Genetic structure of *Albizia gummifera* and its local adaptation to the associated arbuscular mycorrhiza

Judith Ssali Nantongo

BSc. Forestry

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DECLARATION

I **Judith Ssali Nantongo** affirm that this work is my own, unless where referenced, and has never been submitted to any institution of higher learning for any award.

Sign…….. Date…….

Judith Ssali Nantongo

Every fragment of this work has been under the supervision of;

Dr. Steven Cavers

Centre for Ecology and Hydrology

Bush Estate, Penicuik, UK

Sign ……

Date……

Dr. John Bosco Lamorris Okullo

Faculty of Forestry and Nature Conservation

Makerere University, Kampala, Uganda

Sign……

Date……

DEDICATION

This work is dedicated to all those who I cherish: my children- Innocent and Immanuel; my dear parents- Mr. and Mrs. Ssali Poly, Bernard and all friends. Above all, to almighty God who saw me through all the challenges of Laboratory work.

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ABSTRACT

The aim of this study was to assess genetic structure and local adaptation of *Albizia gummifera* to associated mycorrhiza using three populations from Uganda, Kenya and Madagascar. Using variation in chloroplast DNA sequences, estimates of genetic diversity and differentiation were obtained. Local adaptation of *A. gummifera* to the associated mycorrhiza was investigated by planting seed from different *A. gummifera* provenances into soils inoculated with soil microbial samples from respective local sites. In addition, the stability of inoculum was tested by comparing the performance of fresh and stored soils as inoculum. Four weeks after seedling emergence, height measurements were initiated and continued for six weeks. Mycorrhizas in the soil inoculum were identified using direct microscopic observation. Genetic data were analysed using GENALEX while greenhouse data were analysed using GENSTAT. The results showed that the species is genetically diverse with 14 cpDNA haplotypes identified (h_{TOT} = 0.803), with Uganda showing most diversity (h =0813) and Kenya the least (h =0.398). Although the majority of variation was distributed within populations (75%), significant population differentiation was observed (Φ_{PT} = 0.249, p > 0.01) and each population contained private haplotypes: Uganda (5), Madagascar (3) and Kenya (1). Greatest genetic distance was observed between Kenya and Madagascar (2.711). The lowest distance was observed between Uganda and Kenya (0.298). The diversity of the mycorrhizal community varied between sites with Ugandan fresh soils being more diverse than Kenyan fresh soils. For the old soils, fungal diversity was highest in Kenya, followed by Madagascar and then Uganda. Based on the growth performance measurements, there was no evidence of adaptation of *A. gummifera* provenances to local mycorrhizas though plant performance for inoculated plants was higher than that of the control. From the study, it appears that the specific kind of fungi the *A. gummifera* plants are exposed to is not important, although they benefit from the exposure. The tree populations seem to have genetically differentiated and transferring them to sites outside their own may pose a genetic threat. More research is however needed to ascertain adaptive differences of *A. gummifera* to abiotic and other biotic factors, the suitable founding genetic diversity and other factors that may affect introductions. The exact mycorrhizas that colonise the plants also need to be identified.

CHAPTER ONE

INTRODUCTION

1.1 Background

The adverse impacts of ecosystem degradation on biodiversity have been widely documented (Balirwa, 2007; Hobbs, 2007; Jardine *et al.,* 2007; Laurance, 2007; Oago and Odada, 2007; Scanlon *et al.,* 2007). Although there has been extensive collection, storage and propagation of several tree species, especially the threatened ones as part of conservation efforts, there is a growing realization that it is not possible to conserve the earth's biological diversity through protection of critical areas and species alone (Early and Thomas, 2007; Lesica and Allendorf, 1999). These efforts need to be augmented by restoration of degraded ecosystems whose success is closely linked to use of genetically diverse, uniform and well adapted local material.

Even if there is empirical evidence that genetic diversity of an individual positively influences its fitness and persistence (Boyce, 1992; Reed and Frankham, 2003), the fact that many local propagules are collected from degraded and/or fragmented areas provides a salient question of the genetic health of these materials. According to Hobbs and Yate (2003) degradation affects genetic diversity through reducing gene flow and enhancement of inbreeding and genetic drift.

Besides genetic structure, the function, rhizospheric properties and hence adaptation of a species may be confounded by mycorrhizal fungi among other functional groups (Chen *et al*., 2005; Ingleby *et al*., 2007; Klironomos *et al*., 2000; Medina *et al*., 2003). While a report by Chen *et al.* (2005) indicates that most known species of mycorrhiza are non specific and ubiquitous in their distribution, the work of Ingleby *et al.* (2007) provides evidence that ecosystems that have been heavily impacted by humans contain fewer fungal isolates, with negative implications on restoration. Besides, it is possible that some symbiosis may be specific. Therefore, generally, key questions considered when planning for restoration are:

a) Can local populations provide sufficient, genetically diverse seed to restore populations?

b) Are local populations isolated or fragmented beyond the scope of gene dispersal?

c) If local material is inappropriate:

- For the target species, what is the spatial scale of gene flow and genetic differentiation?
- At what spatial scale are populations locally adapted?
- How much genetic variation is appropriate in propagules for restoration purposes?
- Is there a risk, to extant local populations, of introduction of exotic material?

d) Are the fungal spores in the field sufficient and non specific?

Against the above background, this study aims at establishing the patterns of genetic structure of *Albizia gummifera* (J.F.Gmel.) and local adaptation to mycorrhizal fungi in East Africa (Uganda & Kenya) and Madagascar.

Albizia gummifera belongs to the family Fabaceae, sub family Mimosoideae. Whereas many activities have relied on well known species, *A. gummifera* is a lesser known leguminous tree species with potential for multifunctional benefits. It is a source of timber and medicine and also has potential as an agroforestry tree species. It forms mycorrhizal associations and has the ability to associate with many crops (Katende *et al.*, 1995). The ability of the species to form mycorrhizal and rhizobial symbiotic associations coupled with fast growth in gaps makes it suitable for use in ecological restoration of degraded forests.

1.2 Statement of the Problem

Although a number of lesser-known species may have a potential to be used for forest restoration, in many cases there are few data to support their use. In addition to the fact that the planting material from within the degraded areas may be genetically impoverished, attempts to

use such species for restoration have often been limited by seed shortage. Thus, to use these lesser-known species, there may be a need to source planting material from outside the restoration area. However, the adaptation and hence performance of most species out of their native range is equivocal. Since genetic diversity influences adaptive potential to the rapid environmental change typical in disturbed forests (Hamrick *et al.*, 1991; Reed and Frankham, 2003), quantifying the genetic diversity of potential species is important. Furthermore, because mycorrhizas are also important in the functioning of the plants in various ecosystems, their specificity and performance outside their local areas needs to be explored.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study was to assess the extent to which genetic diversity and local adaptation of *A. gummifera* are important factors in choice of indigenous plant species material to use for replanting and the extent to which soil mycorrhiza contribute to provenance performance differences.

