pair-Group method (UPGMA) (sokal and Michener, 1958) and clustering using the sequential agglomerative hierarchical nested (SAHN) program and tree plot of Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1.

CHAPTER FOUR

RESULTS

4.1 Cooking Time and Organoleptic Traits of the Genotypes

There was a significant difference in cooking time ($P \leq 0.001$) among the genotypes evaluated (Table 4). Cooking time for the genotypes ranged from 87 minutes to 159 minutes with genotypes M5 taking the shortest time to cook while the local variety taking the longest time to cook. Five out of eight genotypes had cooking time lower than the general mean (122.21 minutes), all of them being the new varieties. Genotype M5 took an average of 87 minutes to cook which is less than 1hour and 30 minutes, genotypes B1, LG1, G2 and G1 took an average of 99, 107, 117 and 121 minutes to cook respectively, which is within 2 hours. However, genotypes W7, DL1002 and Local variety took an average of 131, 154 and 159.33 minutes to cook which is more than 2 hours. Therefore there was a notable significance difference between the new varieties and the checks (a local variety and DL1002) with the new varieties taking a shorter cooking time as indicated by table 4.1.

Table 4.1: Mean Cooking Time of Eight Dolichos Genotypes

Genotype	Cooking time
W7	131.67e
G2	117.0d
M5	87.67a
B1	99.33b
LG1	107.67c
G1	121.0d
Local Variety	159.33g
DL1002	154.0f
Grand mean	122.21
$MS_{(Genotype)}$	1900.7***
$MS_{(error)}$	2.6
SD	256.64
CV (%)	2.1

NB *** = significant at $P \leq 0.001$. The means were separated by the Duncan's Multiple Range Test (DMRT).

4.2 Organoleptic Traits of Eight Dolichos Genotypes.

The results obtained from the organoleptic traits, i.e. appearance, taste, texture and acceptability, evaluated were highly significant, due to the differences in their means, at $P \leq 0.001$ (Table 5). The local variety was ranked lowest in all the traits evaluated whereas genotype G2 and G1 were ranked highly in all the traits evaluated. Despite genotype W7 being ranked highly in appearance, it was ranked average in terms of acceptability. There was a deviation from the expected where some brown genotypes B1 and M5 were ranked poorly and given low scores for appearance, 3.75 and 3.38 respectively. Genotypes G1,

G2, M5 and B1 received the highest overall acceptability scores of 4.54, 4.25, 4.0 and 4.0 respectively. This is due to the fact that they were highly scored in all of the traits evaluated except for genotype M5 and B1 which received low scores of 3.37 and 3.75 for appearance. This high level of significance in the organoleptic traits evaluated ($P \leq 0.001$) depicts the importance of organoleptic evaluation and thus should also be incorporated in other breeding programs as an important aspect in breeding and selection.

Table 4.2: Means for organoleptic traits of eight dolichos genotypes

Entry	Genotype	Appearance	Taste	Texture	Acceptability
1	**W7**	4cd	3.4bc	3.8bc	3.8bc
2	**G2**	4.4de	4.1de	4.3cd	4.3de
3	**M5**	3.4ab	4.6e	4bc	4cd
4	**B1**	3.8bc	4cde	4.3cd	4cd
5	**LG1**	3.3ab	3.6bcd	3.5b	3.5b
6	**G1**	4.9e	4cde	4.8d	4.5e
	Local				
7	**Variety**	3.1a	2.6a	2.9a	2.9a
8	**DL1002**	4cd	3.3ab	3.9bc	3.7bc
	Grand mean	3.8	3.7	3.0	3.8
	$MS_{(Genotype)}$	2.9***	3.1***	2.5***	2.1***
	$MS_{(error)}$	0.5	0.7	0.6	0.4
	CV (%)	13.6	18.5	15.7	10.9

N/B*** = Significant at $P \leq 0.001$. Means followed by the same letter are not significantly different, according to DMRT

4.3 Qualitative Traits

The dolichos genotypes were evaluated for 15 qualitative traits as listed in Table 6. There was no variation in seven of the qualitative traits evaluated in terms of leaf hairiness which was low pubescent, ovate leaf shape, their pod curvature was slightly curved, medium pod beak, green pod color, the attachment of unripe pod was erect and that of ripe pods was intermediate. However, there was some variation in some other traits. Genotypes W7, G1, B1, LG1, M5, Local Variety and DL1002 had extensive stem pigmentation, presence of leaf anthocyanin and green leaf color whereas genotype M5 had no stem pigmentation, no leaf anthocyanin and pale green leaf color. Genotype M5 had white flowers while all the other seven genotypes exhibited purple flower color. On the color of the seed, there was variation in that M5 had brown seeds, B1 had brown seeds with black dots while the other six genotypes had black seeds. There was also a variation in terms of leaf color and leaf anthocyanin in that, M5 had pale green leaves while the other seven genotypes had green leaves, M5 had no leaf anthocyanin which was present in all the other seven genotypes. There was variation in terms of seed shape in that seven of the varieties had oval seed shape expect LG1 which had flat seeds. There was also variation in the growth habit in that, genotype W7, M5, LG1, and G1 were semi determinate whereas G2, B1, Local variety and DL1002 were indeterminate. Due to the fact that dolichos is largely intercropped with other crops especially maize, most farmers like the determinate genotypes (Shivachi *et al.,* 2012; Gowda, 2008). Lastly, there was variation among the genotypes in terms of branch orientation where genotypes W7, G2, B1, and G1 had their first lateral branches long and spreading over the ground where as in Genotypes G2, B1,

Local variety and DL1002 their first lateral branches were perpendicular to the main stem. The qualitative traits evaluated were used to develop descriptors for the genotypes (Appendix II-VII). These variations in qualitative traits can be used in the selection of genotypes for further breeding programs.

