

**GENETIC DIVERSITY OF *FAIDHERBIA ALBIDA* (Del.) A. Chev COLLECTIONS  
HELD AT THE WORLD AGROFORESTRY CENTRE (ICRAF) NAIROBI, KENYA**

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**A thesis submitted in partial fulfillment of the requirements for the award of the degree of  
Master of Science (Genetics) in the School of Pure and Applied Sciences of Kenyatta  
University**

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**DECLARATION**

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

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**DEDICATION**

To my family, for their inspiration which keeps my quest for knowledge alive.

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**ABBREVIATIONS AND ACRONYMS**

AFLP	Amplified Fragment Length Polymorphism
CI	Confidence Interval
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetra-acetic acid
EtOH	Ethanol
FAO	Food and Agriculture Organization
ICRAF	International Center for Research in Agroforestry
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RPM	Revolutions per minute
TE	Tris Ethylenediamine Tetra-acetic acid
TTPGA	Tools for Population Genetic Analysis.
UPGMA	Unweighed Pair Group Method with Arithmetic Mean
UV	Ultraviolet

**DEFINITION OF TERMS**

Accession	A collection of plant material from a particular location.
Biobank	A facility that stores biological samples for use in research.
Cultivar	A race or variety of a plant that has been created or selected intentionally for desirable characteristics and maintained through cultivation.
Ecotype	Genetically distinct geographic variety, population or race with species which is adapted to the specific environmental condition.
Founder effects	The loss of genetic variation that occurs when a new population is established from a very small number of individuals from a large population.
Gene flow	The introduction of genetic material, by interbreeding from one population of a species to another thereby changing the composition of the gene pool of the receiving population.
Hardy Weinbergs equilibrium	This is a genetics principle that models an ideal population based on the statement that, in the absence of disturbing forces both allele and genotype frequencies in a population remain constant from generation to generation.
Ligation	This refers to the joining of linear DNA fragments together with covalent bonds using T4 DNA ligase.
Microclimate	A local atmospheric zone where the climate differs from the surrounding area.
Phytochoria	A phytochorion is a geographical area with a relatively uniform composition of species. These areas usually possess a large number of endemic taxa.
Propagule	Any material that is used for the purpose of propagating an organism to the next stage in their life cycle via dispersal.
Provenance	Refers to the specific area in which plants that produced seed are located or were derived.
Restriction	Cutting of DNA at specific recognition nucleotide sequences known as restriction sites by enzymes called endonucleases.
Riparian	This is the interface between land and a river or stream.

**ABSTRACT**

*Faidherbia albida* (Del.) A. Chev is a tree species native to Africa and the Middle East. It is an important agro-silvo pastoral species owing to its morphological and physiological dynamics that mediate improvement of soil structure and fertility while offering several other benefits to farmers and the environment. Key among these is its reverse phenology, a phenomenon that allows the tree to compete minimally with crops while providing a favorable microclimate for their growth. It is also a chief source of fodder, wood, medicine and nectar for apiculture in the dry season. Sustainable incorporation of *F. albida* in African farming systems will only be underpinned by proper management of its genetic resources. Molecular information has become indispensable in the contemporary management practices of agroforestry plant species. Genetic structure data is crucial in conservation, breeding and introduction. *Faidherbia albida* accessions in ICRAF seed bank are not characterized and they are gradually losing viability. This study assessed the genetic diversity of seventeen *Faidherbia albida* provenances from 10 African countries by employing amplified fragment length polymorphism (AFLP) with an aim of providing crucial background genetic diversity information for *in situ* and *ex situ* management and utilization of the collections. Plant materials consisted of *F. albida* accessions held at World Agroforestry Centre (ICRAF) seed bank. Five primer sets (EcoRI-ACA/MseI-CAT, EcoRI-ACT/MseI-CTC, ECoRI-AAG/MseI-CTT, ECoRI-ACC/MseI-CAT and ECoRI-AGC/MseI-CTC) that produced clear and scorable bands were utilized for the analysis. Fragments were resolved using the ABI 3730 DNA analyzer. A total of 676 bands ranging from 50 to 499 base pairs were produced. GenALEx 6.41 was employed to reveal partitioning of variation across the populations and to display molecular relationships among the populations. Genetic diversity estimates were computed using TFPGA software. EcoRI-ACT/MseI-CTC was the most informative of the five markers (mean PIC 0.24). The average percentage of polymorphic loci over all populations was 34 %. Collections from the Taveta (Kenya) provenance had the highest percentage of polymorphic loci (69.53 %) while those from Manapools (Zimbabwe) had the lowest percentage of polymorphic loci (13.46 %). The average heterozygosity ranged from 0.05-0.28 with a mean of 0.17 across all the provenances. There was high and quite significant population differentiation among the populations (PhipT =0.583, p=0.01). Amova revealed that 58 % of the total molecular variation was partitioned among the populations while 42 % was within the populations. UPGMA clustering to a great extent reflected the geographical origins and similarity of the germplasm save for Bolgatonga (Ghana) and Bignona (Senegal) whose positions could explain the origin and evolutionary trends that have shaped the population structure and distribution of the species. The results show that the germplasm held at ICRAF seed bank is of low genetic variability with the Western and some of the Eastern Africa provenances having the highest diversity. These can be used as seed sources for introduction and domestication programs. More collections need to be done to cover the entire distribution range of this species to capture more diversity and enrich this genepool.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

*Faidherbia albida*, formerly widely included in the genus *Acacia*, is a tree species native to Africa and the Middle East. It is a leguminous woody species belonging to the *Mimosoidae* subfamily and commonly known as Apple-ring *Acacia*, Ana tree or Winter Thorn (Fagg and Barnes, 2003). *Faidherbia albida* is an important agroforestry tree that has been used throughout arid and semiarid zones of Africa for soil conservation and soil fertility improvement. Its importance is underscored by a peculiar inverted (reverse) phenology, a phenomenon whereby the tree undergoes a physiological dormancy and sheds its nitrogen rich leaves during the early rainy season – when seeds are being planted and need the nutrients and then regrow its leaves when the dry season begins and the crops are dormant. This makes it highly compatible with food crops since it does not compete with them for nutrients and light (ICRAF, 1989). Shedding leaves during the rainy season at the time of higher microbial activity in the soil improves the soil structure, permeability while retaining leaves in the dry season provides shade and mulch reducing evaporation thus conserving the available soil moisture (Dangasuk *et al.*, 2006).

Besides the reverse phenology benefits, the tree is a good Nitrogen fixer which improves the soil fertility (Payne *et al.*, 1998). Its leaves and pods provide crucial source of fodder in the dry season for livestock and it is also a chief source of wood and medicine. The species is also crucial in agroforestry systems and in desertification control through stabilization of sand dunes and as wind breakers thus controlling soil erosion (Dancete and Poulaine, 1969; Cossalter, 1991). Due to these benefits and the potential of this species in sustainable agro-silvopastoral practices,

the challenge remains that of conservation, breeding and broadening the utilization range of the species. As a result of threats to parts of its genotype, its importance in rural development and scope for domestication and improvement, *F. albida* was listed as a priority species by the FAO Panel of Experts on Forestry Gene Resources since 1974 (FAO, 1974). Following this, a comprehensive seed collection mission was initiated by the Oxford Forestry Institute (OFI) in early 1990. The seeds were distributed to various seed banks for international provenance and progeny trials and molecular studies. In June 2001, all seeds held at OFI seed bank were transferred to ICRAF seed store in Nairobi.

A number of studies have been done towards understanding the ecology, growth and genetic dynamics of the species. Dangasuk *et al.* (2001) observed that early growth performance variance in *F. albida* populations was a factor of climatic selection pressure and soil properties. Sustainable management of *F. albida* requires an overall evaluation of its genetic resources. Molecular techniques like isozyme genotyping, molecular marker fingerprinting, DNA sequencing and expression profiling are commonly used for such studies. This study was based on one of the most widely used PCR based marker technology, AFLP, to assess the genetic diversity of *F. albida* provenances in ICRAF seed bank. The marker has been employed to assess genetic structuring in tree species such as *Warbugia* (Muchugi *et al.*, 2008) and *Pinus pinaster* (Mariette *et al.*, 2001). The success of any genetic conservation and breeding is dependent on understanding the amount and distribution of genetic diversity present in the gene pool. For example, information on diversity distribution and vegetation history is crucial for construction of core collections.

## 1.2 Problem statement

Inadequate land management systems have led to deforestation over most of the arid and semiarid zones of Africa. This has in turn affected the ecosystem balance leading to massive erosion of genetic diversity which has adversely impaired ecological adaptability of trees. The sustainable solution to this lies in the proper management and cultivation of indigenous tree species especially the pioneer legumes that are the natural colonizers of disturbed and degraded lands (Fagg and Barnes, 1995). The extent of this need has become more apparent with the increasing costs of agricultural inputs and animal feeds. Incorporation of trees into farming systems can however be readily embraced if the targeted tree species has practical benefits to farmers. *F. albida* has an advantage in this perspective in that its reverse phenology allows it to compete minimally with crops among many other benefits to the farming systems.

Successful management of *F. albida* in agroforestry systems requires a thorough understanding of its morphology, biochemical and genetic details. Part of the management strategies of *F. albida* is *ex situ* conservation of germplasm as is the case in ICRAF seed bank. These accessions were collected in 1993 and due to long storage their viability has become low. Low viability and seed death leads to further loss of genetic diversity (Karin *et al.*, 2010) and uninformed regeneration of germplasm is uneconomical and increases the chances of homozygosity and sampling effects. There is therefore a dire need for regeneration of this germplasm and to enrich the collection by targeting unrepresented provenances. These measures could be guided by proper molecular characterization and evaluation of the genetic structure of the accessions.

### 1.3 Justification

A remarkable part of the sub-Saharan Africa is covered by arid and semi arid areas. *F. albida*'s drought tolerance and reverse phenology qualities enables farmers in these regions to improve agricultural productivity, sustainable agricultural practices and environmental resilience hence food security. There is therefore need to conserve collections of a wide diversity as part of management of this species. Knowledge of the structure of genetic diversity of *F. albida* accessions conserved *ex situ* in ICRAF seed bank is paramount in their management. This knowledge is however still limited and adequate management methodologies require further development. The establishment of AFLP fingerprints of these accessions will go a long way in informing management activities to ensure diversity of the collections. This will guide in enrichment of the collections and resampling for future collections once the germination rate goes below the accepted 55 percentage as confirmed (Mwaura 2012, pers. comm., 20 May). Information generated will be essential in germplasm collection and establishing populations of a rich gene pool by averting the possibilities of founder effects. It will help in development of better procedures for regeneration of germplasm. Through improved characterization and development of core collections based on diversity information, it will be possible to exploit *Faidherbia* genetic resources valuably and reduce the number of redundant accessions.

### 1.4 Research questions

- i. What is the level of heterozygosity within *F. albida* populations?
- ii. How does genetic variation partition among *F. albida* provenances?
- iii. Are there distinct *F. albida* molecular clusters to suggest population differentiation?

## **1.5 Hypotheses**

- i. There is low genetic polymorphism among and within *F. albida* provenances from Africa.
- ii. There is no partitioning of genetic diversity in *F. albida* populations.

## **1.6 Objectives**

### **1.6.1 General objective**

To establish the extent and distribution of genetic diversity in *F. albida* provenances from 10 African countries using AFLP-PCR fingerprints.

### **1.6.2 Specific objectives**

- i. To assess the level of genetic variation among *F. albida* provenances from ten African countries.
- ii. To establish the level of heterozygosity of the *F. albida* provenances.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Origin and distribution of *Faidherbia albida*

The origin of this species is not well understood. Chevalier (1934) and Wickens (1969), were of the view that it originated in North Africa as evidenced by fossil records and spread southwards. The other school of thought maintains that the species originated in Eastern and Southern Africa and spread northwards through human and animal grazing activities. Giffard (1965) and Vandenbeldt (1991), proposed the Sudan and Ethiopian regions as the center of origin and that the subsequent migration of the species to Northwest and South East Africa resulted in the formation of the upland and riparian ecotypes.

The key distribution zones of this plant species are found in the dry uplands of Northwest Africa and the riparian regions of Southeast Africa. Largest populations of the species occur on the sandy alluvial soils of the Jabel Marra highlands in Sudan (Wickens, 1969). It also extends westwards to Chad, Niger, Mali to Senegal and northwards to Ethiopia and Egypt. From Jabel Marra center the species also extends southwards to Somalia, Tanzania and Kenya forming the East African population. Further south the species occur in Malawi, Zambia, Zimbabwe, Mozambique, Botswana, South Africa and Namibia (Wickens, 1969; CTFT, 1989), see Figure 1. In Kenya, it is common in Rift valley along river Turkwel, around Oloitoktok and also in Taveta. Outside Africa spontaneous occurrences of the species have been found in Yemen, Israel, Jordan and Lebanon where the Northern most is 30 km north of Beirut. This species does not occur in Madagascar. The species has also been introduced to Ascension Island, the Cape Verde island (Wickens, 1969), Cyprus and Pakistan (Brenan, 1983) and more recently in Peru and India.

