ANALYSIS OF FLAVOUR AND MOLECULAR DIVERSITY OF KENYAN LABLAB BEAN (*Lablab purpureus* (L.) Sweet) ACCESSIONS

By

ESTHER NYAMBURA KIMANI

A thesis submitted to the Graduate School in partial fulfilment for the requirements of the Master of Science degree in Biochemistry and Molecular Biology of Egerton University

EGERTON UNIVERSITY

DECEMBER 2010

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and it has not been presented in this or any other University for the award of a degree of diploma

Kimani Esther Nyambura
SM14/1712/06

Sign:..... Date: 29/12/2010

Recommendation

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

Francis N. Wachira
Associate Professor,
Department of Biochemistry and Molecular Biology, Egerton University

Signature..... Date..29/12/2010

Miriam G. Kinyua
Associate Professor,
Department of Biotechnology, Moi University, Eldoret

Signature..... Date..29/12/10

COPYRIGHT

DEDICATION

This work is dedicated to my dear parents Joseph Kimani Wanjama (the late) and Esther Wangare Kimani Wanjama.

To God,

my ever present help in time of need, to You be the glory.

ACKNOWLEDGEMENT

I would like to thank the Almighty God for seeing me through the challenging times during this study and the life lessons I had to go through. I am greatly indebted to my supervisors, Prof. Francis N. Wachira, Prof Miriam Kinyua, for their encouragement, effort, supervision, insightful criticism and guidance into deep understanding and knowledge of this study and the preparation of this thesis. I wish to express my sincere appreciation to the following institutions for providing support in the course of my study: Kirk House Trust for the financial support, Egerton University and Government Chemist for the laboratory facilities. My gratitude is extended to the Centre Director KARI, Njoro for availing the laboratory facilities, financial support and enabling environment for me to conduct the research.

I am grateful to my AWARD mentor- Dr Santie deVillers, for the invaluable advice and effort in scientific writing, and Prof Matasyoh (Chemistry department-Egerton University), who contributed enormously to the analysis of volatile compounds. I also extend my gratitude to the following for their unfailing support during the project; Kimani Paul, Mr. Otieno, Dr. Maling'a, Dr. Karanja, Evans Nyaboga, Loice Kariuki and Kevin Kamanyi for the assistance in the laboratory; Mr Kariuki (Chemistry department, Egerton University) for the support with GC analysis, Dan Orwa for providing the genebank accessions; Dr. Bridigitte Maass for the publications on lablab; Nathan Muasya and Steve Kimno (Moi University) for all the correspondence and Mr. John Kamundia and Bramwel Wanjala for the help with statistical analysis. To all my friends who encouraged me and kept me going to finish this work especially Nelson Ndegwa, John Ndungu, Mwaura Kiarie, Faith Muniale; and Dr. Franklin Nyabuga who made sure I did not lack any scientific paper that I needed, despite his busy schedule.

I owe a lot of gratitude to my parents and my siblings for tolerating me during those times I spent long hours in the laboratory, and when the work pressure was too high. You are the best family ever. Last but not least, John Kageche, for the love, spiritual and emotional support and encouragement in the last leg of this work.

ABSTRACT

The legume species *Lablab purpureus* L. Sweet grows in most tropical environments. It is used as a cover crop and green manure and provides a high–protein food for humans and livestock feed. The study was carried out to analyse flavour components and molecular diversity of Kenyan lablab accessions. Twenty four accessions from the National genebank and farmers were evaluated for odour and bitter taste intensities using sensory tests. Analysis of cyanogenic glycosides was carried out using the picrate method and volatile compounds were isolated and separated using gas chromatography. The genetic diversity of 50 accessions was studied using Amplified fragment length polymorphism (AFLP) markers. The sensory evaluations showed significant ($p \leq 0.05$) differences for the bitter taste but none for odour. Accession 10706 and 13096 exhibited the highest and lowest means respectively for both bitter and odour taste. The levels of cyanogenic glycosides were not different for the 24 accessions, but significant ($p \leq 0.05$) differences were observed in the volatile compounds isolated from the accessions with upto 89% similarity of the accessions. Two hundred and sixty two volatile compounds were identified using literature databases. The molecular study revealed a total of 180 polymorphic bands. The overall mean expected heterozygosity (H_e) for all the populations was 0.189. The Eastern population had the highest H_e of 0.297. The plot of the first and second principal coordinates for cluster analysis revealed an overlap of the accessions forming a tight cluster, with the exception of four; namely Mwingi-3 and 12000 from Eastern population, 12187R3 and 10706R1 from Coast and Rift Valley populations. The Unweighted pair group using mathematical arithmetic averages (UPGMA) cluster analysis generated from the distance matrix revealed three major groups. Group 1 had accessions 10706R1 and Mwingi-3, group 2 had accessions 12187R3 and 12000, while group 3 had the rest of the accessions. The low diversity revealed from these results may be due to the narrow genetic base for breeding stocks, and exchange of germplasm across the country. Results obtained from this study will be of great help in lablab accession management by ensuring maximization of exploitation of this vital resource as well as in developing breeding strategies for *Lablab purpureus.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AFLP- Amplified Fragment Length Polymorphism

ANOVA- Analysis of variance

AMOVA- Analysis of molecular variance

bp- base pair

CTAB- Cetyltrimethylammonium bromide

DNA- Deoxyribonucleic Acid

EDTA- Ethylenediaminetetraacetic acid

HCl- Hydrochloric acid

HCN- Hydrogen cyanide

GC- Gas chromatography

KARI- Kenya Agricultural Research Institute

KI- Kovats index

µl- microlitre

µg- microgram

ng- nanogram

NaCl- Sodium chloride

NaOH- Sodium hydroxide

NEB- New England Biolabs

OD- Optical density

PCR- Polymerase Chain Reaction

PVP- Polyvinylpyrolidone

RAPD- Random Amplified Polymorphic DNA

RFLP- Restriction Fragment Length Polymorphism

rpm- Revolutions per minute

SAS- Statistical Analysis System

SSR- Simple Sequence Repeats

TBE- Tris Boric acid EDTA

TEMED- N,N,N',N'-Tetramethylethylenediamine

UPGMA- Unweighted pair group method of arithmetic averages

CHAPTER ONE
INTRODUCTION

1.1 Background

Lablab bean, *Lablab purpureus* L. Sweet (2n = 22) belongs to the family Fabaceae, that includes *Medicago truncatula* (barrel medic), *Phaseolus vulgaris* (common bean), *Glycine max* (soybean), *Pisum sativum* (garden pea) and *Arachis hypogaea* (peanut [groundnut]) among others. It is among one of the ancient cultivated plants. It is a legume species that grows in the tropics and the subtropics of the world, and is presently grown through out the tropical regions of Asia and Africa. The bean is known by different names in different geographical areas (Table 1.1). In this study, the common name used is lablab, as it reflects the scientific name. To date, it remains a minor crop in most of these regions (Engle and Altoveris, 2000). The main lablab producing areas in Kenya are Eastern (Meru), Central (Nyeri, Thika) and Coast (Lamu) provinces where it is grown either as a pure stand or as an intercrop especially with maize. The crop has also been introduced in other parts, such as Mwingi and Machakos. Lablab yield of 980kg/ha has been reported in a study testing the effect of improved legumes (Lelei *et al*., 2009). The species is cultivated either as a pure crop or intercropped with maize, finger millet, groundnut, castor, or sorghum in tropical regions.

Lablab combines a great number of qualities that can be used successfully under various conditions because of its adaptability. It is reported to have grain yields that are higher than cowpeas (Adebisi and Bosch, 2004). Not only is it drought resistant, but it is also able to grow in a diverse range of environmental conditions. It can be used advantageously as a cover crop since its dense green cover protects the soil against desiccation and decreases erosion by wind or rain. It has been known to provide up to six tonnes of dry matter/ha as green manure (Murphy and Colucci, 1999). It also biologically fixes nitrogen into the soil thereby improving yields of subsequent crops in an economic and environmentally friendly manner (McDonald *et al*., 2001).

Lablab out-yields most conventional crops, especially during the dry season, and its enhanced nutritive value and thus it is also used as a fodder crop (Maundu *et al*., 1999). It is palatable to livestock and is a rich source of protein. It is grazed in a pasture setting or as a companion crop to maize, cut as hay, or mixed with corn silage. It has been observed to increase livestock weight and milk production during the dry season (Maundu *et al*., 1999). Lablab is also

used as human food and it is eaten as green pods or mature seeds. The leaves have also been reported to be eaten as vegetables.

Lablab has the capability of being an outstanding resource for tropical agricultural systems and in improving human food and animal feedstuffs as a vegetable, pulse and/or forage crop in the tropical regions (Pengelly and Lisson, 2003). However, it is not being used to its full potential. In many areas where it could be beneficial, its production is restricted by the inability to buy seed, economic constraints and producers' unwillingness to take the risk in trying a new crop practice. Effort is being devoted to conducting more research to extend both technical and practical knowledge about the bean so that its full potential may be achieved. In most instances the lablab bean has been promoted in many areas as forage. Most of the research on improving lablab as a food crop is currently in Asia, with limited research in Africa (in Kenya and Tanzania) (Maass *et al.,* 2010). Results obtained from this study will be of great help in making decisions on accession management, maximizing the exploitation of accession resources as well as in developing breeding strategies for *Lablab purpureus* for different uses.

Table 1.1: Different names used for *Lablab purpureus*

Njahe (Kikuyu)	Nchabi (Meru)	Garbanzo	Mbumbu (Kamba)	Tonga Bean
Dolichos lablab	Lablab	Hyacinth Bean	India Butter Bean	Sim Bean
Country Bean	Frijol dólicho	Egyptian Bean	*Lablab niger*	Field Bean
Dolichos Bean	Caballero	Siem Bean	Poor-man's Bean	Gallinita
Lablab vulgaris	Lubia Bean	Poroto japonés	Chimbolo verde	
Hierba de Conejo	Frijol jacinto	Bonavist Bean	Frijol de la Tierra	

1.2 Statement of the Problem

Lablab is one of the few crops that have the capacity to provide grain, vegetable, and forage to farmers. In spite of these qualities, Lablab has not been utilised extensively. Like other legumes, lablab seeds contain anti-nutritional factors which include trypsin and chymotrypsin inhibitors, tannins, phytohemagglutinins (lectins), lathyrogens, cyanogenic glycosides and goiterogenic factors, saponins and alkaloids (Vijayakumari *et al*., 1995). These substances are

reported to be generally eliminated by prolonged soaking and subsequent discarding of the liquid and/or by heat treatment at relatively elevated temperatures (Vijayakumari *et al*., 1995).

Prolonged cooking time, however, increases the cost of utilizing the bean and the presence of cyanogenic glycosides has been reported to confer a bitter taste in lablab (Duke *et al*., 1981). The bitter taste, in some cases, persists even after cooking especially in dark-seeded types (Wanjekeche *et al*., 2000). Thus, evaluation and selection for improvement of flavour is necessary considering the time and cost that is involved in eliminating the bitter taste in the lablab bean. Farmers preferred other legumes over the lablab bean because of the bitter taste (Wanjekeche *et al.*, 2000). In addition, the volatile components of cooked beans, which are responsible for the odour, may also affect its acceptance (Kim and Chung, 2008). Studies to evaluate the genetic diversity of the lablab accession in Kenya have not been reported. A sustainable agricultural system requires that components of genetic diversity be used in a way and at a rate that will not lead to a long term decline of diversity, thus maintaining its potential to meet the needs and aspirations of present and future generations. It is therefore essential to determine this diversity.

1.3 Justification

There has been a world wide interest in searching for new and potential uses of unconventional legumes. Because of its already well-established use as a pulse, vegetable and forage, lablab should be a priority genus in developing multi-purpose legumes in both commercial and small holder farming systems in the tropics (Pengelly and Maass, 2001). Sensory factors are a major determinant of the consumers' subsequent purchasing behaviour (Watts *et al*., 1989). Some of the most important characteristics considered in selecting dry bean varieties for production and consumption are fast cooking and good flavour quality traits (Scott and Maiden, 1998). However, there are problems with the sensory (organoleptic) detection of quality since there is considerable variation between analysts in their ability to detect flavour. Thus chemical and biochemical analyses are required. Research has been focused on the nutritional values and health benefits of various beans and limited data is available on the volatile components of lablab beans which may affect its acceptance. Indeed in Kenya, no study has been carried out to evaluate the quality traits of the local lablab germplasm.

Further, despite its potential in Kenya, no effort has been made to genetically improve lablab bean. Progress in genetic improvement relies on the extent of genetic diversity of existing gemplasm and breeding stocks. Though genetic diversity can be assayed using phenotypic traits, these are greatly influenced by the environment and do not correctly reflect genetic relatedness between accessions. Molecular markers now provide a robust tool that is neutral to environmental effect and phenology for estimation of genetic diversity in plant accessions. The extent of genetic diversity in Kenyan *L. purpureus* germplasm is has not been determined.

1.4 Objectives

1.4.1 General Objective

This study aims to determine the diversity of sensory characteristics and volatile components of *Lablab purpureus* L. Sweet in Kenyan accession, and to characterize the accessions grown in Kenya using molecular markers.

1.4.2 Specific Objectives

1. To assess the flavour traits in twenty four Kenyan *L. purpureus* accessions
2. To determine the levels of cyanogenic glycosides responsible for bitter taste in twenty four *L. purpureus* accessions from Kenya.
3. To assess the volatile flavour compounds responsible for the odour characteristics of twenty four *L. purpureus* accessions from Kenya.
4. To determine the level of molecular diversity in fifty Kenyan *L. purpureus* accessions based on Amplified Fragment Length Polymorphism (AFLP) markers.

1.5 Null Hypotheses

To meet the objectives of this study, the following null hypotheses (H_o) were postulated.

1. The twenty four *L. purpureus* accessions from Kenya have similar flavour.
2. The level of cyanogenic glycosides is similar in twenty four Kenyan lablab accessions.
3. Volatile flavour compounds of twenty four Kenyan lablab accessions are similar.
4. There is no molecular diversity in fifty Kenyan lablab *accessions*.

CHAPTER TWO
LITERATURE REVIEW

2.1 Lablab bean crop

Lablab is a legume suited to grow in most tropical environments as it is adaptable to a wide range of rainfall, temperature and altitudes. It is reported to grow well under warm and humid conditions at temperatures ranging between 18^{o} and 30^{o}C. It is a drought hardy crop grown in semi-arid and humid regions with rainfalls between 200-2500mm (Murphy and Colucci, 1999). It continues to grow producing flowers and seeds for many months, and remains green even where the weather becomes dry and cool. Lablab is a climbing perennial crop with thick foliage. The species is propagated by seed and can be sowed alone or intercropped; staked or planted near hedges or near other less leafy plants to climb on. Lablab has a short-day flowering response, with early and late flowering types available. Some landraces flower as early as 55 days after sowing. It is predominantly self pollinated although some out crossing is known to occur.

Its leaves have three leaflets which are up to 15cm long and a well-developed tap root and adventitious roots. The flowers are either purple or cream (Plate 2.1a&b) while the pod is variable in shape. It can be flat or inflated, straight or curved, long or short and usually has 3-6 seeds (Maundu *et al*., 1999). Cultivated or semi-domesticated cultivars have a wide variation in size, colour, and shape (Maass and Usongo, 2007). The seeds may be black, brown, white, speckled red, plain red, or mottled (Plate 2.1c). The red type is rare in Kenya and its seeds are reported to be poisonous after a few generations (Maundu *et al.*, 1999). The seeds of the wild cultivars are greyish brown in coulour and relatively small in size (Maass and Usongo, 2007). The pods are harvested at any time since they do not shatter but are mainly harvested when dry (Maundu *et al*., 1999). Average mature seed yields of 450kg/ha have been reported when lablab bean is grown as a mixed crop and 1,600kg/ha when grown alone (Murphy and Colucci, 1999). Production of legumes and other traditional food crops has declined in Kenya due to a number of factors, such as heavy rains, pests and diseases and lack of planting materials, low interest by seed companies and changes in eating habits. These factors have also led to low production of lablab in Kenya (Kamotho *et al*., 2010; Waldmueller, 1992).

Plate 2.1: Flowers and seed colours. (a) purple flower (b) white flower and (c) different seed colours of *L. purpureus*

Duke *et al*., (1981) described the major pests and diseases of lablab to be pod boring noctuid caterpillars (*Adisura atkinsoni*), the gram caterpillar (*Heliothis armigera*) the plume moth (*Exelastis atomosa*) and the spotted pod borer (*Maruca testulalis*). The flowers are destroyed by the *Mylabris* beetles while young seedlings are attacked by cock-shaver larvae (*Schizonycha* sp). Other major pests of economic importance to lablab are the bean leaf beetle (*Cerotoma ruficormis*), aphids and stinkbugs (*Coptasoma eribraria*) and bruchid beetles which form neat holes in lablab seed. Lablab is attacked by viral diseases such as mosaic disease, ring spot virus, alfalfa mosaic, alfalfa yellowing, bean chlorotic ringspot, Brazilian tobacco streak, white clover mosaic and yellow mosaic virus disease. Diseases and pests and the high costs of control measures have led to reduced yields of lablab in Kenya (Kamotho *et al.*, 2010).

Marketing in Kenya is another limitation to the production of lablab as farmers lack avenues to sell their produce (Ministry of Agriculture, personal communication). Utilization of the crop for human consumption has also not been extensively studied and is also hampered by its flavour and the colouring effect on food of the black lablab bean varieties (Waldmueller, 1992).

2.2 Importance of Lablab

Lablab has multipurpose uses. The Highworth, Rongai and White cultivars of Lablab have been used successfully as cover crops to suppress weed growth, retard soil erosion and as a green manure (Plate 2.2) (Maundu *et al*., 1999). It is also useful in biologically fixing nitrogen in the soil (McDonald *et al*., 2001), as a legume. Lablab ranks high in terms of crude protein, crude fibre and dry matter digestibility making it a good source of animal feed, either when dry or as green material. It is grazed on by cattle, sheep, pigs and goats, especially during the dry season, as it retains some green growth during drought (Murphy and Colucci, 1999). Dried lablab seeds are a suitable source of protein concentrates. Lablab has been used as a grain legume for more than 3500 years in Asia (Fuller, 2003). The beans have moderately balanced amino acids with high lysine content, and as such complement diets that are heavy on the staples. Crude protein of the *L. purpureus* ranges from 21-34% (Maass and Usongo, 2007). The wild accessions also have relatively high nitrogen content, thus high crude protein levels (Maass and Usongo, 2007). In Kenya, dry and green beans are cooked and eaten by the Kikuyu, Kamba, Maasai, Meru, Embu and Nandi communities. The leaves are also cooked and eaten as vegetable in Central and Coast provinces (Waldmueller, 1992). They are rich in protein (average 28%) and they are a source of iron among legumes (155mg/100g dry weight) (Maundu *et al*., 1999).

2.3 Flavour in lablab

Flavour comprises of odour and taste. It is defined as a perceived attribute resulting from integrated responses to a complex mixture of stimuli on several senses including smell, taste, touch, sight and even hearing (Lawless and Lee, 1993). There are four basic tastes: sweet, bitter, sour and salty while the odour potency of various compounds varies over a wide range, indicating that the compounds may be present in greatly differing quantities.

Plate 2.2: Lablab for soil conservation (a) ground cover by Lablab in Lamu District- Coast province, Kenya (b) Lablab planted on terraces to reduce soil erosion in Mwingi District- Eastern province, Kenya

Some of the factors that are reported to affect flavour are carbohydrates, proteins, phenolic acids and lipids in red kidney beans (van Ruth *et al*., 2004). Volatile components (Kim and Chung, 2008), mastication rates (Buettner and Schieberle, 2000), soaking solutions (De Leon *et al*., 1992; van Ruth *et al*., 2004), enzymatic factors (e.g. lipoxygenase activity), and non-enzymatic factors (e.g. Maillard reactions) (Martins *et al*., 2001), also contribute to flavour differences.

2.3.1 Sensory Evaluation of lablab

In India, lablab is valued for its nutritional and sensory attributes (Venkatachalam and Sathe, 2007). Sensory analyses use human panellists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of food products and other materials (Watts *et al*., 1989). Information on the specific sensory characteristics of a food is obtained by using product-oriented tests in the laboratory using trained sensory panel (Watts *et al*., 1989). Trained panellists are used to identify differences among similar food products or to evaluate intensities of flavour, texture or characteristics of appearances. Tests using sensory panels are conducted under controlled conditions, using appropriate experimental designs, test methods and statistical analyses.

2.3.2 Cyanogenic Glycosides in lablab

Cyanogenic glycosides are carbohydrate derivatives of cyanohydrins (2-hydroxynitriles) produced by plants. All of its known compounds are β-linked, mostly with D-glucose. Cyanogenesis is the ability of some plants to synthesize these cyanogenic glycosides, which when enzymatically hydrolyzed, release cyanohydric acid (HCN), known as prussic acid (Harborne, 1993). In most cases, hydrolysis is accomplished by the β-glucosidase enzyme and leads to the production of sugars and a cyanohydrin that spontaneously decomposes to HCN and a ketone or an aldehyde. The hydrolysis step is also catalyzed by the hydroxynitrile lyase enzyme, which is widespread in cyanogenic plants (Harborne, 1993). In the intact plant, the enzymes and the cyanogenic glycoside remain separated, but if the plant tissue is damaged, the two come into contact and cyanohydric acid is released (Gruhnert *et al.*, 1994). Legumes contain certain cyanogenic glycosides which release hydrogen cyanide (HCN) upon hydrolysis. The linamarin and loustralin, are the cyanogenic glycosides that have been found in the Phaseoeae tribe (Fabaceae Family) (Seigler *et al.*, 1989). Most plants produce a small amount of cyanide associated with ethylene production, but upto 12000 plant species are known to produce sufficient quantities of cyanogenic compounds (Mcmahon *et al*., 1995). Cyanogenic and acyanogenic plants can occur within the same species, and the function of cyanogenesis is revealed through the phenotypic characteristics (Francisco and Pinotti, 2000). The yield of HCN reported for other beans are, limabeans 210.0 to 312.0mg/100g and bengalgram, redgram, peas, kidneybean 0.5 to 2.3 mg/100g (Gupta, 1987).

Apart from cyanogenic glycosides, other factors have also been reported to affect the bitter taste in beans. These include polyphenols e.g. tannins (Bressani and Elias, 1980), minerals e.g. iron (Yang and Lawless (2005); saponins (Heng *et al*., (2004) and Shi *et al.*, (2004); and the malliard reaction (Martins *et al.*, 2001).

2.3.2 Volatile compounds

Volatile compounds are extracted using numerous methods. The methods include the Liquid-liquid extraction (solvent extraction), solid–phase extraction, solid-phase microextraction, super-critical fluid extraction and more sensitive techniques such as the dynamic headspace and static headspace methods (Augusto *et al*., 2003). Solvent extraction is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water

and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. It is a basic technique and it is performed using a separatory funnel. Raw materials are submerged and agitated in a solvent that can dissolve the desired aromatic compounds. Commonly used solvents for extraction include hexane and dichloromethane (Augusto *et al*., 2003). Direct solvent extraction and simultaneous steam distillation–solvent extraction are commonly used for the determination of volatiles in beans (Apriyantono *et al*., 1999; Kim and Chung, 2008). The direct solvent extraction was the method of choice for this study as the extract prepared by this technique usually contains a wide spectrum of volatile components. The choice of solvent for this study was hexane. Hexane, a petroleum-derived product has been extensively used as solvent for the extraction of soya beans because of its low vapourisation temperature (boiling point 63^{o}-69^{o}C), high stability, low corrosiveness, low greasy residual effect, and better aroma and flavour productivity for the milled products (Becker, 1978; Johnson and Lusas, 1983). It is suitable for extraction of non-polar compounds such as aliphatic hydrocarbons. Dichloromethane has high extraction efficiency for a wide rage of non-polar to polar compounds, however, dichloromethane, like benzene, is carcinogenic and it has a low boiling point which would interfere with the recovery of the volatile compounds during concentration uisng a rotavapor.

Lablab has been found to have similar quality but different quantity of most volatile components responsible for the off-flavor in soybean (van Ruth *et al*., 2005). One hundred and five volatile compounds were identified in *L. purpureus* using the gas chromatography- mass spectrometry (GC-MS) technique (Kim and Chung, 2008).

2.4 Molecular Markers and their use in crop diversity

A large and diverse pool of genetic variation is required by plant breeders for crop improvement (McCouch, 2004). For a long time, plant breeders have relied on phenotypic selection to develop new varieties. However, the phenotype is determined by the interaction of genetic and environmental factors. Biochemical markers, like enzymes (isoenzymes/allozymes), are also routinely used to detect differences between individuals (Weeden and Wendel, 1990). These markers only sample actively expressed regions of the genome. This limits their use in certain aspects of plant biology and genetics as co-dominant neutral genetic markers due to lack of adequate polymorphism (Tanksley and Orton, 1983). Molecular markers have the potential to

detect genetic diversity and to aid in the management of plant resources, and are now used to complement phenotypic and protein-based markers (Virk *et al*., 1995; Song *et al*., 2003).