1.3.2 Specific Objectives

1. To evaluate the genetic diversity, gene flow and differentiation among *A. gummifera* tree populations.
2. To assess the diversity of fungal communities associated with *A. gummifera* across experimental sites.
3. To evaluate performance of *A. gummifera* provenances as tested in both home and exotic soils.
4. To test inoculation protocols by comparing the extent of mycorrhizal formation from fresh and old soils.
5. To determine the effect of fungal association on the performance of *A. gummifera* seedlings

1.4 Hypotheses

1. At a regional scale, *Albizia gummifera* populations do not experience significant gene flow and hence are genetically differentiated.
2. The *A. gummifera* tree and fungal populations have not co-adapted to local site conditions such that its seedlings and mycorrhiza perform equally well in all provenances/ecozones.

1.5 Significance of the study

Because natural regeneration may be slow to maintain equilibrium of all of the ecological cycles that depend on the degraded forests, there is a need for intervention through reseeding and planting with the indigenous species before some important attributes are irreversibly lost. The information collected in this study can be used for optimizing strategies for efficient collection and deployment of indigenous tree germplasm in ecosystem restoration. Such efforts can also contribute to increased productivity of degraded land by optimizing restoration efforts with the needs of local people in mind; hence restoration species will be targeted such that the balance between their ecological importance and their socio-economic importance is maximized.

1.7 Scope of the study

This study covered two countries in East Africa (Uganda & Kenya) and Madagascar, which are the working areas of the FOREAIM project. FOREAIM project aims at bridging restoration and multi-functionality in degraded forest landscapes of Eastern Africa and the Indian Ocean Islands. This current research has contributed to work package three of the project, which is: restoration through planting: characterization and silviculture of native and naturalized species to restore environmental and economic function.

CHAPTER TWO

LITERATURE REVIEW

2.1 The need for tropical forest restoration

Maintenance of biodiversity has become an increasingly important management goal, because biodiversity provides a broad array of ecosystem services that directly or indirectly benefit humans. These services include among others; water purification, sustaining and/or increasing primary production as well as storing and cycling nutrients. However, biodiversity conservation is counteracted by the high yet increasing habitat loss most especially of the tropical forests which habit most biodiversity (Laurance, 2007; Pandit *et al*., 2007) . Some of the tropical forests have been severely degraded, hampering colonization and hence natural recovery (Holl and Kappelle, 1999; Hobbs and Harris, 2001; Shono *et al*., 2006). Consequently severe impacts implicating species extinction are documented causing concern. To maintain alpha diversity (species diversity at the local scale), a dynamic balance of local colonization and local extinction is required (Tilman, 1993, Palmer *et al*., 1997).

2.1.1 Causes of slow forest recovery

The direct causes of limited recovery documented by various authors include limited seed rain, unfavorable microclimate and soil degradation, competition with grasses and other non-woody vegetation (Holl and Kappelle, 1999; Yates *et al*., 2000; Renison *et al*., 2004; Shona *et al*., 2006), limited seed dispersal and high seed predation, and production of smaller and less viable seeds (Renison *et al*., 2004; Shona *et al*., 2006). Where these conditions are severe, for example in over-degraded areas, natural recovery/regeneration will be too slow to sustain complete ecosystem function. Human intervention through restoration can thus reverse these conditions and enhance recovery/regeneration. Though a relatively new science, restoration has already been proposed as one of the paradigms for sustainable livelihoods and environmental management especially

become relevant given the recent and anticipated climatic changes. Harris *et al.* (2006) for example documented that ecological restoration should be one of the important responses to climate change as it positively influences the planet's carbon budget. The primary objective of ecological restoration is the re-initiation of natural succession that will lead to the re-establishment of ecosystem form and function (Montalvo *et al.*, 1997).

2.2 Restoration in practice

Clewell *et al.* (2005) define ecological restoration as a process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed. Ecological restoration intends to initiate and/or accelerate ecosystem recovery with respect to its health (functional processes), integrity (species composition and community structure), and sustainability (resistance to disturbance and resilience) (Clewell *et al.*, 2005). Most restoration attempts to return an ecosystem to its historic trajectory. Nevertheless, restoring originality may be impossible since the former dynamics may not be fully understood. Besides, the former disturbances may not function because of the existing environmental, political, economic and social dynamics, of which proactive restoration activities need to take into account (Clewell *et al.*, 1994). Because these factors vary in different areas, a wide spectrum of philosophies can be found among restoration practitioners.

2.2.1 Choice of a restoration strategy

Interventions employed in restoration vary widely among projects, depending on the extent and duration of past disturbances, cultural conditions that have shaped the landscape, and contemporary constraints and opportunities. As reported by Holl and Kappelle (1999), lack of propagules in tropical forests is the most important constraint to secondary forest succession. Therefore, most restoration programs should aim to provide adequate regeneration materials. According to Lamb (1998) various approaches have been developed and applied depending on

the level of ecological disturbance. Of all these, planting in gaps, has been deemed the most plausible approach for large-scale recovery programs. This is especially true in areas where there are aggressive weeds, a typical phenomenon in abandoned agricultural areas, and in wide forest gaps (Holl & Kappele, 1999). There is evidence from various studies that planting accelerates natural forest regeneration.

The regeneration materials can be novel to region, indigenous to the region but novel to the ecosystem or can be indigenous and local (Jones, 2001). Although novel species have been shown to considerably increase vegetation cover, it may not sustain the entire ecosystem (Holl and Kappelle, 1999). As such and due to their other advantages as documented by Holl and Kappelle, (1999); Montalvo and Ellstrand (2000); Bischoff *et al.* (2006); and Jones (2003) many restorations programs have given preference to indigenous species.

Given all the advantages, the introduction of novel and potentially maladapted genotypes to restoration sites is still a major genetic concern in restoration. As demonstrated by Montalvo and Ellstrand (2000) and Keller *et al.* (2000) demonstrated, use of non-local genotypes might reduce the success of restoration projects if they are maladapted and/or negatively affect adjacent native populations adapted to local conditions through gene pollution, out breeding depression and swamping.

2.3 Local adaptation

Local adaptation is where local genotypes express phenotypes that are optimum for current local condition (Kawecki and Ebert, 2004). As noted by Garcia de Leaniz *et al.* (2007), it is a process driven by natural selection, which leads to adaptive variation between populations. Consequently, plants with locally adapted genotypes may show a home-site advantage by growing better in their site of origin than plants from other sites (McGraw & Antonovics, 1983; Kawecki & Ebert,

2004; Bischoff *et al.*, 2006). This form of interaction - referred to as antagonistic pleitropy also implies that no single genotype is superior in all habitats. Therefore, when planning for a restoration, performance in new habitat must be assessed.