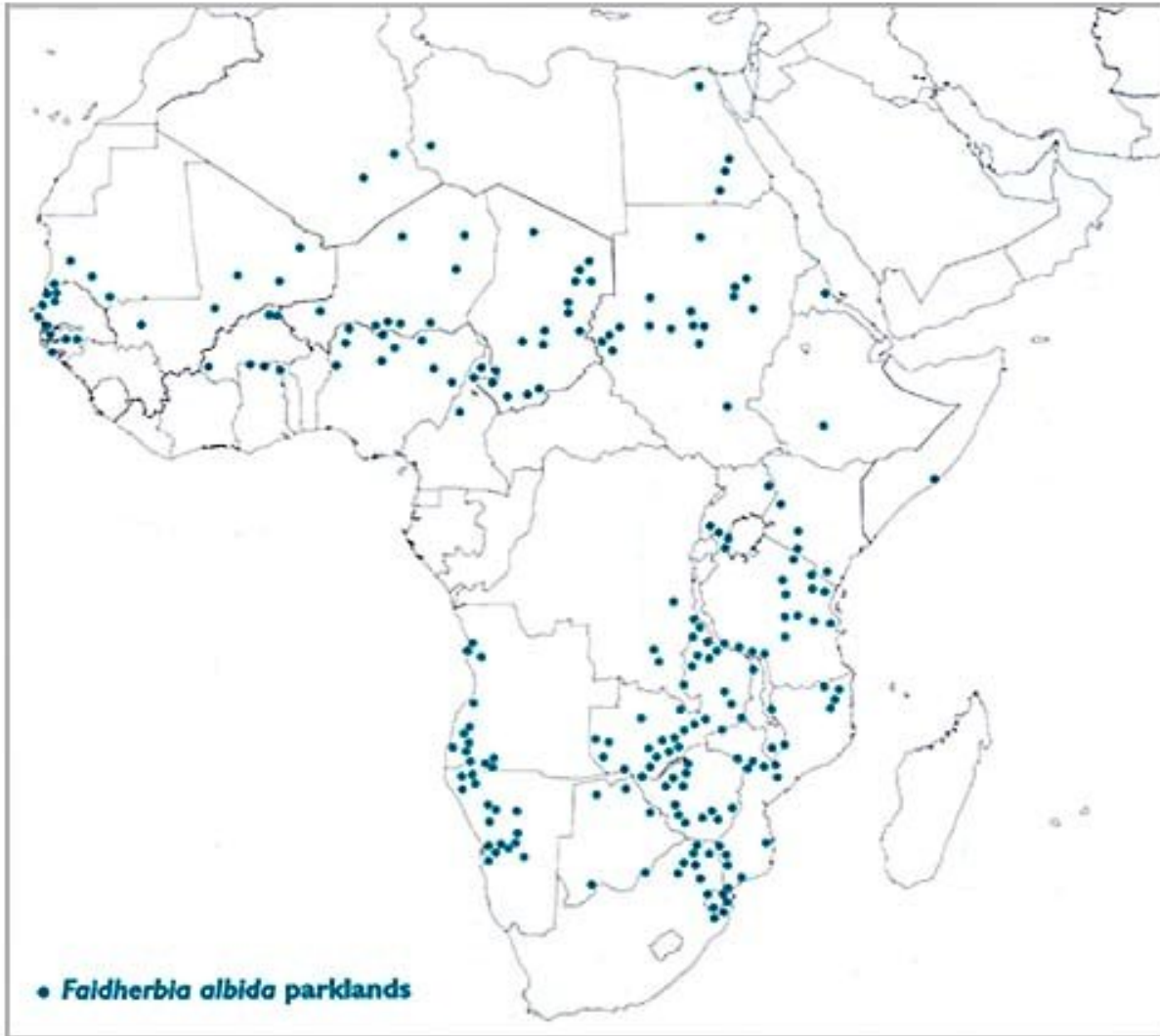


Figure 1: Geographical distribution of *Faidherbia albida* (Boffa, 1999).

## 2.2 Botanic description of *Faidherbia albida*

*F. albida* belongs to the family *leguminosae/fabacae*, subfamily *Mimosoidae* and tribe *Acaciaea*. It is a monotypic genus in this subfamily meaning it is the only genus in this subfamily. It is indigenous to much of Africa and adapted to a wide range of habitats, elevations and soils (Felker, 1978). It can attain up to 30 m height and a diameter of 1.5 meters. More usually it reaches 15-25 meters height and a breast height diameter of up to 1 meter (Saka *et al.*, 1990; Weil and Mughogho, 1993). Roots can reach aquifers up to 80 m below the surface. The leaves are compound and bipinnate with leaflets borne along the pinnae as is typical of *Mimosoidae* (CTFT, 1989). Leaves are glabrous to pubescent, bluish green oblongate and occasionaly submucronate, overlapping slightly, 2.5-12 mm in length and 0.7-5 mm in width (Timberlake, 1999). Thorns occur in pairs at the base of the leaves and are modified spiny stipules. They are straight and robust, thickened at the base and often orange or brown at the tip and are 0.2-3.2 cm long. They may be distinguished from those of *Acacia* species with long thorns, such as *A. tortilis* subspecies *raddiana*, *A. nilotica* or *A. seyal*, by their basal thickening (Vandenbeldt, 1992).

Flowers are borne in dense axillary panicles and are successively white, cream and then yellow and are strongly scented (perfumed) (CTFT, 1989). Panicles are 3.5-169 cm long with a peduncle 2-4 cm long. The calyx is 1-1.7 mm long, glabrous to pubescent, consisting of 5 sepals. The corolla, 3-3.5 mm long, carries 5 free petals. The stamens are about 40-50, 4-6 mm long and their filaments are fused for about 1 mm. Anthers are 0.2-0.4 mm in diameter and lack glands even in the bud. The ovary is slightly stipitate and bears small hairs. The inflorescence in *F.*

*albida* functions as a reproductive unit with the basal flowers attractive to the pollinators because of pollen reward. Apical flowers open more slowly and produce more pods than the basal and median ones. Basal flowers that open first attract the pollinators while apical flowers are well suited to receive pollen from surrounding trees (Gassama-Dia *et al.*, 2003). The same trend has been observed in *Acacia tortilis* with well shaped inflorescence (Tybirk, 1993). Fruits are indehiscent bright orange to reddish brown pods whose surfaces are convex on one side and become concave on the other, and the mesocarp, which is fleshy to begin with, tends to roll into a spiral as it lignifies, giving the appearance of a dried apple peel (El Amin, 1990). The pods contain dark brown shiny ovoid seeds whose seed coat is water proof and leathery. This provides protection, hence maintaining viability for many years.

### **2.3 Ecology of *F. albida***

The species commonly occurs below 1200 m above sea level but it can be found up to 1800-2000 m altitude in Ethiopia and Jebel Marra of Sudan (Wood, 1992). It is found particularly in association with water, that is, along rivers, on the shores of lakes and ravines that are moist. The tree must have access to available sources of water as demonstrated by its vegetative vigor in the dry season, in the total absence of rain, high temperatures, low humidity and maximum evapotranspiration. This is associated with deep and well drained alluvial soils where it has access to ground water. *F. albida* prefer deep sandy-clay soils, particularly alluvial deposits along flood plains of rivers which are easily exploited by its root system (Sanusi *et al.*, 1992). It has however been reported growing on rocky, heavy and cracking clays (Saka *et al.*, 1989). It is associated with low rainfall areas, usually with 500-800 mm annually and mainly distributed in

summer. In South-West Africa it can thrive under desert conditions where the mean annual rainfall is only 20 mm and the mean annual daily temperature 16.8 °C (Fagg and Barnes, 1995). It also grows in areas with mean annual temperatures of 18-30 °C (Webb *et al.*, 1984).

## **2.4 Establishment and growth**

The hard coated *F. albida* seeds store well under dry conditions and are usually extracted by pounding the pods in a mortar. Pretreatment is often needed for rapid uniform germination. Various methods used include; mechanical scarification, dipping in concentrated Sulphuric acid for 5-15 min and dipping in boiling water. Beckman (1992), observed that the physical scarification techniques provided the best germination results (Nicking-26.39 %, cutting and abrasion 18 % each) compared to hot water treatment (1.11 %). There are 7,000-20,000 seeds per kg of *F. albida* seeds. Seeds can be sown directly or planted in nursery, ideally using long poly tubes (30 x 8 cm), 15 mm deep with regular watering and frequent mechanical root or air root pruning (CTFT, 1989). Seedlings can be transplanted 3-6 months later with a common spacing of 10 x 10 m.

## **2.5 Uses of *Faidherbia albida***

### **2.5.1 Agroforestry**

Previous bioclimatic studies indicate that the microclimate created by a stand of *F. albida* is favorable to crops beneath them. It does so by influencing on various climatic conditions like potential evapotranspiration, relative humidity, soil moisture, air temperature, soil temperature,

irradiation, precipitation and insolation (Barnes and Fagg, 2003). Its canopy considerably reduces potential evapotranspiration. Insolation, the transmitted sunlight essential for growth, remains high during the rainy season because of the inverse phenology of *F. albida* and irradiance has been found to be reduced inside the canopy by 0.5 to 1.0 kw m<sup>-2</sup> (Payne *et al.*, 1998). Studies have shown that *F. albida* significantly changes the soil beneath the canopy and that the overall effect of these changes is increased soil fertility (Barnes and Fagg, 2003). The tree cover increases water infiltration and also has a beneficial effect on bulk density, structural stability and chemical and biochemical properties. Kamara and Haque (1992), found that *F. albida* trees on a vertisol in Ethiopia had higher organic matter on the Westside than East due to the accumulation of wind blown litter on the leeward side of the tree. Organic matter Nitrogen, Phosphorous and Potassium levels were higher under the tree canopy than outside for all directions and depths studied. Under the *Faidherbia* canopy, total carbon has been found to be increased by 60-90 %, Mineralizable carbon by 73 %, humus content by 40-47 % and Nitrogen by 100-110 %. Biological activity has been found to be five times higher under the canopy than in the open during the period of cultivation (Barnes and Fagg, 2003).

*F. albida* is a crucial nutrient source and also helps in cycling of nutrients. Dangasuk *et al.* (2001), observed that its deep extensive nodulated root system helps to ensure that a large amount of the nutrients concentrated under the tree do not come from the surface layer of the soil but from the deep horizons and the atmosphere and this results in a net gain to the arable soil layers. Kessler (1990), put forward the possibility that trees extensive root system mine the surface layers of the soil beyond the reaches of its crown and in so doing redistribute the nutrients in the litter that then falls beneath its canopy. Increase in grain yield has generally been

found to be highest close to the bole of the tree and to decrease to the base yield level at 4-8 meters from the bole depending on the size of the trees (Louppe *et al.*, 1996). Increases in yield have been reported for sorghum and other species grown under *F. albida* in various parts of Ethiopia, sub-Saharan Africa and in India. Examples of such cultivation are shown in plate 1 and 2. In studies conducted across Africa, yields were greater under the tree canopies than in the open by amounts varying from 36-250 % (Payne *et al.*, 1998).



Plate 1: Groundnuts under *F. albida* canopy (Adapted from World Agroforestry Centre).



Plate 2: Maize crop under *F. albida* canopy (Adapted from World Agroforestry Centre)

### 2.5.2 Fodder

The nutritive value of leaves and fruits is well known. Pods fall towards the end of the dry season when fodder is scarce; leaves and branchlets are lopped around this time. Fruit production is highly variable between trees and between years depending on the age, size and management of the tree, the soil and climatic conditions (CTFT, 1989). *F. albida* leaves contains high levels of crude protein but due to presence of phenolic compounds, protein digestibility in ruminants is reduced but its however a good protein substitute (Reed *et al.*, 1992). Average pod production ranges from 6-135 kgs per tree in a year in the Sudanian zone. In Zimbabwe (Manapools) two trees averaged 161 kgs per tree in a year (Dunham, 1990). In West Africa pods are sometimes shaken down, collected and fed to animals or sold in the market or road sides. Leaves, pods and

seeds contain 200, 150 and 260 gram total protein /kg respectively of dry matter, with total protein digestibility reaching up to 73 %. Tannins limit digestibility but incorporating into low quality fodder enhances ingestion without reducing digestibility while milling of pods increases digestion of seeds (Fagg, 1995).

### **2.5.3 Other uses**

There are numerous reports across Africa of seeds of *F. albida* being eaten as food by humans during famine (Marunda, 1992; Wickens *et al.*, 1995). The pods are crushed and the seeds separated from the chaff, boiled for several hours before being eaten. Seeds can also be pounded and baked into cakes or mixed with maize meal. *F. albida* extracts are pharmacologically active. They are used as astringents to treat gastrointestinal disorders, particularly diarrhoea (Muhammad *et al.*, 1998). Extracts of the bark, gum and the roots are used as a gum wash to stop bleeding. The leaves are used as an astringent for teeth and may contain fluorine. Medicinal uses specifically for the gum are given as an emollient for inflammation, haemorrhage, diarrhoea and ophthalmia (Wickens *et al.*, 1995). The powdered pods and seeds are widely used to stupefy or poison fish in pools. (Timberlake *et al.*, 1999).