The dolichos genotypes exhibited some degree of phenotypic variation for some traits observed. Plate 1 shows the variation in terms of seed color and flower color.

Plate 1: Selected qualitative traits observed in March-August 2012

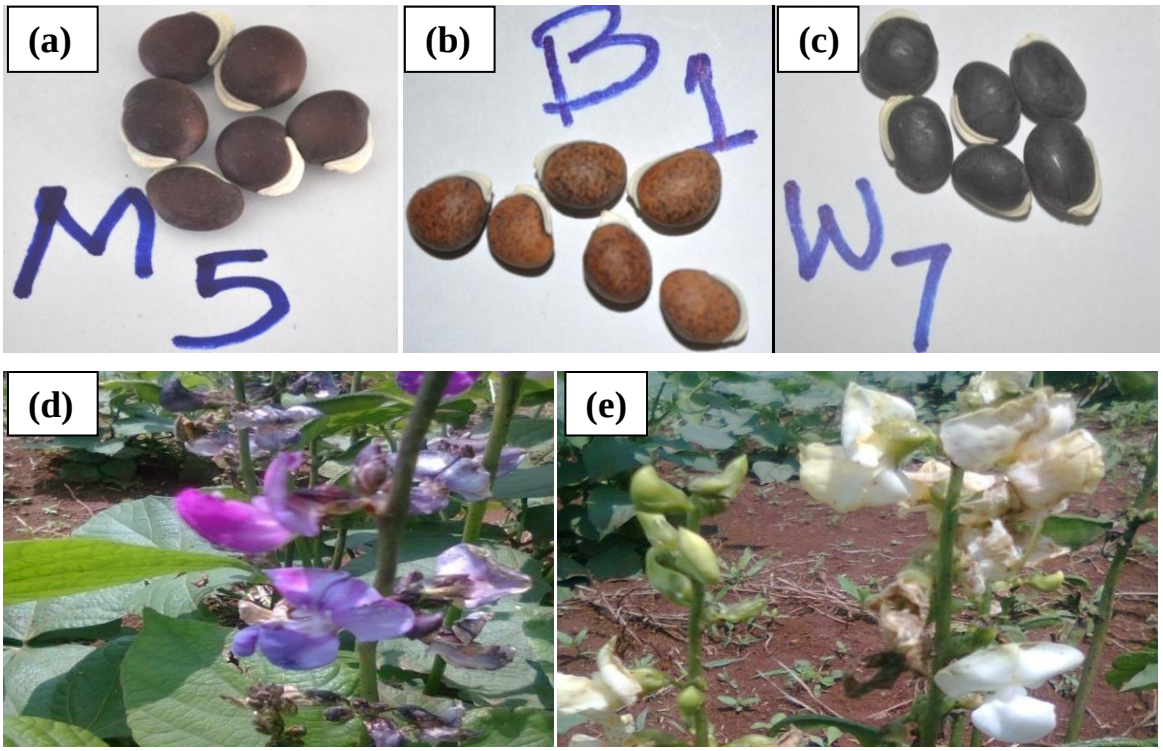

Plate 1: Some selected traits of the dolichos genotypes showing differences in seed color; (a) brown, (b) brown with black dots, (c) black and in flower color; (d) purple, (e) white. (Source: Author, 2013).

Table 4.3: Qualitative traits of eight dolichos genotypes

	Qualitative traits						
	SP	**LA**	**LC**	**LH**	**LS**	**GH**	**BO**
Accession							
W7	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	Semi determinate	First lateral branches long and spreading over ground
G2	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	Indeterminate	First lateral branches long and spreading over ground
M5	No pigmentation	Absent	Pale green	Low pubescent	Ovate	Semi determinate	Perpendicular to main stem
B1	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	Indeterminate	Perpendicular to main stem
LG1	Extensive	present at leaf edges	Green	Low pubescent	Ovate	semi determinate	Perpendicular to main stem
G1	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	semi determinate	First lateral branches long and spreading over ground
Local Variety	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	Indeterminate	First lateral branches long and spreading over ground
DL1002	Extensive	Present at leaf edges	Green	Low pubescent	Ovate	Indeterminate	First lateral branches long and spreading over ground

Key: Stem pigmentation (SP), Leaf anthocyanin (LA), Leaf color (LC), Leaf hairiness (LH), Leaf shape (LS), Growth habit (GH), Branch orientation (BO).

	FC	PC	PB	Pco	UPA	RPA	SCm	SS
Accession								
W7	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Oval
G2	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Oval
M5	White	Slightly curved	Medium beak	Green	Erect	Intermediate	Brown	Oval
B1	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Brown with black dot**s**	Oval
LG1	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Flat
G1	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Oval
Local Variety	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Oval
DL1002	Purple	Slightly curved	Medium beak	Green	Erect	Intermediate	Black	Oval

Key: Flower color (FC), Pod curvature (PC), Pod beak (PB), Pod color (PC), Unripe pod attachment (UPA), Ripe pod attachment (RPA), Seed color {mature} (SCm), Seed shape (SS).