Although substantial evidence is available demonstrating adaptation and relative superiority in performance of the local provenances over the introduced ones (Joshi *et al.*, 2001; Linhart & Grant, 1996; Worrell, 1992), a study by Galloway and Fenster (2000), using *Chamaecristata fasciculata* found no evidence in support of local adaptation except at very large spatial scale of over 1000km. More important than showing no adaptation empirical data is also available to illustrate that some species can actually be maladapted to their local environments. This is cited as the explanation for high mortality and patchy distribution of surviving seedlings in a number of restoration programs despite all efforts devoted to maximizing plant quality (Vallejo *et al.*, 2005). Therefore, given the contradicting results, site and species specific assessment of adaptation is critical for FOREAIM project.

2.3.1 Adaptation Versus no adaptation

According to Bischoff *et al.* (2006) and Garcia de Leaniz *et al.* (2007), it is impossible to have no adaptation given the fact that the environment always varies in space and time. Since at a particular point, an organism will be more suited to specific environmental conditions, there is never a single phenotype that can outperform the others under all environmental conditions. Besides, better adaptation (as seen in invasive species) being only thought to be short term (perhaps a few generations), it is speculated that native populations possess fitness advantages that come into play only after long time- perhaps 50 or 100 years which constrains their performance in the short term. Thus, introduced species may be able to out-perform native species but only in the short term but only in the short term (Allendorf and Lundquist, 2003). A report by Bischoff *et al.* (2006) indicates that sometimes failure to detect local adaptation in

experimental studies results from insufficient simulation of the local environment and the inability to properly delineate a local species.

2.4 Delineating a Local Species

Defining a species that is local to a restoration site is a challenge since the range of many species is not known (Alleaume-Benharira *et al.*, 2006). As reported by Jones (2003), whether material is local or not is a matter of scale which varies from region, ecosystem to population level. A number of practitioners have however, defined local provenances in terms of proximity to the area to be restored. This is because adaptation to a specific site is expected to decrease with increasing geographical distance from the source population as a result of a concomitant increase in genetic isolation and environmental differentiation.

2.4.1 Gene flow and Genetic identity of forest ecosystems

Defined by Endler (1977), gene flow is the proportion of newly immigrant genes moving into a given population. It is the major determinant of genetic structure among populations of sexually reproducing plants. In plants, it occurs mainly by seeds and pollen, the latter being more common due to limited dispersal distances of the former (Oddou-Muratorio *et al.,* 2001; Levin *et al.*, 2003). In some wind-dispersed species though, for example *A. gummifera*, seeds can go up to several kilometres. As documented by Levin *et al.*, (2003), the distance of travel depends on seed characteristics and dispersal mode, which is an adaptation of the plant being dispersed. Similarly the dispersal of pollen grains will depend on the characteristics and the mode of dispersal. However, in general, more seeds or pollen grains are always dispersed within a shorter distance from the source than in a longer distance (leptokurtic distribution) (Levin *et al.*, 2003; Sato *et al.*, 2006). For most organisms the longer distances are usually incidental though a number of studies have demonstrated the significance of long distance gene flow inferred from the unexpectedly low differentiation in out crossing plant species. Patterns and levels of gene flow via pollen and

seed dispersal are one of the most critical determinants in the establishment of genetic structure (Sato *et al.*, 2006)

2.4.1.1 Implications of gene flow dynamics to restoration

The importance of gene flow for selecting restoration material is that populations of the given taxon neighbouring the target site have higher gene exchange and hence genetic identity than separate but related taxa. As documented by Hartl & Clark (1997), gene flow among populations that exceeds about four migrants per generation causes homogenization of neutral alleles among populations, effectively producing panmictic populations. Individuals from such populations are ideal for restoration. Though protected polymorphism in a heterogeneous environment may be maintained between the demes even if dispersal results in complete mixing of the gene pool, in such a case demes will not differentiate genetically and hence there will be no local adaptation (Griswold, 2006).

Wright (1931) demonstrated that species cohesion breaks down only when gene flow is reduced among populations to less than one migrant per generation thereby allowing differentiation. When gene flow is above one but less than four migrants per generation, populations may evolve at the 'major' genes while experiencing uniformity at neutral alleles. Therefore, there is need to ascertain the extent of gene flow among selected species and ecosystems since it varies between different species and ecosystems.

2.4.2 Environmental homogeneity and selection differential

Like genetic similarity, environment homogeneity decreases with increasing geographical distance from the source population. Environmental factors are important in that, even in presence of substantial gene flow (potential gene flow), realized gene flow may not occur where environmental selection is strong (Griswold, 2006; Garant *et al.,* 2007).

In restoration, the implication of this is that use of genetic similarity indices that rely on realized gene flow measures may not be appropriate in delineating which populations have genetic connectivity. This also means that use of geographic distance to deduce genetic similarity may not be factual in presence of other forces of selection. Actually, it is documented that though individuals may be separated geographically, they may tend to develop similar adaptive traits if they are located in similar ecosystems. Therefore, these may be better propagules than neighbouring populations with disparate conditions (Galloway and Fenster, 2000).

2.5 Within-population gene dynamics in degraded forest ecosystems

In degraded areas, a shift in pollinator species composition (Waser *et al.,* 1996; Yates *et al*., 2007) might still be accompanied by changes in patterns of pollen flow for animal pollinated species. This is especially true if the behaviour and pollination efficiency of the new pollinator differ from those of the original species. The consequences of this, however, are a multitude. Inbreeding, allee effects and genetic drift, with a consequent reduction overall in heterozygosity are of great significance in restoration (Hartl and Clark , 1989).

2.5.1 Implications of Genetic drift and Inbreeding to Restoration

Inbreeding, allee effects and genetic drift are phenomenons that are likely to affect small populations in degraded areas, following the models given by Hartl and Clark (1989). The dangers of using inbred germplasm in restoration programs are related to increased susceptibility to demographic and environmental stochasticity (Yates *et al*., 2007). However, the impact of population size reduction will only be apparent where degradation negatively impacts on gene flow. When gene flow occurs, more individuals contribute their genes to the next generation, maintaining genetic diversity and counteracting the negative fitness consequences of small population size and isolation.

The effect of degradation on gene flow has been deemed to differ in species depending on life history traits such as breeding system, dispersal syndrome and longevity of the species concerned (Hobbs & Yate, 2003; Costin *et al.,* 2001; Dick, 2003; Hobbs and Yates, 2003; Yates *et al*., 2007). Although, lack of gene flow between populations may be an incentive for conservation purposes (Alleaume-Benharira *et al*., 2006), there is strong empirical evidence which suggests that genetically diverse populations would be preferred for restoration because of the importance genetic diversity confers on organisms.

2.6 Importance of genetic diversity to forest restoration

Among and within population genetic diversity is required to ensure ecosystem resilience and adaptability (Montalvo and Ellstrand, 2001). Empirical evidence and genetic theory suggest that genetic diversity plays an important role in the survival of living organisms especially in surges of rapid changes. A report by Frankel & Soul´e (1981) indicates that genetic variation is the raw material for evolutionary change since it allows populations to evolve in response to environmental change, whether that be new/changed diseases, parasites, predators and competitors, or greenhouse warming, ozone layer depletions, or other results of pollution. Pre-existing genetic variation is also critical for short-term evolutionary change.