The wood of *F. albida* has been described as light yellow to white and is reputed to be of good quality, soft and easy to work with (Wickens *et al.*, 1995). It is used to make dugout canoes, mortars and pestles and light carpentry work. The Maasai use it as flat wood upon which the firestick is twirled to make fire. In apiculture, the species produces flowers at the end of the rains

while most sahelian species flower just before or during the rains hence a main source of pollen and nectar at that time (Maundu and Tengnas, 2005).

## **2.6 Genetic diversity and its measurements**

Genetic diversity is the sum of genetic characteristics within any species or genus (Rao and Hodgikin, 2002). It refers to the level of heterozygosity in a species which is the probability that, at a single locus, any two alleles, chosen at random from the population, are different from each other (Sokal and Rohlf, 1994). Genetic diversity serves as a way populations adapt to changing environments. The more the variation in a population the more the probability that individuals will possess variations of alleles suited for a variable environment (Groom *et al.*, 2006). Genetic diversity is important for sustainable production in tree species since greater losses of characteristics in any population may limit its chances of survival and requires greater human efforts for successful production (Trethowan and Kazi, 2008). Different studies have shown the value of genetic diversity in providing adaptability to different biotic and abiotic stresses; Hughes *et al.* (2004) and Hajjar *et al.* (2008) showed that increasing genetic diversity is important for pest and disease management and provides chances for further improvement of the species.

Genetic diversity of a population can be assessed by some simple measures which include; Proportion of polymorphic loci. Many loci are characterized by a number of relatively common alleles that allow members of a naturally occurring population to be categorized into sharply distinct phenotypes. Genetic polymorphism is the occurrence of multiple alleles at a locus where

at least two alleles appear with frequencies greater than 1 percent (Satoh, 2007). By convention, polymorphic loci are those at which at least 2 percent of the population is heterozygous. However, because many polymorphic loci are characterized by a large number of different alleles, the proportion of heterozygote at some loci is much greater. Alleles with frequencies of less than 1 percent are called rare variants. (Groom *et al.*, 2006). Heterozygosity is also used to measure genetic diversity. This is the mean number of individuals with polymorphic loci. The maximum heterozygosity is 0.5. This signifies a high genetic diversity. Heterozygosity is zero at fixation. Genetic diversity can also be demonstrated by the number of alleles per locus (allelic diversity).

Genetic diversity has been conventionally estimated on the basis of different biometrical techniques (Meteroglyph,  $D^2$ , divergence analysis and principal component analysis) such as phenotypic diversity index (H) or coefficient of parentage utilizing morphological, agronomical and biochemical data (Matus and Hayes, 2002; Mohammadi and Prassana, 2003; Ahmed and Khaled, 2008). However, evaluation based on these phenotypic data is laborious and can take years to draw a conclusion. The advent of different molecular techniques led researchers to estimate genetic diversity on the basis of data generated by different molecular markers, which provide a means of rapid analysis of germplasm and estimates of genetic diversity. These markers are categorized in various ways; PCR or Non PCR based, dominant or codominant markers, microsatellites or minisatelites, mainly highlighting the technique or target DNA fragments. These techniques are based either on DNA –DNA hybridization like Restriction fragment polymorphism (RFLP), non PCR marker (Beckman and Soller, 1983) or on the amplification of specific DNA fragments using specific or random primers like Random

Amplified polymorphic DNA (RAPD), a PCR based marker, (Williams *et al.*, 1990) and Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995). These marker techniques can easily be performed on species with a small to moderately large genome size such as barley (1500 MB) and mammal genomes (3000 MB). PCR based techniques require at least 1000 times less template DNA needed for amplification than other techniques. They are given popularity due to their simplicity, high probability of success and because they provide flexibility in detecting genetic variation since a variety of primers can be used which are designed to reveal particular types of polymorphism based on selective amplification of DNA (Dangasuk, 2006).

One of the most reliable PCR based DNA fingerprinting technique, AFLP (Vos *et al.*, 1995), allows the selective amplification of subsets of genomic restriction fragments. AFLP has been used for construction of linkage maps as in barley (Qi *et al.*, 1998); for saturation of specific genomic regions in potato (Van Voort *et al.*, 1997); for analysis of genetic diversity in nematodes (Forkertsma *et al.*, 1996) and for molecular phylogeny studies in potato (Kardolus *et al.*, 1998). AFLP can be tailored according to the complexity of the pool of the restriction fragments by varying the number of selective nucleotides added to the core primers which hybridize to adaptors ligated to the restriction fragment. This method includes additional steps that permit high-resolution interrogation of the entire genome, and it yields highly specific, reproducible genotypic data. Although AFLP can only be used to study dominant genetic markers, it does not rely on any previous knowledge of the genome sequence.

## **2.7 Genetic marker methods for plants**

A genetic marker is any visible character or otherwise assayable phenotype, for which alleles at individual loci segregate in a Mendelian manner. It is a chromosomal landmark or allele that allows for the tracing of a specific region of DNA (Semagn, 2006). King and Stansfield (1990), defined it as a gene whose phenotypic expression is usually easily discerned, used to identify an individual or a cell that carries it, or as a probe to mark a nucleus, chromosomes or locus. Genetic markers are used to study the genetics of organisms, including trees, at the level of single genes. They are also used to study the genetics of natural and domesticated populations of trees and the forces that bring about change in these populations.

Some of the more important applications of genetic markers include: Describing mating systems, levels of inbreeding, and temporal and spatial patterns of genetic variation within tree stands, describing geographic patterns of genetic variation and inferring taxonomic and phylogenetic relationships among species. They are also used for evaluating the impacts of domestication practices, including forest management and tree improvement, on genetic diversity. In conservation and plant breeding they are crucial in fingerprinting and germplasm identification for marker assisted breeding, propagation of populations and construction of linkage maps. Genetic markers are classified into three broad groups; those based on visually observable traits (morphological and agronomic markers), those based on transcriptional products (biochemical markers) and those based on DNA analysis (molecular markers) (Semagn, 2006).

### **2.7.1 Morphological markers**

These are markers based on visually assessable characters. There is however very few characters inherited in a classical Mendelian fashion that has been discovered in trees and this poses a problem in forest genetics studies. Many of the identified morphological markers are mutations observed in seedlings such as albino needles, dwarfing and other aberrations (Franklin, 1970; Sorensen, 1973). They are often recessive mutants with rare occurrence and usually confer highly deleterious or even lethal phenotypes to organisms.

### **2.7.2 Biochemical markers**

Biochemical markers can either be at a secondary metabolite level for example monoterpenes or at a protein level such as allozymes. Monoterpenes are a class of the terpenoid substances with a number of structural conformations. They are found in resins and essential oils of plants (Kozlowski and Pallardy, 1979). The concentrations of different monoterpenes were determined by gas chromatography and used as genetic markers. Allozymes have for long been the most important genetic markers in forestry and have been used in many species for different applications. The more general term for allozymes is isozymes. Therefore, allozyme variation is often also referred to as isozyme variations (Kephart, 1990; May, 1992). Isozymes are defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozymes originate through amino acid alterations, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and therefore, their electrophoretic mobility (Kumar *et al.*, 2009). Allozymes are allelic forms of enzymes that can be distinguished by a procedure called electrophoresis.

This technique has been employed to study variation of coniferous trees (Curtu *et al.*, 2009), oleaster (wild olive tree) populations (Lumaret *et al.*, 2004) and resistance and susceptibility of *Fagus grandiflora* trees to beech bark disease (Houston and Houston, 2000). Allozyme markers are favored for their simplicity in that analysis does not require DNA extraction or the availability of sequence information, primers, probes and they are quick and easy to use. The main drawback of allozymes is their relatively low abundance and low level of polymorphism. Moreover, proteins with identical electrophoretic mobility (co-migration) may not be homologous for distantly related germplasm. In addition, their selective neutrality may be in question (Berry and Kreitman, 1993 and Hudson *et al.*, 1994). Allozymes are, strictly speaking, phenotypic markers and as such prone to environmental effects. This is because a gene being expressed in one tissue might not be expressed in another tissue and therefore the banding pattern will change depending on the tissue being analyzed.

### **2.7.3 Molecular markers**

A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily be monitored. The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purposes (Kumar *et al.*, 2009). DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are neutral. They are therefore able to detect high levels of genetic variation such as single nucleotide mutations as well as insertion and deletions (Powell *et al.*, 1996a). Various molecular markers are used to evaluate DNA polymorphisms and are generally categorized as DNA-DNA hybridization markers for example restriction fragment length polymorphism and Polymerase

chain reaction (PCR) based markers such as random amplified polymorphic DNA (RAPD), microsatellites and amplified fragment length polymorphism (AFLP).

### **2.7.3.1 Restriction fragment length polymorphism**

Restriction fragment length polymorphism (RFLP) was the first DNA based genetic markers developed (Botstein *et al.*, 1980), which resulted from the differences in the sequence of nucleotides of different plants. Such differences arise due to mutations occurring over time leading to restriction fragment polymorphisms (variation in the length of restriction fragments). Point mutations, insertions, deletions, translocations, inversions and duplications are the common mutations that cause the variations. Some of the differences in DNA sequence at the restriction sites can result in the gain, loss or relocation of a restriction site. Hence digestion of DNA with restriction enzymes results in fragments whose number and size can vary among individuals, populations and species (Semagn *et al.*, 2006). RFLP involves several steps. These include; extraction of DNA from the plant, digestion of DNA with restriction enzymes, size fractionation on a gel using electrophoresis, transfer of DNA fragments to a hybridization membrane and identification of specific fragments with a labeled probe (Farooq and Azam, 2002). RFLPs were the first to provide the means to directly detect variations present at the DNA level (Diers and Osborn, 1994). They have also been used to document genetic diversity in cultivated plants and their wild relatives (Zschockes *et al.*, 2000; Weising *et al.*, 2005).

RFLP markers are valued for their high reproducibility, codominant inheritance, non reliance on sequence information and the ease of scoring due to the large differences between fragment

sizes. The major drawbacks to RFLP analysis are; it requires high quality and quantity DNA (Roy *et al.*, 1992), it is not amenable to automation and depends on the development of specific probe libraries for each species, the level of polymorphism and multiplexing (loci detected per assay) is low, it is labor intensive and requires use of radioactive materials.

### **2.7.3.2 Random amplification of polymorphic DNA (RAPD)**

RAPD markers have been one of the most widely used molecular marker types in trees to date. They were the first of the PCR-based markers and were developed independently by Welsh and McClelland (1990), and Williams *et al.* (1990). RAPD is a PCR based technology which is based on enzymatic amplification of target or random DNA segments with arbitrary primers. This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence (Kumar *et al.*, 2009). The RAPD reaction involves a single species of primer annealing to the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA amplicon is produced through thermo cyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (William *et al.*, 1993). The technique unlike RFLP requires a very small amount of template DNA, usually about 10 nanograms.

Carlson *et al.* (1991), first demonstrated the use of RAPD markers in trees by showing the inheritance of RAPD markers in F1 families of *Pseudotsuga menziesii* and *Picea glauca*. In a subsequent paper, Tulsieram *et al.* (1992), used RAPD markers and mega-gametophyte segregation analysis to construct a partial genetic linkage map for *Picea glauca*. RAPDs have

since been used among others, to study genetic variation in the medicinal tree (*Prunus africana*) (Muchugi *et al.*, 2006); reveal polymorphism in *Commiphora wightii* (Medicinal tree) (Suthar *et al.*, 2008) and for population studies in tree legumes (Gomez *et al.*, 2011). RAPDs are easy and quick to use. They require a small amount of starting DNA per reaction since they are PCR based. Since random primers are commercially available, no sequence data for primer construction are needed. Moreover, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome. They are dominant markers and hence have drawbacks in their use as markers for mapping, which can be overcome to some extent by selecting those markers that are linked in coupling (Williams *et al.*, 1993). The main drawback of RAPDs is their low reproducibility (Schierwater and Ender, 1993), which makes them unsuitable markers for comparison of results from similar species between different research groups. RAPD assays also require pure and high molecular DNA.

### **2.7.3.3 Microsatellite DNA markers**

Microsatellites (Litt and Luty, 1989) also known as simple sequence repeats (SSRs) are sections of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species (Powell *et al.*, 1996b). The repeat motifs can range between 10 to 100 times. These markers often present high levels of inter and intra specific polymorphism, particularly when tandem repeats number is ten or greater (Queller *et al.*, 1993). Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favoured in population studies (Smith and Devey, 1994) and for the identification of closely related cultivars (Vosman *et al.*, 1992). If nucleotide sequences in the flanking regions of the microsatellite are known,

specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR (Kumar *et al.*, 2009). Primers that have already been designed for closely related species can also be used for SSR assays. Polymerase slippage during DNA replication, or slipped strand mispairing, is considered to be the main cause of variation in the number of repeat units of a microsatellite, resulting in length polymorphisms that can be detected by gel electrophoresis. Other causes have also been reported (Matsuoka *et al.*, 2002).

Microsatellite loci are more common in some organisms than others and screening may produce few useful loci in some species (Cooper, 1995). The efficiency of microsatellite marker development depends on the abundance of repeats in the target species and the ease with which these repeats can be developed into informative markers. SSR molecular markers have proven useful for assessment of genetic variation in germplasm collections (Mohammadi and Prassana, 2003). SSRs are the most preferred in the contemporary molecular genetic studies since they require very low amount of template DNA, they are highly polymorphic even between closely related organisms, are amenable to automation for high throughput analysis and are highly reproducible. SSRs are also codominant markers and very suitable for population genetic studies and mapping. The major constraint of using SSR markers is the high development cost and effort required to obtain working primers for a given study species (Semagn *et al.*, 2006).