Molecular markers have been established in many plants (Ribaut and Hoisington, 1998). These molecular markers include Restriction Fragment Length Polymorphism (RFLPs), and PCR-based molecular markers which include Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Microsatellites/ Simple Sequence Repeats (SSRs) and Sequence Characterized Amplified Regions (SCARs). These markers offer scientists the potential of making plant genetic improvement progress more precisely and more rapidly than through phenotypic selection. The markers of choice for this study are the AFLP.

2.4.1 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) technology was developed for the detection and evaluation of genetic variation in accession collections and in the screening of biodiversity (Zabeau, 1993; Vos *et al,* 1995). The technique is based on the principle of selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Genomic DNA of an organism is digested with two different restriction enzymes, of which one has a 4-basepair (bp) and the other a 6-bp recognition sequence (Zabeau, 1993). The ends of the resulting restriction fragments are then modified by adding oligonucleotide linkers/adapters about 12-20bp long (Zabeau, 1993). This yields a complex banding pattern when the resticted DNA fragments are separated.

The complexity of the banding pattern is reduced by designing PCR primers in such a way that adjacent to the sequence homologous to the linkers, additional selective nucleotides are added to the 3' end. These 'selective nucleotides' allow the primers to recognize only those restriction fragments which have perfectly matching sequences to the linker and the adjacent nucleotides (Sommer and Tautz, 1989). Polymorphism is then detected by differences in the length of the amplified fragments by denaturing polyacrylamide gel electrophoresis (PAGE). The advantages of amplified fragment length polymorphism (AFLP) markers are that small DNA quantities are used and no prior information on the sequence is required. Unlike RFLPs, AFLP markers are faster, less labour intensive and provide more information. They have an additional advantage over RAPDs as they are reproducible (Steiger *et al.,* 2002), which is essential if

effective screening networks are to be established and results compared between different laboratories. AFLPs have been successfully used to elucidate structures and geographic patterns of diversity as well as pathways of evolution in a wide range of crop species such as common beans (Thome *et al*., 1996), soyabeans (Maughan *et al.*, 1996), azuki beans (Yee *et al.*, 1999), eggplant (Mace *et al*., 1999), grapes (Goto-Yamamoto, 2000), sweet potato (Zhang *et al*., 2000), tea (Wachira *et al.*, 2001) and coffee (Steiger *et al*., 2002).

AFLPs have been used to show genetic diversity in lablab accessions from other countries; India Commonwealth Scientific and Industrial Research Organisation (CSIRO) 103 germplasm accessions (Maass *et al.*, 2005), 62 landraces collected from southern India, (Venkatesha *et al*., 2007), and 40 other accessions across India (Patil *et al*., 2009). Other markers that have been used to show genetic diversity in lablab bean are the simple sequence repeats on 47 accessions from United States Department of Agriculture (USDA) collections (Wang *et al.*, 2007), gene specific primers and expressed sequence tags (EST) generated from related species on 62 landraces collected from Southern India (Venkatesha *et al*., 2007); Random amplified polymorphic DNA (RAPDs) on 40 accessions from CSIRO collections (Liu 1996), 60 accessions from Bangladesh/Japan and CSIRO germplasm (Sultana *et al*., 2000); and 11 varieties from China (Tian *et al*., 2005).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Plant material

A total of 50 lablab accessions were sourced from the National Repository Centre at the Kenya Agriculture Research Institute (KARI) -Muguga genebank and farmers fields in Eastern, Central, Coast, and Rift Valley provinces, creating a diverse collection for the study. Twenty two accessions from the genebank and two varieties from farmers were used in the sensory tests and biochemical analysis (Table 3.1) while the total 50 germplasm accessions (Table 3.2), were characterized using AFLP molecular markers. The accessions were bulked at KARI-Njoro in the field, in Njoro District of Rift Valley province (0 20'S; 35 56'E; 2166m above sea level (asl). The accessions were selected based on their high yields, they were then cleaned and dusted with actellic super and stored at room temperature awaiting use. The seeds were washed and air dried to remove the pesticide before the tests were carried out.

3.2 Sensory Evaluation (Organoleptic Tests)

Dry seeds were harvested from the 24 lablab bean accessions selected for this study (Table 3.1). The seeds were cleaned and cooked at the Egerton University Food Science Department sensory testing facilities. A random panel of 15 trained tasters (panellists) was used to evaluate the samples. The panel comprised of male and female students of Food Science department at Egerton University, who had been trained on tasting. Panellists were seated in individual booths equipped with white fluorescent light. The samples were boiled in distilled water until cooked and 5 seeds of each sample were served to each panellist while warm (about 40^{o}C) in identical containers. The experiment was used a random complete block design. The samples were coded with 3-digit random numbers and presented in a random order. Water was provided for rinsing the palate between the samples.

Table 3.1: *Lablab purpureus* accessions used for sensory and biochemical tests

Accession No.	Accession Name	Province of Collection
1.	10702*	Eastern
2.	10695*	Eastern
3.	11719*	Eastern
4.	10706*	Rift Valley
5.	12230*	Eastern
6.	13129*	Eastern
7.	12000*	Eastern
8.	11705*	Coast
9.	Njoro#	Rift Valley
10.	Bahati#	Rift Valley
11.	11723*	Nairobi
12.	11741*	Eastern
13.	13086*	Eastern
14.	10822*	Coast
15.	26932*	Eastern
16.	28663*	Eastern
17.	12158*	Eastern
18.	13083*	Eastern
19.	10703*	Eastern
20.	12187*	Coast
21.	11722*	Eastern
22.	11736*	Coast
23.	27007*	Eastern
24.	13096*	Eastern

*#sourced from farmers; *sourced from the National genebank*

Each sample was evaluated by each panellist (blocks) three times (on three different days). The sensory characteristics, i.e. bitter taste and odour, were evaluated through quantitative descriptive analyses. The panellist were instructed to evaluate each sample, and indicate the intensity of the specified characteristic by checking an appropriate category (for bitter taste), and ordering them using five descriptive terms; trace, slightly intense, moderately intense, very intense and extremely intense, and by making a vertical mark on a 15cm line scale used to order odour intensity as suggested by Quirien and Keith (2005). Trace checked was if no bitter taste was detected; slightly intense was for the presence of a mild bitter taste, while a strong was for the extremely intense bitter taste.

3.2.1 Statistical analysis of organoleptic data

For analysis of category scale for the bitter taste data, the categories were converted to numerical scores by assigning successive numbers to each category; 0 was assigned to the lowest intensity (trace), and 5 to highest intensity (extremely intense). For analysis of line scale odour data, panellists' marks were converted to numerical scores by measuring the distance in centimetres from the left or lowest intensity point on the scale to the panellists' mark. The scores were converted using 0.5cm=1unit score as suggested by Quirien and Keith (2005). The data was subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedure, which is the most common parametric test for interval scale sensory data to determine if significant differences existed among the samples. Differences between the means were ranked by Fisher's least significant difference (LSD) test, where using the SAS software version 9.1.3 (SAS Institute, Inc., 2004). This was performed on those that showed significant differences. Correlation coefficients were determined to establish the relationship between the variables (Bower, 2000).

3.3 Biochemical Assays

3.3.1 Chemical assay of cyanogenic glycosides

In this study, cyanogenic glycosides were detected qualitatively using the picrate-impregnated paper technique described by Harborne (1972) and modified by (Williams and Edwards, 1980). It was performed in triplicate in a complete block design and carried out for the 24 lablab accessions shown in Table 3.1. The seeds were ground using a blender and one gram of the sample placed inside a test tube. For leaves, five 1cm diameter discs were punched from young leaves of lablab plants. Five drops of toluene were added to the tube and a filter paper strip, saturated with alkaline picrate, was suspended above the sample by holding it tightly against a rubber cork. The filter paper strips (5.0 X 1.5cm) were treated soaked in an aqueous solution of 0.05M picric acid that was previously neutralized with sodium bicarbonate, filtered and left to dry at ambient temperature. Contact between the strip and the sample inside the tube was avoided. The contents were left at room temperature. Observations were made after 4, 6, 24 and 48 hours.

Table 3.2: Lablab accessions used for molecular characterization and the province of collection

Accession No.	Accession Name	Province	Accession No.	Accession Name	Province
1	45349*	Eastern	26	12230*	Eastern
2	Mwingi-1#	Eastern	27	10841*	Coast
3	Mwingi-2#	Eastern	28	12038*	Coast
4	Meru Central-1#	Eastern	29	Lamu-1#	Coast
5	Mbeere#	Eastern	30	Lamu-2#	Coast
6	Machakos-1#	Eastern	31	10699*	Coast
7	10707*	Eastern	32	Lamu-3#	Coast
8	Machakos-2#	Eastern	33	11736*	Coast
9	Mwingi-3#	Eastern	34	12187R3*	Coast
10	Machakos-3#	Eastern	35	12187R2*	Coast
11	Meru Central#	Eastern	36	11705*	Coast
12	27007*	Eastern	37	Thika-1#	Central
13	10703*	Eastern	38	Thika-2#	Central
14	13083*	Eastern	39	Thika-3#	Central
15	11719R2*	Eastern	40	Thika-4#	Central
16	13096*	Eastern	41	Thika-5#	Central
17	13129*	Eastern	42	Maragwa#	Central
18	12000*	Eastern	43	10824*	Rift Valley
19	28663*	Eastern	44	Njoro-1#	Rift Valley
20	12230R3*	Eastern	45	Bahati#	Rift Valley
21	12158*	Eastern	46	10706R1*	Rift Valley
22	10702*	Eastern	47	Njoro-2#	Rift Valley
23	26932*	Eastern	48	10706*	Rift Valley
24	11741*	Eastern	49	11723*	Nairobi
25	13129R1*	Eastern	50	11723 R1*	Nairobi

*#sourced from farmers; *sourced from the National Genebank*

Cassava leaves and roots from variety 990072 with reported values of 59.17 and 94.23 ppm average HCN amount (Ndung'u *et al*., 2008) were used as the positive control. Sodium picrate (yellow) is converted to sodium isopurpurate (brick-red) by free hydrocyanic acid. The colour change on the picrate strip was compared with that of a pre-set colour scale of 1-9 depending on the basis of intensity of yellow to red colour.

3.3.1.1 Analysis of cyanogenic glycosides data

The score structure ranged from 1-9 representing >10, 10-15, 15-25, 25-40, 40-60, 60-85, 85-115, 115-150 and >150ppm (Williams and Edwards, 1980). A brown-red coloration within 2

hours indicated the presence of cyanogenic glycoside and the respective hydrolytic enzyme, while a brown-red color appearing within 48 hours indicated that the cyanogenic glycoside spontaneously released hydrogen cyanide (HCN) without the action of enzyme. No colour change after 48 hours indicated that the test was negative for cyanogenic glycosides. Data was subjected to Analysis of variance (ANOVA) using General linear model using SAS software Version 9.1.3 (SAS Institute, Inc., 2004).

3.3.2 Analysis of volatile compounds of Kenyan *Lablab purpureus*

Identification of the volatile components in the raw beans was carried out to ascertain the compound classes present in lablab. Raw beans were used to establish the genetically fixed aroma profile traits. Volatile compounds were cold extracted using GC grade hexane (BDH, England) to collect preliminary data as suggested by Mestres *et al*. (2000). Dry lablab seeds of the 24 samples (Table 3.1) were ground into powder with a blender. Fifty grams of ground seed for each of the sample was put into a separating funnel and to it added 100ml of analytical grade hexane. The separating funnel was corked and the sample shaken vigorously, while releasing pressure by opening and closing the valve. The separating funnel was then clamped on a stand and the cork removed. The solution was filtered slowly into a 250ml conical flask through a filter paper (Whatman 1, diameter 125mm). Any sample that passed through the filter paper was put back into the flask. The extraction was repeated twice by adding 50ml of hexane to the separating funnel each time and shaking it vigorously as earlier and filtering through a new whatman filter paper. The filtrate collected in the conical flask was sealed with parafilm and stored in the dark awaiting concentration.

The extracts were concentrated by evaporating the hexane using a rotavapor (BÜCHI Rotavapor R-205, Labortechnik GmbH, Essen, Germany). The water bath was cleaned and filled with clean water, before it was switched on to a temperature of 60^{o}C. The extract was put into the evaporating flask and fixed to the rotavapor. The flask was lowered into the waterbath, and the pump tube fixed to the condenser, cooled with tap water. The rotation knob was set to a speed of 42rpm and the pressure pump switched on. Evaporation was allowed to continue until about 1ml remained. The rotation knob was then set to zero and the tube for the pump removed to release any pressure, before switching off the pump. The evaporation flask was lifted from the water bath using the button on the handle upwards the flask and unscrewed. About 1ml of the

concentrated sample was transferred into a 5ml sample bottle and loosely closed to allow evaporation of the remaining hexane in the dark at room temperature for two days. The samples were weighed and stored at -20^{o}C before gas chromatography (GC) analysis.

One hundred microliters of hexane were added to the sample bottle the night before GC analysis to dissolve the extracts. Five (5) microliters of the sample was then injected into the GC. A Shimadzu GC (Model GC2010, Tokyo, Japan) fitted with a 30-m fused silica open-tubular column (ZB-5, 0.25 mm i.d., 0.25mm film thickness, Phenomenex,) with phase composition of 5% phenyl and 95% dimethylpolysiloxane was used. The GC was operated under the following conditions: initial and final temperatures and holding times were 32^{o}C for 5 min and 195^{o}C for 5min, respectively; the ramp rate was 2^{o}C/min. Flame ionisation detector (FID) was used at 250^{o}C and injector temperature was 220^{o}C. The carrier gas was helium with a flow rate of 10.5ml/min.

3.3.2.1 Analysis of chromatogram

The identification of compounds from gas chromatogram was carried out by comparing the peak kovats/retention indices with those found in available literature (Adams, 1995) and online database, Pherobase (El-Sayed, 2005) as discussed by Babushok *et al.,* (2007). Kovats index was calculated by drawing a calibration line using the retention times (in seconds) of the n-alkanes, and their kovats indices reported in literature (Adams, 1995). The data was subjected to analysis of variance (ANOVA) by the general linear models (GLM) procedure, and differences which were statistically significant were ranked by least significant differences (LSD) using the SAS software version 9.1.3 (SAS Institute, Inc., 2004). Similarity levels were obtained from the retention times of all the major peak areas of the volatile compounds using MINITAB 11.12 statistical analysis software (MINITAB Inc, State College, Pennsylvania, USA, 1996).

3.4 Molecular Characterization

3.4.1 Genomic DNA Isolation

Seven DNA isolation protocols (summarized in Table 3.3) were evaluated on a sub-sample of six lablab accessions. The major differences in the protocols evaluated mainly concerned the contents of the extraction buffer. This included the use of cetyltrimethylammonium bromide (CTAB) or sodim dodecyl sulphate (SDS) as detergents and

use of dithiothreitol (DTT) or β-mercaptoethanol as reducing agents. Other differences were in the incubation time at 65C and in purification of the DNA either using chloroform: isoamyl alcohol (24:1), Phenol: chloroform: isoamyl alcohol (25:24:1), or 5M potassium acetate (Table 3.3). A modification of the cetyltrimethylammonium bromide (CTAB) method described by Gawel and Jarret (1991) and modified by James *et al*., (unpublished) yielded the best results of intact high molecular weight DNA and was carried out as detailed below. Four hundred milligrams fresh leaf material was ground using a mortar and pestle in 3ml of 2x CTAB extraction buffer (2% CTAB, 100mM Tris- Hydrochloric acid (HCl) pH 8.0, 1.4M Sodium chloride (NaCl), 50mM Ethylenediaminetetraacetic acid (EDTA), 2% Polyvinylpyrolidone (PVP) 10, 2% β-mercaptoethanol). The slurry was transferred to 2, 1.5ml microfuge tubes and incubated at 65^{o}C for 15minutes in a water bath with constant shaking, then centrifuged at 13000rpm for five minutes (Eppendorf 5415C, Germany). Seven hundred and fifty microliters (750µl) of the supernatant were transferred to a fresh 1.5ml eppendorf tube and an equal volume of chloroform:isoamyl alcohol (24:1) was added. The eppendorf tubes were shaken well before separating the contents in a centrifuge at 13000rpm for 5 minutes. Six hundred microliters (600µl) of the aqueous phase was transferred into a fresh tube and an equal volume of chloroform: isoamyl alcohol (24:1) added. The tubes were shaken well as before and then centrifuged for 5 minutes at 13000rpm. About 450µl of the aqueous phase was transferred to a fresh tube and an equal volume of ice-cold isopropanol added, and mixed by inverting several times to precipitate the DNA. The tubes were centrifuged at 13000rpm for 5 minutes. The supernatant was decanted, leaving the DNA pellet at the bottom of the tube. The pellet was washed using 500µl of 70% ethanol, and spun for 1minute before they were air dried for 1hour. The dried pellet was re-suspended in 50µl of sterile distilled water. RNA was removed by adding two microliters of pancreatic ribonuclease A (RNase A) (10mg/ml) and incubating the samples for 1 hour at 37^{o}C. The samples were stored at -20^{o}C.

3.4.2 Estimation of DNA Concentration and Quality

The quantity and quality (intactness) of genomic DNA was examined by comparing the isolated DNA samples with uncut, unmethylated lambda (λ) DNA standards of known concentrations in a 0.8% agarose (Sigma, UK) gel in 1x TBE buffer (89.2mM Tris, 89.0mM Boric acid, 1.25mM EDTA pH 8.0). The spectrophotometric method based on optical density

(OD) readings at 260nm and 280nm was also used to determine yield and quality of isolated DNA using a biophotometer (Eppendorf, bioPhotometer, Germany). DNA has a maximal absorbance at 260nm and an OD of 1.0 is equivalent to 50µg/ml of DNA (Maniatis et al., 1982). An OD ratio of 260/280nm was used to establish the purity of DNA samples. Pure preparations of DNA have OD260/OD280 value of 1.8 and a deviation from this signifies the presence of contaminants in the DNA that may inhibit PCR.

3.4.3 Amplified Fragment Length Polymorphism

3.4.3.1 Restriction of DNA, adaptor-ligation, and Pre-selective PCR

The isolated DNA was digested, adaptor ligated and pre-selective PCR carried out as described by Waugh (1994). The digestion was carried out using two restriction enzymes (*Mse*I and *EcoR*I). *Mse*I is a 4-base (frequent) cutter with a T/TAA cutting site, whereas *EcoR*I is a 6-base (rare) cutter with a G/AATTC recognition cutting site. The digestion reaction comprised of 2µg of template genomic DNA, 10µl of restriction-ligation buffer (5xRL) (50mM TrisAc pH 7.5, 50mM MgAc, 250mM KAc, 25mM DTT), 2.5% w/v Bovine Serum albumin (BSA), 4 units of *EcoR*I enzyme and 4 units *Mse*I enzyme. The digest was made upto 30µl with sterile distilled water, and incubated at 37^{o}C overnight. Five microlitres of the digest was run in a 1.5% agarose gel in 1xTBE buffer (89.2mM Tris, 89.0mM Boric acid, 1.25mM EDTA pH 8.0), to establish whether all samples were completely digested. Sterile double distilled water was used as the negative control. The digested DNA was ligated to adaptors of the enzymes in a restriction-ligation reaction mix containing, 25µl of the digested DNA, 2µl of 5xRL buffer, 1µl of 5pmol/µl *EcoR*I adaptor, 1µl of 50pmol/µl *Mse*I, 1µl of 10mM adenosine triphosphate (ATP) and 1µl of 1unit/µl T4 DNA ligase. These were made upto 35µl with sterile distilled water. The tube was then tapped to mix the contents before incubating overnight at 37^{o}C.

After incubation, a pre-selective PCR was carried out using the method described by IAEA (2002). The restriction-ligation reaction (2.0µl) was added into a PCR tube and in it added 10.8µl sterile distilled water, 5µl of 5x PCR buffer, 1µl *EcoR*I (75ng/µl) preselective primer (E00), 1µl *Mse*I (75ng/µl) preselective primer (M00) (Table 3.4), 2.5µl of 2mM deoxynucleotides triphosphates (dNTPs- dATP, dCTP, dGTP, dTTP), and 0.02µl of Hotstart*Taq* (5U/µl) polymerase (Promega, Madison, Wisconsin, USA). These were mixed by slight vortexing of the tube.

Table 3.3: DNA Isolation protocols evaluated

Method	Summarized Detail	Reference
SDS minipreparation method with β-mercaptoethanol	200mg of fresh leaf tissue, SDS extraction buffer (100mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0, 500mM NaCl, 20% SDS) 0.07% β- mercaptoethanol; Incubation at 65^{o}C for 15minutes; Purification: 5M potassium acetate, treated with RNase A	Dellaporta *et al*., 1983
CTAB large scale method with 0.1% Dithiothreitol (DTT)	2g of fresh leaf tissue, 2x CTAB extraction buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 1.4M NaCl, 20mM EDTA), 0.1% DTT; Incubation at 65^{o}C for 30minutes; Purification using chloroform:Isoamyl alcohol (24:1), treated with RNase A	Gawel and Jarret (1991)
CTAB small scale method with 0.1% DTT	0.5g of fresh leaf tissue, 2x CTAB extraction buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 1.4M NaCl, 20mM EDTA), 0.1% DTT; Incubation at 65^{o}C for 30minutes; Purification using chloroform:Isoamyl alcohol (24:1): treated with RNase A	Gawel and Jarret (1991)
CTAB method with 0.4% β-mercaptoethanol	0.8g fresh leaf tissue, 2x CTAB extraction buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl) , 0.4% β-mercaptoethanol; Incubation at 65^{o}C for 1 hour; Purification: chloroform:isoamyl alcohol (24:1) and $MgCl_2$, and further precipitation using sodium acetate and isopropanol, treated with RNase A	University of Agricultural Sciences (2004)
CTAB method with 0.2% β-mercaptoethanol	0.1g fresh leaf tissue, 2X CTAB extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris HCl 8.0, 1% PVP), 0.2% β-mercaptoethanol; Incubation at 65°C for 45 min, Purification using: chloroform:isoamyl alcohol (24:1) and reextraction using 10% CTAB (in 0.7 M NaCl) and further purification using chloroform:isoamyl alcohol (24:1).	Doyle and Doyle, 1990 (modified at NCSU Forest Biotechnology Laboratory)

Table 3.3: Continued

Method	Summarized Detail	Reference
SDS minipreparation method with 6.5mM DTT	50mg fresh leaf tissue, SDS extraction buffer (200mM Tris-HCl pH 7.5, 25mM EDTA pH 8.0, 250mM NaCl, 10% SDS) 6.5mM DTT; No incubation at 65°C; Purification: phenol:chloroform:IAA (25:24:1); treated with RNase A	Matasyoh *et al*., (2008)
CTAB method with 2% β-mercaptoethanol	0.4g of fresh leaf tissue, 2x CTAB extraction buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 1.4M NaCl, 50mM EDTA, 2% PVP 10), 2% β-mercaptoethanol; Incubation at 65^{o}C for 30 minutes. Purification using chloroform:iosamyl alcohol (24:1) twice; treated with RNase A	Gawel and Jarret (1991) modified by (James *et al*., unpublished

The tube was placed in the PCR machine (Bioneer, MyGenie 96, Daejeon, South Korea) and DNA amplified, using the following temperature profile (94ºC for 30 secs; 60ºC for 30secs; 72ºC for 1 min) times 30 cycles and a final extension at 72ºC for 10 minutes. Five microlitres of the resultant PCR-amplified product was loaded on a 1.5% agarose gel in 1x TBE buffer, and electrophoresis carried out at 80V for 30 minutes, and visualized in a UV-transilluminator (SYNGENE, Synoptics Ltd, Cambridge UK).

3.4.3.2 Selective Amplification

The pre-selectively amplified DNA was diluted by adding 100µl of $T_{0.1}E$ (10mM Tris HCl pH 8.0, 0.1mM EDTA) buffer) (referred to as test DNA) and used in the selective amplification step (IAEA, 2002). The test DNA (2.5µl) was added in a 0.2ml PCR tube to 1xPCR buffer, 0.75µl *EcoR*I and 0.75µl *Mse*I selective primers (50ng/µl) (Table 3.4), 2µl of 2mM each dNTP and 0.05µl of 5U/µl Hotstart*Taq* DNA polymerase (Promega, Madison, Wisconsin, USA). The volume was made upto 10µl with sterile distilled water. These components were mixed by gentle vortexing before placing the tube in the PCR thermal cycler (Bioneer, MyGenie 96) for amplification using the following temperature profile (94ºC for 30 secs; 65ºC for 30secs reducing by 0.7ºC each subsequent cycle to 56ºC; 72ºC for 1 min) times 11

cycles; (94ºC for 30 secs; 56ºC for 30secs; 72ºC for 1 min) times 24 cycles and then held at 4ºC. A negative control was included in the assay. This included all the components of the selective AFLP-PCR except for the template DNA which was replaced with sterile double distilled water. The AFLP primer pairs were selected on the basis of previous lablab studies (Maass *et al.*, 2005).