In addition, several authors (Hamrick *et al.,* 1991; Mckay *et al*., 2005 and Carnus *et al*., 2006) have also reported that genetic diversity is important in performance of organisms since genotypes partly determine organisms' physical form and function. Genes regulate size, shape, physiological processes, behavioral traits, reproductive characteristics, tolerance of environmental extremes, dispersal and colonizing ability, the timing of seasonal and annual cycles and disease resistance, among others.

2.7 Categories of Restoration Gene Pools

Populations from the target site plus those from adjacent areas that are genetically connected to the site via gene flow (metapopulations) are referred to, according to Jones (2003) as the primary restoration gene pool (RGP). Jones (2003) also suggested that the Primary RGP material would be the most appropriate material for restoration, if it is available or when the ecological function of the target site has not been fundamentally altered in a manner that makes such material no longer adapted.

The populations that are genetically disconnected from the target site are referred to as secondary restoration gene pools (Jones, 2003). Jones suggested that these should be the next alternative where the primary gene pool is lacking or where the target area is disturbed. Though its use may be associated with some risk, Jones (2003) proposed that the attached risk may be low in species whose genetic distribution follows a normal distribution curve. Transplant studies which especially take into account environmental variation can help predict the risk of failure in the field.

2.8 Micro-scale variation

Besides selection of the best local species for restoration, there is growing appreciation of the importance of the effects of micro scale variation on recruitment and spatial structure of the populations. Ecosystems are very heterogeneous in biotic and abiotic terms. However, functional heterogeneity (variability of a system property affecting ecological processes) is only a fraction of the available structural heterogeneity (variability of a property measured without reference to ecological effects) (Gomez *et al.*, 2004; Valladares and Gianoli, 2007).

Nevertheless, in view of the fact that the most limiting factor for growth in most tropical environments is the limited availability of soil nutrients, mycorrhiza should be very important in

establishment and survival of plants. Actually, mycorrhizas are an important soil factor that may influence both abiotic and other biotic properties of an ecosystem (Onguene & Kuyper, 2002) and hence plant and ecosystem productivity. As documented by (Palmer *et al.*, 1997), restoration of function of a system may require restoration of key linkages related to food web structure that is a number of trophic levels and their connectence or taxa critical to material processing such as decomposers. Due to this, fungi are highly envisaged as critical in a successful restoration.

2.8.1 Effect of mycorrhiza on plant performance and adaptation

According to Klironomos *et al.* (2000), arbuscular mycorrhiza form intimate relationships with around 85% of the terrestrial plants. Although mineral nutrient accumulation and acquisition from soil is considered to be the primary function of mycorrhizas, other roles for these fungi have been suggested. These include; provision of protection from parasitic fungi and nematodes and suppression of competing non-host plants, non-nutritional benefits to plants due to changes in water relations, phytohormone levels, carbon assimilation and growth form changes to root architecture and vascular tissue (Azcon-Aguilar *et al.*, 2003; Ingleby *et al.,* 2007).

As reported by Azcon-Aguilar *et al.* (2003), Klironomos *et al.* (2000) and Robertson *et al.* (2007), the importance of arbscular mycorrhiza in ecosystems translates into reduction in competition between plants and preventing nutrient losses from the system, which in turn contribute to the stability and diversity of ecosystems. Besides, there is increased reproductive success as networks of hyphae supported by dominant trees may help seedlings become established or contribute to the growth of shaded under storey plants (Chen *et al.,* 2005). Mycorrhizal roots and fungal fruiting bodies are also important as food sources and habitats for other micro and macro fauna, which are also important in ecosystems. Ecto-hyphae are important in maintaining soil physical and biological properties.

Lastly and of great value to restoration is that hyphae may transport carbon from plant roots to other organisms involved in the ecosystem cooperating with other members of the decomposition soil food-web (Chen *et al.*, 2005; Ehlers and Thompson, 2004). It is because of this function that the presumption that some mycorrhizal plants can act as nurse plants or keystone species can hold. Functional relationships associated with nurse plants is an area in many ecosystems that has not been fully explored though a few studies have demonstrated co-adaptation of some species (Ehlers and Thompson, 2004). Given these functions, numerous researchers have suggested a relationship between the recovery time of disturbed ecosystems and the abundance of infective propagules of mycorrhizal fungi.

Various studies have also demonstrated the importance of mycorrhiza in plant performance although the degree of dependency varies within species. Whereas some plants have obligatory mycorrhizal associations, others can have facultative, or no mycorrhizae at all. In natural ecosystems, plants with facultative mycorrhizal associations or non-mycorrhizal roots are more common in very dry, wet or cold habitats where plant productivity is limited by soil/environmental conditions, or in disturbed habitats where mycorrhizal fungus inoculum is limited (Brundrett, 1991). Non-mycorrhizal trees are however, rare.

Though most of them are thought to be ubiquitous (Menoyo, 2007; Chen *et al.,* 2005) and non specific in infestation, the response of plants to mycorrhiza in a given soil or vice versa may vary depending on soil and climatic characteristics and mycorrhizal status of the dominant species (Klironomos *et al.,* 2000; Menoyo *et al.,* 2007). For example, Baer (2004) demonstrated that chronic disturbance can alter availability and/or spatial distribution of mycorrhizas, which can in turn strongly influence vegetation pattern, community structure, and diversity in terrestrial ecosystems.

It is also being speculated that mycorrhiza from a particular place or host may not perform well in new places or on a new host due to adaptation even though the converse may also be true. For instance Klironomos *et al.* (2002) indicated that within a given habitat, new plants are usually associated with positive microbial feedback interactions, while natives displayed a more stable negative relationship with soil flora and fauna. In addition Klironomos *et al.* (2000) documented that these fungal communities can comprise of genotypes that are mutualists as well as cheaters (parasites). However, like for many fungi, their position along the parasitism-mutualism continuum will depend on the plant symbiont as well as edaphic factors. Brundrett *et al.* (1996) supposed that mycorrhiza can probably be (i) a single generalist phenotype showing a similar degree of adaptation to all habitats; (ii) a single specialist phenotype optimally adapted to one habitat (usually the habitat that is most frequently encountered or of highest quality) and poorly adapted to other habitats; and (iii) a set of specialist phenotypes each maximizing fitness in one habitat type. Local adaptation requires an outcome close to (iii).

In general terms, relationships between genetic diversity, habitat heterogeneity and the scale of adaptation in trees are complex, involving a variety of factors. One other unresolved issue involves ascertaining whether all the variation observed in organisms is adaptive or not. As Garcia de Leanize *et al.* (2007) documented, not all genetic variation is adaptive, and yet not all phenotypic variation is inherited. Therefore, there is need to distinguish between adaptive and non- adaptive variation.