#### **2.7.3.4 Amplified fragment length polymorphism (AFLP)**

AFLP (Vos *et al.*, 1995) is an intermediate technique between RFLP and RAPD. It combines the power of RFLP with the flexibility of PCR based technology by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998). The first report of the use of AFLPs in trees was by Cervera *et al.* (1996), who used this marker system to genetically map a disease resistance gene in *Populus*. Since then the technique has been widely employed in molecular and population genetic studies. Genetic linkage maps based on AFLPs have been constructed in *Eucalyptus globulus* and *E. tereticornis* (Marques *et al.*, 1998) and in *Pinus taeda* (Remington *et al.*, 1998).

AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. The first step in AFLP analysis involves restriction digestion of DNA about (350-500 ng) with a combination of a rare cutter for example ECoRI or PstI and a frequent cutter such as MseI or TaqI restriction enzymes (Semagn *et al.*, 2006). Here the optimum working conditions for various enzymes should be factored in for successful restriction. For example, EcoRI exhibits star activity due to prolonged restriction time. Double stranded oligonucleotide adaptors are then ligated to both ends of the restriction fragments to provide known sequences which act as the priming sites for PCR amplification, see Figure 2. Vos *et al.* (1995), observed that amplification will only occur where the primers are able to anneal to fragments which have the adaptor sequence plus the complementary base pairs to the additional nucleotides called selective nucleotides. The first PCR (pre amplification) is performed by using primer sets with a

single selective nucleotide. This ensures there is a reasonable amount of template for the final PCR.

The final PCR (selective) reaction is performed using primer pairs with up to three base pairs extension. Selective nucleotides serve to reduce the number of amplified fragments so that not too many bands are amplified to cause smears or high levels of band comigration during electrophoresis but sufficient enough to reveal polymorphism (Vos *et al.*, 1995). AFLP fragments are then visualized by denaturing polyacrylamide gel electrophoresis (PAGE) with autoradiography (Matthes *et al.*, 1998) or automatic DNA sequences. Semagn *et al.*, (2006) observed that PAGE provides maximum resolution of AFLP banding patterns to the level of single nucleotide length differences while fragments lengths of less than 10 nucleotides are difficult to score on agarose gels. Automatic AFLP product resolution systems such as the ABI prism require that one of the primers be labeled with a florescent dye to be detected by the sequencer.



#### **2.7.3.4.2 Limitations of AFLP**

The major disadvantage of AFLP is that they are dominant markers hence they cannot differentiate dominant homozygotes from heterozygotes. This reduces their accuracy in genetic mapping, population genetic studies and marker assisted selection. AFLP also requires purified and high molecular weight DNA that is free of compounds that would interfere with the restriction digest. Complete digestion is essential in AFLP since incomplete digestion generates partial fragments of high molecular weight that generate an altered banding pattern, which may be misinterpreted as false polymorphisms (Vos *et al.*, 1995). AFLP is also labor intensive and involves more number of steps to produce results. There is also always a possibility of non-homology of comigrating fragments belonging to different loci (Kumar *et al.*, 2009).

### **2.8 Genetic diversity of *Faidherbia albida***

*F. albida* is a diploid species ( $2n = 26$ ) over most of its range; a polyploid ( $2n = 52$ ) has been recorded from Israel (Halevy, 1971). A handful of genetic studies have been conducted on *F. albida* with varying observations. Dangasuk and Gudu (2000), using isozyme analysis showed a significant deviation from the Hardy Weinberg equilibrium and deficiency in heterozygotes which was associated with inbreeding. However a high rate of inbreeding or selfing would be expected to generate considerable genetic differentiation between provenances, which was not detected in the isozyme data. Studies conducted by Joly (1992), using isozyme as genetic markers revealed that *F. albida* populations were highly variable for the loci examined. In most

cases the percentage polymorphic loci was 90 %, only the population of ManaPools, Zimbabwe exhibited a low level of heterozygosity. The level of heterozygosity, which can be considered a measure of allelic diversity (Nei, 1977), was very high for the West African populations (0.400) as compared to the East African one (0.265). RAPD analysis done by Dangasuk *et al.* (2006) reported a high degree of polymorphism among 16 African provenances with Tot and Kainuk provenances from Kenya having the least polymorphism in the East African region. A dendrogram based on six primers was able to categorize the provenances into three distinct regional clusters representing East Africa region.

## **2.9 Genetic diversity and germplasm conservation**

The sum total of all the genes present in a plant and its related species constitutes its germplasm. It is ordinarily represented by a collection of various strains and species. Germplasm provides the raw materials (genes), which the breeder uses to develop plant varieties. Therefore, germplasm is the basic indispensable ingredient of all plant management programmes, and a great emphasis is placed on its collection, evaluation and conservation (Rao, 2008). Conventionally, germplasm is conserved as seeds stored at ambient temperature, low temperature or ultra low temperature. Direct threats to plant survival are a combination of habitat loss, aggressive alien species, over exploitation and climate change. Conservation of species, particularly the agroforestry ones which have an impact on human well being, has now attained paramount importance, in the efforts to provide for the sustainable utilization of biological resources, by preventing further loss. The genetic material of many organisms is of immense

value and needs to be preserved for the benefit of the future generations. Plant genetic resources should be available for use in research and breeding in agriculture and forestry.

There are two main approaches to conservation of plant genetic resources; In *in situ* conservation the species is in the natural habitats, where evolutionary progression continues (Rao, 2008). In this system, wild species and the associated natural ecosystems are preserved together to maintain the genetic integrity of the population and to allow natural evolution (Chopra, 1989). Over a period of time and several generations, the species/variety may change its genetic and morphological composition and even the desired traits may be lost, if they do not have any advantage to the survival of species in that environment. In *ex situ* conservation the species is conserved away from the natural habitats, which requires appropriate techniques for long term preservation of the seed or other material in biobanks, botanical gardens or field genebanks (Engelmann and Engels, 2002). On account of removal from the natural habitat, there is cessation of natural evolutionary progression, but the desired genes would be preserved. The problem with this strategy is that seed and other propagules may lose their viability sooner or later. Compared with *ex situ* conservation approach, *in situ* has the important advantage in that it maintains natural genetic interactions between plants, their wild relatives and the local environment, while *ex situ* techniques freeze adaptive evolutionary development, especially in the context of pest and disease resistance (Hawkes *et al.*, 2000). Complementarity between *in situ* and *ex situ* is commonly presented as essential to secure a sustainable conservation of plant genetic resources. Adopting complementary strategies for resources conservation is useful to overcome the advantages and impediments of both strategies (Maxted *et al.*, 1997).

In the recent past, genetic diversity studies have become a cornerstone of germplasm conservation. The extent and distribution of genetic diversity in a plant species depends on its evolution, mating system, ecological and geographical factors, past bottlenecks and often by many human factors. A better understanding of genetic diversity and its distribution is essential for its conservation and use. It helps in determining what to conserve as well as where to conserve and improves understanding of the taxonomy and the origin and evolution of plant species of interest (Rao and Hodgkin, 2002). In order to manage conserved germplasm better, there is also need to understand the genetic diversity that is present in the collections. This helps in rationalizing collections, developing and adopting better protocols for regeneration of germplasm seed. It also ensures economic efficiency in conservation through elimination of redundancies, i.e., material with similar characteristics that wastes resources through increased cost of management (Rao and Hodgkin, 2002).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Plant material

Seventeen provenances of *F. albida* accessions held at World Agroforestry Centre seed bank were used. Selection was based on the regional distribution of the material. Seeds were selected to cover varying elevations of the major phytochoria of Africa where *F. albida* is endemic, see table 1. Seeds from each provenance were randomly selected for sowing. The seeds were pretreated by mechanical scarification, nicking with a nail clipper as proposed by Beckman (1992). They were then sown in polythene tubes at 15 mm depth. Leaves from 20 day old seedlings were harvested for DNA extraction.

#### 3.2 DNA isolation

Genomic DNA was extracted from approximately 500 mg fresh leaves according to Doyle and Doyle (1987). The leaf tissue were washed in 70 % Ethanol and rinsed with distilled water. The fresh sterilized leaves were ground into powder by the use of a Minibeater (Biospec products) and the DNA extracted with 550 µl of CTAB extraction buffer ( 2 % CTAB), 100 mM Tris-HCL (pH 7.5), 1.4 M NaCl, 20 mM EDTA (pH 8.0) 0.2 % mercaptoethanol and a pinch of polyvinyl pyrrolidone (PVP) preheated at 65 °C. The ground tissue and buffer mixture was incubated at 65°C for 30 minutes with occasional shaking. An equal volume (550µl) of chloroform/isoamylalcohol (24:1) was then added to the mixture, inverted ten times and centrifuged at 14000 rpm for 15 minutes at room temperature using Eppendorf 5417R centrifuge (Hamburg,Germany). The supernatant was recovered and 0.7 volume of cold isopropanol was

added and chilling was carried out overnight at -20°C. The mixture was centrifuged for 15 min at 14000 rpm at 4°C to pellet the DNA. The supernatant was carefully poured off and the pellet suspended in 100 µl of TE.

Table 1: Details of provenances sampled

<b>Country</b>	<b>Provenance</b>	<b>Location</b>	<b>Sample size</b>	<b>Seed source No.</b>	<b>Population designation</b>
Mozambique	Guija limpopo	24.30 S,33.02E	15	140/94	1
Zimbabwe	Manapools 1	15.45 S 29.20E	15	24/90	2
Malawi	Bolero	10.58 S,33.43 E	15	78/90	3
Malawi	Chawanje	14.39 S,34.48 E	15	7/92	4
Ethiopia	South harar	9.17 N, 42.06 E	14	120/94	5
Ethiopia	Lake koka	8.20 N, 38.59 E	15	119/94	6
Tanzania	Wagingombe	8.51 S, 34.38 E	15	5/92	7
Zimbabwe	Manapools 2	15.45 S 29.20 E	15	19/92	8
Senegal	Bignona	12.45 N,16.25W	15	8/92	9
Malawi	Lupaso	9.55 S, 33.53 E	14	77/90	10
Namibia	Okangwati	17.25 S,13.17 E	11	16/94	11
Zambia	Chizombo	13.08 S, 32.45 E	9	75/90	12
Kenya	Maseno	0.01 S, 34.6 E	15	-	13
Kenya	Taveta	3.24 S, 37.42 E	15	179/92	14
Ghana	Bolgatonga	10.46 N, 1.00 W	15	74/90	15
Ethiopia	Gelemso	8.45 N, 40.27 E	14	117/94	16
Zimbabwe	Bubye river	21.42S, 30.29E	14	27/93	17

Four microlitre of RNase Cocktail, RNase A (500 U/ml) and RNase T1 (20,000 U/ml (Life technologies, New York, USA) was added into the dissolved DNA and incubated at 37 °C for 30 minutes in a water bath to digest RNA. The suspended DNA was then mixed with 315 µl of wash buffer (Ethanol sodium acetate, 20:1) and the mixture chilled for at least three hours before centrifugation at 14000 rpm at 4 °C for 15 minutes. The DNA pellet was then washed with 500 µl of 70 % EtOH by centrifuging at 14000 rpm for 5 minutes. Alcohol was poured off and pellet allowed to air dry before being suspended in 50 µl TE. The isolated DNA was stored at 4 °C.

### **3.3 DNA quantification and quality analysis**

DNA quantification and quality analysis was carried out using agarose gel electrophoresis. In this, 2 µl of sample, 2 ul loading buffer (bromophenol blue, xylene cyanol and sucrose) and 1 ul of SYBR® green fluorescent dye, mixed gently by pipetting up and down and then loaded into each well of 0.8 % agarose gel and electrophoresis carried out at 80V power for 1 hour. Band intensities from the UV electrograph were compared with those of a 50 ng/µl standard molecular weight marker (Lambda DNA, Promega USA). Gels were viewed under UV illumination (312 nm) and photographed using Uvitec gel documentation system (Cambridge, UK).

DNA concentration and purity was evaluated spectrophotometrically by use of the Thermo Scientific NanoDrop 2000 system (Wilmington, USA). The ratio of the absorbance at 260 and 280nm ( $A_{260/280}$ ) was used to assess the purity of the DNA. For pure DNA,  $A_{260/280}$  is

approximately 1.8. DNA was then diluted to the required experimental concentration of 100 ng/ $\mu$ l.

### **3.4 AFLP analysis**

Techniques for the AFLP analysis of *F. albida* were adapted from those described by Vos *et al.* (1995). The same procedure has been adapted in the AFLP™ Plant mapping protocol of the Applied Biosystems (ABI) and by Hemeida *et al.* (2004).