3.4.3.3 Preparation of the electrophoresis plates and 6% polyacrylamide gel

Vertical gel electrophoresis plates were prepared by rinsing and drying the upper surfaces with 100% ethanol. Repellant (Rain repellant, Halford,UK) was applied to the upper surface of the long plate and spread evenly using a cloth and allowed to dry. Five hundred microlitres of bind silane (Promega, Madison, Wisconsin, USA) was applied to the upper surface of the short plate, with attached spacers (1.5mm thick) and spread evenly using a cloth. The plates were clamped together. Gel solution was prepared using 150ml acrylamide/ bis solution 19:1, 100ml 10x Tris Borate EDTA (TBE) buffer, 420g urea 6M and made upto one litre with distilled water that was stored at 4^{o}C. Twenty microlitres of TEMED and 200µl of 10% freshly prepared ammonium persulphate solution were added to 20ml of gel solution just before the gel was poured. This was mixed and the gel poured in between the plates. The comb was inserted and the gel left to polymerise for approximately 1 hour. The comb was gently removed and the plates clamped into the electrophoresis tank. Two litres of 1xTBE buffer were added into the tank to full level. The wells were cleaned using distilled water before the rig was connected to a power supply, switched on and pre-run for 30 minutes at 100volts. The samples were prepared by adding 10µl of formamide dye mix to 10µl of the PCR amplification products and denatured for 3 minutes at 95^{o}C and placed on ice. Five microlitres (5µl) of each sample was loaded into individual wells of the gel, and run alongside 3µl of 100bp molecular weight size standards (2-log DNA ladder, New England Biolabs [NEB] Ipswich, Massachusetts, USA). The gel was run until the blue front of the dye ran off the bottom of the gel. DNA was visualized using silver staining (CIMMYT, 2005).

Table 3.4: Preselective and selective primer combinations (selective bases on the 3'end)

Pre-selective Primers
EcoRI + (A)
MseI + (C)
Selective Primer Combinations
EcoRI + (ACA)/MseI + (CAC)
EcoRI + (ACC)/MseI + (CTA)
EcoRI + (AGC)/MseI + (CTA)
EcoRI + (ACA)/MseI + (CTC)
EcoRI + (ACC)/MseI + (CTC)
EcoRI + (ACC)/MseI + (CAC)
EcoRI + (ACT)/MseI + (CTC)
EcoRI + (AGC)/MseI + (CGC)
EcoRI + (ACT)/MseI + (CAT)
EcoRI + (AAC)/MseI + (CTA)
EcoRI + (AGC)/MseI + (CAT)
EcoRI + (AAC)/MseI + (CAC)
EcoRI + (AGC)/MseI + (CAC)
EcoRI + (ACA)/MseI + (CGC)
EcoRI + (ACC)/MseI + (CAT)

3.4.3.4 Silver staining of the polyacrylamide gel

After electrophoresis, the plates were separated and the shorter plate with the gel placed in a container. The DNA bands were fixed with 200ml of fixer solution (10% ethanol with 0.5ml/100ml acetic acid) for 5 minutes while shaking, poured and the gel rinsed with distilled water. 0.2% silver-stain (2g silver nitrate in one litre distilled water) was added to the container and left shaking for 10 minutes. The silver nitrate solution was poured off and the gel and container thoroughly rinsed with distilled water. Prior to developing the gel, developer was prepared from 1ml of 40% formaldehyde solution added to 200ml of the 3% sodium hydroxide (3% NaOH and 0.5ml/100ml formaldehyde). The gel was agitated in developer until the bands near the bottom of the gel were visible. The developer was poured off and the gel rinsed with distilled water, before stopping the reaction by adding 200ml of stop solution (10% acetic acid) for 5 minutes (CIMMYT, 2005). The gel was rinsed in distilled water before it was photographed against white light (White Transilluminator, UVP, USA), by placing the glass plate with the gel on the white box and using the light to visualise the bands clearly.

3.4.3.5 AFLP Data Analysis

Amplified Fragment Length Polymorphism (AFLP) data was scored twice manually in a binary form, as presence (1) and absence (0) of band. The band sizes were estimated by comparison with 100bp standard (2-log DNA ladder, NEB). Markers ambiguous in a few genotypes were treated as missing data. Only those fragments that could be clearly scored were used. The binary file was then configured as an input file in an Excel spreadsheet and analyzed using Genetic Analysis in Excel (GenAlEx) version 6.2 software (Peakall, and Smouse, 2006) which computed allele frequencies, expected heterozygosity, genetic distance between pairs of populations, using the method of Nei (1978), analysis of molecular variance (AMOVA), cluster analysis and principal coordinate analysis (PCoA). Populations were delineated based in source.

Genetic diversity within each population was calculated as the mean genetic diversity over all loci from all populations. The expected heterozygosity (H_e) for binary data followed the method of Lynch and Milligan (1994) assuming complete selfing.

$$H_e = 1 - \Sigma p^2$$

where assuming random mating:

Presence represented both genotypes *AA* or *Aa*,

Absence represented the genotype *aa*

Allele *A* has Frequency (Freq.) p = 1-q; - Allele *a* has Freq. q = 1-p

Freq. of genotype *aa* is q^2 = Freq. of absence= 1- Freq. of presence. So, q = √(Freq. of absence).

Shannon information index (Shannon and Weaver, 1949) was also used as a measure of gene diversity.

GenAlEx offered the calculation of standard genetic distance (Nei, 1978) between pairs of populations. Nei's Genetic Distance (Nei_D) which was calculated as

$$Nei\_D = -\ln(I)$$

Where *I* is Nei's Genetic Identity (Nei_I). Nei_I was calculated as below:

$$Nei\_I = \frac{J_{xy}}{\sqrt{(J_x J_y)}}$$

$$J_{xy} = \sum_{i=1}^{k} p_{ix} p_{iy}$$

$$J_x = \sum_{i=1}^{k} p_{ix}^2,$$

$$J_y = \sum_{i=1}^{k} p_{iy}^2$$

Where p_{ix} and p_{iy} were the frequencies of the *i*-th allele in populations *x* and *y*. For multiple loci, J_{xy}, J_x and J_y are calculated by summing over all loci and alleles and dividing by the number of loci. These average values were then used to calculate *I*.

The analysis of molecular variance (AMOVA) was done using GenAlEx version 6.2. It allowed the hierarchical partitioning of genetic variation into within and among population and components. The estimate of PHIpt (Φpt), an analog of F_{st} for binary data, which also estimates partition of genetic diversity within and among populations, was derived. AMOVA procedure followed the methods of Excoffier *et al*., (1992) and Huff *et al*., (1993). Φpt via AMOVA without regional data structure was calculated by:

$$\phi_{PT} = \frac{V_{AP}}{(V_{AP} + V_{WP})}$$

Where: V_{AP} was the variance among populations and V_{WP} the variance within populations. Φ*PT* was calculated as the proportion of the variance among populations, relative to the total variance.

Genetic difference between the *L. purpureus* accessions was determined through derivative of average expected heterozygosity (H_e) of the accessions using the POPGENE version 1.32, (Yeh *et al*., 2000) software assuming Hardy-Weinberg equilibrium and no population structure. A genetic identity distance matrix was derived and a dendogram based on the unbiased Nei's (1978) genetic distances matrix was constructed using unweighted pair group method of arithmetic averages (UPGMA) to reveal genetic relatedness among the accessions. The UPGMA option constructed a tree by successive (agglomerative) clustering using an

average-linkage method of clustering (Sneath and Sokal, 1973), which is the most commonly adapted clustering algorithm.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results

4.1.1 Sensory Evaluation

The results from sensory evaluation were obtained from eleven of the fifteen panellists and were used for analyses because some panellists did not turn up for all the evaluation sessions. The mean data scores for odour intensity and bitter taste are presented in Table 4.1 below. The mean odour intensity scores for the 24 accessions ranged from 2.68 to 3.60 and no significant ($P>0.05$) difference were observed (Table 4.1). However, significant ($P<0.05$) differences were observed in the level of bitterness of the 24 accessions. The score for bitterness ranged from 1.42 to 2.42. From the separation of the means for bitterness, accession 10706 ranked highest. The same accession also ranked highest for odour intensity. Accession 13096 ranked lowest for the two quality traits.

Statistically significant positive correlation was observed between odour intensity and bitter taste ($r = 0.510$, $P<0.05$) of the 24 *L. purpureus* accessions. There was insignificant positive correlation between the colour of the accession and the odour intensity ($r=0.046$, $P>0.05$) and bitter taste ($r=0.0271$, $P>0.05$). The colour of the seeds therefore did not show any relationship with the level of bitterness or odour intensity. From the data (Table 4.1), the black, brown and speckled (dark coloured seeds with black spots) coloured accessions had varying intensities (high, medium and low) of both odour intensity and taste. Both the accessions that had the highest and lowest intensity for odour and bitter taste, 10706 and 13096, were brown in colour. Accession 13096 ranked the lowest, with the lowest mean value for both the odour and bitter taste, while 10706, ranked the highest (Table 4.2). The brown accessions 11705, 11736, 12000, 12187, and 10703 also showed low intensity mean values for the two parameters and had lower ranks. The black accessions ranked 14-18, with 13083, ranking at 23 and 10822 and Njoro accessions with lower rankings of number 9 and 10. The speckled accessions ranked 7 and 8. However, it was noted that the beans change colour during cooking due to the solubilisation of the colour pigments, and the speckled do not appear as speckled after cooking, tending to dark brown.

Table 4.1: The mean scores and standard deviations for odour intensity and bitter taste of *L. purpureus* accessions

Accessions	Colour	Mean odour*	Mean bitter taste
10706	Brown	3.60 ± 1.30	2.42^{a} ± 1.31
Bahati	Black	3.18 ± 1.21	2.09^{ab} ± 1.01
11741	Speckled	2.78 ± 1.44	2.09^{ab} ±1.18
10702	Brown	3.09 ± 1.23	2.03^{abc} ± 1.16
10695	Brown	3.31 ± 1.32	2.00^{abc} ± 1.11
13083	Black	3.56 ± 1.36	2.00^{abc} ± 1.08
11719	Brown	3.24 ± 1.57	1.94^{bc} ± 0.97
26932	Black	3.15 ± 1.28	1.91^{bcd} ± 1.18
13086	Black	3.31 ± 1.41	1.88^{bcde} ± 0.99
12158	Black	3.19 ± 1.42	1.84^{bcdef} ± 0.72
27007	Black	3.17 ± 1.38	1.84^{bcdef} ± 1.02
11723	Black	3.25 ± 1.23	1.82^{bcdef} ± 0.88
28663	Speckled	3.03 ± 1.21	1.79^{bcdef} ± 0.89
11736	Brown	2.80 ± 1.32	1.78^{bcdef} ± 0.79
12000	Brown	2.79 ± 1.40	1.73^{bcdef} ± 0.91
Njoro	Black	3.01 ± 1.39	1.73^{bcdef} ± 0.88
10703	Brown	2.90 ± 1.23	1.72^{bcdef} ± 0.81
10822	Black	3.08 ± 1.16	1.70^{bcdef} ± 0.85
12230	Brown	3.18 ± 1.21	1.69^{bcdef} ± 0.74
13129	Brown	3.11 ± 1.25	1.67^{bcdef} ± 0.89
11705	Brown	2.80 ± 1.28	1.61^{cdef} ± 0.79
12187	Brown	2.94 ± 1.17	1.50^{def} ± 0.72
11722	Light Brown	3.39 ± 1.37	1.47^{ef} ± 0.72
13096	Brown	2.68 ± 1.14	1.42^{f} ± 0.67
Mean		3.11 ± 0.22	1.82 ± 0.16
LSD		n/s	0.05

*Means within a column followed by the same letter are not significantly (p>0.05) different according to the LSD) test. n/s= Not significant. *- data has been transformed by square root method {SQRT = (Y+1)}*

Table 4.2: Rankings for the *L. purpureus* accessions for odour intensity and bitter taste and the mean ranking of the two parameters (1-lowest intensity, 24- highest intensity)

	Parameters		Mean for Parameters
Rankings	Taste	Odour	
1	13096	13096	13096
2	11722	11736	11705
3	12187	11705	11736
4	11705	12000	12000
5	12230	10703	12187
6	13129	11741	10703
7	10822	12187	11741
8	10703	28663	28663
9	Njoro	10822	10822
10	12000	Njoro	Njoro
11	28663	10702	13129
12	11736	13129	12230
13	11723	12230	10702
14	12158	26932	26932
15	27007	Bahati	11723
16	13086	11723	Bahati
17	26932	27007	27007
18	11719	12158	12158
19	10695	13086	13086
20	13083	10695	10695
21	10702	11719	11719
22	11741	11722	11722
23	Bahati	13083	13083
24	10706	10706	10706

4.1.2 Detection of cyanogenic glycosides

Sodium picrate (yellow) is converted to sodium isopurpurate (brick-red) by free hydrocyanic acid. The lack of a colour change on the picrate strip in tests carried out in this study was therefore an indication of lack of significant levels of cyanogenic glycosides in the seeds of the 24 accessions of *Lablab purpureus*. Indeed, the colour remained yellow on the picrate strip for all the test accessions after 0, 2, 4, 8, 24 and 48 hours, which corresponded to a concentration

of 0-10ppm of cyanogenic glycosides on the colour chart. The positive control in this test showed a colour range of 3 for cassava leaves and 4 for roots (cassava variety 990072). The colour change occurred after 2 hours (Plate 4.1), indicating the presence of cyanogenic glycosides and the respective hydrolytic enzyme in the cassava control. It was not possible to see the differences in the levels of cyanogenic glycosides between samples.

Plate 4.1: Analysis of cyanogenic glycosides for *L. purpureus* seed samples using the picrate method: cassava positive controls are shown at each end of the test tube rack

4.1.3 Identification of volatile compounds in *Lablab purpureus* accessions

Volatile compounds associated with odour were extracted from the lablab beans accessions and analyzed using gas chromatography. A representative sample chromatogram is shown in Figure 4.1. The ANOVA of the retention times revealed that there were significant differences ($p<0.05$) among the accessions (Table 4.3). This implied that the samples significantly affected the relative retention times. The Kovats indices were calculated from the retention times using the equation generated on the calibration curve (Figure 4.2), where y= kovats index and x=retention time in seconds.

Table 4.3: Mean squares of the retention times of the volatile compounds for the 24 *L. purpureus* accessions

Source	df	Squares	Mean Square	F Value	Pr>F
Accession	23	90149.7	3919.6	16.05	0.0001
Error	6200	1514369.1	244.3		
Total	6223	1604518.8			

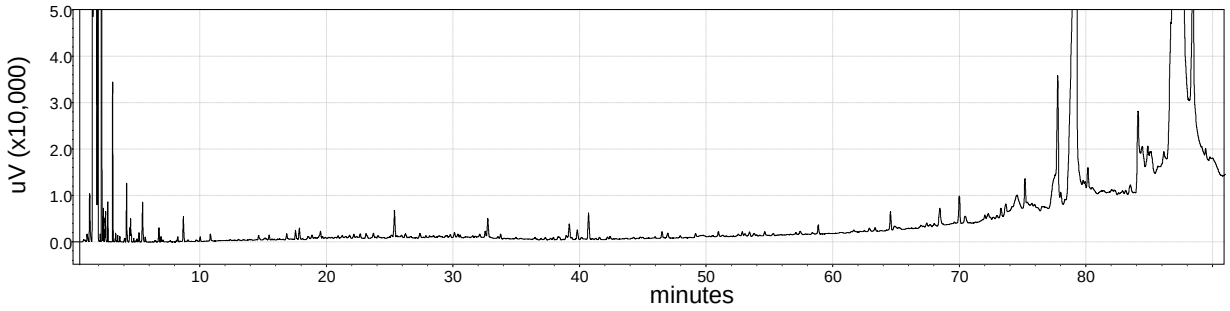

Figure 4.1: An example of a chromatogram for volatile compounds from *L.purpureus* seeds of accession Machakos-1, obtained from a Shimadzu gas chromatograph

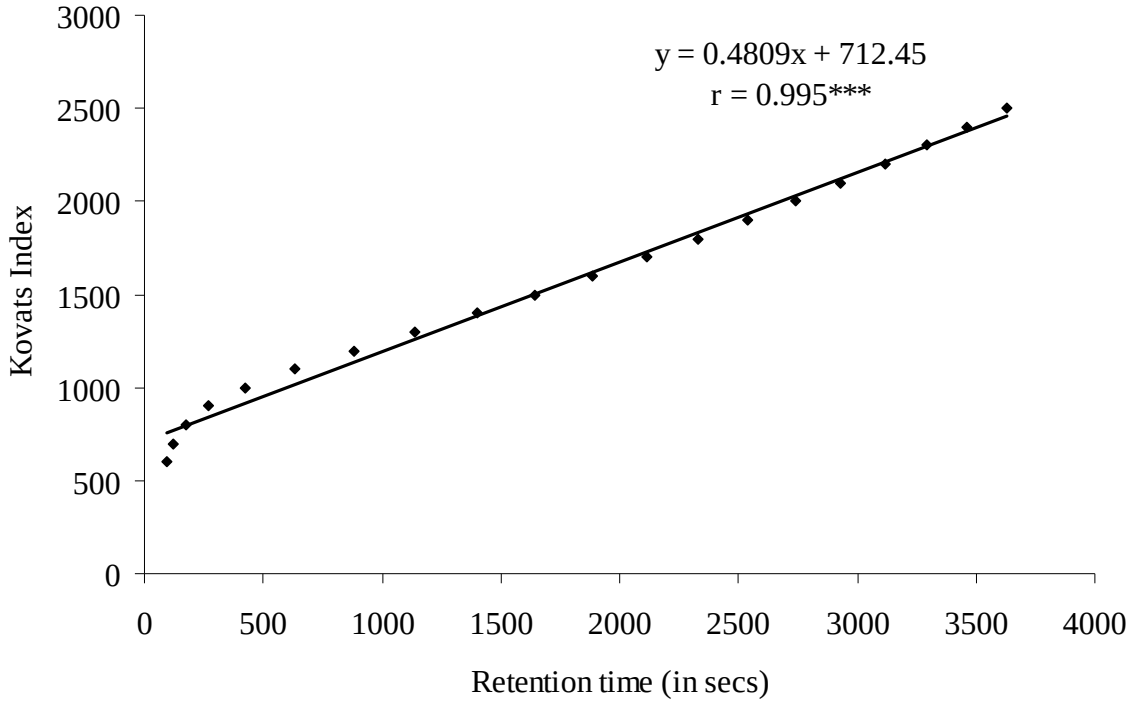

Figure 4.2: Calibration curve obtained from retention times and kovats indices of n-alkanes for calculation of the Kovats indices of the volatile compounds

From the peaks on the chromatograms, a total of 262 compounds with area measurement above 100,000 were identified using literature (Adams, 1995) and the online database, Pherobase (El-Sayed, 2005) (Table 4.4). The volatile constituents were dominated by volatile terpenes and terpenoids, and their derivatives, which accounted for 46% of all the detected odour compounds. The detected compounds were separated into 12 classes namely; alcohols (28), aldehydes (10), ketones (19), esters (46), acids (7), oxygen heterocycles (1), pyrazines (5), thiazoles (4), hydrocarbons (57), terpenes and terpenoids (59), phenols (5) and miscellaneous compounds. The branched long hydrocarbons also dominated and showed the predominance of odd-number homologs. The most common individual compounds were Isopentyl alcohol, 3,7,11-Trimethylhentriacontane, (E)-2-Octene, 7,11,17,21-Tetramethylhentriacontane/ 7,11,17,25-Tetramethylhentriacontane, 6-Methyldotriacontane, Norbornene, Pentanol, 4-methyl thiazole, 5,9,13-Trimethylnonacosane/5,9,15-Trimethylnonacosane/5,9,19-Trimethylnonacosane, 3,7,11,15-Tetramethylhentriacontane, Methyl Butyrate, Isopentyl formate, 13,17-Dimethylnonacosane, 13-Methylhentriacontane, 9-Methylhentriacontane, 7-

Methylhentriacontane, Santene, Heptanal/n-Nonane, 5-Methylnonacosane, 5-Methylhentriacontane, 3,11,19-Trimethylhentriacontane and 3,7-Dimethylhentriacontane.

A similarity graph (Figure 4.3) derived from the retention times of the abundant compounds using MINITAB 11.12 software (MINITAB Inc, State College, Pennsylvania, USA, 1996) showed a mean similarity coefficient of 89.53% between the test *Lablab purpureus* accessions. Accession 11719, 11723, 13086 and 11741 were distinct, with accession 11741 being the most distinct and isolated in the dendogram. The rest of the accessions, showed a similarity of 98%, with accessions 10695, 10706, 27007, 13096, 11705, Njoro, 10702, 26932, 10703, and 11736 forming a tight clade in the dendogram and were most similar (almost 100%).