2.9 Adaptive versus non-adaptive variation

There are three strategies that organisms may use to exploit new areas. These are genetic differentiation, phenotypic plasticity (the ability of an organism with a given genotype to change its phenotype in response to changes in the environment) and genetic polymorphism (existence of two or more forms of individuals in the same species (Dybdahl and Kane, 2005; Valladares

and Gianoli, 2007). If two genotypes express the same phenotype in the same environment, phenotypic differences observed under natural condition result from phenotypic plasticity. Conversely, if phenotypic differences between genotypes are maintained across different environments, the variability comes from a genetic polymorphism. Whereas the genetic differentiation is adaptive, plasticity and polymorphism may or may not have a genetic component. It is thus important to differentiate whether the observed variations have a genetic basis in order to guarantee survival over generations.

CHAPTER THREE

METHODS

3.1 Study Sites: Location and Biophysical Conditions

3.1.1 Mabira Forest Reserve, Uganda

Mabira forest is located in South Central Uganda between 0° 22' - 0° 35'N and 32°56' - 33° 02'E. The forest covers an area of 30,600 ha (Nature Uganda, 2006). It is a mid elevation forest located between 1070 and 1340 m above sea level, occupying gently undulating plains with numerous flat-topped hills and wide shallow valleys. The reserve is isolated from other protected areas by agricultural land (Natureuganda, 2006). Within the forest, there are 27 official village enclaves inhabited by local people involved in subsistence farming.

The degradation history is such that they are areas that have been very recently (0-20yrs) encroached although some were encroached on in the 1950s – 1980s. There is also an undisturbed nature reserve. The climate is tropical with two peaks of rainfall from March to May and September to November with mean annual rainfall of 1200-1500 mm, and temperatures that rarely exceed 28^0C (Komutunga and Musiitwa, 2001). About 95% of the area is covered by medium altitude moist semi-deciduous forest (Langdale-Brown, 1960). The remaining portion is occupied with medium altitude moist evergreen forest. However, the forest has been greatly influenced by human activities making some areas characteristic of sub climaxes. The three recognized sub climaxes are: colonizing forest, mature mixed forest and *Celtis* mixed forest.

3.1.2 Kedowa Forest, Kenya

Kedowa is located on the South Western part of Mau Forest Complex which is located between 0°30' South and 35°20' East and in the Rift Valley Province of Kenya. The rainfall pattern at the western flanks is governed by the moist monsoon winds from the Indian Ocean and dry winds

from the Great Rift Valley. Mean annual rainfall varies from 1,000 to 1,500 mm with peaks in April and August. The western flanks are under the influence of the Lake Victoria macroclimatic region and are generally wetter. Rainfall is above 2,000 mm and more evenly distributed (www.ifrikenya.org/kedowa.htm.).

The forest being a reservoir of unique biological diversity plays a significant role in water catchment, feeding the major rivers and streams that make up the hydrological systems of Lake Victoria. The complex has been under pressure by adjacent communities and immigrants from other areas. The settlers are clearing land for cultivation removing remnants of the forests and opening up new forest areas through non-residential cultivation. Although the forest is legally registered as a forest reserve where human activities are controlled through enactment of rules and regulations, there have been cases of illegal encroachment and harvesting of various forest products such as timber, poles, posts, charcoal, grass, building stones among others. There is also overgrazing and excision all of which have led to a notable decrease in forest cover (www.ifrikenya.org/kedowa.htm.)

3.1.3 Madagascar: Vohimana Site

Vohimana Forest Reserve is representative of the middle altitude evergreen forests in eastern Madagascar. It is located between longitudes 48°30'22" E and 48°31'22" E and latitudes 18°55'12" S and 18°56'24" S, at an altitude of between 705 and 936 m. The annual rainfall is between 1,500 and 2,500 mm, with the possibility of an ecologically dry season in September/October (Brand, 1997). Vohimana forest is an important rain forest in eastern Madagascar. It is endowed with globally unique amphibians and orchids, medicinal plants and endemic birds in the region. It also has the biggest and the most beautiful lemurs of the country, in particular 'Indri' and 'Syfaka à diadème'. The forest is, however, extremely threatened by

anthropological pressures although it represents the last key link of the corridor of the park of Mantadia towards forests more to the South.

3.2 Assessing genetic structure of *A. gummifera*

3.2.1 Sampling design

Genetic structure of *A. gummifera* and the status associated mycorrhiza status of the three sites were compared. Within each site a minimum of 30 mature *A. gummifera* individuals separated by at least 100 meters were sampled from each population. The 30 individuals were required for the mean to fall in the 95% confidence interval, for variance stabilization and for inclusion of rare alleles (Sokal and Rohlf, 1995). The individual tree locations were geo-referenced with a Global Positioning System (GPS) for easy monitoring. Leaves were collected from all individuals and seeds were taken from at least five trees. Leaves were dried on silica gel prior to DNA extraction.

3.2.2 Selection of markers

Chloroplast markers were used because of being haploid and are considered good indicators of mutations and other historical bottlenecks, founder effects and genetic drift. Chloroplasts evolve slowly and exhibit little variation at the intraspecific level (Clegg *et al.*, 1991). The chloroplast DNA (cpDNA) markers have also been widely favored for phylogenetic inference (Olmstead *et al.,* 1992) and to some extent, for within-species genetic studies. Since chloroplast DNA is also maternally inherited, therefore reflecting seed dispersal and maternal gene flow, it is an effective tool for genetic variation studies and for identifying postglacial migration routes (McCauley, 1994). In addition, the geographically structured cpDNA variations permit the elucidation of evolutionary history and the study of intraspecific phylogeography (Soltis *et al.*, 1997). Therefore, in relation to the study, cpDNA had the ability to provide evidence for whether the populations under study have evolved as a single population and whether they can be treated as a single population in restoration.

3.2.3 RFLP analysis

DNA was extracted using a DNeasy plant kit (QIAGEN). For amplification of chloroplast RFLP regions, an initial screening of more than 20 RFLP primers was done. Although some failed to amplify through all the populations, a number of them (Table 1) gave good amplification (Fig 1) and hence were screened. However, only RPS/RPL, CCMP5R/CCMP5F and TFC/TGF showed variation.

Table 1: cpDNA primers that were used in initial screening

Primer	Sequence 5'-3'		Reference
RPS	GTCGAGGAACATGTACTAGG	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
RPL	TTTGTTCTACGTCTCCGAGC	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
CCMP5R	TTTGTTCTACGTCTCCGAGC	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
CCMP5F	TGTTCCAATATCTTCTTGTCATTT	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
TRNVR	CCGAGAAGGTCTACGGTTCG	Forward	Demesure *et al.* (1995) molecular ecology 4 129-131
TRNF	CTCGTGTCACCAGTTCAAAT	Reverse	Demesure *et al.* (1995) molecular ecology 4 129-131
TFC	CGAAATCGGTAGACGCTACG	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
TGF	GGGGATAGAGGGACTTGAAC	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
PSBCE	GGTTCGAATCCCTCTCTCTC	Forward	Demesure *et al.* (1995) molecular ecology 4 129-131
TRNSE2	GGTCGTGACCAAGAAACCAC	Reverse	Demesure *et al.* (1995) molecular ecology 4 129-131
PSBF	CGCAGTTCGTCTTGGACCAG	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
PSBB	GTTTACTTTTGGGCATGCTTCG	reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
CCMP10R	TTCGTCGDCGTAGTAAATAG	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
CCMP10F	TTTTTTTTTAGTGAACGTGTC	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
PSBA	CGAAGCTCCATCTACAAATGG	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
TRNH	ACTGCCTTGATCCACTTGCC	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
TRNS	GCCGCTTTAGTCCACTCAGC	Forward	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525
TRNG	GAACGAATCACACTTTACAC	Reverse	Hamilton, M.B.(1999) Molecular ecology, 8, 513-525