4.4 Quantitative traits

A total of eight quantitative traits were evaluated across three sites (University of Eldoret, Njoro and in Mabanga in Bungoma). Sites (locations) were significant at P≤ 0.001 in; days to 50% flowering, days to maturity, flower buds per raceme, plant height and 1000 seed weight, a significant difference of P≤ 0.01 for racemes per plant and seeds per pod and a significant difference of P≤ 0.05 for number of pods per plant. There was also a high significant difference in the means for the genotypes, P≤ 0.001, in terms of to 50% flowering, days to maturity, flower buds per raceme and plant height. The interaction between genotypes and sites were significant at P≤ 0.01 in; days to flowering, days to maturity and plant height. The other traits; Flower buds per raceme, raceme per plant, pods per plant, seeds per pod and 1000 seed weight had a significance of P≤ 0.05. From the results, days to maturity was highly significant at P≤ 0.001, the new improved genotypes took shorter time to mature than the two checks. Genotype B1 had the shortest maturity time of 139 days whereas Local variety had the longest maturity time of 166 days, a difference of 27 days. The number of flower buds per raceme ranged from 12 in local variety to 18 in genotypes G2 and LG1, plant height ranged from 122cm in G1 to 163 in LG1, this is because genotype G1 is semi determinate while genotype LG1 is indeterminate. There was no correlation between number of flower buds per raceme and the number of pods per plant e.g. in genotype G2 and LG1 both had an average of 18.11 flower buds per raceme but had different number of pods per plant where genotype G2 had an average of 68.58 and genotype LG1 had an average of 46.18. The high variation among the genotypes under study can be exploited further in future breeding program.

Table 4.4: Quantitative traits of eight dolichos genotypes

Site	Genotype	Days to 50% flowering	Days to maturity	No. of flower buds/raceme	Racemes/plant	pods per plant	Seeds/pod	Plant height (cm)	1000 seed weight
Combined	**W7**	71.11bcd	147b	15.78bc	12.78bc	51.7a	2.978ab	129.4ab	236.7bcd
	G2	72.78de	142.2a	18.11d	14.22c	68.58b	3.311b	147.9bcd	255.1d
	M5	68.56a	141.1a	14.89b	12.67bc	51.49a	3.144b	136.6abc	244.8cd
	B1	69.56ab	139.78a	17.22cd	12.11abc	45.19a	2.967ab	121.5a	237.4bcd
	LG1	70.33abc	147.33b	18.78d	13.67c	46.18a	2.967ab	163.8d	217.1a
	G1	73.67f	150.1b	14.33ab	11ab	41.65a	3.3b	122.5a	235.2abc
	Local Variety	80.33f	166.89d	12.22a	9.89a	45.57a	2.689a	149.1cd	223ab
	DL1002	71.89cde	155.33c	14.22ab	11.11ab	46.82a	2.956ab	128.3a	226.5abc
	UoE	74.17b	153.54b	17.42b	12.17ab	47.16a	2.987a	152.4b	247.8b
	Mabanga FTC	75.63c	155.08b	12.67a	10.96a	45.03a	2.85a	107.8a	211.3a
	Njoro	67.04a	137.54a	17b	13.42b	56.75b	3.279b	151.8b	244.3a
	Grand mean	72.28	148.72	15.69	12.18	49.6	3.039	137.4	234.5
	$MS_{(site)}$	506.3***	2264.3***	166.056***	36.264**	936.5*	1.1526**	15696.1***	9730***

$MS_{(Genotype)}$	120***	720.95***	44.246***	18.982**	625.5*	0.3819*	2010.9***	1343.7**
$MS_{(Genotype*site)}$	16.28**	75.22**	8.579*	6.518*	183.2*	0.1395*	480.3**	188.9*
CV (%)	3.36	2.97	14.7	19.2	29.8	12.8	14.4	8.7

NB* = significant at P≤0.001, ** = significant at P≤ 0.01, * = significant at P≤0.05; Mean square (MS), Coefficient of variation (CV). Genotype and site means having the same letter are not significantly different at the 5% level of significance according to Duncan's Multiple Range Test (DMRT).

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4.5 Cluster analysis

The genetic distance based on simple matching cluster analysis constructed from quantitative traits revealed that the closest genotypes were B1 and G1 while the longest distance was observed between G2 and W7&M5 (Figure 1). Using the mean similarity as cutoff, the genotypes were clustered in three groups; the first consisted of genotypes W7, M5, B1, G1 and DL1002; the second LG1 and Local Variety, and the third only with G2, which showed high divergence compared to the other genotypes, particularly due to higher means of raceme per plant, pods per plant and 1000 seed weight as seen in Table 7.

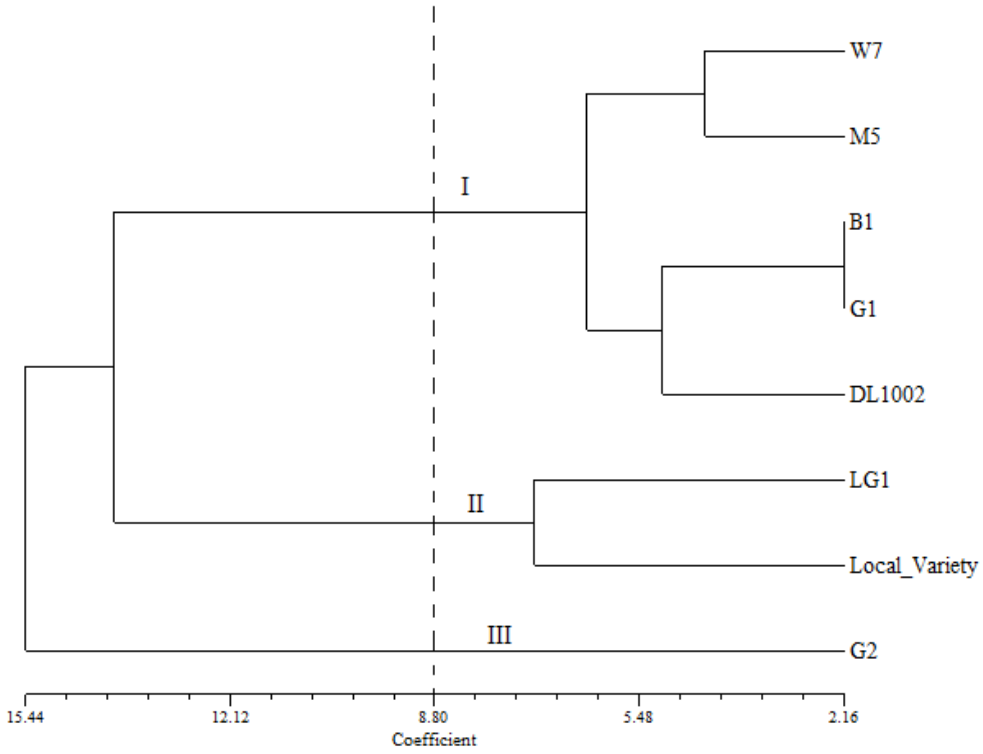

Figure 1: Dendogram generated, by NTSYSpc version 2.1, based on the simple matching coefficient, UPGMA clustering using quantitative traits.