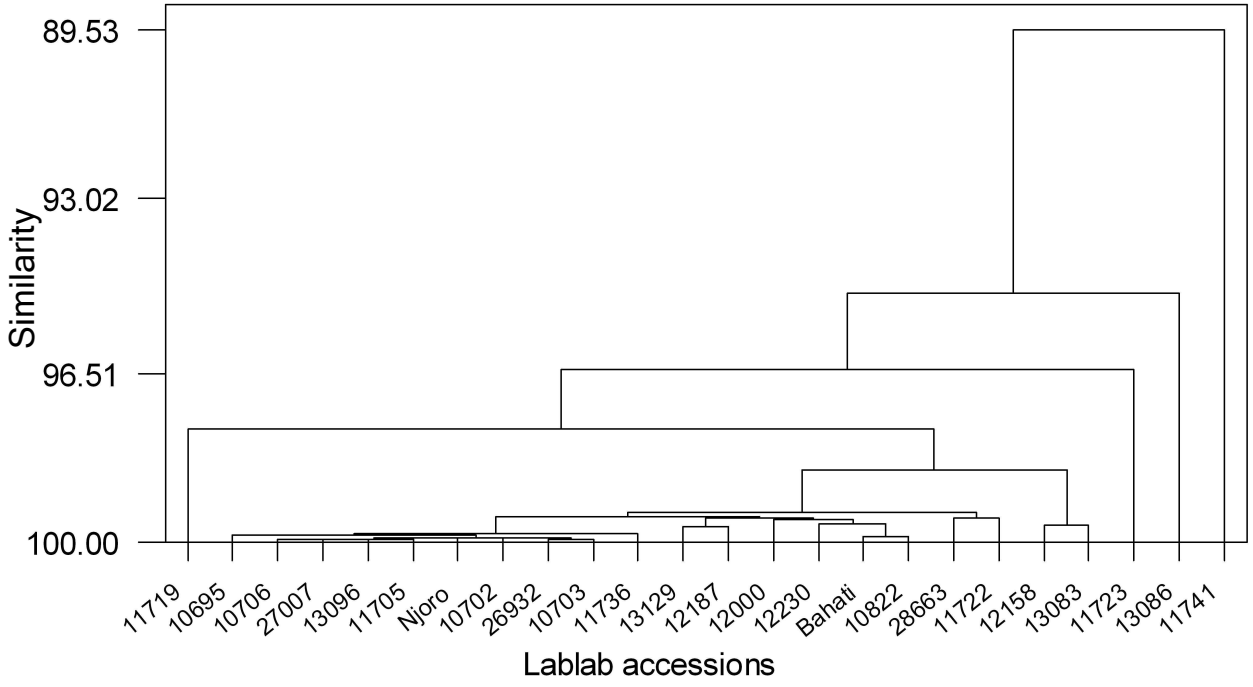

Figure 4.3: Similarity graph of 24 *L. purpureus* accessions based on seed volatile compounds

Table 4.4: Volatile compounds identified in 24 *L. purpureus* accessions and Kovats indices (KI)

Compound	KI	Class	Compound	KI	Class
Pentanal	697[a]	Aldehyde	α-Gurjunene	1409[a]	terpenoid (sesquiterpoid)
Ethyl propanoate	714[a]	Ester	ethyl Anthranilate	1410[a]	ester
Methyl Butyrate	724[a]	Ester	1,7-di-Epi-β-Cedrene	1410[a]	Terpene
Isopentyl alcohol	734[a]	Alcohol	para-menth-1-en-9-ol acetate	1420[a]	Ester
Thiazole	740[a]	Thiazole	(E)-Isoeugenol	1447[a]	Alcohol
Norbornene	747[a]	cyclic HC	α-Himachalene	1447[a]	terpene HC
Pentanol	768[a]	Alcohol	EPI- β-Santalene	1449[a]	terpenes (seisquiterpenes)
2,3-Butanediol	769[a]	Alcohol	Ethyl Vanillin	1452[a]	Misc
2-methyl 3-Buten-2-ol acetate	774[a]	Ester	Geranyl acetone	1453[a]	terpene ketone
Cyclopentanol	781[a]	Alcohol	Neo-Mentyl lactate	1465[a]	Ester
1-Octene	792[a]	Alkene	β-Acoradiene	1466[a]	terpene HC
Isopentyl formate	792[a]	Ester	Isobornyl n-Butyrate	1471[a]	Ester
3,4-Hexanedione	793[a]	Ketone	β-Thujaplicin	1472[a]	Misc
n-Butylmethylether	816[a]	Ether	γ-Gurjunene	1473[a]	terpenes (seisquiterpenes)
(E)-2-Octene	818[a]	Alkene	Citronellyl Isobutyrate	1482[a]	Ester
4-methyl thiazole	818[a]	Thiazole	α-Muurolene	1499[a]	Terpene
Methyl pentanoate	825[a]	Ester	β-Himachalene	1499[a]	terpene HC
Methyl-Pyrazine	826[a]	Pyrazine	α-Chamigrene	1500[a]	terpenes (seisquiterpenes)
Isovaleric acid	834[a]	Acid	Lavandulyl isovalerate	1510[a]	Ester
Isopropyl butyrate	842[a]	Ester	6-methyl-α-(E)-ionone	1518[a]	Ketone
(E)-3-Hexenol	851[a]	Alcohol	(E)-dihydro Apofarnesal	1518[a]	Misc

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
Ethyl isovalerate	856[a]	Ester	β-Vetivenene	1526[a]	Terpene
(Z)-3-Hexenol	857[a]	Alcohol	γ- dehydo-AR-Himachalene	1526[a]	terpene HC
2-methyl butyl acetate	880[a]	Ester	α-Cadinene	1538[a]	Terpene
3-methyl-3-Buten-1-ol acetate	883[a]	Ester	Laciniata Furanone H	1539[a]	Lactone
allyl butyrate	883[a]	Ester	Epi-Longipinanol	1561[a]	terpenoids sesquiterpenoids
Santene	888[a]	HC	Caryophyllene alcohol	1568[a]	Alcohol
5-hydroxypentanal	890[a]	Aldehyde	n-Tridecanol	1575[a]	Alcohol
Propyl butyrate	896[a]	Ester	Spathulenol	1576[a]	alcohol (sesquiterpene)
Ethyl pentanoate	898[a]	Ester	Carotol	1594[a]	alcohol (sesquiterpenoid)
Heptanal	899[a]	Aldehyde	5-Cedranone	1618[a]	Terpenoid
n-Nonane	899[a]	HC	1-EPI-Cubenol	1627[a]	alcohol (sesquiterpene)
2-ethyl Pyrazine	906[a]	Pyrazine	(Z)-3-Hexenyl Phenyl acetate	1631[a]	Ester
Tricyclene	926[a]	Terpene	(E)-Sesquilavandulol	1632[a]	terpenoid (sesquiterpoid)
4,5-dimethyl-Thiazole	934[a]	Thiazole	β-Acorenol	1634[a]	Terpenoid
5-methyl-3-Heptanone	943[a]	Ketone	α-Acorenol	1634[a]	Terpenoid
Exo-5-Norbonen-2-ol	945[a]	Alcohol	6-methyl-6-(3-methylphenyl)-heptan-2-one	1637[a]	Ketone
3-methyl valeric acid	947[a]	Acid	3-Iso-Thujopsanone	1637[a]	Ketone
β-Citronellene	947[a]	Terpene	Valeranone	1672[a]	Ketone
2,6-dimethyl-2-heptanol	991[a]	Alcohol	Khusimol	1736[a]	Misc
2,4,5-trimethyl thiazole	995[a]	Thiazole	β-Eudesmol acetate	1786[a]	Ester

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
n-decane	999[a]	Alkane HC	Dodecanoic acid, butylester	1786[a]	Ester
2,3,5-Trimethyl pyrazine	999[a]	Pyrazine	β-Bisabolenol	1786[a]	terpene
2-acetyl-pyrazine	1020[a]	Pyrazine	Iso-acorone	1806[a]	Terpene
Propyl tiglate	1034[a]	Ester	cyclopentadecanolide	1828[a]	Misc
Lavender lactone	1039[a]	Lactone	Canellal	2036[a]	terpene (sesquiterpene dialdehyde)
(E)-β-Ocimene	1050[a]	Terpene	Oroselone	2146[a]	Misc
Ortho-cresol	1053 [a]	Phenol	Isoincensole acetate	2152[b]	Ester
Artemisia ketone	1062 [a]	Ketone	(Z)-9-Octadecenoic acid	2161[b]	Acid
γ-Terpinene	1062[a]	Terpene	Intermedine	2185[a]	Misc
Cis-vertocitral	1078[a]	Misc	n-docosane	2200[a]	Hydrocarbon
2-acetyl-2-methyl pyrazine	1080[a]	Pyrazine	Phyllocladanol	2200[a]	Terpenoids
Ortho-Guaiacol	1086[a]	Phenol	(Z,Z,Z)-3,6,9-Tricosatriene	2270[b]	Hydrocarbon
para-mentha-2,4 (8)-diene	1086[a]	Terpene	(2S,12S)-2,12-Diacetoxyheptadecane	2273[b]	Hydrocarbon
endo-5-Norbornen-2-ol acetate	1092[a]	Ester	Incensole oxide	2290[a]	terpene (diterpene)
ethyl Heptanoate	1095[a]	Ester	Isopimarol	2301[a]	terpene (diterpene)
α-pinene oxide	1095[a]	Terpenoid	trans-14-Isopropylpodocarpa-8,11,13-trien-13-ol	2303[b]	Alcohol
Maltol	1108[a]	Oxygen heterocycles	trans-Totarol	2303[a]	Terpene
Veratrole	1147[a]	Benzene	Palustrol	2314[a]	terpenoid (sesquiterpoid)

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
Ipsdienol	1147[a]	terpenoid (monoterpenoid)	trans-Ferruginol	2325[a]	Terpene
Iso-isopulegol	1156[a]	Terpene alcohol	3-α-14,15-dihydro-Manool Oxide	2332[a]	terpenoid (diterpenoid)
β-pinene oxide	1156[a]	Terpenoid	4-EPI-Abietol	2341[a]	terpenoid (diterpenoid)
meta-cresol acetate	1163[a]	Ester	7-Methyltricosane	2342[b]	Hydrocarbon
Menthol	1173[a]	Alcohol	Neo-Abietol	2345[a]	terpenoid (diterpenoid)
cis-Pinocamphone	1173[a]	Ketone	5-Methyltricosane	2351[b]	Hydrocarbon
cis-pinocarveol	1183[a]	Alcohol	dehydroAbietol	2359[a]	terpenoid (diterpenoid)
para-Cymen-8-ol	1183[a]	Alcohol	cis-Ferruginol	2362[a]	Terpene
(Z)-3-Hexenyl Butyrate	1186[a]	Ester	3-Methyltricosane	2372[b]	Hydrocarbon
3-decanone	1186[a]	Ketone	methyl Strictate	2388[a]	terpene (sesquiterpene)
Myrtenol	1194[a]	Alcohol	Methyl nidoresedate	2388[a]	Terpenoids
n-Dodecane	1196[a]	Alkane HC	Abietol	2391[a]	terpenoid (diterpenoid)
dihydro Citronellol	1196[a]	Terpenoid	n-Tetracosane	2400[a]	Hydrocarbon
Octanol acetate	1211[a]	Ester	Integerrimine	2402[b]	misc alkaloid
(Z)-Cinnamaldehyde	1214[a]	Aldehyde	cis-Ferruginol acetate	2406[a]	Terpene
cis-Sabinene hydrate acetate	1219[a]	Terpene	Labd-13E-8,15-diol	2412[a]	Terpenoids
Neo-iso-dihydro Carveol	1226[a]	Alcohol	trans-totarol acetate	2417[a]	ester (diterpene)
Citronellol	1228[a]	terpenoid	Heneicosanoic acid	2424[b]	Acid
cis-Carveol	1229[a]	terpenoid alcohol	11-Methyltetracosane	2435[b]	Hydrocarbon

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
Nordavanone	1229[a]	terpenoid ketone	1,2,3-Benzenetriol	2486[b]	Phenol
(z)-Ocimenone	1231[a]	Terpenoid	Docosenoic acid	2495[b]	Acid
cis-Ascaridole	1237[a]	Terpene	Jacobine	2495[b]	misc alkaloid
(E)-Ocimenone	1239[a]	Terpenoid	n-Pentacosane	2500[a]	Hydrocarbon
Neral	1240[a]	Aldehyde	Docosanoic acid	2526[b]	Acid
Butyrophenone	1251[a]	Ketone	1,3,5-Benzenetriol	2692[b]	Phenol
(Z)-Anethole	1251[a]	Misc phenylpropanoid	3-Methylheptacosane	2773[b]	Hydrocarbon
Carvenone	1252[a]	Ketone	(6E,10E,14E,18Z)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	2790[b]	hydrocarbon
Piperitone	1252[a]	Ketone	3,15-Dimethylheptacosane	2806[b]	Hydrocarbon
Chavicol	1253[a]	Alcohol	Pentacosanoic acid	2829[b]	Acid
trans-Sabinene hydrate acetate	1253[a]	Ester	14-Methyloctacosane	2832[b]	Hydrocarbon
Dec-9-en-1-ol	1263[a]	Alcohol	12,16-Dimethyloctacosane	2860[b]	Hydrocarbon
Ambersage	1263[a]	Misc	13-Methylnonacosane	2931[b]	Hydrocarbon
Isopulegol acetate	1273[a]	Ester	15-Methylnonacosane	2931[b]	Hydrocarbon
dihydro-Linalool acetate	1275[a]	Ester	9-Methylnonacosane	2936[b]	Hydrocarbon
Neo-Menthyl acetate	1275[a]	Ester	5-Methylnonacosane	2951[b]	Hydrocarbon
Citronellyl formate	1275[a]	Terpene ester	13,17-Dimethylnonacosane	2960[b]	Hydrocarbon
trans-Carvone oxide	1277[a]	Terpenoid	5,17-Dimethylnonacosane	2981[b]	Hydrocarbon
Undec-10-en-1-al	1296[a]	Aldehyde	9,13,19-Trimethylnonacosane	2987[b]	Hydrocarbon
Carvacrol ethyl ether	1297[a]	terpenoid	9,15,19-Trimethylnonacosane	2987[b]	Hydrocarbon
Geranyl Formate	1300[a]	Ester	11,15,19-Trimethylnonacosane	2987[b]	Hydrocarbon
Iso-3-Thujyl acetate	1301 [a]	Ester	5,9,13-Trimethylnonacosane	3009[b]	Hydrocarbon
n-Nonanol acetate	1312[a]	Ester	5,9,15-Trimethylnonacosane	3009[b]	Hydrocarbon

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
Sesamol	1312[a]	Phenol	5,9,19-Trimethylnonacosane	3009[b]	Hydrocarbon
(E,E)-2,4-Decadienal	1314[a]	Aldehyde	15-Methyltriacontane	3032[b]	Hydrocarbon
Dimethoxy-(Z)-Citral	1316[a]	Terpenoid	7-Methyltriacontane	3041[b]	Hydrocarbon
cis-2,3-Pinanediol	1316[a]	terpenoid alcohol	5-beta-cholestan-3alpha-ol	3098[b]	Cholesterol
Methyl Geranate	1323[a]	Ester	Hentriacontane	3100[b]	Hydrocarbon
Piperonal	1329[a]	Aldehyde	13-Methylhentriacontane	3129[b]	Hydrocarbon
Benzyl Butyrate	1345[a]	Ester	15-Methylhentriacontane	3130[b]	Hydrocarbon
Citronellyl acetate	1354[a]	Ester	11-Methylhentriacontane	3133[b]	Hydrocarbon
(Z)-α-damascone	1354[a]	Ketone	9-Methylhentriacontane	3135[b]	Hydrocarbon
γ- Nonalactone	1360[a]	Ester	7-Methylhentriacontane	3141[b]	Hydrocarbon
trans-2,3-Pinanediol	1360[a]	terpenoid alcohol	5-Methylhentriacontane	3150[b]	Hydrocarbon
α-cyclogeraniol	1361[a]	terpenoid alcohol	9,21-Dimethylhentriacontane	3163[b]	Hydrocarbon
Furfuryl Hexanoate	1367[a]	Ester	7,11-Dimethylhentriacontane	3168[b]	Hydrocarbon
Cyclosativene	1368[a]	Terpene HC	3-Methylhentriacontane	3174[b]	Hydrocarbon
(Z)-ethyl cinnamate	1374[a]	Ester	Cholest-5-en-3beta-ol	3192[b]	Cholesterol
3,4-dihydro-coumarin	1376[a]	phenylpropanoid	3,15-Dimethylhentriacontane	3201[b]	Hydrocarbon
β-Patchoulene	1380[a]	HC	7,11,21-Trimethylhentriacontane	3203[b]	Hydrocarbon
1-phenyl-4-methyl-Pentan-3-one	1380[a]	Ketone	7,11,25-Trimethylhentriacontane	3203[b]	Hydrocarbon
(E)-β-Damascenone	1380[a]	Ketone	3,7-Dimethylhentriacontane	3208[b]	Hydrocarbon
β-Maaliene	1380[a]	Terpene HC	3,7-Dimethylhentriacontane	3208[b]	Hydrocarbon
Daucene	1380[a]	Terpene HC	3,11,19-Trimethylhentriacontane	3229[b]	Hydrocarbon
(Z)-Cinnamyl acetate	1386[a]	Ester	3,7,11-Trimethylhentriacontane	3234[b]	Hydrocarbon

Table 4.4: Continued

Compound	KI	Class	Compound	KI	Class
3-Dodecanone	1386[a]	HC	7,11,17,21-Tetramethylhentriacontane	3240[b]	Hydrocarbon
Isocomene	1386[a]	Terpene	7,11,17,25-Tetramethylhentriacontane	3240[b]	Hydrocarbon
(Z)-Jasmone	1394[a]	Ketone	6-Methyldotriacontane	3245[b]	Hydrocarbon
(Z)-Trimenal	1395[a]	Aldehyde	3,7,11,15-Tetramethylhentriacontane	3258[b]	Hydrocarbon
1,7-di-Epi-α-Cedrene	1397[a]	Terpene	Tritriacontane	3300[b]	Hydrocarbon
Iso-italicene	1397[a]	Terpene	Cholest-5-en-24-methyl-3beta-ol	3305[b]	Cholesterol
(E)-β-damascone	1409[a]	Ketone	Tetratriacontane	3400[b]	Hydrocarbon
α-Cedrene	1409[a]	Terpene	Cholest-5-en-24-ethyl-3beta-ol	3408[b]	Cholesterol

Identification references [a] = Adams, (1995) and [b] = The Pherobase (El-Sayed, 2005)

4.1.4 Molecular characterisation

a) Estimation of DNA concentration and Quality

Optimization of the method for isolation of genomic DNA from *Lablab purpureus* leaves was carried out by comparing the following methods: Plant DNA Sodium dodecyl sulphate (SDS) minipreparation with β-mercaptoethanol (Dellaporta *et al.*, 1983), 2x cetyltrimethylammonium bromide (CTAB) large and small scale methods (CIMMYT, 2005), SDS minipreparation method with 6.5mM dithiothreitol (DTT) (Matasyoh *et al*., 2008), 2x CTAB method (Doyle and Doyle, 1990, modified at NCSU Forest Biotechnology Laboratory), 2x CTAB method modified by University of Agricultural Sciences (2004), used for lablab; 2x CTAB protocol described by (Gawel and Jarret, 1991) and modified by (James *et al*., unpublished). Except for the 2xCTAB method of Gawel and Jarret (1991) the other methods gave inconsistent results with sub optimal DNA concentrations and degraded DNA.

The total genome DNA isolated from the 50 lablab accessions was of high quality and intactness with a concentration ranged from 271ng/µl to 3305ng/µl as determined by biophotometer (Eppendorf, bioPhotometer, Hamburg, Germany) method. The concentration of the DNA was also estimated by comparing the band size with 1µl of uncut, unmethylated lambda DNA standards (2000ng, 1000ng, 750ng, 500ng, 100ng) (Plate 4.3). Two micrograms of each of the template genomic DNA was double digested with EcoRI and MseI and used for the amplification in the AFLP analysis.

b) Selective amplification and gel electrophoresis

An example of typical AFLP profiles on 6% silver stained polyacrylamide gel is shown in Plate 4.4. The fifteen primer pairs revealed a total of 227 different AFLP bands out of which 180 were polymorphic. The sizes of the fragments ranged from 40 base pairs (bp) to 5000bp. The primers differed in their ability to reveal positive amplicons, with the number of markers ranging from 11-21 per primer set. The primers also differed in their ability to detect polymorphic markers which ranged from 4-19. The most informative set was E(ACA)/M(CGC) with 19 polymorphic loci. The percentage polymorphism ranged for 36.36% to 100% (Table 4.5).

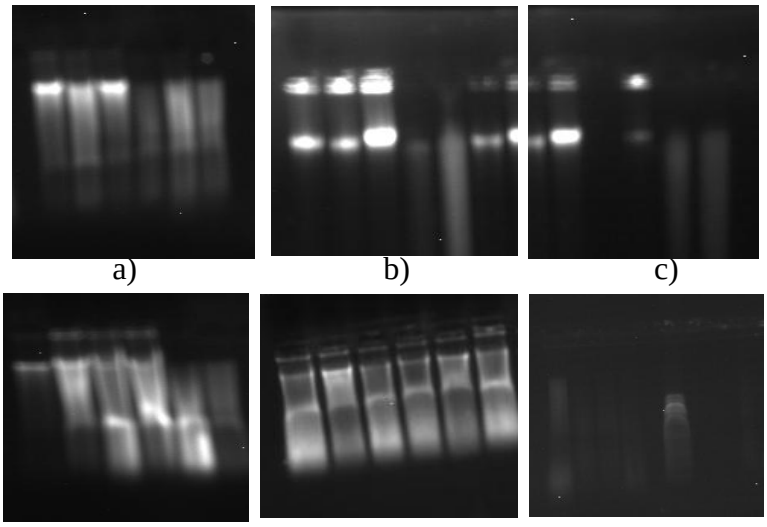

Plate 4.2: DNA isolation protocols: a) SDS minipreparation with β-mercaptoethanol (Dellaporta *et al*., (1983), b) CTAB large scale method with DTT (Gawel and Jarret, (1991) c) CTAB small scale method with DTT (Gawel and Jarret, (1991), d) CTAB method by University of Agricultural Sciences (2004), e) CTAB method by Doyle and Doyle, (1990) f) SDS minipreparation method with 6.5mM DTT (Matasyoh *et al*., 2008)

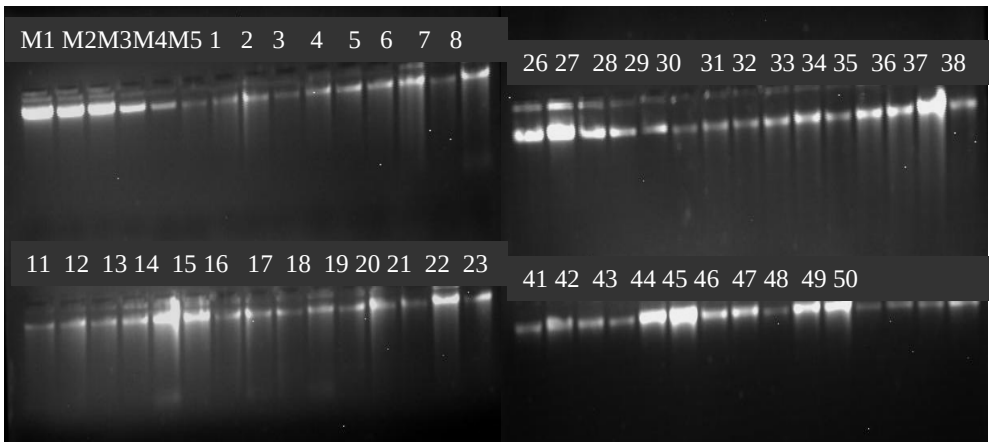

Plate 4.3: DNA extracted from *L. purpureus* accessions using the 2x CTAB (Gawel and Jarret, 1991) modified method with β-mercaptoethanol, electrophoresed on 0.8% agarose gel in 1xTBE buffer stained with ethidium bromide and visualized under UV light. M1-M5 Lambda DNA at concentration 2000, 1000, 750, 500 and 100ng/µl. 1-50 (Lablab accessions in Table 3.2)

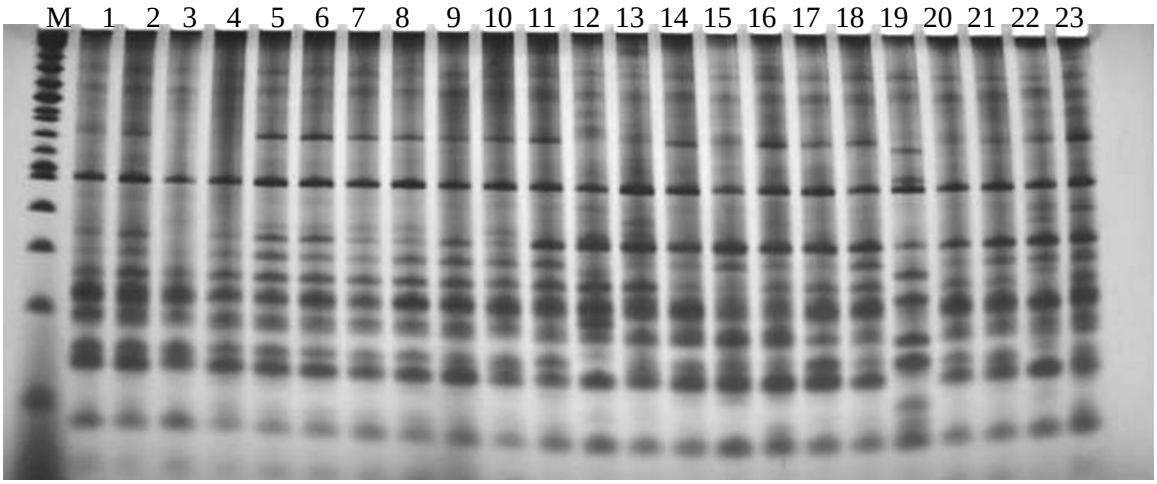

Plate 4.4: A silver stained AFLP profile for *L. purpureus* accessions separated on 6% polyacrylamide gel using EcoRI (AGC)/MseI(CAT). (M- ladder, 1-23 Lablab accessions in Table 3.2)

4.1.4.1 Analysis of the five populations

The AFLP banding patterns across the 5 sampling populations of *L. purpureus*; Eastern, Coast, Central, Rift valley and Nairobi were studied (Figure 4.4). Accessions from Eastern had the largest number of amplified bands and the highest percentage of polymorphic loci (93.33%). The population also had the highest heterozygosity (H_e) of 0.297 (Table 4.6). Populations from Rift Valley, Coast, Central and Nairobi had 67.78%, 55.56%, 34.44% and 13.33% percentage of polymorphic loci, respectively. The highest number of effective loci was observed in the Rift Valley population (1.507) and the least in Nairobi population (1.094), while the mean over all the loci and populations was 1.331. The mean expected heterozygosity estimate (H_e) for polymorphic markers for each primer pair ranged from 0.055 to 0.297, with the overall mean expected heterozygosity estimate (H_e) for the 180 polymorphic AFLP markers for all the populations was 0.189. The highest and lowest mean expected heterozygosity and Shannon index were for Eastern and Nairobi populations, respectively. The mean expected heterozygosity (H_e) for the populations from Eastern and Nairobi were 0.297 and 0.055, respectively, while their Shannons' index were 0.448 and 0.081, respectively (Table 4.6).