#### **3.4.1 Template preparation and adapter ligation**

Prior to carrying out the AFLP analysis, the appropriateness of the restriction enzymes that was used to digest the DNA was checked. This was done by digesting the genomic DNA with MseI (frequent cutter) and EcoRI (rare cutter) (New England Biolabs, Ipswich, USA) independently and then with combination of both. One microgram of genomic DNA was incubated overnight at 37°C in a 20  $\mu$ l reaction mixture consisting of 1  $\mu$ l of 20 U/ $\mu$ l EcoRI, 1  $\mu$ l of 4 U/ $\mu$ l MseI, 2  $\mu$ l of 5X T4 DNA ligase buffer ( 50 mM Tris-HCl (pH 7.8), 10 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 1 mM ATP, 25  $\mu$ g/ml bovine serum albumin) and 11  $\mu$ l of sterile triple distilled water. Ten  $\mu$ l of each of the digested products together with 2  $\mu$ l of 6X loading buffer and 1  $\mu$ l of SYBR® green fluorescent dye was run on 1.5 % agarose gel to check for complete digestion with 100 bp DNA size marker (New England Biolabs, Ipswich, USA).

In accordance with the ABI AFLP™ protocol, the restriction and ligation steps were carried out together. A restriction ligation master mix was first prepared by combining 0.2 µl 5X T4 DNA ligase buffer with ATP, 0.1 µl 1mg/mL NaCl, 0.05 µl 1mg/mL BSA, 1 unit of MseI, 5 units of EcoRI and 1 weiss unit of T4 DNA ligase and topped up with sterile triple distilled water to a total volume of 1µl per sample. The adaptor pairs were first annealed by heating at 95 °C for 5 minutes and the tubes allowed to cool at room temperature for 10 minutes. The restriction ligation reaction was then prepared by mixing 2.0 µL of 5X T4 DNA ligase buffer, 1.0 µL 0.5M NaCl, 0.5 µL 1.0 mg/mL BSA, 1.0 µL MseI adaptor, 1.0 µL EcoRI adaptor and 1.0 µL Enzyme Master Mix.

About 500ng of genomic DNA in 5.5 µl sterile distilled water was then added, mixed gently and spun for 10 seconds. The reaction mixture was incubated at 37 °C for 3 hours. Overnight incubation was avoided to prevent EcoRI star activity. The restriction ligation products were diluted 20 fold using 1X TE to obtain the right template concentration for the subsequent PCR. The diluted products were stored at 4 °C for use within one month and at -20 °C in the cases of longer storage periods.

Table 2: Oligonucleotide adaptors used for AFLP analysis

Name	Sequence
EcoRI adapter	5'-CTCGTAGACTGCGTACC-3'
	3'-CTGACGCATGGTTAA-5'
MseI adapter	5'-GACGATGAGTCCTGAG-3'
	3'-TACTCAGGACTCAT-5'

### 3.4.2 Pre-selective amplification

PCR pre-selective amplification of the restriction/ligation product was performed. The threshold of selection here was low since only a single selective nucleotide was employed. Large quantities of fragments (final PCR products) which form templates for selective amplification are therefore generated. The PCR reactions were performed with a GeneAmp 9700 PCR system (PE Applied Biosystems, foster city, CA.) in a 20  $\mu$ l reaction containing 4  $\mu$ l of the restriction / ligated DNA and 16  $\mu$ l of a mixture containing 1  $\mu$ l of EcoRI and MseI AFLP pre-selective primers and 15  $\mu$ l of AFLP core mix (Applied Biosystems). The PCR amplification protocol consisted of 94 °C for 30 min followed by 20 cycles of the following profile; 94 °C for 30 sec, 56 °C for 30 sec and 72 °C for 2 min with a final hold of 60 °C for 30 min. To check the success of the preamplification reaction, 10  $\mu$ l of the preselective amplification product were mixed with 2  $\mu$ l of 6X loading buffer and 1  $\mu$ l SYBR green florescent dye and run on 2.0 % agarose gel in 1X TBE buffer at 4 V/cm for 3-4 hours.

The gel was viewed on a UV trans-illuminator. Successful amplification was confirmed by presence of a smear in the range of 100-1500 bp. The amplified products were diluted 20 fold using 15 mM Tris-Hcl buffer pH 8.0 containing EDTA and stored at 4 °C.

Table 3: Primers used in preselective amplification

Name		Sequence
EcoRI +1-A	E-A	5'-GACTGCGTAACCAATTC+A-3'
MseI+1-C	M-C	5'-GATGAGTCCTGAGTAA+C-3'

### 3.4.3 Selective amplification

Selective amplification was conducted using five primer sets, each primer of a set specific for MseI and the other EcoRI adaptors on a test panel of representative samples. The reaction mixture for each selective amplification contained 3 µl of the diluted pre-selective amplification products, 15 µl AFLP core mix, 1 µl AFLP EcoRI dye labeled primer with 3 additional user selected nucleotides and 1 µl AFLP MseI unlabeled primer with 3 user selected nucleotides. The selective amplification PCR profile consisted of an initial denaturation at 94 °C for 2 min then one cycle of 94 °C for 20 sec, 66 °C for 30 sec, and 72 °C for 2 min, followed by 10 cycles of each with 1 °C lowering of annealing temperature and finally 25 cycles of 94 °C for 20 sec, 56 °C for 30 sec and 72 °C for 2 min with a final hold of 60 °C for 30 min.

A total of 15 primer combinations which were adapted from previous work by Muchugi *et al.*, (2008), were used for trials (Table 4). Five combinations that showed good amplification and acceptable polymorphism across all the provenances were selected for full runs (Table 5).

Table 4: Primer combinations tested for AFLP analysis

	<b>EcoRI</b>		<b>MseI</b>			
	<b>CAA</b>	<b>CTT</b>	<b>CAG</b>	<b>CAT</b>	<b>CTA</b>	<b>CTC</b>
AAC			√		√	
AAG		√	√			
ACA	√			√		
ACC				√		
ACG						√
ACT	√			√		√
AGC		√	√			√
AGG		√				

Table 5: Primer combinations used for AFLP analysis

<b>Name</b>		<b>Sequence</b>
EcoRI+3-ACT	E-ACT	5'-GACTGCGTACCAATTC+ACT-3'
MseI+3-CTC	M-CTC	5'-GATGAGTCCTGAGTAA+CTC-3'
EcoRI+3-ACC	E-ACC	5'-GACTGCGTACCAATTC+ACC-3'
MseI+ 3-CAT	M-CAT	5'-GATGAGTCCTGAGTAA+CAT-3'
EcoRI+3-ACA	E-ACA	5'-GACTGCGTACCAATTC+ACA-3'
MseI+ 3-CAT	M-CAT	5'-GATGAGTCCTGAGTAA+CAT-3'
EcoRI+3-AGC	E-AGC	5'-GACTGCGTACCAATTC+AGC-3'
MseI+3-CTC	M-CTC	5'-GATGAGTCCTGAGTAA+CTC-3'
EcoRI+3-AAG	E-AAG	5'-GACTGCGTACCAATTC+AAG-3'
MseI+3-CTT	M-CTT	5'-GATGAGTCCTGAGTAA+CTT-3'

### 3.5 Selective amplification product resolution

To check for the success of selective amplification, SYBR Green stained amplicons were resolved on 2 % agarose gels buffered in 1× TBE. The gels were then viewed on a trans-illuminator and photographed. Following a successful amplification, the AFLP products were prepared for analysis on ABI prism 3730 DNA analyzer. A loading buffer mix was prepared by adding 12 µl of gene scan 500 LIZ® internal size standard to 800 µl deionised formamide. Eight microlitres of the size standard mix was added to 1.5 µl of the selective amplification products in a micro-Amp 96 PCR plate.

The amplification products were denatured by heating at 95 °C for five minutes and then snap cooled in ice before loading into the genetic analyzer. On loading the samples, the first step involved electrokinetic injection. The capillary and electrode were placed in the sample and voltage applied. The DNA fragments along with the size standards were then separated as they moved through the polymer filled capillary electrodes with illumination of capillaries at the detection cell. During the electrophoresis run, the ABI Genetic Analyzer recorded the fluorescence intensity as a function of time and wavelength from regions on a CCD camera that correspond to different detection wavelength ranges. The signals were relayed into a computer that has Data Collection Software version 4.1.

The separation parameters include; the length of the capillary, separation medium, separation voltage, separation temperature and the electro kinetic injection. From the ABI PRISM 3730, the

data was further analyzed using the GeneMapper™ software to display the fragment sizing results as electrophoregrams and tabulate the binary data.

### **3.6 Analysis of molecular data**

The fragment data from the ABI Prism 3730 automated sequencer was scored using GeneMapper® 3.7 software which generated data in a binary form (1=allele presence, 0=allele absence) and AFLP electrophoregrams of DNA fragments produced by the most optimal primer combinations.

#### **3.6.1 Analysis of Molecular variance (Amova)**

The analysis of molecular variance (Excoffier *et al.*, 1992) was run by employing Genalex 6.41 (Peakall and Smouse, 2009) software to reveal the partitioning of the variation across the populations. The variance components were used to calculate PhipT statistics which are analogous to F-statistics and useful for analysis of dominant markers. PhipT values were used to summarize the degree of differentiation among the populations and to attach significance to the estimates.

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

Principal coordinate analysis based on distance matrix was conducted using GenALEx 6.41 (Peakall and Smouse, 2009) software to display provenance relationships by interpreting patterns in the composition of the samples.

### **3.6.3 Cluster analysis**

Genetic relationships among the populations were displayed by phenetic classification using similarity index. The similarity matrix was based on Nei's genetic distances (Nei, 1978) generated with TFPGA 1.3 (Miller, 1997) software. Cluster analysis on the basis of these distances was conducted by employing the unweighed pair-group method with arithmetic averaging (UPGMA) (Sneath and Sokal, 1973), to generate a dendrogram showing relationship among the populations. Validation of the cluster analysis was done by computing 5000 bootstrap samples using TFPGA.

### **3.6.4 Measures of genetic diversity**

To establish genetic diversity of the provenances, the percentage of polymorphic loci at 99 % CI and unbiased average heterozygosity were computed using TFPGA (Miller, 1997). Here the allele frequencies were estimated based on the square root of the null genotype and Hardy Weinberg equilibrium was assumed.

**CHAPTER FOUR****RESULTS****4.1 DNA extraction**

The success of AFLP is underpinned by clean and high molecular weight DNA. SYBR® Green stained DNA, extracted based on CTAB procedure was run on 0.8% agarose gel against Lambda DNA standards and gel photo taken, see Plate 3.

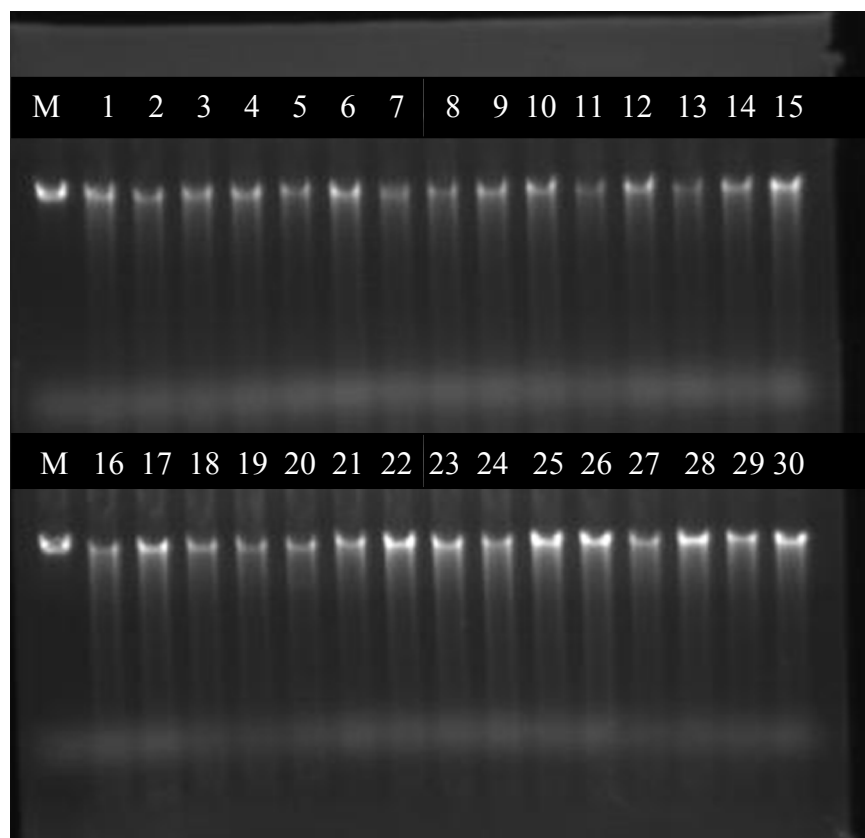


Plate 3: SYBR® Green stained agarose gel (0.8 % w/v) showing CTAB DNA extraction of thirty *F. albida* samples. Guija limpompo (Lanes 1-15) and Manapools (Lanes 16-30). First lane (M) is the  $\lambda$  DNA size marker of 100 ng/ $\mu$ l (New England Biolabs, Ipswich, USA).

The average DNA concentration and purity were determined by spectrophotometry, see Fig 3. DNA contamination by proteins was assessed at 260:280 absorbance ratio while that of other organic contaminants was assessed at 260:230 ratio.