4.6 Microsatellite (SSR) Analysis

Ten primer sets were used to amplify DNA extracts from the 8 genotypes. A total of 6 out of the 10 primers gave polymorphic bands (Table 8), 3 primers failed to amplify any product and 1 primer was monomorphic and therefore were not considered for further analysis.

A total of 19 alleles were detected ranging from 2 to 5 per locus with a mean of 2 alleles per locus (Table 8). The polymorphic information content (PIC) values also ranged from 0.195 in LABRRT 83 to 0.746 in LABRRT52 with an average of 0.489. Based on the PIC values, the most polymorphic primers were LABRRT52, LABRRT2 (Plate 2) and LABBRT77. Genetic diversity was high ranging from 0.219 in LABRRT83 to 0.781 in LABRRT52 with a mean value of 0.542.

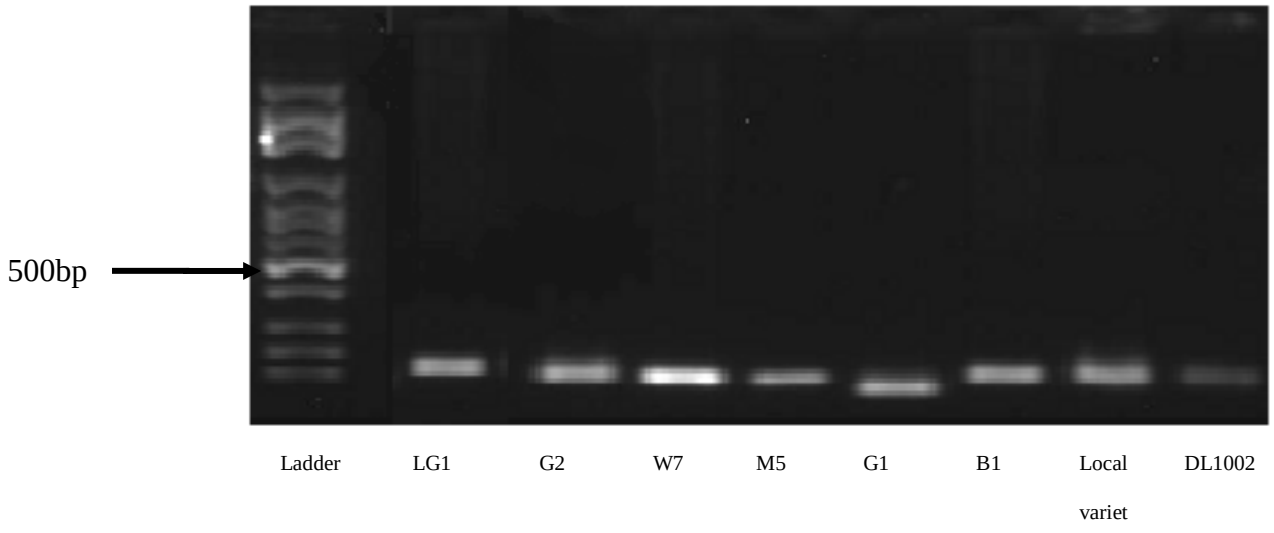

Plate 2: Simple sequence repeat (SSR) markers profile of 8 dolichos genotypes generated by primer LABRRT2(Auther, 2014).

4.6.1 Genetic diversity between the eight genotypes

Markers LABRRT52, LABRRT2, LABRRT77 and LABRRT50 uncovered high gene diversity of 0.781, 0.719, 0.625 and 0.531. The markers that uncovered diversities of less than 0.5 were LABRRT 83 and LABRRT23 which had a gene diversity of 0.219 and 0.375 respectively (Table 8). These diversities in the genotypes under this study should be exploited further in future breeding program.

4.6.2 Polymorphism information content (PIC) of markers

Polymorphism information content is a closely related diversity measure (Botstein *et al.*, 1980). The more the number of alleles uncovered by a marker, the higher its PIC. The markers with high polymorphism information content of more than 0.5 were LABRRT52, LABRRT2 and LABRRT77 with 0.746, 0.668 and 0.555 PIC respectively. Markes LABRRT 50, LABRRT23 and LABRRT83 were the least informative with a PIC of 0.468, 0.305 and 0.195 respectively (Table 8).

Table 4.5: Polymorphism, diversity and frequency results

Markers	Sample size	Major Allele Frequency	Allele No	Gene Diversity	PIC
LABRRT2	8	0.375	4	0.719	0.668
LABRRT23	8	0.750	2	0.375	0.305
LABRRT50	8	0.625	3	0.531	0.468
LABRRT52	8	0.250	5	0.781	0.746
LABRRT77	8	0.500	3	0.625	0.555
LABRRT83	8	0.875	2	0.219	0.195
Mean	**8**	**0.563**	**3.167**	**0.542**	**0.489**

Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendogram of 8 Dolichos genotypes based on SSR data (Figure 2). The genotypes were clustered into four groups; (I) consisted of M5, LG1 and W7, (II) B1 and G1, (III) G2, (IV) Local variety and DL1002. The genetic distance revealed that the closest genotypes were LG1 and W7 with the longest distance being genotype LG1&W7 and DL1002.