Table 4.5: Number of loci amplified by AFLP primers in *L. purpureus* accessions

Primer Set	Total No. of loci	No. of polymorphic loci	Percent polymorphism (P)
E(ACA)/M(CAC)	15	15	100
E(ACC)/M(CTA)	14	9	64.3
E(AGC)/M(CTA)	16	14	87.5
E(ACA)/M(CTC)	12	8	66.7
E(ACC)/M(CTC)	15	12	80.0
E(ACC)/M(CAC)	10	7	70.0
E(ACT)/M(CTC)	20	16	80.0
E(AGC)/M(CGC)	12	6	50.0
E(ACT)/M(CAT)	14	13	92.9
E(AAC)/M(CTA)	17	12	70.6
E(AGC)/M(CAT)	21	17	81.0
E(AAC)/M(CAC)	16	14	87.5
E(AGC)/M(CAC)	15	14	93.3
E(ACA)/M(CGC)	19	19	100.0
E(ACC)/M(CAT)	11	4	36.4
Mean	15.13	12	77.3

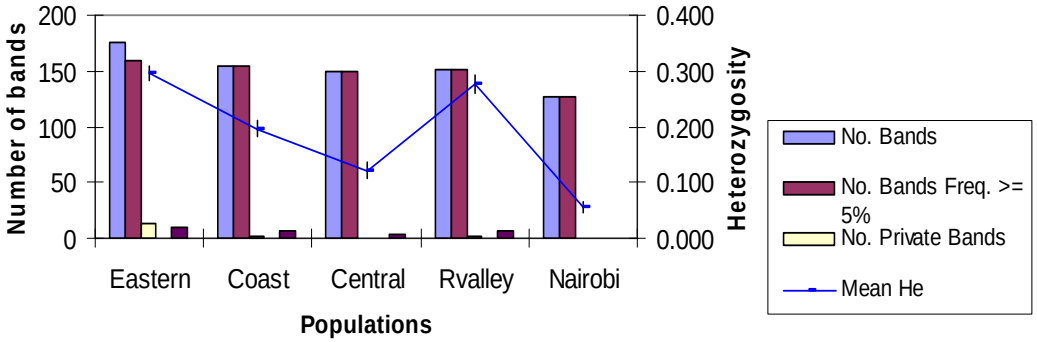

Figure 4.4: Band patterns across *L. purpureus* populations in Kenya

No. Bands = total number of amplified bands; No. Bands Freq. >= 5% = number of different bands with a frequency >= 5%; No. Private Bands = number of bands unique to a single population; No. LComm Bands (<=25%) = number of locally common bands (Freq. >= 5%) found in 25% or fewer populations; No. LComm bands (<=50%) = number of locally common bands (Freq. >= 5%) found in 50% or fewer populations
H_e = mean expected heterozygosity

Table 4.6: Mean expected heterozygosity (He), number of different loci, number of effective loci and Shannon Index over Loci for five populations of *L. purpureus* in Kenya

Population	**No. of different loci**	**No. Effective loci**	**Expected Heterozygosity (H_e)**	**Shannon Index**
Eastern	1.911±0.026	1.502±0.025	0.297 ±013	0.448±0.017
Coast	1.417±0.054	1.344±0.029	0.196±0.015	0.292±0.022
Central	1.172±0.052	1.207±0.025	0.121±0.014	0.182±0.02
Rift Valley	1.522±0.056	1.507±0.032	0.276±0.016	0.4±0.022
Nairobi	0.839±0.047	1.094±0.018	0.055±0.011	0.081±0.015
Mean over all loci and populations	1.372±0.025	1.331±0.013	0.189±0.007	0.28±0.01

Table showing means followed by the standard errors

The level of relatedness between the 5 populations was established through a genetic identity and distance matrix based on the proportion of shared (common) loci (Nei, 1978), derived using GenAlEx 6.2 data analysis software. Pairwise comparison of Nei's unbiased genetic distance among the 5 populations ranged from a low of 0.0333, between Eastern and Rift Valley populations, to a high of 0.1138, between Rift Valley and Nairobi populations (Table 4.7).

Table 4.7: Nei's genetic identity (above diagonal) and distance (below diagonal) matrices for 5 populations of *L. purpureus* based on 180 polymorphic loci

Eastern	Coast	Central	Rift Valley	Nairobi	
****	0.963	0.942	0.967	0.929	Eastern
0.0375	****	0.957	0.944	0.931	Coast
0.0598	0.0437	****	0.918	0.938	Central
0.0333	0.0573	0.0855	****	0.893	Rift Valley
0.0738	0.0711	0.0644	0.1138	****	Nairobi

The populations exhibited a high level of unbiased genetic identity of greater than 0.89, with the Rift Valley and Eastern populations displaying the highest genetic identity of 0.967. Rift Valley and Nairobi populations had the least genetic identity of 0.893. The distance matrix was used to derive a dendogram of the populations using Nei's (1978)

unbiased measures of genetic distance, and unweighted pair group method using arithmetic averages (UPGMA) (Figure 4.5). Nairobi revealed the highest genetic distance from the other populations and could sampled because it may have germplasm with unique alleles.

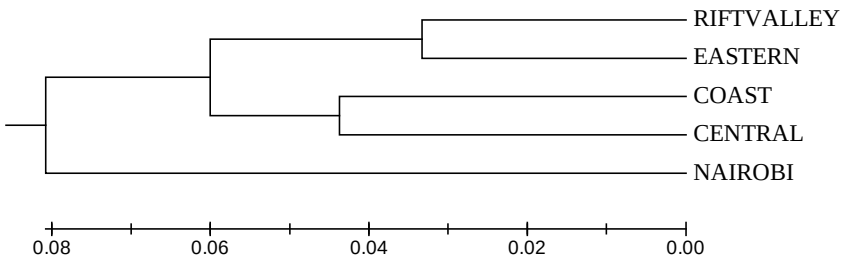

Figure 4.5: A dendogram of five populations of *L. purpureus* Kenyan accessions based on AFLP analysis of fifteen primers based on Nei's (1978) unbiased measures of genetic distance

The estimation of the variance components among and within populations using nested analysis of molecular variance (AMOVA) was not significant (P>0.05) (Table 4.8). Partitioning of the genetic diversity revealed that overall, most genetic variation resided within populations (99%) and only 1% variance among the populations. A low value of Φ_{PT} of 0.007 was observed showing a small extent of differentiation among the populations.

Table 4.8: Nested AMOVA for 50 accessions of *L. purpureus* and partitioning of the total diversity into population components

Variance Components	**df**	**SS**	**Variance**	**% Total Variance**	**P**	**PhiPT (Φ_{PT})**
Among population	4	79.35	0.14	1%	0.347	0.007
Within population	45	842.41	18.72	99%		

df= degrees of freedom, SS- sum of squares

4.1.4.2 Analysis of the 50 *L. purpureus* accessions

Principal coordinate analysis plot of the first two coordinates was used to display the multidimensional relationship of the 50 accessions assayed in this study. The first principal coordinate accounted for 61.81% of the variance of the AFLP scored data, while the second and third coordinates accounted for 13.38% and 7.20% variance, respectively, giving a cumulative variance of 82.39%. The plot of the first and second coordinates, which accounted for 75% of the total variance, showed a high degree of overlap (clustering) between accessions from different geographic origins, apart from four accessions (Figure 4.6). The four accessions that were outside the tight cluster were from Eastern (Mwingi-3 and 12000), Coast (12187R3) and Rift Valley (10706R1) populations.

Genetic distance and genetic identity matrix (Appendix 1) was generated to establish the level of relatedness of the 50 *L. purpureus* accessions listed in Table 3.2. The estimated genetic identity values for the 50 accessions ranged from 0.41 to 0.93, with the minimum genetic identity value of 0.41 observed between the accessions Njoro-2 and Mwingi-3. Mwingi-3 had a genetic identity of 0.42 with Meru Central-2, Lamu-1, 10699, Lamu-3 and Thika-2. The maximum genetic identity value of 0.93 was observed between accession 10841 and 45349, Mwingi-1 and 12038; Lamu-2 and Mwingi-2, and Lamu-2 and Meru Central-1; accession 11736 and Meru Central-2 and 27007.

The genetic distance values ranged from 0.07 to 0.89. The minimum genetic distance value of 0.07 was observed between the accessions 12038 and 10841, 10841 and Mwingi-1, Lamu-2 and Mwingi-2, and Meru Central-1 and Lamu-2. Those with genetic distance of 0.08 were observed between accession 45349 and Mwingi-2, 10841 and Thika-1; between accession 11736 and Machakos-3 and Meru Central-2; 13129R1 and 11741, and Thika-1 and Lamu-2. Accessions, Njoro-2 and Mwingi-3 had the largest genetic distance of 0.89. Mwingi-3 also had large genetic distances of 0.88 with Meru Central-2, Lamu-3, and Thika-2; 0.86 with Lamu-1 and 10699; and 0.84 with Meru Central-1, 11719R2, 13096, 13129R1, Thika-5, 10824, and 11723R1. Accessions collected from farmers fields exhibited a higher genetic distance of 0.89, between Njoro-2 and Mwingi-3, and a lowest distance of 0.07, between Mwingi-2 and Lamu-2 and Meru Central-1 and Lamu-2. Those collected from the National genebank had a range of

genetic distance from 0.08 between accessions 45349 and 10841, to 0.84 between accessions 10824 and 10706R1. The black coloured accessions are the most widely cultivated accession in Kenya and in this study, they exhibited genetic distance ranging from 0.081 between Thika-1 and 45349, to 0.287 between Meru central-1 and Thika-3.

The relationship between the accessions was examined further by subjecting the 50 accessions that consisted of 28 accessions from the genebank and 22 from farmers' fields, to cluster analysis using the distance matrix data and the unweighted pair group method using arithmetic averages (UPGMA) linkage analysis. The dendogram resulting from the cluster analysis revealed three major groups (Figure 4.7). Group one consisted of Mwingi-3 and 10706R1, group 2 had 12187R3 and 12000 and group 3 was the largest group consisting of the rest of the accessions, and subdivided into two subgroups. The subgroups consisted of Thika-3 in group 3a and the rest of the accessions in group3b, which was further subdivided to two groups with accessions 13129 and 12230R3 in group 3b(i) and the other accessions in group 3b(ii). The grouping of the *L. purpureus* accessions did not exhibit any relationship to the geographic regions of origin, showing random dispersion of the accessions from the different regions. Accessions 10706R1 from Rift Valley population and Mwingi-3 from Eastern population were the most distant in the dendogram.

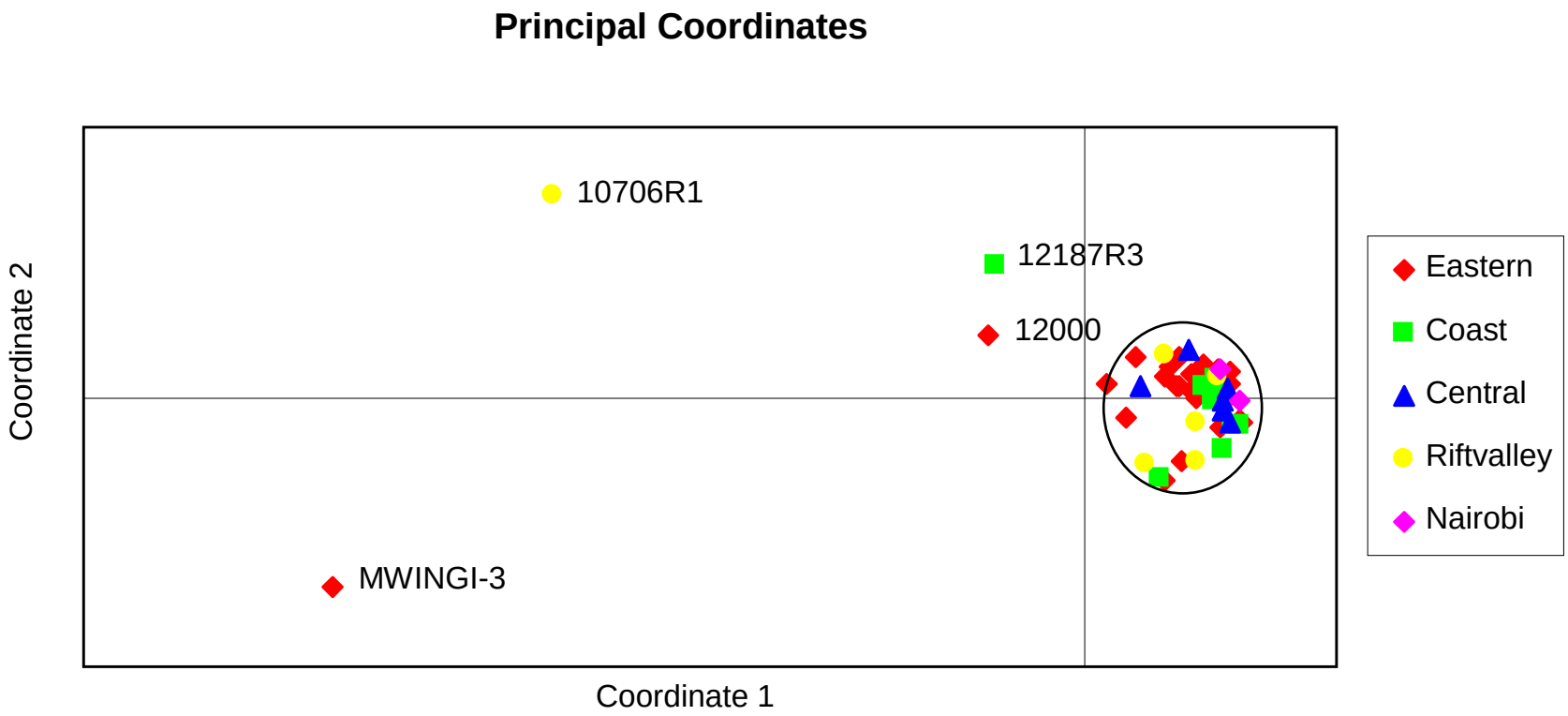

Figure 4.6: Principal coordinate analysis (PCA) plot of molecular genetic diversity of 50 *L. purpureus* accessions with 180 AFLP markers

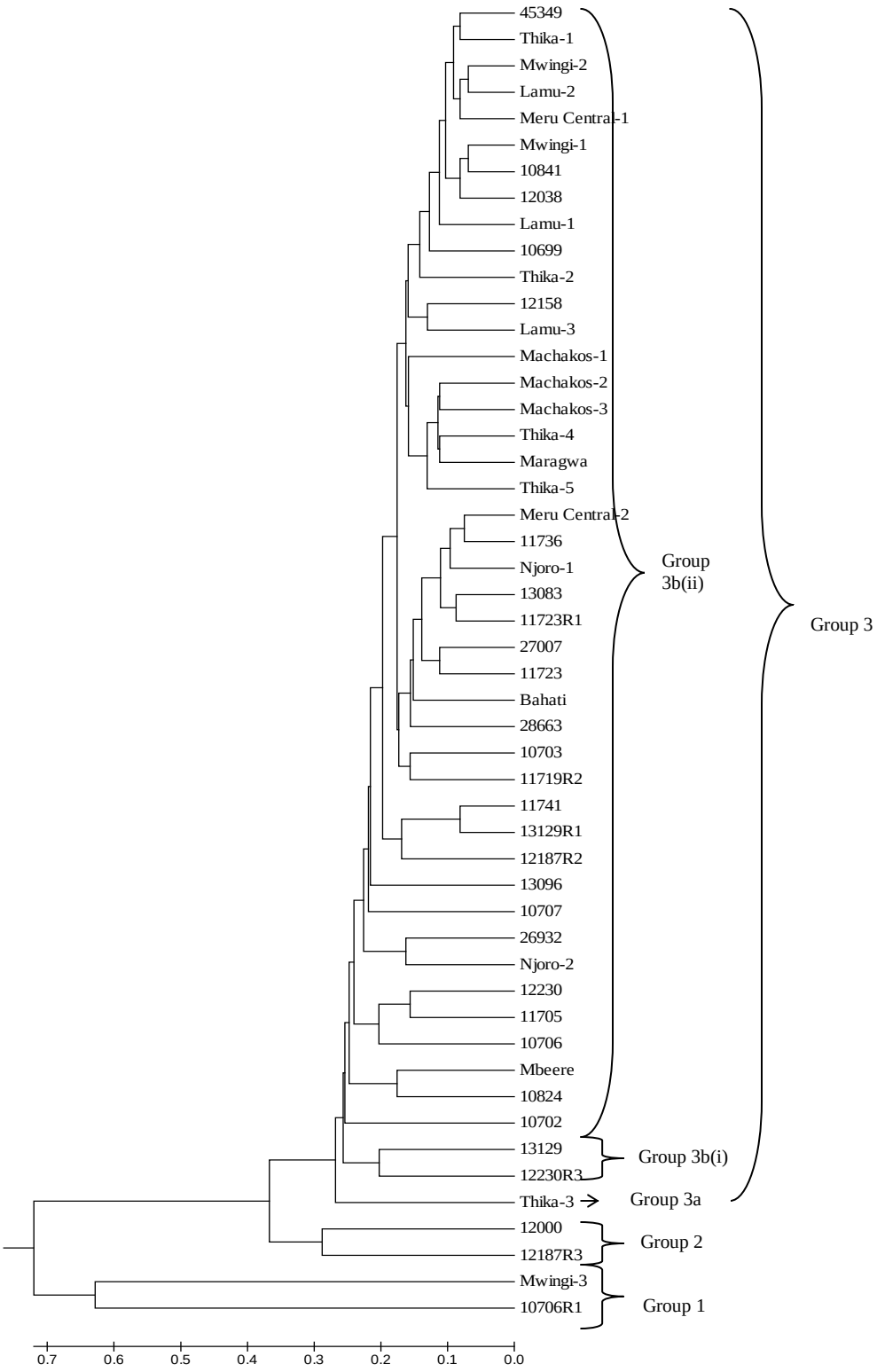

Figure 4.7: Relationships between 50 Kenyan *L. purpureus* accessions based on Nei's (1978) genetic distance using UPGMA method

4.2 Discussion

4.2.1 Sensory Evaluation of Kenya *L. purpureus*

Though numerous studies have been carried out worldwide to determine the organoleptic properties of legumes, this study was the first of its kind in Kenya to evaluate the quality traits of Kenya *L. purpureus* accessions. From a study of organoleptic properties of green beans, it was observed that high overall impression of the bean was closely related to colour and odour among other characteristics (Khah and Arvanitoyannis, 2003). In this study, the taste of the Kenyan accessions was different, with some of the accessions being reported as more bitter. The means were not clearly separated due the narrow diversity of the lablab accessions as determined using molecular markers in this study. This was similar to results by Wanjekeche *et al*., (2000), who reported that the bitter taste of lablab bean in some cases persists even after cooking especially in dark-seeded types. The colour of the seeds did not show any relationship with the bitter taste in this study. The accessions with highest and lowest scores for taste were brown in colour. Dark stripped beans were reported to be more bitter than the lighter coloured ones in a sensory study of common beans (Mkanda *et al.,* 2007).

A study on common beans reported that bitter taste contributes to consumers' dislike of bean varieties, alongside other sensory characteristics (Mkanda *et al.,* 2007). The correlation between the taste and the odour in this study may be explained by the fact that the both parameters contribute to food flavour (Nursten, 1977). However, it should be noted that the correlation is not strong ($r<0.8$), and further analysis on more accessions may be carried out to confirm the relationship. Other studies on organoleptic aspects of lablab were carried out on leaves and pod meal of 29 accessions and 4 landraces in Tanzania (Tefera *et al*., 2006). The accessions ranked differently in their acceptability with some of the accessions being more preferred for vegetable than for pod meal. Watts *et al*, (1989) explains that the differences in the sensory rankings of the lablab accessions are as a result of the considerable variation between analysts in their ability to detect the parameters.

Sensory evaluation is a very useful parameter to consider, since the consumption of the bean determines the purchasing and thus production of the bean (Watts *et al*., 1989). There is no one instrument that can replicate or replace the human response, making the sensory evaluation component of any food study essential, especially in the introduction of a food where it is not common in the diet. In this study differences in ranking of the accessions for the odour and bitter

taste intensity were noted and may be attributed to the fact that sensory evaluation is a subjective test and depends on the consumers' preferences. The differences may also be attributed to the changes in the proportions of the compounds over time which affects the balance of the flavour during the eating process causing different sensory experiences (van Ruth *et al.,* 2005).

4.2.2 Detection of cyanogenic glycosides in *L. purpureus*

The Kenyan accessions analysed for cyanogenic glycosides in this study using the picrate method all showed levels below 10ppm. This corroborated similar studies carried out on *D. lablab* var *vulgaris*, which observed that the content of hydrogen cyanide (HCN) level in raw seeds was 3.6ppm (Oke, 1969). In another study on *L. purpureus* from Philippine (Laurena *et al*., 1994) none of the levels of cyanide in the seeds, immature pods and mature leaves was 50ppm and above. This is unlike for the black lima bean (*Phaseolus lunatus*) that has been reported to taste bitter with a yield of more than 200ppm hydrogen cyanide (Fenwick *et al*., 1990). Results from this study indicate that the cyanogenic glycosides in the Kenyan *L. purpureus* do not pose a health risk to consumers as HCN levels of 50ppm or µg/g and less are considered nontoxic (Bolhuis, 1954, Ndung'u *et al.,* 2010). Despite this, it has been reported that the presence of these cyanogenic glycosides also confers a bitter taste in lablab (Duke *et al*., 1981, Smartt, 1985).

Apart from cyanogenic glycosides, other factors have also been reported to affect the bitter taste in beans. In a study on consumer preferences of different types of beans, those that were classified as bitter had darker stripes than the lighter ones (Mkanda *et al.,* 2007). In another study, white or cream-coloured seeds were also preferred to dark-seeded types because of the bitter taste (Duke *et al*., 1981; Smartt, 1985). Bressani and Elias (1980) reported that dark coloured seeds had higher contents of phenolics that may contribute to bitterness. Large amounts of tannins, the most common polyphenols in beans, have been found in coloured bean seed coats (red, black or bronze), compared to none in the white bean varieties (Guzmán- Madondo *et al.,* 1996). However, further studies have revealed that total polyphenol content alone may not fully account for the bitterness found in common beans. Indeed, beans with high amounts of total polyphenol content were not considered as bitter by Mkanda *et al.,* (2007). The possible association of polyphenols with bitterness in the *L. purpureus* accessions assessed for quality in this study was not investigated.

Yang and Lawless (2005), reported that the presence of minerals such as iron in a food product may confer some undesirable flavours such as metallic and bitter tastes. They suggested that presence of some iron compounds such as ferrous sulphate, ferrous chloride and ferrous gluconate, may contribute significantly to bitter taste. However, low iron content in bean samples classified as bitter, did not support this suggestion (Mkanda *et al.,* 2007). The malliard reactions take place during cooking (Martins *et al.,* 2001) when lysine, and depending on the amount of reducing sugars available, reacts with free amino groups resulting in formation of N-substituted glycosylamines. The instability of the N-glycosylamines causes further reactions producing ketosamines, which with further reactions may produce brown nitrogenous polymers and copolymers called mellanoidins, which may produce different flavours including bitterness (Martins *et al.,* 2001). The amount of lysine in *L. purpureus* has nonetheless been found to be high at 63.1mg/g of the whole seed (Chau *et al.,* 1998). The role of minerals and lysine in production of bitter taste in the Kenyan *L. purpureus* is yet to be determined.

The amounts of cyanogenic glycosides in this study were below the limits to cause a colour change in the picrate paper. However, it is not possible to eliminate the glycosides from causing the bitter taste, and further sensitive tests would correlate the amount of cyanogenic glycosides and the bitter taste. The contribution of other compounds discussed above would provide relevant information on the effects of bitter taste in lablab.

4.2.3 Identification of volatile compounds in *L. purpureus* accessions

Flavour is an important sensory aspect of food. Basic knowledge of volatile compounds constituting the lablab bean flavour can help product developers meet the challenges that they commonly face when promoting the lablab bean. The volatile compounds identified in the Kenyan *L. purpureus* accessions included alcohols, aldehydes, ketones, esters, acids, oxygen heterocycles, pyrazines, thiazoles, hydrocarbons (57), terpenes and terpenoids, phenols and miscellaneous compounds. These were slightly different from those identified by Kim and Chung (2008) in *Lablab purpureus*. The difference between the two studies may be accounted for by use of different extraction methods. The method of extraction used in this study was cold solvent while Kim and Chung (2008) used the simultaneous distillation and extraction procedure using a Likens and Nickerson apparatus. Differences in assayed germplasm, growing conditions and GC conditions e.g. temperatures and separating columns may also have contributed to the

noted differences in profiles of identified volatile compounds. The compounds identified that were common in these two studies nonetheless were pentanal, geranylacetone, heptanal, pentanol, (Z)-3-hexenol. Unlike in this study, Kim and Chung (2008) did not identify any presence of branched and unbranched alkanes.

The presence of the odd numbered long alkanes in the Kenyan accessions unlike in the other studies is due to the fact that the alkanes have poor volatility in steam (Radulovic *et al*., 2006) which was predominantly used for extraction by Kim and Chung (2008). Some of the n-alkanes identified in the Kenyan *L. purpureus* accessions were also found in vanilla beans (Ramaroson-Raonizafinimanana *et al*., 1997). These were n-docosane, hentriacontane, triacontane and tetracontane. Pentanal, geranylacetone, heptanal, n-nonane, n-decane and n-docecane, were also extracted and identified from dry beans (*Phaseolus vulgaris*), isolated by headspace solid phase microextraction (HS-SPME) (Oomah *et al.,* 2007). Further, (Z)-3-hexenol was also identified in French beans (Hinterholzer *et al*., 1998) using gas chromatography/ olfactometry (GC/O), a useful tool used to focus on the identification of those odorants contributing to the food flavour (Schieberle, 1995). Maltol, a γ-pyrone, was also identified in Thai soy (Wanakhachornkrai and Lertisiri, 2003), and it is recognized as an important volatile particularly in foods which have undergone heating.