Reactions were carried out in 25 µL of solution consisting of 5µl of template DNA, 1.6 µL of dNTP (Promega), 0.4 µL of each primer (Thermo), 2µL of buffer and 0.2µL of *Taq* polymerase (New England Biolabs). Polymerase chain reaction was performed using MBS satellite 0.2G thermal cycler with the following programme. An initial 3-minute denaturation at 94°C was followed by 40 cycles of 94°C for 1 min and 1 min of annealing at 57°C and extension at 72°C for 10 min. 5µL of the PCR product were then checked on agarose for successful amplification (Figure 1). With successful amplification, 5µL of each sample were digested at 37°C for 6hrs with 0.1µL H*inf* I in a 20µL volume with 2µL of the corresponding buffer. The digest was then visualised by Polyacrylamide Gel Electrophoresis (Figure 2). Fragments were scored by comparison to the standards.

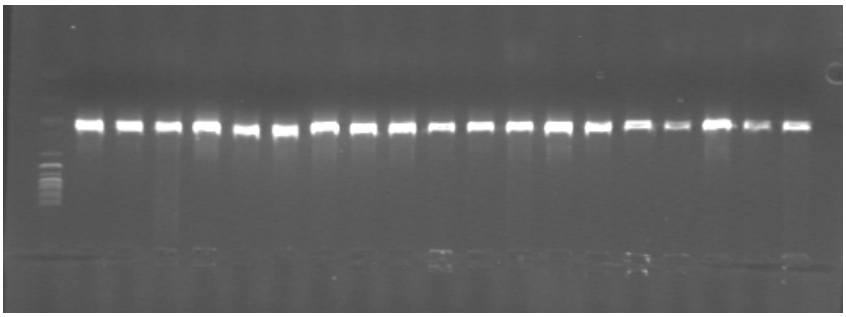

Figure 1: Typical agarose gel produced

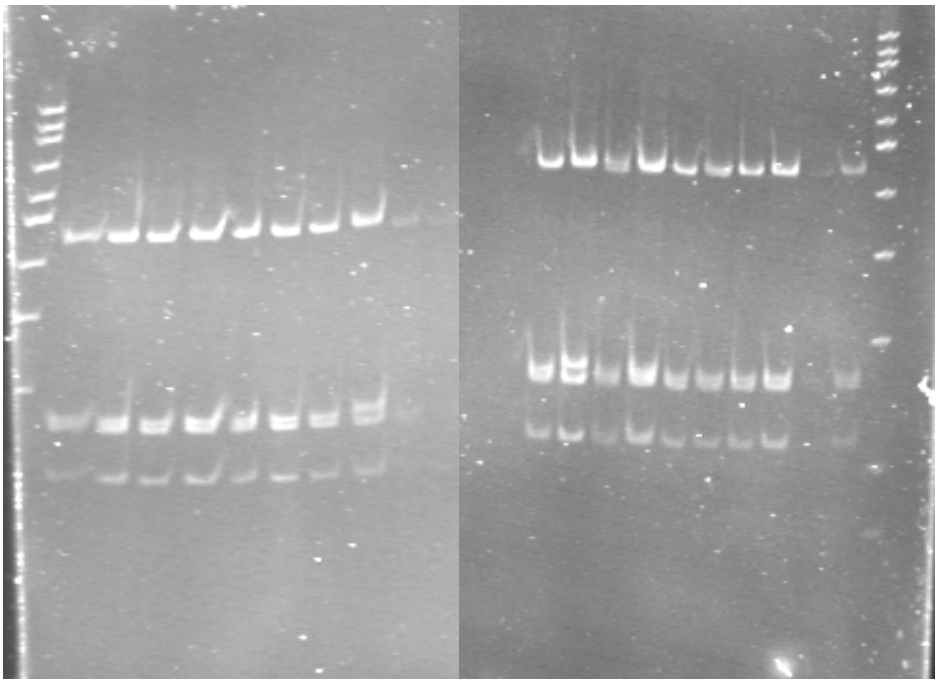

Figure 2: A mutation produced with RPS primer on PAGE gel

3.3 Assessing the abundance and diversity of Mycorrhiza

3.3.1 Collection of soil samples

New and old soils were collected near the trees selected for seed collection. Four sub samples were taken from the north, east, west and southern directions of the base of the tree, not more than 2 m away. These were mixed to obtain a composite sample from which 2 kg were taken for spore analysis and for inoculating the experiment. Five samples were taken from the five trees which were not less than 200 m from one another.

3.3.2 Spore Extraction and Identification

Fungal spores of arbuscular mycorrhiza were extracted from soil by wet sieving and decanting, followed by sucrose centrifugation following the methodology proposed by Gerdemann and Nicolson (1963) and Walker *et al.* (1982) respectively. The supernatant was poured through a 50 µm mesh and quickly rinsed with tap water. Spores were counted using a Doncaster dish under the dissecting microscope, and grouped according to morphological characteristics. Counting was done twice for every sample to eliminate error. Permanent slides were prepared for each different spore morphotype using polyvinyl alcohol and polyvinyl alcohol plus Melzer's solution (1:1). After confirming the uniformity of the morphological groups under the optical microscope, the different morphotypes were identified to genus and where possible to species levels. Spore identification was mainly based on spore size and color, wall structure, presence or absence of germination shield and hyphal attachment. These observations were corroborated with observations of freshly formed AMF spores in trap cultures.

3.4 Dependence of *A. gummifera* on Mycorrhiza

3.4.1 Green house experimental design

Figure 3: Experimental set up

1) Treatments

a) Seeds

Two seed lots both from Kenya. One collected from Kedowa, (Londiani) in February 2006 and the other from Tavete (Batch 05100905) in June 2006. A total 250 seeds of each seed lot were scarified on sandpaper to enhance germination before sowing in the pots.

b) Inocula

Five different soil inocula were used. The soils were categorized on basis of origin and time of collection. In March 2007, soils were collected from Kenya, Uganda and Madagascar and stored at 4 ^{o}C. In September 2007, fresh soil was collected from the same sites in Kenya and Uganda. New soils were collected not more than two weeks before the experiment was set up, whereas the old soils had been in cold storage for at least six months. The sixth soil treatment was a control treatment which comprised an autoclaved (1 hour at 121 ^{o}C) mixture of the 5 different soil inocula. Each of the 12 treatments (6 soil inocula x 2 seed lots) was replicated eight times.