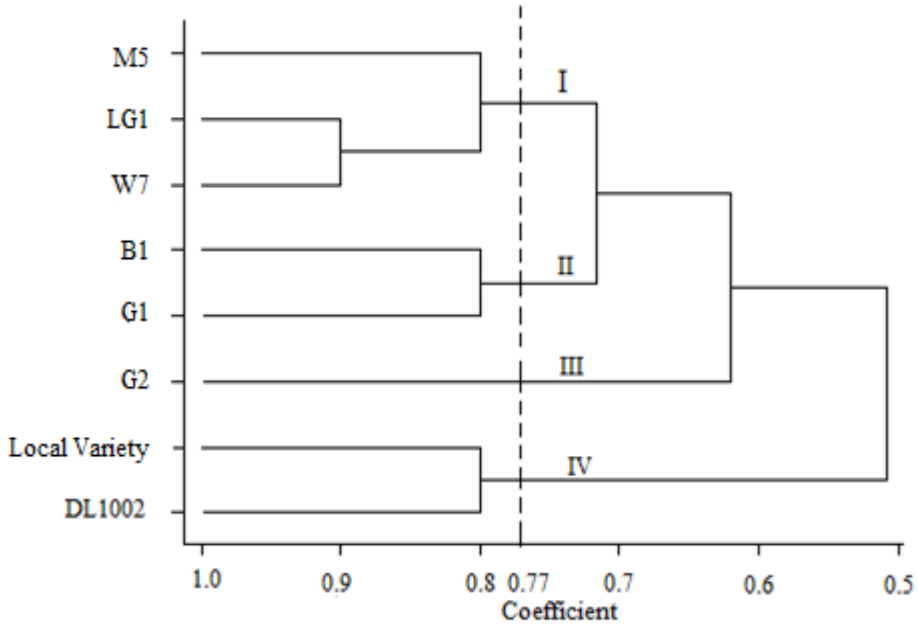

Figure 2: Dendogram of eight dolichos genotypes based on microsatellite (SSR) markers.

A dendogram was generated based on the simple matching coefficient, UPGMA clustering using combined morphological and SSR data (Figure 3). The eight dolichos genotypes were grouped into four groups; (I) B1 and LG1, (II) G2, (III) G1, W7 and M5 and lastly (IV) DL1002 and Local Variety. The dendogram also revealed that the closest genotypes were G1 and W7 (Figure 3).

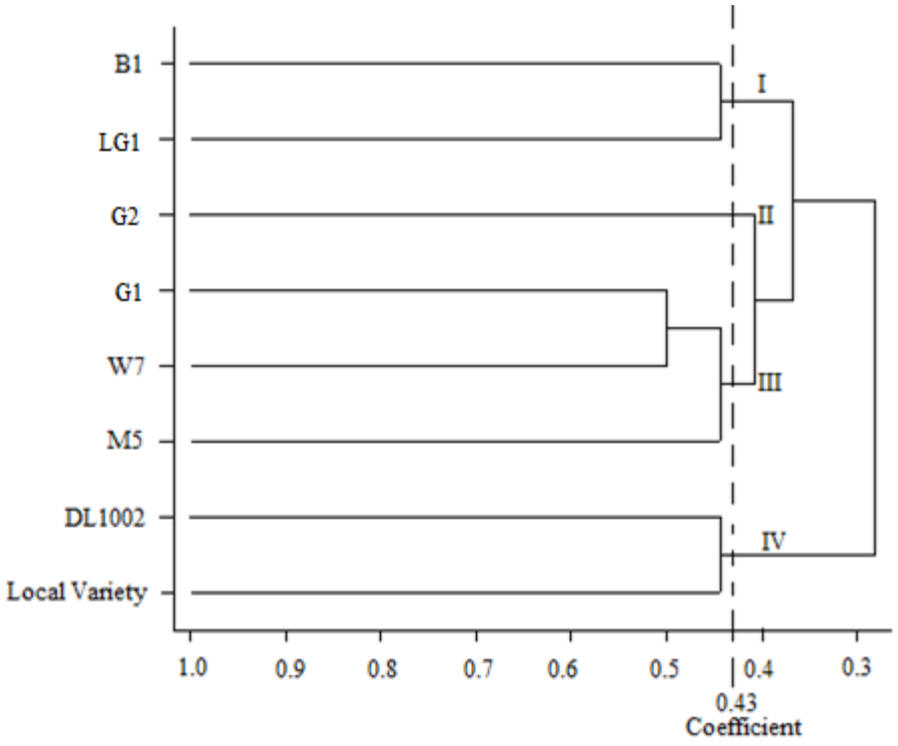

Figure 3: A dendogram of eight genotypes based on combined morphological and SSR markers

CHAPTER FIVE

DISCUSSIONS

5.1 Cooking Time and Organoleptic Traits of the Genotypes

There was variation in cooking time among the eight genotypes ranging from 87 minutes to 159 minutes. The cooking times recorded in this study are lower than findings form Shivachi *et al.,* (2012) who reported cooking time of thirteen genotypes to be between 70-197 minutes. Comparatively, the two checks (DL1002 and Local variety) took relatively long time to cook than the new improved genotypes. This was expected since the new genotypes had been bred to improve on their cooking time as well as organoleptic traits. The shortest cooking time was 87 minutes recorded by M5 which is a brown seeded genotype followed by B1 which is also brown seeded but has black dots and the longest cooking time of 159 minutes was recorded by Local variety which is black seeded and a common land race grown by farmers in Ruiri-Meru.