Among the compounds identified in this study, 2,3-Butanediol, heptanal and pentanal were found to be present in the volatile isolate of kidney beans (van Ruth *et al.*, 2004; van Ruth *et al*., 2005), and pentanol and heptanal were identified in soybean. These were isolated in a model mouth system and sampling of the headspace (van Ruth and Roozen, 2000). It should be noted that the perception of flavour is not a static experience during the course of eating and the overall perception of food is based on its initial impact, perception during chewing and perception of residual flavour (Lindinger *et al*., 1998). It is a dynamic process and should be measured in real time. A large number of volatile compounds were identified in the Kenyan lablab bean. Results have shown that less than 5% of the volatiles identified in foods contribute to the aroma or odour (Grosch, 2000). Methods that are used in analysis of the smaller and more volatile compounds and reflect more accurately the profile as experienced during eating e.g. isolation by model mouth system and sampling of the headspace (van Ruth and Roozen, 2000) can be used.

Apart from the volatile compounds, Drumm (1989) indicated that the other major components of beans that would potentially affect its flavour include lipids, carbohydrates, proteins and phenolic acids which were not assayed in this study. The compounds identified in this study would affect flavour since they have been identified to affect flavour in lablab and other beans. Bean cultivars have been reported to differ in abundance and profile of volatiles (Oomah, *et al*., 2007). This is similar to the results in this study where differences in the volatile compounds in the accessions were observed. Since the similarities were calculated from the retention times of the abundant compounds, the accessions that were distinct showed more abundance of other compounds like the rest of the accessions. The high level similarity (98%) of most of the Kenyan *L. purpureus* accessions, however, was expected since the accessions are from the same species.

4.2.4 Molecular characterization

The analysis of genetic variation in breeding materials is of fundamental interest to the plant breeders. It contributes to selection, monitoring of germplasm and prediction of potential genetic gain (Chakravarthi and Naravaneni, 2006). The characterization of genetic diversity within a closely related crop germplasm is an essential tool for rational use of genetic resources. The AFLP technique used in this study provided a basis for detecting molecular diversity within and among the Kenyan populations of *L. purpureus* for the first time, thus determining genetic affinities.

Overall, this study revealed that *L. purpureus* accessions studied are based on material of narrow genetic base (with a mean expected heterozygosity H_e=0.189). The low level of the species diversity may be attributed to self pollinating nature of *L. purpureus*, though some outcrossing has also been reported (Hacker and Hanson, 1999); and increased gene flow as a result of exchange of germplasm by farmers across the regions, which are geographically close to one other. In most cultivated plant species, mean heterozygosity (H_e) has been reported to be 0.30 in soyabean (Ude *et al*., 2003), 0.32 in common beans using AFLP markers (Maras *et al*., 2008), 0.361 in green beans using SSR markers (Sarıkamış *et al*., 2009), mung bean 0.444 using SSR markers (Gwag *et al*., 2006), 0.361 in tea using AFLP markers (Wachira *et al*., 2001), 0.313 in rice using SSR markers (Cao *et al*., 2006) and 0.37 in wheat with SSR markers (Zhang *et al*., 2006).

Among the populations, the highest diversity was resident in Eastern population (H_e =0.297) with the least in Nairobi population (H_e =0.055). The expected heterozygosity accounts for the frequency of the different types of alleles or loci in the population (Mohammadi and Prasanna, 2003). This implies that sampling for conservation of the Kenyan *L. purpureus* population can be carried out extensively in the Eastern population across ecological amplitude, to capture as many loci as possible, in contrast to screening from different many populations. Maass *et al*., (2005) used AFLP markers to determine the sources of diversity in cultivated and wild *L. purpureus* accessions from various African, Asian and other countries. The African countries included Angola, Egypt, Ethiopia, Kenya, Mozambique, Malawi, Nigeria, Sudan, Tanzania, Uganda, South Africa, Zambia and Zimbabwe. The accessions from Kenya were the old cultivar, Rongai, 100602 and CPI 16882. Moderate genetic diversity was displayed for the landraces from Africa and Asia, and the accessions were clustered according to their subspecific taxonomic organization and as cultivated and wild forms. The Kenyan *L. purpureus* revealed that the existing variations in cultivated forms had no geographic basis. Indeed, clustering of the accessions was not dependent on the geographical area of collection. The same results were obtained by Maass *et al.*, (2005). It may be hypothesized that the modest diversity in *L. purpureus* is attributed to the movement of accessions between the regions resulting in a narrow genetic base. Maass *et al*., (2005) suggested that continuous exchange and selection from a narrow set of landraces may have resulted in a reduction in the genetic base of the crop in the Indian and Africa continents.

The low diversity within the Kenyan *L. purpureus* accessions was also captured in the principal coordinate analysis plot (Figure 4.6) tight cluster of points. This is similar to the tight cluster of points on a PCA plot for the first and second coordinates reported by Venkatesha *et al*., (2007), on accessions from UAS and *L. purpureus* collected from Southern states of India. More diversity however was revealed from a wider scatter PCA plot when accessions from Africa were included. The use of expressed sequence tag (EST) markers and gene-specific primers pairs, designed from legumes within the Phaseoleae tribe, i.e. *M. truncatula, Glycine max, Pisum sativum, V. unguiculata, P. coccineus, P. lunatus, P. vulgaris* and *V. radiate,* have also given similar results as AFLP analyses of moderate diversity (Venkatesha *et al*., 2007). Liu (1996) reported a high level of genetic variation in *L. purpureus* using random amplified polymorphic DNA (RAPD). However, the differences observed were mainly between the cultivated and wild

forms, with the cultivated genotypes showing only moderate dissimilarities. Great diversity has also been reported for the wild forms (Maass *et al*., 2005). The wild and cultivated forms can be crossed to produce variability of high fertile hybrids in the Kenyan breeding program. A large agro-morphological diversity of *Lablab purpureus* has been reported in South Asia (Maass *et al.*, 2010), and these can also be included in the breeding programs to expand the genetic base. However, breeding can also be carried out between the most diverse accessions Mwingi-3, 10706R1, 12000 and 12187R3 with the accessions in the other cluster groups.

AMOVA revealed that most of the diversity is partitioned within populations (99% variance) and only 1% between populations, with F-statistics analogous measure Φ_{PT} value of 0.007. This shows little genetic differentiation between the populations assayed in the Kenyan *L. purpureus* accessions. Genetic differentiation is a function of the geneflow among populations via pollen and/or seed dispersal (Mohammadi and Prasanna, 2003). The low genetic differentiation also supports the high levels of population genetic identity recorded (ranging from 0.893 to 0.967), and low genetic distances (0.033 to 0.1138).

Nairobi population was the most distinct population on the dendogram (Figure 4.5). This uniqueness may be explained by the fact that Nairobi is commercial region and receives seed from all the parts of the country. The inflow to Nairobi from other regions is much higher than to other population regions and population may access most alleles from within country and outside the country since it is a point of entry into the country. There is thus need to sample from Nairobi as well in order to capture the unique alleles that are not present in the other populations.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Sensory characteristics such as bitter taste and odour contribute to the consumers preferences of a *L. purpureus* accessions. The results from this study revealed significant ($p<0.05$) differences in the bitter taste of the 24 lablab accessions, with accession 10706 reporting highest bitter taste and 13096 ranked lowest. In the overall rankings of the two parameters assayed, accession 10706 ranked highest in both parameters while 13096 ranked lowest. The differences in rankings of the accessions are due to the subjective nature of sensory evaluations and ability of analysts to detect the parameters. The bitter taste in some of the *L. purpureus* accessions has been found to persist despite the heat treatment during cooking. No significant ($p>0.05$) differences were observed for the odour of the accessions. The accessions that had lower levels of bitter taste are recommended where lablab is being introduced and this can contribute to food security.

The cyanogenic glycosides in the accessions studied were found to be below detectable levels using the picrate method. However, chemosensory stimulation is rarely the result of the action of a single substance and the cause of bitter taste of lablab bean should be investigated further by analysing other compounds, alongside the cyanogenic glycosides, and also their interactions towards contributing to the bitter taste. The Kenyan accessions assayed had volatile compounds that were very similar and accession 11741 was the most distinct. The most common compounds identified were esters, alcohols and hydrocarbons.

Molecular diversity analysis can identify lines that are genetically dissimilar and can be used to generate heterotic crosses and to identify novel alleles for genes of agronomic and biochemical importance. The AFLP markers used in this study were a useful tool for detecting genetic diversity within Kenyan lablab bean, since there are no specific markers for lablab. Diversity analysis identified accessions Mwingi-3, 10706R1, 12000 and 12187R3, as genetically diverse and these can be used to generate crosses and to identify novel alleles for genes of agronomic and biochemical importance. Following the moderate to low genetic diversity, there is need to diversify the genetic base of the Kenyan accessions by including the wild cultivars and exotic germplasm from other countries, like South Asia in the breeding programs. Genetic polymorphisms detected with AFLP are mainly inherited as dominant and thus are not able to

identify heterozygous loci, limiting their use in estimating gene flow and studying mating systems. Since lablab has been considered as a promising crop because of its wide spectrum of adaptability to different ecological conditions than other legumes, participatory evaluation at an early breeding stage could shape the variety being developed to increase the productivity and other traits as well as for utilization as food.

5.2 Recommendations

From the study, the following recommendations were made:

1. That the accessions 13096, 11705 and 11736 are recommended for cultivation by farmers for food as they ranked overall lowest for the mean scores of bitter taste and odour.
2. That a comprehensive descriptive analysis be carried out to profile the sensory characteristics of lablab accessions, including the texture characteristics, appearance of the bean, flavour, physiochemical attributes and overall acceptability.
3. That the Kenyan lablab accessions studied are safe for human consumption and are low in cyanogenic glycosides (below 10ppm).
4. That more sensitive techniques be used for isolation and identification of the volatile compounds contributing heavily to the characteristic odour of Kenyan lablab accessions.
5. That Kenyan *L. purpureus* accessions have low genetic diversity and conservation efforts should be enhanced. Collection strategies within the country should focus on sampling from one population preferably from Eastern province ensuring that wide ecological amplitude of the cultivation area, as is possible, is covered. Nairobi could also be sampled because it may have germplasm with unique alleles.
6. That farmers should grow divergent accessions in their farms to cushion themselves against attendant risks associated with diseases, pests and changing weather and climate. The following combinations of disparate accessions are recommended Mwingi-3 and 45349 or 10706R1 and Thika-1.
7. That *L. purpureus* accessions from other countries and wild cultivars should be incorporated into the Kenyan breeding programs to increase the genetic diversity and offer new potential for enhancing desired traits.

REFERENCES

Adams, R. P. (1995). Identification of essential oil components by gas-chromatography/mass spectroscopy. Allured Publishing Corp, Carol Stream, Illinois, USA.

Adebisi, A.A. and Bosch, C.H. (2004). *Lablab purpureus* (L.) Sweet. In: Grubben, G.J.H. and Denton, O.A. (eds) Plant Resources of Tropical Africa (PROTA), no. 2, vegetables. PROTA Foundation, Wageningen, The Netherlands / Backhuys, Leiden, The Netherlands / CTA, Wageningen, Netherlands pp 343-348

Apriyantono, A., Husain, H., Lie, L., Jodoamidjojo, M., & Puspitasari-Nienaber, N. L. (1999). Flavor characteristics of Indonesian soy sauce (Kecap manis). In F. Shahidi, & C. T. Ho (eds.), *Flavor Chemistry of Ethnic Foods* Plenum Press, New York, USA pp. 15–31

Augusto, F., Leite e Lopes, A., Zini, C. A. (2003). Sampling and sample preparation for analysis of aromas and fragrances. *Trends in Analytical Chemistry* **22**(3):160-169

Babushok, V.I., Linstrom, P.J., Reed, J.J., Zenkevich, I.G., Brown R.L., Mallard, W.G. and Stein S.E. (2007). Development of a database of gas chromatographic retention properties of organic compounds. *Journal of Chromatography A* **1157**:414-421

Becker, W. (1978). Solvent extraction of soybean. *Journal of American Oil Chemists' Society* **55** (11):754-761

Bolhuis, G.G. (1954). The toxicity of cassava roots. *Netherlands Journal of Agriculture and Science* **2**:176-185

Bower, J. A. (2000). Statistics for Food Science – Correlation and regression (part B). *Journal of Nutrition and Food Science* **2**:80-85

Bressani, R and Elias, L.G. (1980). The nutriotional role of polyphenols in beans. *Proceedings of the 36th Institute of Food Technologists Symposium,* 10-13 June 1979. St. Louis, Missouri., Hulse, J.H. (Ed). International Development Research Centre, Ottawa, Canada, pp 72-111

Buettner, A. and Schieberle, P. (2000). Influence of mastication on the concentrations of odour volatiles- Some aspects of flavour release and flavour perception. *Food Chemistry* **71**:347-354

Cao, Q., Lu, B.R., Xia, H., Rong, J., Sala, F., Spada, A. and Grassi F. (2006). Genetic diversity and origin of weedy rice (*Oryza sativa* f. *spontanea*) populations found in North-Eastern

China revealed by simple sequence repeat (SSR) markers. *Annals of Botany* **98**: 1241–1252

Cameron, D.G. (1988). Tropical and subtropical pasture legumes. *Queensland Agricultural Journal*. March-April: 110-113

Chakravarthi, B.K. and Naravaneni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). *African Journal of Biotechnology* **8**:684-688

Chau, C.F., Cheung, P.C.K. and Wong, Y.S. (1998). Chemical composition of three underutilized legume seeds grown in China. *Food Chemistry* **61**:505–509

CIMMYT. (2005). Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. 3rd Edition. Mexico, D.F.: CIMMYT pp43

De Leon, L.F., Elias, L.G. and Bressani, R. (1992). Effect of salt solutions on the cooking time, nutritional and sensory characteristics of common beans (*Phaseolus vulgaris* L.). *Food Research International* **25**:429-436

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter* **1** (4):19-21

Doyle, J.J. and Doyle J.L. (1990) (modified at the NCSU Forest Biotechnology Laboratory). Isolation of DNA from small amounts of plant tissues. *Focus* **12**:13-15.

Drumm, T. D. (1989). Characterization of the major components of dry beans and their potential relationship to flavor. *Dissertation Abstracts International* **49:**3524–3525.

Duke, J.A., Kretschmer, A.E. Jr., Reed, C.F and Weder, J.K.P. (1981). *Lablab purpureus* (L.) *Sweet*. In: Duke J.A. (ed). *Handbook of legumes of world economic importance,* Plenum Press, New York, USA and London, UK. pp102-106

El-Sayed, A.M. (2010). The Pherobase: Database of Insect Pheromones and Semiochemicals. Lincoln, New Zealand, (2005). http://www.pherobase.com/database/kovats/kovats-index.php

Engle, L.M. and Altoveris, N.C. (eds) (2000).Collection, conservation and utilization of indigenous vegetables. In: *Proceedings of a workshop, AVRDC, Shanhua, Tainan, Taiwan, 16-18 August 1999,* Asian Vegetable Research and Development Centre (AVRDC), Shanhus, Taiwan, ROC, 142pp

Excoffier, L., Smouse, P.E., and Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitocondrial DNA restriction sites. *Genetics*. **131**:479-491.

Fenwick, G.R., Curl, C.L., Griffiths, N.M., Heaney, R.K. and Price, K.R. (1990). Bitter principles in food plants. In: Rouseff RL, ed. *Bitterness in foods and beverages; developments in food science* Elsevier, Amsterdam, the Netherlands pp 205–250

Francisco, I.A. and Pinotti, M.H.P. (2000). Cyanogenic Glycosides in Plants. *Brazilian Archives of Biology and Technology* **43**(5):487-492

Fuller, D.Q. (2003). African crops in prehistoric South Asia: A critical review. *Africa Praehistorica* **15**:239-271

Gawel, N.J., and Jarret, R.L. (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomea*. *Plant Molecular Biology Reports* **9**:262-266

Goto-Yamamoto, N. (2000). Phenetic clustering of grapes (*Vitis* spp.) by AFLP Analysis. *Breeding Science* **50**: 53–57.

Grosch, W. (2000). Specificity of the human nose in perceiving food odorants. In. Schieberle P and Engel K.H. (eds), *Frontiers of Flavour Science, Proceedings of the Ninth Weurman Flavour Research Symposium.* Deutshe Forchungsanstalt für Lebensmittelchemie, Garching, Germany pp 213-219

Gruhnert, C., Biehl, B. and Selmar, D**.** (1994), Compartmentation of cyanogenic glucosides and their degrading enzymes. *Planta* **195**:36-42

Gupta, Y.P. (1987). Anti-nutritional and toxic factors in food legumes: a review. *Plant Foods for Human Nutrition* **37**:201-228

Guzmán-Madondo, H., Castellana, J. and González de Mejía, E. (1996). Relationship between theoretical and experimentally detected tannin content of common beans (*Phaseolus vulgaris* L.). *Food Chemistry* **55**:333-335

Gwag, J.G., Chung J.W., Chung H.K., Lee J. H., Ma, K.H., Dixit A., Park Y.J., Cho, E.G., Kim, T.S. and Lee, S.H. (2006). Characterization of new microsatellite markers in mung bean, *Vigna radiata* (L.). *Molecular Ecology Notes* **6**: 1132–1134

Hacker, J.B. and Hanson, J. (1999). Crop growth and development: Reproduction. In: Loch D.S. and Ferguson J.E. (eds). *Forage Seed production 2. Tropical and Subtropical Species.* CABI Publishing. Wallingford, UK. pp 93-111

Harborne, J.B. (1972). Cyanogenic glucosides and their function. In: *Phytochemical Ecology*. Academic Press, London pp 104-123

Harborne, J.B. (1993). Plant toxins and their effects on animals. In: *Introduction to Ecological Biochemistry*. Academic Press, London pp 71-103

Heng, L., van Koningsveld, G. A., Gruppen, H., van Boekel, M.A.J.S., Vincken, J.-P., Roozen, J.P. and Voragen, A.G.J. (2004). Protein-Flavour interactions in relation to development of novel protein foods. *Trends in Food Science and Technology* **15**:217:224

Hinterholzer, A., Lemos, T. and Schieberle P. (1998). Identification of the key odorants in raw French beans and changes during cooking. *Zeitschrift für Lebensmitteluntersuchung und - Forschung A* **207**:219–222

Huff, D.R., Peakall, R. and Smouse, P.E. (1993). RAPD variation within and among natural populations of outcrossing buffalograss *Buchloe dactyloides* (Nutt) Engelm. *Theoretical and Applied Genetics*. **86**:927-934.

International Atomic Energy Agency (IAEA). (2002). Mutant accession characterization using molecular markers- Manual. *Training Course Series 19,* Vienna, Austria pp7.1-7.7

Johnson, L. A. and Lusas, E. W. (1983). Comparison of alternative solvents for oil extraction. *Journal of American oil Chemists' Society* **60** (2):229-241

Kamotho, G.N., Kinyua, M.G., Muasya, R.M., Orwa, D.O. and Kimani, E.N. (2010). Baseline Survey on Production Practices, Constraints and Utilization of Dolichos Bean in Kenya: Implications for Dolichos Bean Improvement. *International Journal of Professional Practice* **1**:21-29

Khah, E. M. and Arvanitoyannis, I. S. (2003) Yield, nutrient content and physico-chemical and organoleptic properties in green bean are affected by N:K ratios. *Food, Agriculture & Environment* **1**(3&4):17-26

Kim, J. S. and Chung, H. Y. (2008). Characterization of volatile components in field bean (*Dolichos lablab*) obtained by simultaneous steam distillation and solvent extraction. *Journal of Food Science and Nutrition* **13**:18-22

Laurena, A. C., Revilleza, M. J. R. and Mendoza, E. M. T. (1994). Polyphenols, phytate, cyanogenic glycosides and trypsin inhibitor activity of several Philippine indigenous food legumes. *Journal of Food Composition and Analysis* **7**:194-202

Lawless, H. T. and Lee, C. B. (1993). Common chemical sense in food flavour. In: T. E. Acree and R. Teranishi (ed), *Flavour science*, American Chemical Society, Washington, USA pp 21-65

Lelei, J.J., Onwonga, R.N. and Freyer, B. (2009). Organic based nutrient management strategies: effect on soil nutrient availability and maize (*Zea mays* L.) performance in Njoro, Kenya. *African Journal of Agricultural Research* **4** (2):92-99

Lindinger, W., Hansel, A. and Jordan, A. (1998). On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer reaction-mass spectrometry (PTR-MS): Medical application, food control and environmental research. *International Journal of Mass Spectrom and Ion Process* **173**: 191-241

Liu, C.J. (1996). Genetic diversity and relationships among *Lablab purpureus* genotypes evaluated using RAPD markers. *Euphytica* **90**:115–119

Lynch, M. and Milligan, B.G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology* **3:**91-99.

Maass, B.L., Jamnadass, R.H., Hanson J. and Pengelly B.C. (2005). Determining sources of diversity in cultivated and wild *Lablab purpureus* related to provenance of accession by using amplified fragment length polymorphism. *Genetic Resources and Crop Evolution* **52**: 683-695

Maass, B.L. and Usongo, M.F. (2007). Changes in seed characteristics during the domestication of the lablab bean (*Lablab purpureus* (L.) Sweet: Papilionoideae) *Australian Journal of Agricultural Research* **58**:9–19

Maass, B.L., Knox, M.R., Venkatesha, S.C., Tefera, T.A., Ramme, S. and Pengelly, B.C. (2010) *Lablab purpureus*- A crop lost for Africa? *Tropical Plant Biology* **3** (3):123-135

Mace, E.S., Lester, R.N. and Gebhardt, C.G. (1999). AFLP analysis of genetic relationships among the cultivated eggplant, *Solanum melongena* L. and wild relatives (Solanaceae). *Theoretical and Applied Genetics* **99**:626–633.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A laboratory manual-Cold Spring Habor Publications, New York, USA. pp 6.11

Maras, M., Šuštar-vozli, J., Javornik, B. and Megli, V. (2008). The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (*Phaseolus vulgaris* L.). *Acta agriculturae Slovenica* **91** (1): 87-96

Martins, S.I.F.S., Jongen, W.M.F. and Van Boekel, M.A.J.S. (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology* **11**:364-373

Matasyoh, L.G., Wachira, F.N., Kinyua, M.G., Muigai, A.W.T, and Mukiama, T.K. (2008). Leaf storage conditions and genomic DNA isolation efficiency in *Ocimum gratissimum* L. from Kenya. *African Journal of Biotechnology* **7** (5):557-564

Maughan, P.J., Saghai Maroof, M.A., Buss, G.R. and Huestis, G.M. (1996). Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical and Applied Genetic* **93** (3)**:**392-401

Maundu, P.M., Ngugi, G.W. and Kabuye, C.H.S. (eds) (1999). *Lablab purpureus* In: Traditional food plants of Kenya. English Press Ltd. Nairobi, Kenya pp154-155

McCouch, S. (2004). Diversifying selection in Plant Breeding. *PLoS Biology* **2**(10):e347

McDonald, L.M., Wright, P. and MacLeod, D.A. (2001). Nitrogen fixation by lablab (*Lablab purpureus*) and Lucerne (*Medicago sativa*) rotation crops in an irrigated cotton farming system. *Australian Journal of Experimental Agriculture* **41**:219-225

Mcmahon, J.M., White, W.L.B., Sayre, R.T. (1995). Cyanogenesis in cassava (*Manihot esculenta* Crantz). *Journal of Experimental Botany* **46:**731-741

Mestres, M., Busto, O. and Guasch J. (2000). Analysis of organic sulfur compounds in wine odour. *Journal of Chromatography A* **881**:569–581

Mkanda, A. V., Minnaar, A. and Henriëtte L. de K. (2007). Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris*). *Journal of the Science of Food and Agriculture* **87**(15):2868 – 2879

Mohammadi, S.A. and Prasanna, B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Science* **43**:1235-1248

Murphy, A.M. and Colucci, P.E. (1999). A tropical forage solution to poor quality diets: A review of *Lablab purpureus*. *Livestock Research for Rural Development* **11**(2) http://www.cipav.org.co/lrrd/index.html

Ndung'u, J.N., Wachira, F.N. and Kinyua M.G. (2008). Influence of the environment on Cassava Quality Traits in Central Rift Valley, Kenya. *3rd Annual Research Week and International Conference* 16th-18th September. Egerton University, Njoro, Kenya.