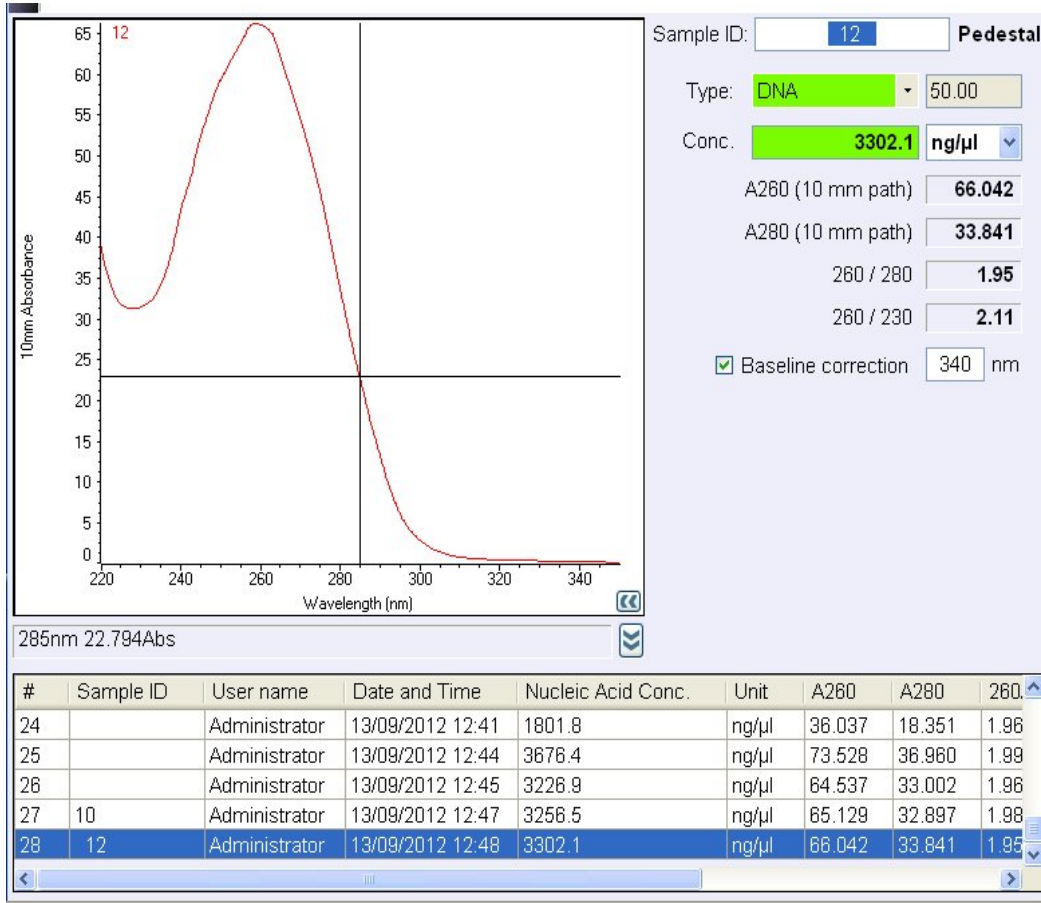


Figure 3: DNA spectrophotometer curve depicting absorbance against wavelength of a pure 3.3 µg DNA sample from Bolero.

## 4.2 AFLP analysis

Proper preparation of template is crucial for a reliable AFLP procedure. Part of this is restriction and ligation of MseI and EcoRI adaptors which produced visible smears of up to 1100 bp, see Plate 4.

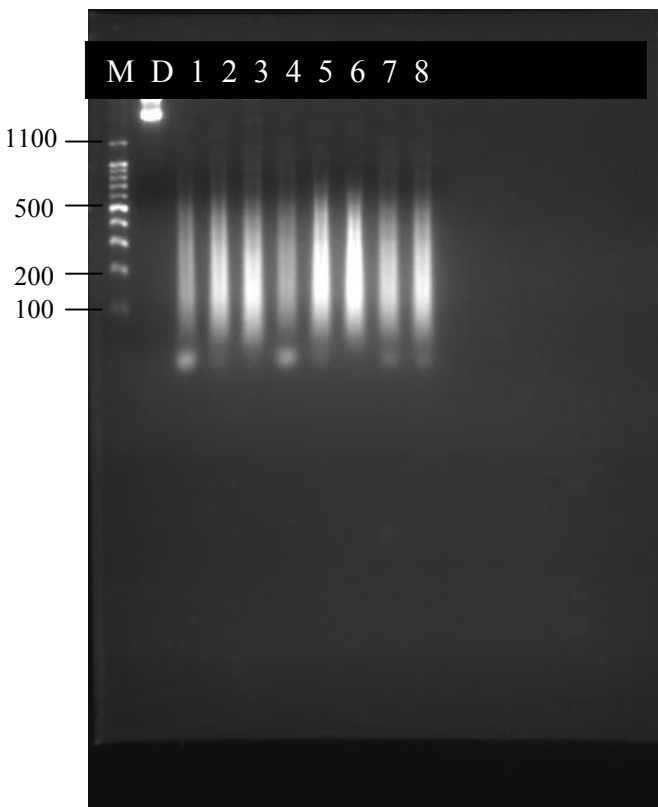


Plate 4: SYBR® Green stained agarose gel (2 % w/v) showing restriction/ligation of eight samples from Taveta, Kenya. M designates a 100 bp molecular ladder; Lane D is undigested DNA.

Preselective amplification products created a clearly visible smear in the range of 100-600 bp when run on 2 % (w/v) agarose gel, see Plate 5.

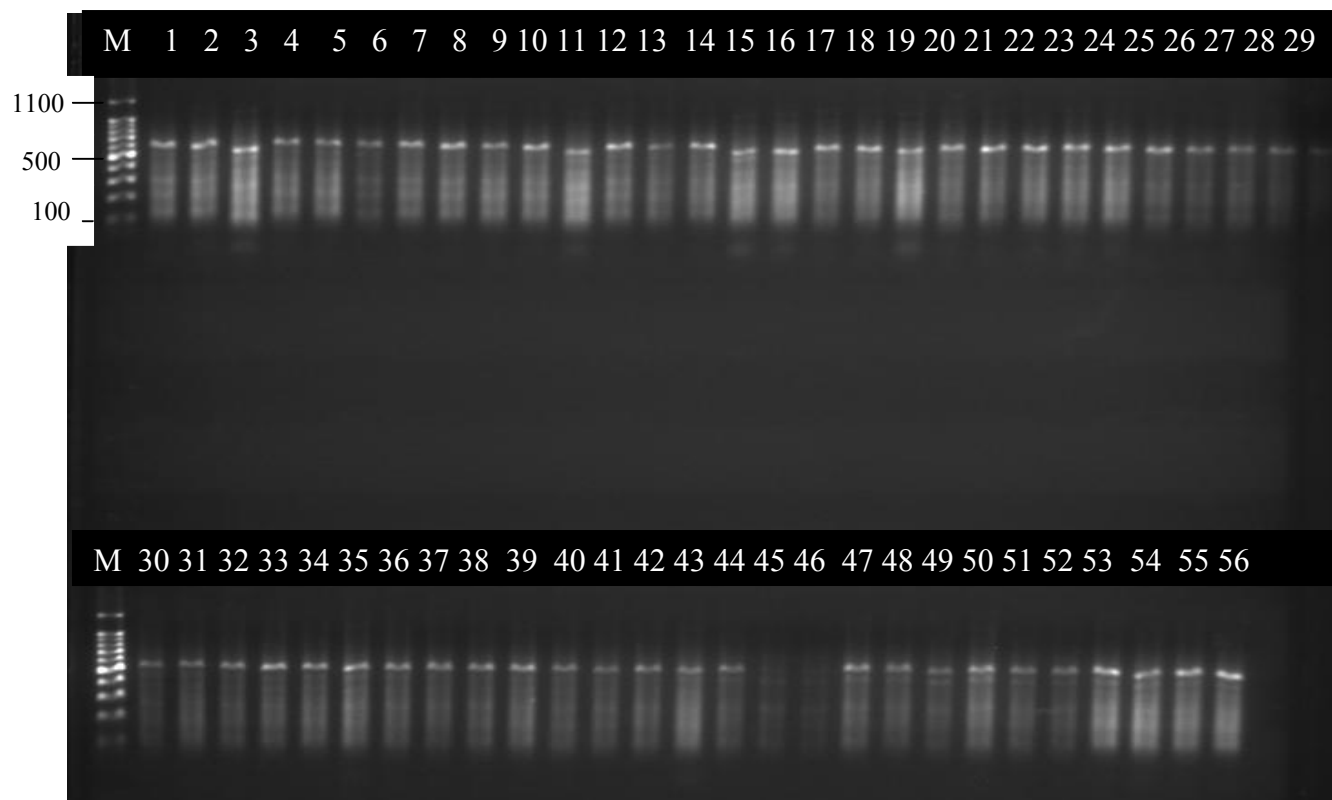


Plate 5: SYBR® Green stained agarose gel (2% w/v) showing preselective amplification of 56 samples. Chawanje (Lanes 1-15), Lake Koka (Lanes 16-30), Manapools (Lanes 31-45) and Bignona (lanes 46-54). M designates a 100 bp ladder.

Successful selective amplification was checked by running the amplicons on 2 % (w/v) agarose gel, see Plate 6.

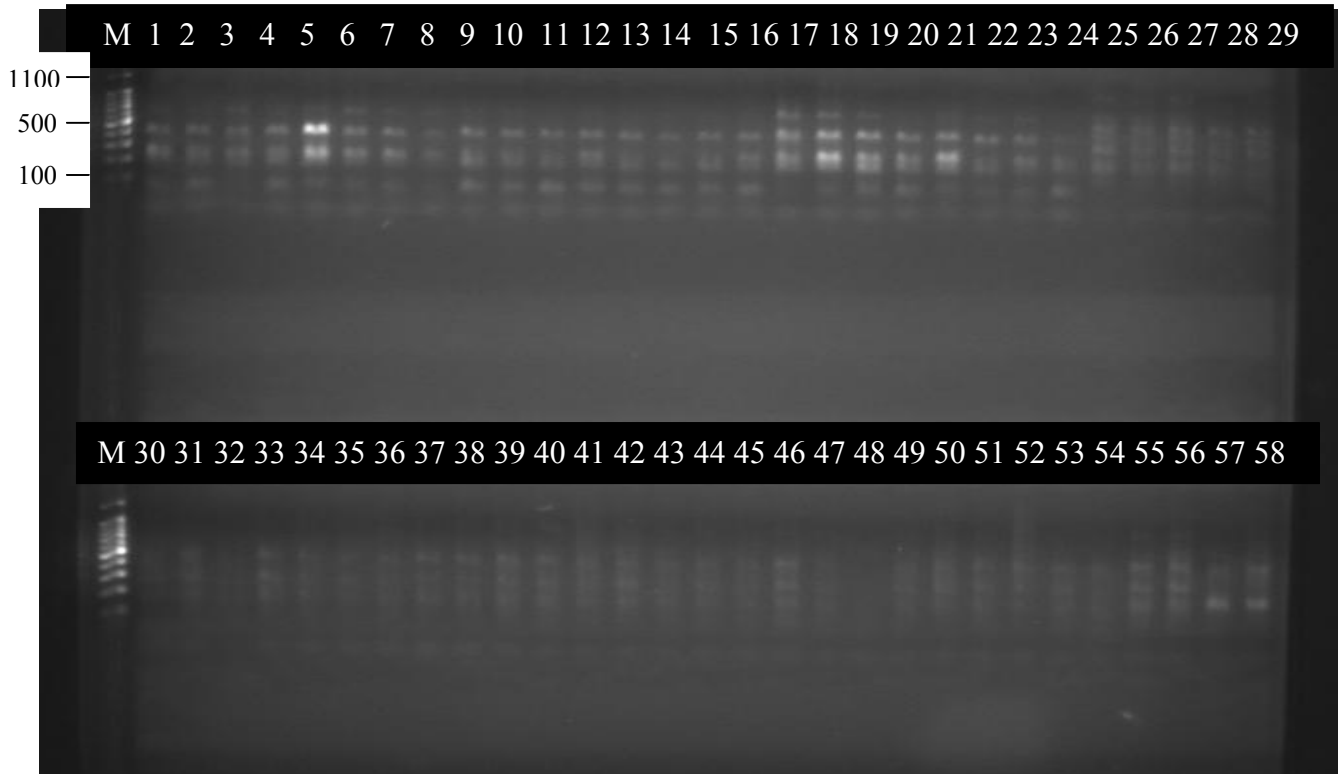


Plate 6: SYBR® Green stained agarose gel (2 % w/v) showing selective amplification of fifty eight *F. albida* samples from Guija limpompo, Manapools, Bolero and Chawanje respectively using primer combination E-ACT/M-CTC. M designates the 100 bp DNA ladder.

Allele sizing results were analyzed and displayed in form of electrophoregrams using the GeneMapper software , see Figure 4.