Variation in cooking time is caused by many factors among them being; genetic, energy source, type of water, size and age of the beans among others (Shivachi *et al.,* 2012). However, because most of these factors i.e. heat supply, water type, source of heat, age and size of bean, were kept constant during the experimentation, it can therefore be concluded that; the difference in cooking time among the genotypes could be attributed to their genetic makeup (Bitjoka, 2008; David *et al.,* 2004; Ngwira and Mwangwela, 2001). The black seeded genotypes took longer to cook than the brown seeded genotypes, this finding also concurred with findings from Shivachi *et al.,* (2012). This result could be attributed to

high anti nutrient levels in their seed coats. Maass and Usongo, (2007); Pengelly and Maass, (2001) related lablab color to anti nutrient levels, and found dark seeded types to contain high amounts of these substances than white or cream seeded types. A large amount of heat is thus required to eliminate these compounds resulting in prolonged cooking of these genotypes, Shivachi *et al.,* (2012). Adeboye, (2006) and Fasoyiro *et al.,* (2005), also concluded that dark seeded pigeon pea and mucuna varieties took long to cook owing to large amounts of anti-nutritional factors contained in their seed. From the organoleptic results gotten we can also conclude that anti nutritional factors are responsible for bitter taste, i.e. dark/black genotypes received low scores for the taste attributes. These genotypes are thus associated with extended cooking time so as to eliminate their bitter taste. Osman *et al.,* (2002) also made similar observations. However, in this study, anti-nutritional factors were neither qualified nor quantified, and thus need further investigation to ascertain their contribution to cooking time.

Results across genotypes for sensory parameters evaluated were significant. From the findings, it was clear that the sensory panelist had clear preference when it came to the specific genotypes. A major finding from the panelist was that; the quality traits of appearance, taste and texture are fundamental and greatly affect consumers' preference for particular lablab genotypes. With regards to appearance, genotype G2 was rated highest and Local variety was lowest. This may be attributed to the fact that G2 has uniform, round and well filled seeds as opposed to the local variety that has flat and the seeds are not well filled and thus not appealing. In terms of taste, genotype M5 was rated highest while the local variety was rated lowest. This could be attributed to the anti-nutritional content of the

genotypes, since M5 is brown seeded as opposed to the Local variety which black seeded. These results were similar to Shivachi *et al.,* (2012); Mkanda *et al.,* (2007) who reported that black seeded genotypes were more bitter than the brown seeded genotypes. In pulse, white or cream genotypes are highly preferred to dark once because the latter, contain relatively high amounts of anti-nutritional factors giving them a bitter taste (Shivachi *et al.,* 2012). In terms of texture, B1 was rated highest while Local variety was rated lowest. Genotype G1 was rated the highest and local variety lowest in terms of the overall acceptability. This could be attributed to the fact that despite the seeds being black in color, they are large, smooth, uniform size and well filled, thus the farmer preference. Local variety was ranked least in nearly all the traits that were evaluated. This was a clear indication that most of the genotypes that are grown by farmers need to be improved.

From the studies, we can conclude that, organoleptic traits i.e. appearance, texture and taste, affect the general acceptance of the lablab genotypes and that farmers adopt genotypes based on all these factors i.e. desirable agronomic attributes like growth habit, yield and adaptation. Similar observations have also been sighted by Kinyua *et al.,* 2007.

5.2 Discussion on Morphological Characterization

Morphological traits are useful tools for preliminary evaluation of genotypes since they offer a fast and useful approach for assessing the extent of diversity. The estimation of different morphological traits studied among the improved dolichos genotypes and their checks in the present study revealed the existence of some level of morphological diversity. This implies that the new genotypes constitute a pool of germplasm with adequate genetic variability.

5.2.1 Discussion on Qualitative Traits

The study revealed that there was variation in most of the qualitative traits evaluated though some qualitative traits did not differ significantly across the three sites. These include; stem pigmentation, leaf anthocyanin, leaf color leaf hairiness, branch orientation, flower color, pod curvature, pod color, pod beak, pod attachment, seed color and seed shape. These findings also concur with findings by Shivachi *et al.,* 2010. From the results, there could be a correlation between stem pigmentation and leaf anthocyanin i.e. M5 which had no stem pigmentation did not have leaf anthocyanin while the other genotypes had extensive stem pigmentation and subsequently had leaf anthocyanin at the leaf edges, this was also reported by Shivachi *et al.,* 2010; Kar *et al.*, 2006; Adebisi and Bosch, 2004). All the genotypes that were purple flowered were also black seeds while white flowered genotype (M5), was brown seeded. These results confirm those of Ewanisiha *et al.,* (2007). There was no correlation between growth habit and branch orientation i.e. genotypes W7 and G1 had their first lateral branches long and spreading over ground and semi determinate growth habit whereas genotypes G2, Local variety and DL1002 had First lateral braches long and spreading over ground and indeterminate growth habit (See table 6). All of the genotypes showed great resistance to pests and diseases except local variety which showed mild resistance. This observation could be explained by the fact that the six genotypes (M5, G1, G2, LG1, B1 and W7) had been bred for pest and disease resistance.