Ndung'u, J.N., Wachira, F.N., Kinyua, M.G. Lelgut, D.K. and Obiero, H. (2010). Biochemical nalysis of cassava quality traits in Central Rift valley of Kenya. *12th KARI Biennial Scientific Conference* 8th-12th November. KARI Heaquaters Complex, Nairobi, Kenya

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583-590.

Nursten, H.E. (1977). The important volatile flavour components of foods. In: Birch, G. G., Brennan, J. G., Parker, K. J. (eds). *Sensory Properties of Foods* Applied science Publishers Ltd. London, UK pp 151-167.

Oke, O.L. (1969). The role of hydrocyanic acid in nutrition. World *Review of* Nutrition *and Dietetis* **11**:118-147

Oomah, B.D., Liang, L.S.Y. and Balasubramanian, P. (2007). Volatile compounds of dry beans (*Phaseolus vulgaris* L.). *Plant Foods and Human Nutrition* **62:**177–183

Patil, P., Venkatesha, S.C., Ashok, T.H., Gowda, T.K.S., Byre, Gowda, M. (2009). Genetic diversity in field bean as revealed with AFLP markers. *Journal of Food Legumes* **22**(1):18–22

Peakall, R. and Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. **6**:288-295. http://www.anu.edu.au/BoZo/GenAlEx/

Pengelly, B.C. and Maass, B.L. (2001). *Lablab purpureus* (L.) Sweet- diversity, potential use and determination of a core collection of this multi-purpose tropical legume. *Genetic Resources and Crop Evolution,* **48**:261-272

Pengelly, B.C. and Lisson, S.N. (2003). Strategies for using improved forages to enhance production in Bali cattle. In: Entwistle, K. and Lindsay, D.R. (eds). *Strategies to improve Bali cattle in eastern Indonesia. Proceedings of a workshop* 4-7 February 2002, Bali, Indonesia. ACIAR Proceedings no. 110. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia pp 29-33

Quirien, van O. and Keith, T. (2005). Sensory evaluation techniques In: *Methods in Development Research: Combining Qualitative and Quantitative Approaches* (eds) Campbell J. R., Holland J. Kent, Great Britain. pp107-118

Radulovic N., Stojanovic G., Palic R., and Sladana, A. (2006). Chemical composition of the ether and ethyl acetate extracts of serbian selected tobacco types: Yaka, Prilep and Otlja. *The Journal of Essential Oil Research* **18** (5):562-565

Ramaroson-Raonizafinimanana, B., Gaydou, É. M. and Bombarda, I. (1997). Hydrocarbons from three *Vanilla* bean species: *V. fragrans*, *V.madagascariensis*, and *V. tahitensis. Journal of Agriculture and Food Chemistry* **45**:2542-2545

Ribaut, J.M and Hoisington, D.A. (1998). Marker–assisted Selection: New tools and strategies. *Trends in Plant Science* **3**:236-239

SAS 9.1.3. (2004). SAS Institute Inc., Cary, North Carolina, USA.

Sarıkamış, G., Yaşar, F. Bakır, M., Kazan, K. and Ergül, A. (2009). Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from Eastern Turkey. *Genetics and Molecular Research* **8** (3): 880-887

Schieberle, P. (1995). New developments in methods for analysis of volatile flavour compounds and their precursors. In: Gaonkar AG (ed) *Characterization of food: emerging methods*. Elsevier, Amsterdam, Netherlands pp 403–431

Scott, J. and Maiden, M. (1998). Socio-economic survey of three bean growing areas in Malawi. *Occasional Paper Series* No. 24, CIAT, Kampala, Uganda

Seigler, D.S., Maslin, B.R., Conn, E.E. (1989). Cyanogenesis in the Leguminosae. *Monographs in Systematic Botany from the Missouri Botanical Garden* **29**:645-672.

Shannon, C.E. and Weaver, W. (1949). The mathematical theory of communication. University of Illinois Press, Urbana, Illinois, USA

Shi, J., Arunasalam,K., Yeung, D., Kakuda, Y., Mittal, G. A. and Jiang, Y. (2004). Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medical Food* **7**:67-78

Smartt, J. (1985). Evolution of grain legumes II. Old and new world pulses of lesser economic importance. *Experimental Agriculture* **21**:1-18

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical taxonomy. Freeman, San Francisco, California, USA

Sommer, R. and Tautz, D. (1989). Minimal homology requirements for PCR primers. *Nucleic Acids Research*, **17**: 6749.

Song, Z.P., Xu X., Wang, B. and Chen, J.K. (2003). Genetic diversity in the northernmost Oryza rufipogon populations estimated by SSR markers. *Theoretical and Applied Genetics* **107**:1492-1499

Steiger, D.L., Naggai, C., Moore, P.H., Morden, C.W., Osgood, R.V. and Ming, R. (2002). AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. *Theoretical and Applied Genetics* **105**:209-215

Sultana, N., Ozaki, Y. and Okubo, H. (2000). The use of RAPD markers in lablab bean (*Lablab purpureus* (L.) Sweet) phylogeny. *Bulletin of Institute of Tropical Agriculture Kyushu University* **23**:45–51

Tanksley, S.D. and Orton, T.J. (1983). Isozymes in plant genetics and breeding. Part B. In: Tansksley, S. D. and Orton, T. J. (eds). *Developments in plant genetics and breeding.* Elsevier Science Publishers B V, Amsterdam, Netherlands pp. 401-421

Tefera, T., Oluoch, M. and Maass, B.L. (2006). Identifying vegetable lablab types by participatory assessment: panelists' perceptions of morphological traits and organoleptic taste assessment. Poster presented at the *13th Australian Society of Agronomy Conference,* 11-14 September 2006, Perth, Australia. http://www.regional.org.au/au/asa/2006/poster/systems/4688_tolerat.htm

Thome, J., Orlando, G.D., Beebe, S. and Duque, M.C (1996). AFLP analysis of gene pools of a wild bean core collection. *Crop Science* **36:**1375-1384

Tian, Z., Wang, S., Wang, W. and Liu, L. (2005). Study on the diversity of germplasm resources of *Dolichos lablab* (L.). *Natural Science Journal of Hainan University* **23**(1):53–60

Ude, G. N., Kenworthy, W.J., Costa, J. M., Cregan, P. B. and Alvernaz, J. (2003). Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. *Crop Science* **43**:1858–1867

University of Agricultural Sciences. (2004). Molecular Marker Techniques for Crop Improvement: Course Manual. Bangalore, India pp 20-24

van Ruth, S.M. and Roozen, J.P. (2000). Influence of mastication and artificial saliva on odour release in a model mouth system. *Food Chemistry* **71**:339-345

van Ruth, S.M., Dings, L., Buhr, K. and Posthumus, M.A. (2004). *In vitro* and *in vivo* volatile flavour analysis of red kidney beans by proton transfer reaction–mass spectrometry

Food Research International **37**:785–791

van Ruth, S.M., Dings, L., Aprea, E. and Odake, S. (2005). Comparison of Volatile Flavour Profiles of Kidney Beans and Soybeans by GC-MS and PTR-MS. *Food Science and Technology Research* **11** (1):63-70

Venkatachalam, M. and Sathe, S.K. (2007). *Val* bean (*Lablab purpureus* L.) proteins: Composition and biochemical properties *Journal of the Science of Food and Agriculture* **87**:1539–1549.

Venkatesha, S. C., Gowda M. B., Mahadevu, P., Rao, A. M., Kim, D.-J., Ellis, T. H. N., and Knox, M. R. (2007). Genetic diversity within *Lablab purpureus* and the application of gene-specific markers from a range of legume species. *Plant Genetic Resources: Characterization and Utilization* **5** (3):154–171.

Vijayakumari, K., Siddhuraju, P. and Janardhanan K. (1995). Effects of various water or hydrothermal treatments on certain anti nutritional compounds in the seeds of the tribal pulse, *Lablab lablab* var *vulgaris* L. *Plant Foods for Human Nutrition* **48**:17-29.

Virk, P. S., Ford-Lloyd, B.V., Jackson, M.T., and Newbury, H.J. (1995). Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* **74** (2):170-179

Vos, P., Hogers, R., Bleeker, M., Rijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pt J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for fingerprinting. *Nuclei Acids Research* **23:**4407-4414

Wachira, F.N., Tanaka, J., and Takeda, Y. (2001). Genetic variation and differentiation in tea (*Camellia sinesis*) accession revealed by RAPD and AFLP variation. *Journal of Horticultural Science and Biotechnology* **76:** 557-563

Waldmueller, L.F. (1992). *Lablab purpureus* In: *Agricultural Extension Handbook for Lamu District*. Lamu, Kenya pp 145-146

Wanakhachornkrai, P. and Lertsiri, S. (2003). Comparison of determination method for volatile compounds in Thai soy sauce. *Food Chemistry* **83**:619–629

Wang, M.L., Morris, J.B., Barkley, N.A., Dean, R.E., Jenkins, T.M. and Pederson, G.A. (2007). Evaluation of genetic diversity of the USDA *Lablab purpureus* germplasm collection using simple sequence repeat markers. *Journal of Horticultural Science Biotechnology* **82** (4):571–578

Wanjekeche, E., Mwangi, J., Kamidi, M., Powon, P. and Khaemba, J. (2000). Farmer participation in the evaluation of grain legumes in North-Western Kenya. In: KARI. Participatory technology development for soil management by small holders in Kenya.

Proceedings of the 2nd Scientific Conference of the Soil Management and Legume Research Network Projects, Mombasa, Kenya pp 273-279

Waugh, R. (1994). Lab Protocols: Plant Genome analysis group, Cell and Molecular Genetics Department. Scottish Crop Research Institute, Invergowrie, Dundee, Scotland pp 52-57

Watts, B.M, Ylimaki, L.E., Jeffery, L.E. and Elias, L.G. (1989). In: *Basic Sensory methods for food evaluation*. International Development Research Centre, Ottawa, Canada

Weeden, N.F. and Wendel J.F. (1990). Genetics of plant isozymes. In: D. E. Soltis and P. S. Soltis (eds). *Isozymes in plant biology*. Chapman and Hall, London, UK pp 46-72

Williams, H.J. and Edwards, T.G. (1980). Estimation of cyanide with alkaline picrate. *Journal of Science Food and Agriculture* **31**:15-22

Yang, H.H. and Lawless, H.T. (2005). Time intensity characteristics of iron compounds. *Food Quality and Preference* **17**:337-343

Yee, E., Kidwell, K.K., Sills, G.R. and Lumpkin, T.A. (1999). Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Science* **39**:268-275

Yeh, F.C., Yang, R.T., Boyle, J., Ye, Z. and Xiyan, J.M. (2000). PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Zabeau M. (1993). *European Patent Application No*. 92402629.7

Zhang, D.P., Cervantes, J., Huama´ n Z., Carey, E. and Ghislain, M. (2000). Assessing genetic diversity of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars from tropical America using AFLP. *Genetic Resource Crop Evolution* **47**:659–665.

Zhang, P., Dreisigacker, S., Buerkert, A., Alkhanjari, S., Melchinger, A.E. and Warburton, M.L. (2006). Genetic Diversity and Relationships of Wheat Landraces from Oman Investigated with SSR Markers. *Genetic Resources and Crop Evolution* **53**:1351-1360

APPENDICES

Appendix 1: Nei's genetic identity matrix for 50 Lablab accessions based on 180 polymorphic AFLP markers. Above diagonal is identity and below diagonal is distance. Names of accessions are as indicated in Table 3.2.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	***	0.92	0.92	0.9	0.82	0.87	0.84	0.86	0.48	0.86	0.85	0.85	0.84	0.84	0.86	0.81	0.77	0.72	0.83	0.79
2	0.09	***	0.92	0.87	0.77	0.83	0.84	0.82	0.44	0.84	0.86	0.87	0.81	0.84	0.83	0.81	0.76	0.69	0.81	0.82
3	0.08	0.09	***	0.91	0.77	0.87	0.84	0.84	0.44	0.86	0.85	0.85	0.84	0.83	0.86	0.82	0.76	0.71	0.83	0.82
4	0.11	0.14	0.09	***	0.77	0.87	0.81	0.86	0.43	0.86	0.83	0.85	0.83	0.83	0.86	0.81	0.79	0.71	0.83	0.82
5	0.2	0.27	0.26	0.26	***	0.78	0.79	0.8	0.46	0.77	0.73	0.77	0.76	0.74	0.74	0.69	0.71	0.66	0.77	0.73
6	0.14	0.19	0.14	0.14	0.24	***	0.82	0.87	0.46	0.88	0.82	0.85	0.8	0.84	0.81	0.79	0.78	0.74	0.84	0.79
7	0.17	0.18	0.17	0.21	0.23	0.2	***	0.81	0.48	0.81	0.79	0.81	0.84	0.77	0.76	0.76	0.72	0.68	0.79	0.77
8	0.15	0.2	0.18	0.15	0.22	0.14	0.22	***	0.47	0.89	0.82	0.87	0.85	0.84	0.84	0.79	0.77	0.68	0.83	0.8
9	0.74	0.82	0.81	0.84	0.77	0.79	0.74	0.75	***	0.47	0.42	0.45	0.47	0.45	0.43	0.43	0.49	0.52	0.48	0.46
10	0.16	0.18	0.16	0.16	0.26	0.13	0.21	0.11	0.76	***	0.86	0.89	0.83	0.88	0.83	0.83	0.79	0.74	0.87	0.84
11	0.16	0.16	0.16	0.19	0.31	0.2	0.23	0.2	0.88	0.15	***	0.87	0.83	0.88	0.84	0.82	0.78	0.68	0.84	0.84
12	0.16	0.14	0.16	0.16	0.27	0.16	0.22	0.14	0.8	0.11	0.14	***	0.84	0.88	0.83	0.83	0.81	0.72	0.84	0.84
13	0.17	0.22	0.17	0.18	0.27	0.22	0.17	0.16	0.76	0.18	0.19	0.18	***	0.83	0.86	0.81	0.78	0.72	0.85	0.81
14	0.18	0.17	0.19	0.19	0.3	0.18	0.26	0.17	0.8	0.12	0.13	0.13	0.19	***	0.87	0.84	0.79	0.72	0.84	0.81
15	0.16	0.19	0.16	0.16	0.3	0.21	0.28	0.18	0.84	0.18	0.18	0.19	0.16	0.14	***	0.82	0.76	0.71	0.81	0.76
16	0.21	0.22	0.2	0.21	0.36	0.24	0.28	0.23	0.84	0.18	0.2	0.19	0.21	0.18	0.2	***	0.72	0.66	0.78	0.76
17	0.27	0.27	0.28	0.24	0.35	0.25	0.33	0.26	0.72	0.24	0.24	0.22	0.25	0.23	0.28	0.33	***	0.66	0.78	0.82
18	0.33	0.37	0.35	0.35	0.42	0.3	0.38	0.39	0.66	0.3	0.39	0.33	0.33	0.33	0.35	0.41	0.41	***	0.72	0.69
19	0.19	0.21	0.19	0.19	0.27	0.18	0.23	0.18	0.73	0.14	0.17	0.17	0.16	0.17	0.22	0.24	0.24	0.33	***	0.86
20	0.23	0.2	0.2	0.2	0.31	0.23	0.26	0.22	0.77	0.18	0.17	0.17	0.22	0.21	0.27	0.27	0.2	0.37	0.16	***
21	0.16	0.2	0.18	0.19	0.25	0.19	0.27	0.2	0.82	0.18	0.22	0.16	0.18	0.16	0.2	0.23	0.27	0.33	0.2	0.25
22	0.26	0.27	0.23	0.27	0.31	0.27	0.29	0.21	0.73	0.26	0.25	0.25	0.2	0.2	0.23	0.27	0.38	0.46	0.24	0.3
23	0.24	0.2	0.24	0.27	0.34	0.27	0.3	0.24	0.82	0.19	0.21	0.22	0.22	0.17	0.22	0.27	0.33	0.41	0.25	0.27
24	0.17	0.18	0.2	0.2	0.23	0.22	0.28	0.27	0.81	0.24	0.24	0.19	0.21	0.22	0.22	0.25	0.34	0.35	0.23	0.27
25	0.17	0.15	0.2	0.2	0.29	0.24	0.28	0.26	0.84	0.22	0.24	0.19	0.22	0.19	0.2	0.22	0.34	0.38	0.26	0.27

Appendix 1: Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
26	0.18	0.19	0.18	0.16	0.3	0.25	0.27	0.18	0.79	0.18	0.19	0.16	0.2	0.16	0.22	0.24	0.33	0.35	0.19	0.26
27	0.08	0.07	0.09	0.12	0.22	0.15	0.16	0.17	0.77	0.14	0.14	0.14	0.19	0.16	0.19	0.22	0.24	0.31	0.17	0.18
28	0.09	0.09	0.1	0.11	0.22	0.19	0.19	0.2	0.75	0.19	0.2	0.17	0.19	0.18	0.18	0.24	0.29	0.31	0.2	0.22
29	0.09	0.11	0.13	0.12	0.19	0.18	0.18	0.18	0.86	0.17	0.18	0.15	0.22	0.2	0.24	0.24	0.3	0.4	0.22	0.22
30	0.09	0.12	0.07	0.07	0.24	0.13	0.18	0.14	0.76	0.14	0.18	0.15	0.16	0.16	0.13	0.2	0.22	0.32	0.16	0.22
31	0.13	0.14	0.11	0.11	0.27	0.18	0.21	0.2	0.86	0.18	0.18	0.18	0.24	0.19	0.21	0.24	0.31	0.4	0.23	0.24
32	0.12	0.16	0.15	0.16	0.18	0.22	0.23	0.13	0.88	0.18	0.24	0.16	0.23	0.22	0.2	0.26	0.3	0.41	0.24	0.28
33	0.14	0.15	0.14	0.16	0.26	0.16	0.22	0.15	0.76	0.12	0.08	0.08	0.17	0.09	0.2	0.2	0.22	0.33	0.12	0.16
34	0.37	0.36	0.37	0.36	0.43	0.31	0.41	0.35	0.79	0.31	0.33	0.3	0.36	0.33	0.39	0.34	0.46	0.29	0.32	0.32
35	0.16	0.16	0.16	0.14	0.34	0.22	0.26	0.22	0.82	0.19	0.18	0.17	0.22	0.16	0.22	0.22	0.3	0.33	0.21	0.21
36	0.25	0.23	0.24	0.24	0.41	0.3	0.3	0.24	0.76	0.24	0.23	0.24	0.3	0.22	0.3	0.25	0.41	0.47	0.29	0.27
37	0.08	0.11	0.09	0.09	0.23	0.16	0.18	0.14	0.79	0.16	0.18	0.16	0.14	0.18	0.17	0.21	0.25	0.32	0.19	0.22
38	0.12	0.17	0.15	0.12	0.25	0.19	0.24	0.14	0.88	0.22	0.22	0.18	0.22	0.21	0.18	0.24	0.29	0.42	0.25	0.28
39	0.22	0.25	0.26	0.29	0.25	0.27	0.24	0.25	0.82	0.26	0.22	0.22	0.23	0.25	0.3	0.33	0.29	0.42	0.28	0.3
40	0.11	0.12	0.12	0.12	0.19	0.17	0.2	0.11	0.76	0.12	0.18	0.14	0.18	0.16	0.18	0.22	0.27	0.33	0.19	0.2
41	0.16	0.15	0.13	0.13	0.27	0.17	0.17	0.16	0.84	0.13	0.19	0.18	0.18	0.16	0.21	0.18	0.28	0.33	0.19	0.2
42	0.19	0.2	0.18	0.16	0.27	0.19	0.22	0.12	0.8	0.11	0.2	0.14	0.19	0.2	0.2	0.23	0.26	0.34	0.2	0.21
43	0.16	0.19	0.17	0.22	0.18	0.21	0.21	0.24	0.84	0.27	0.23	0.2	0.25	0.24	0.27	0.31	0.36	0.43	0.26	0.26
44	0.18	0.18	0.16	0.16	0.25	0.19	0.24	0.16	0.82	0.14	0.11	0.14	0.19	0.11	0.19	0.18	0.23	0.37	0.14	0.17
45	0.15	0.21	0.19	0.16	0.25	0.2	0.24	0.14	0.73	0.15	0.14	0.18	0.19	0.14	0.18	0.27	0.24	0.39	0.17	0.24
46	0.65	0.7	0.69	0.61	0.66	0.61	0.74	0.56	0.63	0.57	0.64	0.62	0.61	0.7	0.67	0.59	0.59	0.54	0.54	0.66
47	0.21	0.2	0.2	0.21	0.32	0.21	0.24	0.2	0.89	0.17	0.18	0.19	0.2	0.16	0.24	0.22	0.3	0.38	0.23	0.26
48	0.24	0.25	0.22	0.24	0.36	0.27	0.3	0.21	0.77	0.23	0.22	0.27	0.27	0.25	0.29	0.32	0.36	0.49	0.31	0.31
49	0.2	0.16	0.14	0.16	0.27	0.17	0.22	0.15	0.81	0.13	0.18	0.11	0.14	0.14	0.18	0.21	0.24	0.3	0.14	0.16
50	0.14	0.14	0.14	0.16	0.27	0.2	0.24	0.18	0.84	0.16	0.12	0.18	0.16	0.09	0.09	0.17	0.28	0.35	0.16	0.23

Appendix 1: Continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	0.85	0.77	0.78	0.84	0.84	0.83	0.93	0.92	0.91	0.91	0.88	0.88	0.87	0.69	0.85	0.78	0.92	0.88	0.81
2	0.82	0.77	0.82	0.84	0.86	0.83	0.93	0.91	0.89	0.88	0.87	0.86	0.86	0.69	0.86	0.79	0.89	0.84	0.78
3	0.84	0.79	0.78	0.82	0.82	0.83	0.92	0.91	0.88	0.93	0.9	0.86	0.87	0.69	0.85	0.79	0.91	0.86	0.77
4	0.83	0.76	0.76	0.82	0.82	0.86	0.88	0.89	0.89	0.93	0.9	0.85	0.86	0.7	0.87	0.79	0.91	0.88	0.75
5	0.78	0.73	0.71	0.79	0.75	0.74	0.8	0.8	0.83	0.78	0.76	0.83	0.77	0.65	0.71	0.66	0.79	0.78	0.78
6	0.83	0.76	0.76	0.8	0.79	0.78	0.86	0.83	0.83	0.88	0.83	0.81	0.86	0.73	0.81	0.74	0.86	0.83	0.76
7	0.76	0.75	0.74	0.76	0.76	0.77	0.85	0.83	0.83	0.83	0.81	0.79	0.8	0.67	0.77	0.74	0.83	0.78	0.78
8	0.82	0.81	0.79	0.76	0.77	0.84	0.84	0.82	0.84	0.87	0.82	0.88	0.86	0.71	0.8	0.78	0.87	0.87	0.78
9	0.44	0.48	0.44	0.44	0.43	0.46	0.46	0.47	0.42	0.47	0.42	0.42	0.47	0.46	0.44	0.47	0.46	0.42	0.44
10	0.84	0.77	0.83	0.79	0.8	0.83	0.87	0.83	0.84	0.87	0.83	0.84	0.89	0.73	0.83	0.79	0.86	0.81	0.77
11	0.8	0.78	0.81	0.78	0.78	0.83	0.87	0.82	0.84	0.84	0.84	0.79	0.93	0.72	0.83	0.79	0.84	0.8	0.8
12	0.86	0.78	0.8	0.83	0.83	0.85	0.87	0.84	0.86	0.86	0.84	0.86	0.93	0.74	0.84	0.78	0.85	0.83	0.8
13	0.84	0.82	0.81	0.81	0.8	0.82	0.83	0.83	0.8	0.86	0.79	0.79	0.84	0.7	0.81	0.74	0.87	0.81	0.79
14	0.86	0.82	0.84	0.81	0.83	0.85	0.86	0.83	0.82	0.85	0.83	0.8	0.92	0.72	0.86	0.81	0.84	0.81	0.78
15	0.82	0.79	0.81	0.8	0.82	0.8	0.83	0.84	0.79	0.88	0.81	0.82	0.82	0.68	0.81	0.74	0.84	0.84	0.74
16	0.79	0.76	0.76	0.78	0.8	0.79	0.81	0.78	0.79	0.82	0.79	0.77	0.82	0.71	0.81	0.78	0.81	0.78	0.72
17	0.76	0.68	0.72	0.71	0.71	0.72	0.78	0.75	0.74	0.8	0.73	0.74	0.8	0.63	0.74	0.67	0.78	0.75	0.75
18	0.72	0.63	0.67	0.71	0.68	0.71	0.73	0.73	0.67	0.73	0.67	0.67	0.72	0.75	0.72	0.63	0.73	0.66	0.66
19	0.82	0.79	0.78	0.79	0.77	0.83	0.84	0.82	0.81	0.85	0.79	0.79	0.88	0.73	0.81	0.75	0.83	0.78	0.76
20	0.78	0.74	0.77	0.76	0.76	0.77	0.83	0.8	0.81	0.81	0.78	0.76	0.85	0.73	0.81	0.76	0.81	0.76	0.74
21	****	0.77	0.74	0.87	0.85	0.79	0.82	0.82	0.85	0.85	0.85	0.88	0.85	0.72	0.81	0.74	0.86	0.86	0.77
22	0.27	****	0.78	0.74	0.75	0.81	0.78	0.78	0.76	0.78	0.75	0.73	0.81	0.67	0.77	0.75	0.81	0.77	0.71
23	0.3	0.25	****	0.78	0.82	0.79	0.8	0.77	0.77	0.77	0.75	0.73	0.83	0.69	0.76	0.74	0.78	0.72	0.74
24	0.14	0.3	0.24	****	0.92	0.78	0.84	0.84	0.87	0.83	0.83	0.83	0.83	0.67	0.84	0.72	0.86	0.82	0.73
25	0.16	0.29	0.2	0.08	****	0.78	0.83	0.83	0.86	0.82	0.83	0.82	0.82	0.66	0.85	0.74	0.83	0.82	0.72