2. Set up

a) Preparation of pots

Pots were two-thirds filled with a mixture of sand, sterilized loam and gravel mixed in a ratio of 1:1:1. This was followed by approximately 50 g of the soil inoculum, which was then covered with more soil mixture. Control pots (with autoclaved inoculum) were prepared first. This was followed by thorough cleaning and disinfection of hands and all used equipment prior to setting up of each soil inocula to prevent cross-contamination of treatments. Five seeds per pot were then sown so that they were roughly equidistant from one another. Thereafter they were covered with more soil mixture. The pots were then watered immediately.

b) Lay-out: randomization and blocking

A randomized block design was used. Random numbers dictated the position of the block and the position of each pot within the blocks. Each treatment was represented once in each block. Blocking was used to account for some perceived extraneous factors like aspect and edge effect.

c) Glasshouse growing conditions

Glasshouse temperature was maintained at a maximum of 28^{o}C (day) and a minimum of 20^{O}C (night). Irradiance was maintained to a minimum of 14 h. Natural light was supplemented with high pressure mercury vapor lamps of similar wavelength. Watering of the plants was done whenever need was perceived.

3.5 Data collection

3.5.1 Germination

Assessment of germination commenced a week after sowing since more than 50% of the seeds had germinated. A second count was done the following week. Pots without germinated seedlings were planted with spare seedlings transplanted from other pots of the same treatment. After thinning, each pot had one seedling. A spare pot of each treatment was also set up and spare seedlings were transplanted to this pot during the thinning process.

3.5.2 Height and diameter measurement

Height measurements commenced a week after thinning. Weekly measurements were made. The heights were measured from soil level to tip of growing bud. To avoid cross-contamination, the ruler was thoroughly cleaned and disinfected after measuring seedlings from each treatment. Height measurements were done for the first six weeks when plants were still fragile and later weekly consecutive diameter measurements were taken at 1M from radical.

3.6 Data analysis

3.6.1 Genetic diversity and structure

The mutations were identified by presence or absence of an allele at the locus of concern. The following genetic parameters were computed for each population using GENALEX: number of haplotypes, Nei's unbiased haplotypic diversity (he), genetic distance and identity, intrapopulation diversity (HS), total diversity (HT) and PhipT (analogous of Fst fixation index) (Nei, 1973; Excoffier *et al.*, 1992). Gene flow among the populations was estimated on the basis of PhiPT values

3.6.2 Mycorrhiza abundance and diversity

Species richness, spore density and relative abundance of each fungal species in the rhizosphere of each soil were calculated. Mycorrhizal fungal diversity was then calculated by using the Shannon–Wiener index, which combines two components of diversity: species richness and evenness of individuals among the species and Simpson's diversity index (Magurran, 1988).

3.6.3 Heights and Diameters

The mean height and diameters of the seedlings, intervallic increment, correlations and the variances were analysed using GENSTAT. Whereas the heights were treated as absolute values, the increments were square root transformed to achieve normal distribution. Normality was checked using various tests namely Anderson-Darling, Cramer-Von Mises and Watson. Bartlett's test was used to check for homogeneity of variances. Graphs were generated using SIGMAplot. By comparing the seedlings that either were or not exposed to mycorrhizal fungi, the experiments permitted the assessment of the relative dependence on mycorrhizas for survival and growth in the different species. Correlations were assesses using MI NITAB.

CHAPTER FOUR

RESULTS

4.1 Genetic structure of *A. gummifera*

4.1.1 Genetic variation within and between the populations

A total of nine mutations at three loci were detected which allowed the identification of fourteen haplotypes among the three populations (Table 2).

Table 2: Haplotypes identified by PCR-RFLP analysis of the chloroplast genome

Haplotype Code	5R/5F – *HinfI*			RPL - *HinfI*		TFC – *HinfI*			
	1	**2**	**3**	**1**	**2**	**1**	**2**	**3**	**4**
H1	1	0	0	1	0	1	0	0	0
H2	0	1	0	1	0	1	0	0	0
H3	0	0	1	1	0	1	0	0	0
H4	0	1	0	0	1	0	1	0	0
H5	0	0	1	0	1	0	1	0	0
H6	0	0	1	1	0	1	0	0	1
H7	0	0	1	1	0	0	1	0	0
H8	0	0	1	0	1	1	0	0	1
H9	0	0	1	1	0	1	0	1	0
H10	0	1	0	1	0	1	0	1	0
H11	0	1	0	0	1	1	0	0	0
H12	0	1	0	1	0	1	0	0	1
H13	0	1	0	1	0	0	1	0	0
H14	0	1	0	1	0	0	1	0	1

'Code' refers to haplotypes, 5R/5F, RPL, TFC are the chloroplast sequences examined and 1,2,3,4 refer to the character states of different mutations, where 0,1 indicates presence or absence of the fragment.

Whereas Kenyan samples have only five of the haplotypes, Ugandan samples have nine haplotypes while Madagascan populations have six of the haplotypes (Figure 4). In a similar pattern, Ugandan samples have the highest number of private haplotypes (5), followed by Madagascar (3) and then Kenya (1).

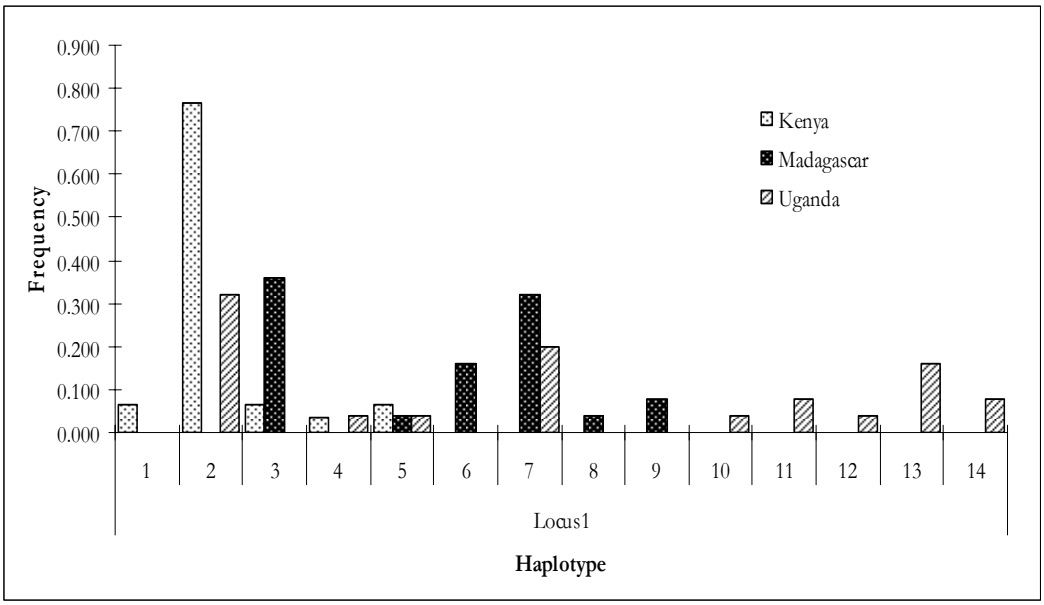

Figure 4: Frequencies of haplotypes in Kenyan, Ugandan and Madagascan populations