5.2.2 Discussion on Quantitative Traits

Most of the quantitative traits that were evaluated were highly significant at ($P \leq 0.001$), except for number of racemes per plant and number of seeds per pod which were significant at $P \leq 0.01$ and number of pods per plant which was significant at $P \leq 0.05$. There was high diversification with notable variations in, days to 50% flowering, maturity period, number of flower buds per raceme, racemes per plant, number of seeds per pods, plant height, and yield among other agronomic traits. This result also conforms to findings from Gowda, (2008). Days to 50% flowering varied from 68-80 days with genotype M5 with the least days and Local variety with the most days respectively. This result conforms to results gotten by Ayisi *et al.,* (2006) who reported 63-75 days. Consequently there was a notable variation in terms of days to maturity ranging from 142-166 days, with genotype M5 taking least days and Local variety taking the most days to mature respectively. Nonetheless, the maturity days lie within the recommended period of between 90-210 days (Adebisi and Bosch, 2004). Number of days to maturity is a key factor that determines farmers' adoption where most farmers prefer early maturing genotypes as opposed to late maturing once. There was also a significant difference in terms of number of flower buds per raceme which ranged from 12-18 with local variety having the least and genotype G2 with the most flower buds per raceme. Farmers prefer genotypes that have high flower buds per raceme since the higher the flower buds per raceme the higher the yields. There was a slight variation in terms of number of racemes per plant which ranged from 9-13, with local variety with the least and genotype LG1 with the highest racemes per plant. There was also a slight variation in terms of number of seeds per pod with a range of 2-3

which represented a significance of 99.95%. Several investigators have reported a seed range of 2-6 seeds per pod of the same crop (Rai *et al.*, 2010; Kar *et al.*, 2006; Nandi *et al.*, 2000). Significant difference was also noted in plant height with a range of 121-163 cm with genotype B1 being the shortest and genotype LG1 being the longest respectively. In Kenya, most farmers' grow lablab as an intercrop with maize (Okumu *et al.*, 2005) thus the short and determinate genotypes are popular. There was also notable significant difference in terms of 1000 seed weight with a range of 217-255g where genotypeLG1 had the lowest weight and genotype G2 had the highest weight. There could be a relationship between seed weight and shape of the seed. This is because, genotype G2 which had the highest weight had rounded seeds that were well filled compared to genotype LG1 which had the least weight and had flat seeds that were not well filled. From the cluster analysis (figure 4.1) based on simple matching coefficient constructed from quantitative traits revealed that the closest genotypes were B1 and G1. Using the mean similarity as cutoff the genotypes were clustered into three groups; (I) W7, M5, B1, G1, and DL1002; (II) LG1 and Local Variety and (III) G2.Cultivated lablab is morphologically diverse with large variations (Newaz, 2005). Such diversity enhances the direct selection of these traits in developing combinations for breeding programs.

5.3 Genetic Diversity Based on SSR Markers

Lack of amplification of an allele in certain genotypes might be due to the result of divergence in the sequences flanking the microsatellite or simply the production of an undetectable amount of PCR product, creating a null allele (Smulders *et al.*, 1997 and Lavi *et al.*, 1994).

The number of alleles produced by different primers from the results ranged from 2 to 5 with an average of 3.2 alleles per primer. This result concurs with findings by Shivachi *et al.,* (2012) and Wang *et al.,* (2007) who reported mean amplifications of 3.5 and 3.6 alleles per primer. In similar studies, on common beans involving microsatellites, Jose *et al.,* (2009); and Maras *et al.,* (2008) reported mean amplifications of 7.8 and 7.1 alleles per primer respectively.

The polymorphic information content (PIC) value was calculated to characterize the capacity of each primer to detect polymorphic loci which ranged from 0.195 to 0.746 in the current study. This concurred with results by Somta *et al.,* (2009) and Cabral *et al.,* (2011) who recorded PIC values of between 0.049-0.883 and 0.11-0.5 respectively. The result showed that most of the primers were found to be highly informative and can be used to study phylogenetic relationship and genetic diversity in future. The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character (Asare *et al.,* 2011).This was slightly lower than findings from Datta *et al.,* (2010) who reported an allelic frequency of 1.3. Gene diversity was high ranging from 0.219 to 0.781 with a mean value of 0.542, comparing favorably with the results obtained by Asare *et al.,* (2011) although the mean value was slightly high (0.58).

5.4 Comparing genetic distance of the dolichos genotypes based on morphological and SSR markers.

The comparison of morphological versus SSR and combined dendograms results suggest that most of the genotypes for both dendograms had similar grouping patterns and that some morphological clusters were confirmed by SSR analysis. These comparison of

morphological and SSR maker clustering pattern gives information on the amount of variability found between the genotypes and hence helps in identifying the best method of assessing diversity. Compared to phenotypic traits, molecular markers have the advantage of not being influenced by the environment, specific, reliable and wider range of genome sampling but have the disadvantage of accessing the genome as a whole and not only the regions responsible for the expression of traits of interest.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

Results from cooking time showed that improved genotypes took less time to cook than the two checks.

The organoleptic study showed that sensory traits of appearance, texture and taste greatly affect consumers' choice and thus influencing the adaptability of bean varieties.

Both morphological and SSR markers were able to group the lablab accessions into distinct groups.

6.2 Recommendation

Since SSR markers specific to dolichos have been developed, it is therefore necessary to characterize all the dolichos accessions in the Country at molecular level which will form a good basis for further development of breeding and selection programs.

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APPENDIX

Appendix I: Participatory farmer evaluation form.

Genotype code ……..

Evaluators name ……. Date……

Trait	**Score/Rank**
Inavyoonekana/ Appearance	
Cooking time	
Ladha/ Taste	
Texture	
Kukubalika/ Overall adaptability	

KEY

1- Mbaya sana	1- Very bad
2- Mbaya	2- Bad
3- Inaridhisha	3- Fair
4- Nzuri	4-Good
5- Nzuri sana	5- Very good

Maoni/ comments

……

……

……

Appendix II: PCR regime used for the ten primers

step 1: initial denaturation:	92^{o}C	3 minutes
step 2: denaturation:	92^{o}C	30 seconds
step 3: annealing:	56^{o}C	30 seconds
step 4: extension:	72 ^{o}C	1 minute
step 5: go to step 2	Repeat setp 2-4	for another 34 cycles
step 6: Final extension:	72 ^{o}C	5 minutes
step 7: hold at	4^{o}C	