Appendix 1: Continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
26	0.23	0.22	0.23	0.25	0.25	***	0.83	0.84	0.82	0.84	0.8	0.79	0.87	0.72	0.84	0.86	0.86	0.81	0.76
27	0.2	0.25	0.22	0.18	0.19	0.19	***	0.93	0.91	0.88	0.87	0.86	0.88	0.69	0.86	0.79	0.92	0.83	0.78
28	0.2	0.25	0.27	0.18	0.19	0.18	0.07	***	0.89	0.91	0.87	0.86	0.85	0.68	0.84	0.78	0.91	0.86	0.77
29	0.16	0.27	0.26	0.14	0.16	0.2	0.1	0.11	***	0.88	0.88	0.88	0.86	0.68	0.84	0.77	0.9	0.88	0.79
30	0.16	0.24	0.26	0.18	0.2	0.17	0.12	0.1	0.13	***	0.89	0.87	0.87	0.7	0.86	0.77	0.92	0.89	0.78
31	0.16	0.29	0.29	0.18	0.18	0.22	0.14	0.14	0.13	0.12	***	0.84	0.86	0.68	0.84	0.79	0.87	0.87	0.74
32	0.13	0.31	0.31	0.19	0.2	0.23	0.16	0.16	0.12	0.14	0.18	***	0.82	0.67	0.79	0.73	0.88	0.88	0.78
33	0.16	0.22	0.19	0.18	0.2	0.14	0.12	0.16	0.16	0.14	0.16	0.2	***	0.72	0.85	0.78	0.87	0.81	0.81
34	0.33	0.4	0.36	0.41	0.42	0.33	0.36	0.38	0.39	0.36	0.39	0.4	0.33	***	0.68	0.64	0.7	0.65	0.67
35	0.21	0.27	0.28	0.18	0.16	0.18	0.16	0.17	0.18	0.15	0.18	0.24	0.16	0.38	***	0.79	0.86	0.81	0.73
36	0.3	0.29	0.3	0.33	0.3	0.16	0.23	0.24	0.27	0.27	0.24	0.32	0.25	0.44	0.23	***	0.78	0.76	0.67
37	0.15	0.22	0.24	0.16	0.18	0.16	0.09	0.1	0.11	0.08	0.14	0.12	0.14	0.36	0.15	0.25	***	0.87	0.77
38	0.16	0.27	0.33	0.2	0.2	0.22	0.18	0.16	0.12	0.11	0.14	0.13	0.22	0.43	0.21	0.27	0.14	***	0.76
39	0.27	0.34	0.3	0.32	0.33	0.27	0.25	0.27	0.23	0.24	0.3	0.25	0.22	0.4	0.31	0.4	0.26	0.28	***
40	0.18	0.27	0.23	0.21	0.21	0.17	0.12	0.12	0.14	0.09	0.17	0.12	0.13	0.37	0.18	0.24	0.11	0.15	0.2
41	0.2	0.26	0.24	0.24	0.22	0.18	0.15	0.15	0.17	0.12	0.14	0.19	0.17	0.36	0.15	0.21	0.14	0.19	0.26
42	0.22	0.28	0.25	0.29	0.29	0.2	0.2	0.2	0.22	0.14	0.22	0.16	0.16	0.32	0.21	0.3	0.16	0.24	0.21
43	0.2	0.27	0.3	0.17	0.2	0.25	0.16	0.16	0.14	0.21	0.18	0.19	0.21	0.41	0.26	0.31	0.2	0.19	0.29
44	0.21	0.21	0.21	0.23	0.24	0.19	0.16	0.2	0.16	0.16	0.2	0.22	0.09	0.36	0.18	0.24	0.19	0.21	0.24
45	0.22	0.22	0.2	0.26	0.24	0.22	0.17	0.2	0.19	0.15	0.18	0.22	0.14	0.36	0.22	0.26	0.16	0.18	0.24
46	0.66	0.8	0.6	0.61	0.65	0.63	0.68	0.73	0.67	0.61	0.74	0.66	0.59	0.44	0.66	0.76	0.61	0.7	0.64
47	0.2	0.24	0.16	0.27	0.24	0.2	0.22	0.24	0.22	0.21	0.21	0.27	0.18	0.33	0.27	0.27	0.21	0.23	0.26
48	0.3	0.34	0.33	0.35	0.33	0.23	0.22	0.27	0.27	0.22	0.26	0.28	0.23	0.47	0.27	0.18	0.23	0.27	0.33
49	0.16	0.2	0.22	0.2	0.21	0.17	0.16	0.19	0.2	0.14	0.2	0.2	0.12	0.3	0.12	0.27	0.16	0.22	0.24
50	0.19	0.18	0.15	0.2	0.2	0.17	0.12	0.16	0.2	0.16	0.18	0.2	0.12	0.36	0.18	0.25	0.16	0.2	0.24

Appendix 1: Continued

	40	41	42	43	44	45	46	47	48	49	50
1	0.9	0.86	0.83	0.86	0.84	0.86	0.52	0.81	0.78	0.82	0.87
2	0.88	0.86	0.82	0.83	0.83	0.81	0.49	0.82	0.78	0.85	0.87
3	0.89	0.88	0.84	0.84	0.85	0.83	0.5	0.82	0.81	0.87	0.87
4	0.89	0.88	0.85	0.8	0.85	0.85	0.54	0.81	0.78	0.86	0.86
5	0.83	0.76	0.77	0.84	0.78	0.78	0.52	0.73	0.7	0.76	0.76
6	0.84	0.84	0.83	0.81	0.83	0.82	0.54	0.81	0.76	0.84	0.82
7	0.82	0.84	0.81	0.81	0.78	0.78	0.48	0.79	0.74	0.8	0.79
8	0.89	0.85	0.89	0.78	0.86	0.87	0.57	0.82	0.81	0.86	0.84
9	0.47	0.43	0.45	0.43	0.44	0.48	0.53	0.41	0.46	0.44	0.43
10	0.89	0.88	0.89	0.77	0.87	0.86	0.57	0.84	0.79	0.88	0.86
11	0.84	0.83	0.82	0.79	0.9	0.87	0.53	0.84	0.8	0.84	0.88
12	0.87	0.84	0.87	0.82	0.87	0.83	0.54	0.83	0.77	0.89	0.84
13	0.83	0.83	0.83	0.78	0.83	0.83	0.54	0.82	0.76	0.87	0.86
14	0.85	0.85	0.82	0.78	0.9	0.87	0.49	0.85	0.78	0.87	0.92
15	0.83	0.81	0.82	0.77	0.83	0.84	0.51	0.79	0.75	0.83	0.91
16	0.8	0.83	0.79	0.73	0.84	0.76	0.56	0.8	0.73	0.81	0.84
17	0.77	0.76	0.77	0.7	0.79	0.78	0.56	0.74	0.69	0.79	0.76
18	0.72	0.72	0.71	0.65	0.69	0.68	0.58	0.68	0.61	0.74	0.71
19	0.83	0.83	0.82	0.77	0.87	0.84	0.58	0.79	0.73	0.87	0.85
20	0.82	0.82	0.81	0.77	0.84	0.79	0.52	0.77	0.73	0.85	0.79
21	0.84	0.82	0.8	0.82	0.81	0.8	0.52	0.82	0.74	0.85	0.83
22	0.76	0.77	0.76	0.76	0.81	0.8	0.45	0.78	0.71	0.82	0.84
23	0.79	0.78	0.78	0.74	0.81	0.82	0.55	0.85	0.72	0.81	0.86
24	0.81	0.79	0.75	0.84	0.79	0.77	0.54	0.77	0.71	0.82	0.82
25	0.81	0.8	0.75	0.82	0.78	0.78	0.52	0.79	0.72	0.81	0.82

Appendix 1: Continued

	40	41	42	43	44	45	46	47	48	49	50
26	0.84	0.83	0.82	0.78	0.83	0.81	0.53	0.82	0.79	0.84	0.84
27	0.88	0.86	0.82	0.85	0.86	0.84	0.51	0.81	0.8	0.85	0.88
28	0.88	0.86	0.82	0.85	0.82	0.82	0.48	0.78	0.77	0.83	0.85
29	0.87	0.84	0.81	0.87	0.85	0.83	0.51	0.8	0.76	0.82	0.82
30	0.91	0.89	0.87	0.81	0.85	0.86	0.54	0.81	0.81	0.87	0.86
31	0.84	0.87	0.81	0.83	0.82	0.84	0.48	0.81	0.77	0.82	0.83
32	0.88	0.83	0.86	0.83	0.8	0.8	0.52	0.76	0.76	0.82	0.82
33	0.88	0.84	0.85	0.81	0.92	0.87	0.56	0.83	0.79	0.89	0.89
34	0.69	0.7	0.73	0.67	0.69	0.69	0.64	0.72	0.63	0.74	0.7
35	0.84	0.86	0.81	0.77	0.83	0.8	0.52	0.76	0.77	0.88	0.84
36	0.79	0.81	0.74	0.73	0.78	0.77	0.47	0.77	0.84	0.77	0.78
37	0.9	0.87	0.85	0.82	0.83	0.85	0.54	0.81	0.79	0.86	0.86
38	0.86	0.83	0.79	0.83	0.81	0.83	0.49	0.79	0.77	0.81	0.82
39	0.82	0.77	0.81	0.75	0.79	0.79	0.53	0.77	0.72	0.78	0.78
40	***	0.89	0.89	0.8	0.85	0.86	0.52	0.81	0.79	0.86	0.84
41	0.12	***	0.89	0.76	0.85	0.83	0.52	0.82	0.78	0.86	0.83
42	0.11	0.11	***	0.72	0.82	0.84	0.57	0.81	0.76	0.88	0.82
43	0.22	0.28	0.33	***	0.81	0.78	0.43	0.78	0.74	0.78	0.81
44	0.16	0.16	0.2	0.22	***	0.86	0.55	0.84	0.79	0.86	0.91
45	0.15	0.19	0.17	0.24	0.16	***	0.52	0.82	0.79	0.86	0.86
46	0.65	0.65	0.56	0.84	0.6	0.66	***	0.56	0.49	0.53	0.5
47	0.21	0.2	0.22	0.25	0.18	0.2	0.59	***	0.76	0.81	0.84
48	0.23	0.24	0.28	0.3	0.24	0.24	0.7	0.27	***	0.77	0.78
49	0.16	0.16	0.12	0.25	0.15	0.15	0.63	0.21	0.26	***	0.87
50	0.17	0.18	0.2	0.21	0.1	0.15	0.69	0.17	0.24	0.14	***

Appendix 2: Nei's genetic identity matrix for 21 *L. purpureus* accessions collected from farmers fields. Above diagonal is identity and below diagonal is distance. Names of accessions are as indicated on Table 3.2.

Acc	2	3	4	5	6	8	9	10	11	29	30	32	37	38	39	40	41	42	44	45	47
2	***	0.92	0.87	0.77	0.83	0.82	0.44	0.84	0.86	0.89	0.88	0.86	0.89	0.84	0.78	0.88	0.86	0.82	0.83	0.81	0.82
3	0.09	***	0.91	0.77	0.87	0.84	0.44	0.86	0.85	0.88	0.93	0.86	0.91	0.86	0.77	0.89	0.88	0.84	0.85	0.83	0.82
4	0.14	0.09	***	0.77	0.87	0.86	0.43	0.86	0.83	0.89	0.93	0.85	0.91	0.88	0.75	0.89	0.88	0.85	0.85	0.85	0.81
5	0.27	0.26	0.26	***	0.78	0.80	0.46	0.77	0.73	0.83	0.78	0.83	0.79	0.78	0.78	0.83	0.76	0.77	0.78	0.78	0.73
6	0.19	0.14	0.14	0.24	***	0.87	0.46	0.88	0.82	0.83	0.88	0.81	0.86	0.83	0.76	0.84	0.84	0.83	0.83	0.82	0.81
8	0.20	0.18	0.15	0.22	0.14	***	0.47	0.89	0.82	0.84	0.87	0.88	0.87	0.87	0.78	0.89	0.85	0.89	0.86	0.87	0.82
9	0.82	0.81	0.84	0.77	0.79	0.75	***	0.47	0.42	0.42	0.47	0.42	0.46	0.42	0.44	0.47	0.43	0.45	0.44	0.48	0.41
10	0.18	0.16	0.16	0.26	0.13	0.11	0.76	***	0.86	0.84	0.87	0.84	0.86	0.81	0.77	0.89	0.88	0.89	0.87	0.86	0.84
11	0.16	0.16	0.19	0.31	0.20	0.20	0.88	0.15	***	0.84	0.84	0.79	0.84	0.80	0.80	0.84	0.83	0.82	0.90	0.87	0.84
29	0.11	0.13	0.12	0.19	0.18	0.18	0.86	0.17	0.18	***	0.88	0.88	0.90	0.88	0.79	0.87	0.84	0.81	0.85	0.83	0.80
30	0.12	0.07	0.07	0.24	0.13	0.14	0.76	0.14	0.18	0.13	***	0.87	0.92	0.89	0.78	0.91	0.89	0.87	0.85	0.86	0.81
32	0.16	0.15	0.16	0.18	0.22	0.13	0.88	0.18	0.24	0.12	0.14	***	0.88	0.88	0.78	0.88	0.83	0.86	0.80	0.80	0.76
37	0.11	0.09	0.09	0.23	0.16	0.14	0.79	0.16	0.18	0.11	0.08	0.12	***	0.87	0.77	0.90	0.87	0.85	0.83	0.85	0.81
38	0.17	0.15	0.12	0.25	0.19	0.14	0.88	0.22	0.22	0.12	0.11	0.13	0.14	***	0.76	0.86	0.83	0.79	0.81	0.83	0.79
39	0.25	0.26	0.29	0.25	0.27	0.25	0.82	0.26	0.22	0.23	0.24	0.25	0.26	0.28	***	0.82	0.77	0.81	0.79	0.79	0.77
40	0.12	0.12	0.12	0.19	0.17	0.11	0.76	0.12	0.18	0.14	0.09	0.12	0.11	0.15	0.20	***	0.89	0.89	0.85	0.86	0.81
41	0.15	0.13	0.13	0.27	0.17	0.16	0.84	0.13	0.19	0.17	0.12	0.19	0.14	0.19	0.26	0.12	***	0.89	0.85	0.83	0.82
42	0.20	0.18	0.16	0.27	0.19	0.12	0.80	0.11	0.20	0.22	0.14	0.16	0.16	0.24	0.21	0.11	0.12	***	0.82	0.84	0.81
44	0.18	0.16	0.16	0.25	0.19	0.16	0.82	0.14	0.11	0.16	0.16	0.22	0.19	0.21	0.24	0.16	0.16	0.20	***	0.86	0.84
45	0.21	0.19	0.16	0.25	0.20	0.14	0.73	0.15	0.14	0.19	0.15	0.22	0.16	0.18	0.24	0.15	0.19	0.17	0.16	***	0.82
47	0.20	0.20	0.21	0.32	0.21	0.20	0.89	0.17	0.18	0.22	0.21	0.27	0.21	0.23	0.26	0.21	0.20	0.22	0.18	0.20	***

Appendix 3: Nei's genetic identity matrix for 29 *L. purpureus* accessions collected from National genebank. Above diagonal is identity and below diagonal is distance. Names of accessions are as indicated on Table 3.2.

	1	7	12	13	14	15	16	17	18	19	20	21	22	23	24
1	***	0.84	0.85	0.84	0.84	0.86	0.81	0.77	0.72	0.83	0.80	0.85	0.77	0.78	0.84
7	0.17	***	0.86	0.84	0.77	0.76	0.76	0.72	0.68	0.79	0.77	0.76	0.75	0.74	0.76
12	0.16	0.22	***	0.84	0.88	0.83	0.83	0.81	0.72	0.84	0.84	0.86	0.78	0.8	0.83
13	0.17	0.17	0.18	***	0.83	0.86	0.81	0.78	0.72	0.85	0.81	0.84	0.82	0.81	0.81
14	0.18	0.26	0.13	0.19	***	0.87	0.84	0.79	0.72	0.84	0.81	0.86	0.82	0.84	0.81
15	0.16	0.28	0.19	0.16	0.14	***	0.82	0.76	0.71	0.81	0.76	0.82	0.79	0.81	0.8
16	0.21	0.28	0.19	0.21	0.18	0.2	***	0.72	0.66	0.78	0.76	0.79	0.76	0.76	0.78
17	0.26	0.33	0.22	0.25	0.23	0.28	0.33	***	0.66	0.78	0.82	0.76	0.68	0.72	0.71
18	0.33	0.38	0.33	0.33	0.33	0.35	0.41	0.41	***	0.72	0.69	0.72	0.63	0.67	0.71
19	0.19	0.23	0.17	0.16	0.17	0.22	0.24	0.24	0.33	***	0.86	0.82	0.79	0.78	0.79
20	0.23	0.26	0.17	0.22	0.21	0.27	0.27	0.2	0.37	0.16	***	0.78	0.74	0.77	0.76
21	0.16	0.27	0.16	0.18	0.16	0.2	0.23	0.27	0.33	0.2	0.25	***	0.77	0.74	0.87
22	0.26	0.29	0.25	0.2	0.2	0.23	0.27	0.38	0.46	0.24	0.30	0.27	***	0.78	0.74
23	0.24	0.3	0.22	0.22	0.17	0.22	0.27	0.33	0.41	0.25	0.27	0.3	0.25	***	0.78
24	0.17	0.28	0.19	0.21	0.22	0.22	0.25	0.34	0.35	0.23	0.27	0.14	0.3	0.24	***
25	0.17	0.28	0.19	0.22	0.19	0.2	0.22	0.34	0.38	0.26	0.27	0.16	0.29	0.2	0.08
26	0.18	0.27	0.16	0.2	0.16	0.22	0.24	0.33	0.35	0.19	0.26	0.23	0.22	0.23	0.25
27	0.08	0.16	0.14	0.19	0.16	0.19	0.22	0.24	0.31	0.17	0.18	0.2	0.25	0.22	0.18
28	0.09	0.19	0.17	0.19	0.18	0.18	0.24	0.29	0.31	0.2	0.22	0.2	0.25	0.27	0.18
31	0.13	0.21	0.18	0.24	0.19	0.21	0.24	0.31	0.4	0.23	0.24	0.16	0.29	0.29	0.18
33	0.14	0.22	0.08	0.17	0.09	0.2	0.2	0.22	0.33	0.12	0.16	0.16	0.22	0.19	0.18
34	0.37	0.41	0.3	0.36	0.33	0.39	0.34	0.46	0.29	0.32	0.32	33	0.4	0.36	0.41
35	0.16	0.26	0.17	0.22	0.16	0.22	0.22	0.3	0.33	0.21	0.21	21	0.27	0.28	0.18
36	0.25	0.3	0.24	0.3	0.22	0.3	0.25	0.41	0.47	0.29	0.27	0.3	0.29	0.3	0.33
43	0.16	0.21	0.2	0.25	0.24	0.27	0.31	0.36	0.43	0.26	0.26	0.2	0.27	0.3	0.17
46	0.65	0.74	0.62	0.61	0.7	0.67	0.59	0.59	0.54	0.54	0.66	0.66	0.8	0.6	0.61
48	0.24	0.3	0.27	0.27	0.25	0.29	0.32	0.37	0.49	0.31	0.31	30	0.35	0.33	0.35
49	0.2	0.22	0.11	0.14	0.14	0.18	0.21	0.24	0.3	0.14	0.16	0.16	0.2	0.22	0.2
50	0.14	0.24	0.18	0.16	0.09	0.09	0.17	0.28	0.35	0.16	0.23	0.19	0.18	0.15	0.2

Appendix 3: Continued

	25	26	27	28	31	33	34	35	36	43	46	48	49	50
1	0.84	0.83	0.93	0.92	0.88	0.87	0.69	0.85	0.78	0.86	0.52	0.78	0.82	0.87
7	0.76	0.77	0.85	0.83	0.81	0.8	0.67	0.77	0.74	0.81	0.48	0.74	0.8	0.79
12	0.83	0.85	0.87	0.84	0.84	0.93	0.74	0.84	0.78	0.82	0.54	0.77	0.89	0.84
13	0.8	0.82	0.83	0.83	0.79	0.84	0.7	0.81	0.74	0.78	0.54	0.76	0.87	0.86
14	0.83	0.85	0.86	0.83	0.83	0.92	0.72	0.86	0.81	0.78	0.49	0.78	0.87	0.92
15	0.82	0.8	0.83	0.84	0.81	0.82	0.68	0.81	0.74	0.77	0.51	0.75	0.83	0.91
16	0.8	0.79	0.81	0.78	0.79	0.82	0.71	0.81	0.78	0.73	0.56	0.73	0.81	0.84
17	0.71	0.72	0.78	0.75	0.73	0.8	0.63	0.74	0.67	0.7	0.56	0.69	0.79	0.76
18	0.68	0.71	0.73	0.73	0.67	0.72	0.75	0.72	0.63	0.65	0.58	0.61	0.74	0.71
19	0.77	0.83	0.84	0.82	0.79	0.88	0.73	0.81	0.75	0.77	0.58	0.73	0.87	0.85
20	0.76	0.77	0.83	0.8	0.78	0.85	0.72	0.81	0.76	0.77	0.52	0.73	0.85	0.79
21	0.85	0.79	0.82	0.82	0.85	0.85	0.72	0.81	0.74	0.82	0.52	0.74	0.85	0.83
22	0.75	0.81	0.78	0.78	0.75	0.81	0.67	0.77	0.75	0.76	0.45	0.71	0.82	0.84
23	0.82	0.79	0.8	0.77	0.75	0.83	0.69	0.76	0.74	0.74	0.55	0.72	0.81	0.86
24	0.92	0.78	0.84	0.84	0.83	0.83	0.67	0.84	0.72	0.84	0.54	0.71	0.82	0.82
25	***	0.78	0.83	0.83	0.83	0.82	0.66	0.85	0.74	0.82	0.52	0.72	0.81	0.82
26	0.25	***	0.83	0.84	0.8	0.87	0.72	0.84	0.86	0.78	0.53	0.79	0.84	0.84
27	0.19	0.19	***	0.93	0.87	0.88	0.69	0.86	0.79	0.85	0.51	0.8	0.85	0.88
28	0.19	0.18	0.07	***	0.87	0.85	0.68	0.84	0.78	0.85	0.48	0.77	0.83	0.85
31	0.18	0.22	0.14	0.14	***	0.86	0.68	0.84	0.79	0.83	0.48	0.77	0.82	0.83
33	0.2	0.14	0.12	0.16	0.16	***	0.72	0.85	0.78	0.81	0.56	0.79	0.89	0.89
34	0.42	0.33	0.36	0.38	0.39	0.33	***	0.68	0.64	0.67	0.64	0.63	0.74	0.7
35	0.16	0.18	0.16	0.17	0.18	0.16	0.38	***	0.79	0.77	0.52	0.77	0.88	0.84
36	0.3	0.16	0.23	0.24	0.24	0.25	0.44	0.23	***	0.73	0.47	0.84	0.77	0.78
43	0.2	0.25	0.16	0.16	0.18	0.21	0.41	0.26	0.31	***	0.43	0.74	0.78	0.81
46	0.65	0.63	0.68	0.73	0.74	0.59	0.44	0.66	0.76	0.84	***	0.49	0.53	0.5
48	0.33	0.23	0.22	0.27	0.26	0.23	0.47	0.27	0.18	0.3	0.7	***	0.77	0.78
49	0.21	0.17	0.16	0.19	0.2	0.12	0.3	0.12	0.27	0.25	0.63	0.26	***	0.87
50	0.2	0.17	0.12	0.16	0.18	0.12	0.36	0.18	0.25	0.21	0.69	0.24	0.14	***