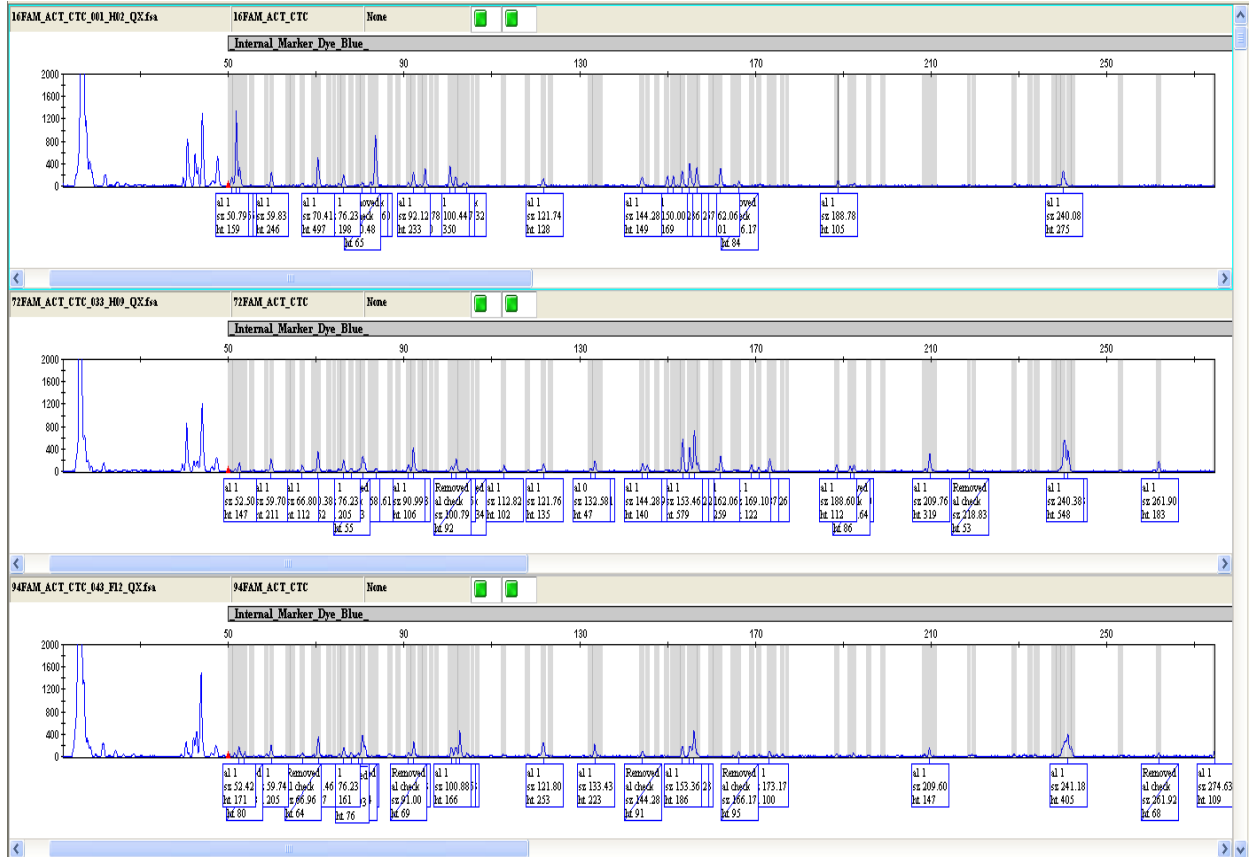


Figure 4: Scored AFLP peaks and allele sizes for three *F. albida* samples for primer set E-ACT/M-CTC.

Table 6: Binary data for automated AFLP peaks in figure 4.

Sample	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5
16FAM_ACT_CTC	1	1	1	0	0
72FAM_ACT_CTC	0	0	1	0	0
94FAM_ACT_CTC	0	0	1	0	0

### 4.3 Analysis of molecular variance (Amova)

The analysis of molecular variance (AMOVA) for provenances showed significant differentiation ( $P= 0.01$ ), with 58 % of the variation attributed to among populations and 42 % to within populations, see Table 7.

Table 7: Summary of the analysis of molecular variance (AMOVA) within and among *Faidherbia albida* provenances.

Source of variation	df	SS	MS	Est.Var	%	Statistic	Value	P-Value
Among populations	16	12408.948	775.559	52.120	58			
Within populations	224	8352.056	37.26	37.286	42			
Total	240	20761.004		89.406	100	phiPT	0.583	0.010

The analysis is based on AFLP genotypes consisting of 676 band states. Levels of significance are based on 99 iterative permutations.

### 4.4 Principle coordinate analysis

Principle coordinate analysis generated 3 general groups (Figure 5) with many of the provenances corresponding to geographical locations. Bolgatonga (Ghana) and Bignona (Senegal) appeared to be a bit distantly related from the rest of the provenances in their respective groups. The first three coordinates explained 86.87 % of the total variation.

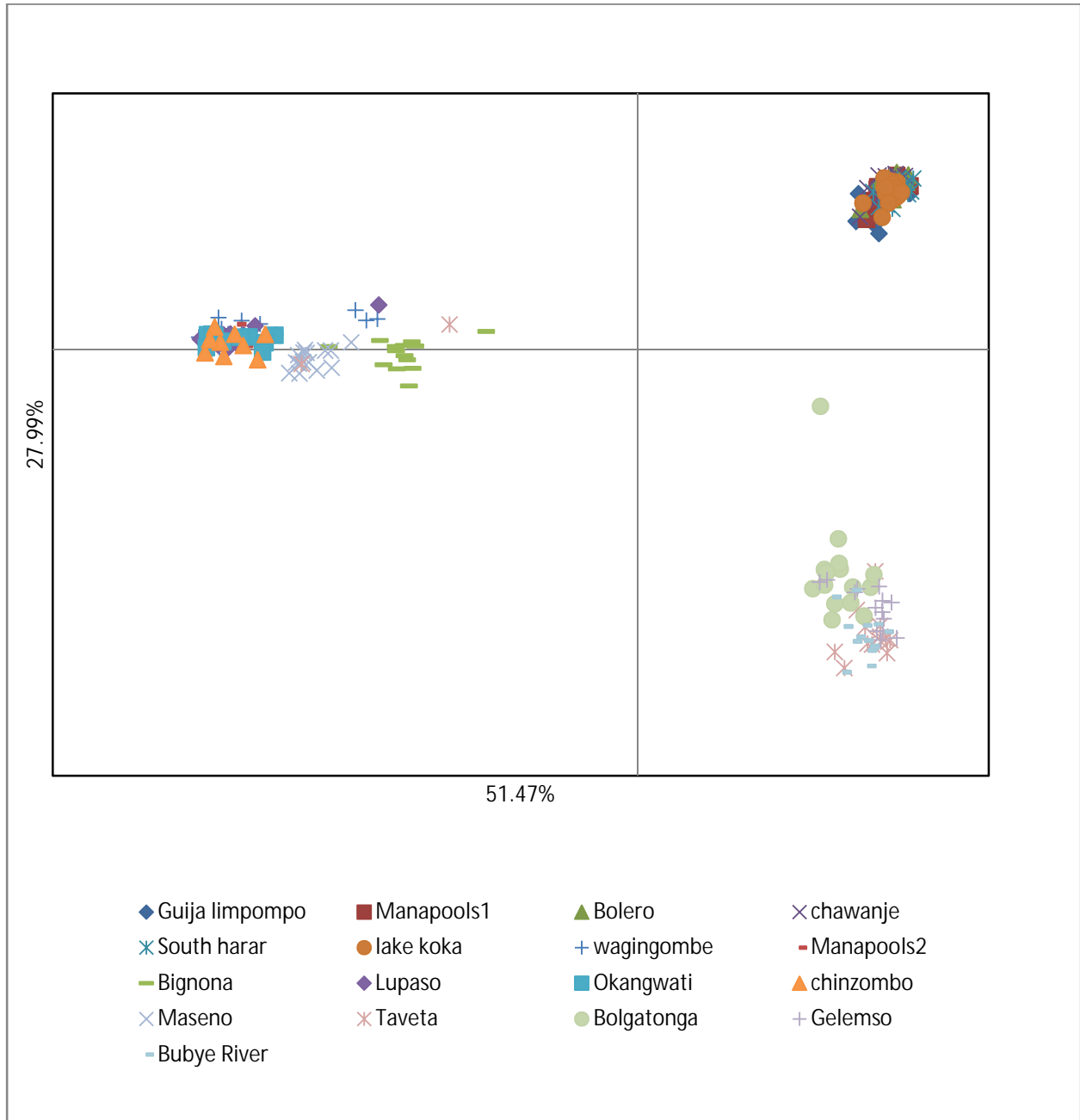


Figure 5: Principle coordinate analysis of the 17 *Faidherbia albida* provenances based on 676 AFLP loci.

#### 4.5 Cluster analysis

Nei's genetic distances, displayed in Table 8, were used to generate genetic similarity estimates based on all the 676 AFLP loci using UPGMA cluster analysis in order to present genetic relationships as a dendrogram as shown in Figure 6. The dendrogram generated showed three major population clusters. The greatest genetic distance (0.488) was observed between Manapools1 and Bubyer river provenances which interestingly are accessions from the same country while the lowest genetic distance (0.012) was observed between Bolero and Chawanje provenances from Malawi as shown in Table 6. Bolgatonga (Ghana) and Bignona (Senegal), though both from West Africa, are in different clusters with a genetic distance of 0.392. Two provenances from Ethiopia (South Harar and Lake Koka) are clustering together with a genetic distance of 0.031 but distantly with Gelemso accessions from the same country with genetic distances of 0.304 and 0.309 respectively. The two Kenyan provenances, Taveta and Maseno, are in different clusters, and quite distantly related (0.308). Taveta is clustering with Gelemso (0.06) and Maseno with Wagingombe, Tanzania, (0.11). Most of the South African provenances are showing a regional similarity. The pattern depicted by the UPGMA tree corresponds well with the PCA ordination, both showing three main clusters with similar grouping.

Table 8: Unbiased genetic distance matrix according to Nei (1978) among 17 *Faidherbia albida* provenances based on 676 AFLP markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1																	
2	0.020																
3	0.025	0.014															
4	0.018	0.013	0.012														
5	0.055	0.036	0.037	0.043													
6	0.061	0.056	0.051	0.058	0.031												
7	0.323	0.332	0.337	0.334	0.358	0.356											
8	0.340	0.347	0.353	0.350	0.376	0.374	0.051										
9	0.320	0.320	0.333	0.329	0.332	0.341	0.135	0.156									
10	0.331	0.335	0.339	0.339	0.356	0.357	0.051	0.049	0.136								
11	0.330	0.329	0.338	0.334	0.356	0.360	0.069	0.042	0.155	0.053							
12	0.337	0.344	0.353	0.351	0.372	0.377	0.054	0.038	0.153	0.043	0.047						
13	0.321	0.326	0.337	0.334	0.343	0.343	0.114	0.138	0.140	0.124	0.143	0.127					
14	0.313	0.326	0.328	0.334	0.334	0.338	0.375	0.377	0.339	0.361	0.382	0.375	0.308				
15	0.280	0.280	0.296	0.291	0.289	0.296	0.421	0.435	0.392	0.425	0.429	0.437	0.408	0.308			
16	0.285	0.293	0.301	0.303	0.304	0.309	0.463	0.468	0.419	0.449	0.457	0.462	0.423	0.408	0.308		
17	0.317	0.323	0.333	0.331	0.345	0.353	0.477	0.488	0.446	0.481	0.479	0.478	0.470	0.408	0.308	0.308	

Numbers 1-17 refer to population designations as shown in Table 1.

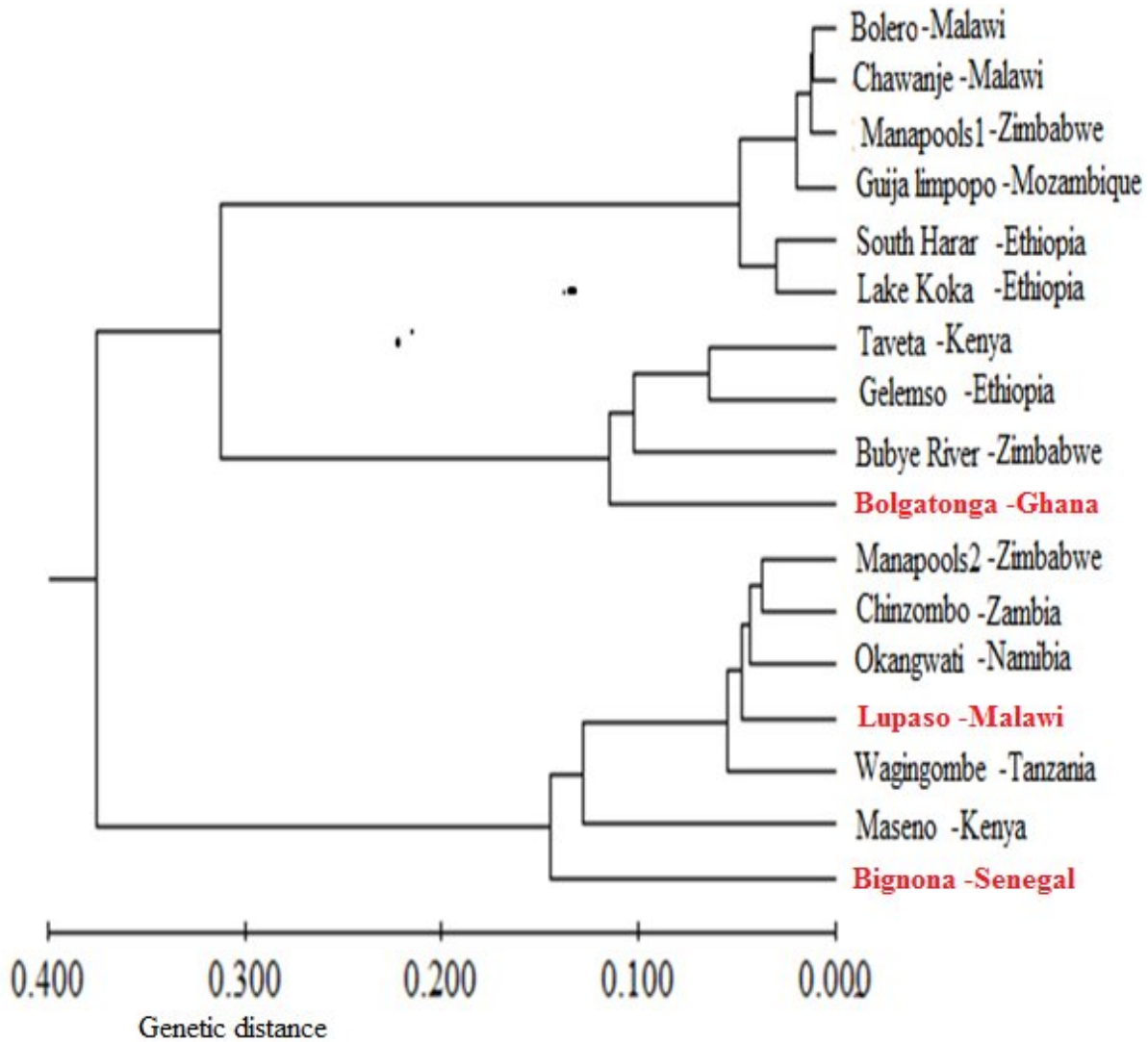


Figure 6: An UPGMA dendrogram showing relationship among 17 provenances of *Faidherbia albida* based on 676 AFLP loci amplified by 5 primer sets.