Appendix III: Dolichos descriptor for LG1

	Trait	**Character**
1	Stem pigmentation	Extensive
2	Leaf anthocyanin	Present at the edges of the leaf
3	Leaf color	Green
4	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	Ovate
6	Plant height	Approx. 163.5cm
7	Growth habit	Semi determinate
8	Branch orientation	Branches tending to be perpendicular to main stem, medium in length
9	No. of flower buds/raceme	18
10	No. of racemes /plant	13
11	Days to 50% flowering	70 days
12	Flower color	Purple
13	Pod curvature	Slightly curved
14	Pod beak	Medium beak
15	Pod color	Green
16	Pod attachment (unripe)	Erect
17	Pod attachment (at maturity)	Erect
18	No. of pods /plant	Approx. 233pods
19	No. of seeds/ pod	3-4 seeds
20	Days to maturity	147 days
21	Seed color (when ripe)	Black
22	Seed shape	Flat
23	1000 seed weight (g)	217.1 (g)

Appendix IV: Dolichos descriptor for G1

	Trait	**Character**
1	Stem pigmentation	Extensive
1	Leaf anthocyanin	Present at the leaf edges
2	Leaf color	Green
3	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	ovate
6	Plant height	Approx. 122.5cm
7	Growth habit	Semi determinate
8	Branch orientation	First lateral branches long and spreading over ground
9	No. of flower buds/raceme	14
10	No. of racemes /plant	11
11	Days to 50% flowering	73 days
12	Flower color	purple
13	Pod curvature	Slightly curved
14	Pod beak	Medium beak
15	Pod color	Green
16	Pod attachment (unripe)	Erect
17	Pod attachment (at maturity)	Intermediate
18	No. of pods /plant	Approx. 41 pods
19	No. of seeds/ pod	3-4 seeds
20	Days to maturity	150 days
21	Seed color (when ripe)	Black
22	Seed shape	oval
23	1000 seed weight (g)	235.2 (g)

Appendix V: Dolichos descriptor for G2

	Trait	**Character**
1	Stem pigmentation	Extensive
2	Leaf anthocyanin	Present at the edges of the leaf
3	Leaf color	Green
4	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	ovate
6	Plant height	Approx. 147.9 cm
7	Growth habit	Indeterminate
8	Branch orientation	First lateral branches long and spreading over ground
9	No. of flower buds/raceme	18
10	No. of racemes /plant	14
11	Days to 50% flowering	72 days
12	Flower color	Purple
13	Pod curvature	Slightly curved
14	Pod beak	Medium beak
15	Pod color	Green
16	Pod attachment (unripe)	Erect
17	Pod attachment (at maturity)	Intermediate
18	No. of pods /plant	Approx. 68 pods
19	No of seeds per pod	3-4 seeds
20	Days to maturity	142 days
21	Seed color (when ripe)	black
22	Seed shape	oval
23	1000 seed weight (g)	255.1 (g)

Appendix VI: Dolichos descriptor for M5

	Trait	**Character**
1	Stem pigmentation	No pigmentation
2	Leaf anthocyanin	Absent
3	Leaf color	Pale green
4	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	Ovate
	Plant height	Approx. 136.6 cm
6	Growth habit	Semi determinate
7	Branch orientation	Branches tending to be perpendicular to main stem, medium in length
8	No. of flower buds/raceme	14
9	No. of racemes /plant	12
10	Days to 50% flowering	68 days
11	Flower color	White
12	Pod curvature	Slightly curved
13	Pod beak	Medium beak
14	Pod color	Green
15	Pod attachment (unripe)	Erect
16	Pod attachment (at maturity)	Intermediate
17	No. of pods /plant	Approx. 51 pods
18	No. of seeds/ pod	3-4 seed
19	Days to maturity	141 days
20	Seed color (when ripe)	Brown
21	Seed shape	Oval
22	1000 seed weight (g)	244.8 (g)

Appendix VII: Dolichos descriptor for W7

	Trait	**CHARACTER**
1	Stem pigmentation	Extensive
2	Leaf anthocyanin	Present at the leaf edges
3	Leaf color	Green
4	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	Ovate
6	Plant height	Approx. 129.4 cm
7	Growth habit	Semi determinate
8	Branch orientation	First lateral branches long and spreading over ground
9	No. of flower buds/raceme	15
10	No. of racemes /plant	12
11	Days to 50% flowering	71 days
12	Flower color	purple
13	Pod curvature	Slightly curved
14	Pod beak	Medium beak
15	Pod color	Green
16	Pod attachment (unripe)	Erect
17	Pod attachment (at maturity)	Intermediate
18	No. of pods /plant	Approx. 51 pods
19	No. of seeds / pod	3-4
20	Days to maturity	147 days
21	Seed color (when ripe)	Black
22	Seed shape	oval
23	1000 seed weight (g)	236.7 (g)

Appendix VIII: Dolichos descriptor for B1

	Trait	**Character**
1	Stem pigmentation	Extensive
2	Leaf anthocyanin	Present at the edges
3	Leaf color	Green
4	Leaf hairiness (on inner surface)	Low pubescent
5	Leaf shape (when 90% of the pods are ripe)	Ovate
6	Plant height	Approx. 121.6cm
7	Growth habit	Indeterminate
8	Branch orientation	Branches tending to be perpendicular to main stem, medium in length
9	No. of flower buds/raceme	17
10	No. of racemes /plant	12
11	Days to 50% flowering	69 days
12	Flower color	purple
13	Pod curvature	Slightly curved
14	Pod beak	Medium beak
15	Pod color	Green
16	Pod attachment (unripe)	Erect
17	Pod attachment (at maturity)	Erect
18	No. of pods /plant	App. 45 pods
19	No. of seeds per pod	3-4 seeds
20	Days to maturity	139 days
21	Seed color (when ripe)	Brown with black dots
22	Seed shape	Oval
23	100 seed weight (g)	237.4 (g)