#### 4.6 Measures of genetic diversity

The mean heterozygosity (Nei's genetic diversity) based on the 676 loci across the 17 populations ranged between 0.050 to 0.279. The percentage of polymorphic loci ranged between 13.462 to 69.526 with Manapools1 accessions having the lowest diversity and percentage of polymorphism and Taveta having the highest, see Table 9. It was observed that the percentage of

polymorphism values and average genetic diversity (H) values are in agreement to the extent that the population showing low percentage polymorphic loci is also showing low H.

Generally, accessions from West and East Africa (Bignona-52.07, Bolgatonga-55.32, Kenya-69.53 and Gelemso-53.25) had higher percentage of polymorphic loci and average heterozygosity compared to accessions from South Africa (Manapools1-13.46, Bolero-15.53 and Chawanje-16.57). Only Bubyer river provenance from Mozambique had a comparatively high genetic diversity (0.17) and percentage of polymorphic loci (45.71). South harar (15.09) and Lake Koka (17.01) accessions from Ethiopia had the lowest estimates among the East African provenances.

Table 9: Mean genetic diversity estimates for 17 *F. albida* provenances based on five primer sets and 676 loci

Country	Provenance	Number of individuals	% Polymorphic loci	Average heterozygosity (H)
Mozambique	Guija Limpopo	15	21.302	0.079
Zimbabwe	Manapools1	15	13.462	0.050
Malawi	Bolero	15	15.533	0.062
Malawi	Chawanje	15	16.568	0.059
Ethiopia	South harar	14	15.089	0.056
Ethiopia	Lake koka	15	17.012	0.063
Tanzania	Wagingombe	15	41.272	0.140
Zimbabwe	Manapools2	15	34.615	0.125
Senegal	Bignona	15	52.071	0.197
Malawi	Lupaso	14	39.053	0.135
Namibia	Okangwati	11	27.663	0.105
Zambia	Chinzombo	9	31.508	0.125
Kenya	Maseno	15	28.698	0.105
Kenya	Taveta	15	69.526	0.279
Ghana	Bolgatonga	15	55.325	0.208
Ethiopia	Gelemso	14	53.254	0.222
Zimbabwe	Bubyer river	14	45.710	0.174

## CHAPTER FIVE

### DISCUSSION

Measures of genetic diversity results based on five primer combinations showed that *Faidherbia albida* accessions held at ICRAF seed bank are generally of fair variability. Taveta provenance from Kenya had the highest diversity estimate values ( $H=0.279$ ) while Manapools1 from Zimbabwe had the least ( $H=0.05$ ). The same trend was manifested by the percentage of polymorphic loci estimates. The mean percentage of polymorphic loci (33.98) and the mean heterozygosity (0.13) for all the populations were in the range of those observed by Harris *et al.* (1997), using isozymes (33.98 and 0.17). On average the West African provenances manifested higher genetic variation followed by Eastern populations and the South African ones had the lowest levels. This is in agreement with the findings of Dangasuk *et al.* (2006) using RAPD analysis and Harris *et al.* (1997) using isozymes. This trend suggest West Africa as the possible centre of origin and centre of diversity of *F. albida* and a subsequent east to south spread. This school of thought was first postulated by Chevalier (1934) and Wickens (1969).

The diversity estimates were however lower than those reported by Dangasuk *et al.* (2006) based on RAPD (West Africa  $H=0.7$ , East Africa= 0.67 and South Africa= 0.47). This could be possible owing to the differences in the marker techniques and the sample size. However the overall trend was virtually similar. The high genetic diversity recorded in West (Bignona and Bolgatonga) and East African (Taveta) accessions makes them potential seed sources for introduction and domestication of *F. albida*. Accessions from South Harar and Lake Koka (Ethiopia ), Bolero and Chawanje (Malawi) and Manapools had the lowest diversity estimates.

This is in agreement with results observed by Harris *et al.* (1997). Indeed he observed that there was absence of heterozygotes in Bolero ( $H=0.00$ ). These patterns of variation point to a possibility of severe founder effects or genetic erosion due to anthropogenic factors acting on these populations long before the time of collection. More collections need to be done from these provenances to capture more diversity and enrich this gene pool.

For a wide range of plant species, the mating system plays a critical role for the patterns of genetic variation both within and among populations (Hamrick *et al.*, 1992). Amova results revealed a considerable level diversity structuring, 58 % percent of which was among population and 42 % within populations. This is contrary to the expected diversity structuring for outcrossing plant species as demonstrated by Rosseto *et al.* (1995) and Chase *et al.* (1995). They showed that out-crossing plant species tend to have 10-20 % of the genetic variation among populations while selfing species have on average, 50 % of the variation among populations. Such structuring means that *F. albida* exhibits some considerable level of selfing. These results are in line with the study done by Joly (1992), where natural populations of *F. albida* revealed a deficit in heterozygotes compared to values expected in open allofertilization confirming a partial selfing. Gassama Dia *et al.* (2003), reaffirmed this view while studying reproductive biology of *F. albida*. Due to the mixed mating system suggested by these results, the conservation implication is that sampling need not be very extensive to capture a large proportion of the variation within this species.

The high and significant differentiation observed among the provenances studied, demonstrated the importance of selection pressure in evolution of ecotypes. Different populations have adapted to immediate ecological conditions like soil type, rainfall patterns and insolation. This coupled with limited gene flow due to long distances between them has led to formation of disjunct distributions of related populations. Studies of population differentiation are therefore clearly important to the conservation of *F. albida* especially where decisions have to be made concerning where and what populations should be conserved and the need for genetic mixing in introduction programs. Without detailed knowledge of the spatial population structure of rare species and closely related more widespread species, the conservation interest of particular populations may be underestimated (Thompson, 1999).

UPGMA cluster analysis on the basis of pairwise genetic distances showed three main distinct groups. This pattern of clustering was in agreement with the principle coordinate analysis which was chosen to complement the cluster analysis information. Cluster analysis is more sensitive to closely related individuals whereas PCA is more informative regarding distances among major groups (Hauser and Crovello, 1982). The dendrogram did not exclusively place provenances according to the geographical origins of the accessions however it gave crucial insights into evolutionary processes that result from patterns of colonization and isolation. The first group was composed of virtually all provenances from Southern Africa except two provenances (South Harar and Lake Koka) with Ethiopian origin. The large genetic distance between the Ethiopian provenances and the four South African provenances in the same cluster points the two as seed sources for the colonization of South African countries through migratory herbivores and other

human activities. South Harar and Lake Koka share a very small genetic distance which is an indication of a recent divergence.

The second group was mainly composed of Eastern and Southern Africa provenances. Only Bolgatonga from Ghana (west) featured in this main cluster. A similar pattern was observed by Harris *et al.* (1997), where Bolgatonga formed part of Ethiopian/Sudanian subcluster rather than the Western subcluster. The same case applied to the third cluster with only Bignona from Senegal (West) appearing in the group. Based on differences in ecological conditions and geographical isolation it would be expected that natural selection and differentiation will make such closeness impossible. This uniqueness of West African populations being genetically closer to east and South African populations was also observed by Dangasuk (2003), where Bignona clustered together with Rama (Ethiopia). However, Vandenbelt (1991) and Joly (1992), reported the uniqueness of the Ethiopian provenances of *F. albida* which made them suspect that Ethiopia is the possible species center of origin containing the greatest genetic diversity of the species. The same observation was made by Dangasuk *et al.* (1997). Although this study did not feature Rama in its analysis, the presence of one of the two West African provenances studied in each cluster suggests that they share considerable characteristic with the east and south population and therefore West Africa is the most likely origin of *F. albida*. A clearer picture about the origin can however be obtained if more West African, Sahelian, and North African provenances could be included in the study.

The large genetic distance between Bolgatonga (Ghana) and Bignona (Senegal) could be explained by the two being located in different phytochoria (White, 1983). Bignona is part of the Sudanian phytochoria while Bolgatonga is part of Guineo Congolian regional centre of floral endemism. These regions are defined by unique ecological conditions which may have subjected the populations to strong selection forces. The large genetic distances and by implication genetic diversity recorded from West African provenances in this study and by Dangasuk (2006), Dangasuk (2003) and Harris *et al.* (1997) presents them as crucial diversity centers for germplasm collection. A peculiarity of big genetic distances between Maseno and Taveta from Kenya, the two Manapools provenances from Zimbabwe and Gelemso from the other Ethiopian provenances was eminent. Such a phenomenon occurs when there is a major barrier to gene flow in terms of pollination and seed dispersal over a number of generations. This could also have been a result of independent colonization events. Going by the GPS coordinates the distance between Maseno and Taveta (both in Kenya), Buby River and Manapools are large enough for any substantial gene flow to have occurred and therefore the observed genetic distance could be a result of isolation and divergent evolution over time. Gelemso is found along the Eastern highlands of Ethiopia while Lake Koka is one of the Great Rift Valley lakes. The genetic distance between these accessions could as well be a result of this isolation.

This study validates the fact that the spatial structuring and diversity of populations does not necessarily reflect their geographical locations (Muchugi *et al.*, 2008; Kadu *et al.*, 2011). Rather the absence of congruence between phylogenetic branching patterns among related taxa and their geographical distributions is a factor of historical associations among populations, the role of isolation in shaping patterns of disjunct distributions of related taxa, the role of evolutionary

forces in shaping traits , the role of human activities on evolution and the interaction between mating systems and the environment.

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

Based on this study, it is clear that AFLP is a reliable tool for studying the genetic diversity of *Faidherbia albida* accessions and information obtained is crucial in management of these germplasm resources. Generally accessions from West African provenances have comparatively higher genetic diversity followed by Eastern Africa and West Africa in that order. These accessions are therefore comparatively variable enough to be used as seed sources for introduction and domestication purposes. The structure of genetic diversity and relatedness of *F. albida* provenances does not entirely reflect their geographical proximity but the historical associations, anthropogenic and evolutionary forces that have shaped their traits. Differences in the ecogeographical conditions defining a regional centre of floral endemism or a transition zone have also been found to spell adaptive variations in adjacent populations. The structuring of genetic diversity revealed that *F.albida* is not entirely outcrossing, a phenomena that has a bearing on the extensiveness of sampling in capturing substantial diversity of accessions. This is because, with the mating system inferred from the structuring of diversity here, it is expected that *F.albida* diversity is fairly distributed within the populations but lacks inter-population homogeneity. This study also confirmed that there are no redundancies among the *F. albida* germplasm analyzed in the seed bank.

## 6.2 Recommendations

The following recommendations were made based on the findings of this study;

- i. Since slightly lower diversity values were obtained here compared with previous studies using RAPD, consideration should be made to use more AFLP primers to cover the *F. albida* genome more extensively or alternatively a codominant marker technique should be employed to make comparisons to the two approaches.
- ii. Accessions from West Africa provenances have the highest genetic diversity and such should be utilized for introduction in cases of domestication and land rehabilitation.
- iii. The exact number of provenances to act as seed source in establishment of a population is crucial due to lack inter-provenance homogeneity.
- iv. In order to have a better picture of genetic diversity and spatial structure of *F. albida* populations in Africa and to draw informed germplasm management strategies, all phytochoria should be well represented in such studies. In this view, germplasm from the Sudanian, Sahelian regional transition zone and Sahara regional transition zone phytochoria need to be collected to fill in the gaps.

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