All the three sites share only one haplotype (haplotype 5). Kenyan and Ugandan populations share the highest number of haplotypes between each other (Figure 5). While 60% of the haplotypes in Kenya are found in Uganda, Kenya and Uganda share only 40% and 22% of their diversity with Madagascar

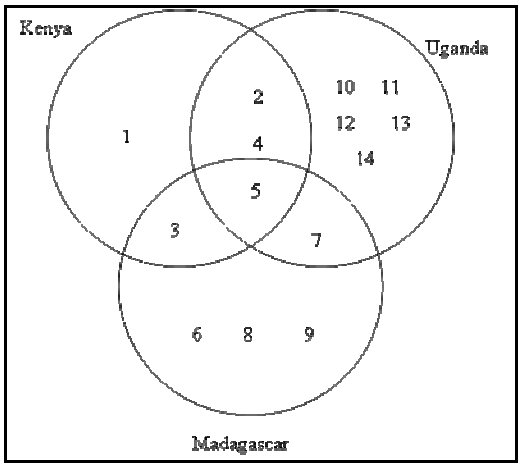

Figure 5: Distribution of haplotypes in the study sites

Overall, analysis of molecular variance (AMOVA), indicated that the majority of diversity was partitioned within populations (75%) with the remainder (25%) partitioned between populations.

4.1.2 Genetic diversity

Total genetic diversity in the dataset was high, h_{TOT} = 0.886 with a range of within-population diversity levels. The Ugandan population was more genetically diverse (h= 0.813) than the populations from Madagascar (h=0.733) and Kenya (h=0.398). Across all measures of diversity (Number of alleles (Na), effective number of alleles (Ne) and number of private alleles), the distribution of haplotypes was such that diversity consistently decreased from Uganda to Madagascar, then to Kenya (Figure 6).

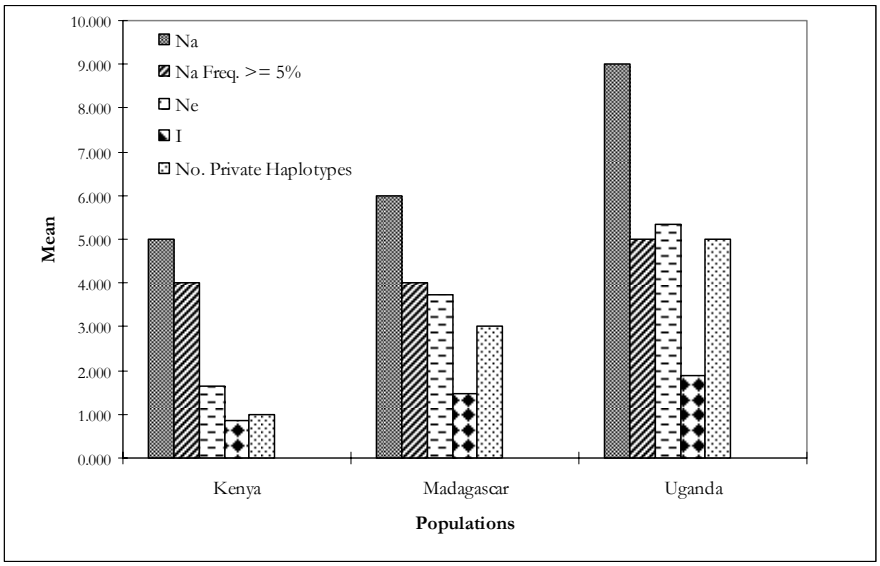

Figure 6: Mean Haplotypic Patterns Across Populations.
Na represents the total number of alleles, Ne is the effective number of alleles

4.1.3 Genetic divergence among populations

The higher within population variation (75%) compared to the between populations variation (25%) was reflected in the total fixation index estimation, (Φ_{ST} = 0.249, p > 0.01). Nei's genetic distance showed the strongest differentiation to be between Kenyan and Madagascan populations (d= 2.711), followed by that between Ugandan and Madagascan populations (1.227). Uganda and Kenya had the least genetic distance (0.298). The fixation index between Kenyan and Madagascan populations was highest (0.404) (Table 3).

Table 3: Population variability and estimate of gene flow (Nem) based on in φST value

Source of variation	d.f.	SS	Percentage of total variation	p-value	φST	NeM
Among populations	2	6.451	25	<0.01	0.249	1.506
Within Populations	77	25.287	75	<0.01	-	-
Total	80	31.738	-	-	-	-

Table 4: Between-populations gene flow

Populations	PhiPT values	Gene flow
Uganda Vs. Madagascar	0.138	3.123
Uganda Vs. Kenya	0.168	2.976
Kenya Vs. Madagascar	0.404	0.747

The relationship between geographical distance and genetic similarity is as illustrated in the map below (Figure 7). The pie charts represent the total size proportional to population sample size with partitions showing numbers of different haplotypes within each population. The similarity between Ugandan and Kenyan populations is evident, with common dominance of the red haplotype and several other shared haplotypes. The reduced diversity in the Kenyan population relative to that in the Ugandan and Madagascan populations is also clear.

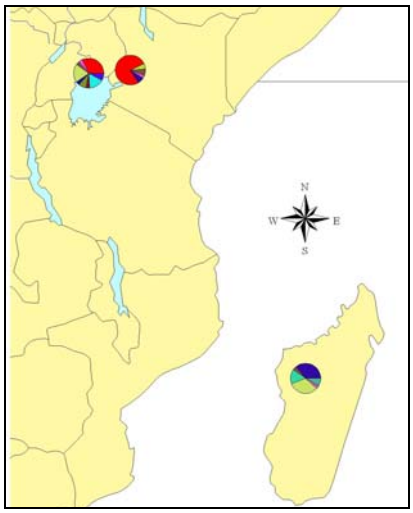

Figure 7: Geographical distance between the sites and haplotypes distribution.
Each color represents a different haplotype

4.2 Mycorrhizal diversity

4.2.1 Spore status in both new and old soils

Typical spores were clearly observed under the dissecting microscope and were therefore easy to enumerate. Storage had a significant effect on the status of the spores. In the Ugandan samples, the new soils had 91% more spores than the old soils, yet the Kenyan new soils had 73% more spores than the old ones. Observing these spores under compound microscopes further reduced the overall effective spore samples since some of these had lost the basic parts critical for identification. There were many cases of spore parasitism especially in the Ugandan new soils. Of the total spores in the old Ugandan soils, only 58% of them were clearly identifiable (it was the proportion of live spores that had all the features needed for proper identification). In the new Ugandan samples 46%was identifiable compared to 56% in old Kenya samples, 92% of spores from new Kenyan samples and 49% of spores from Madagascan soil (Figure 8).

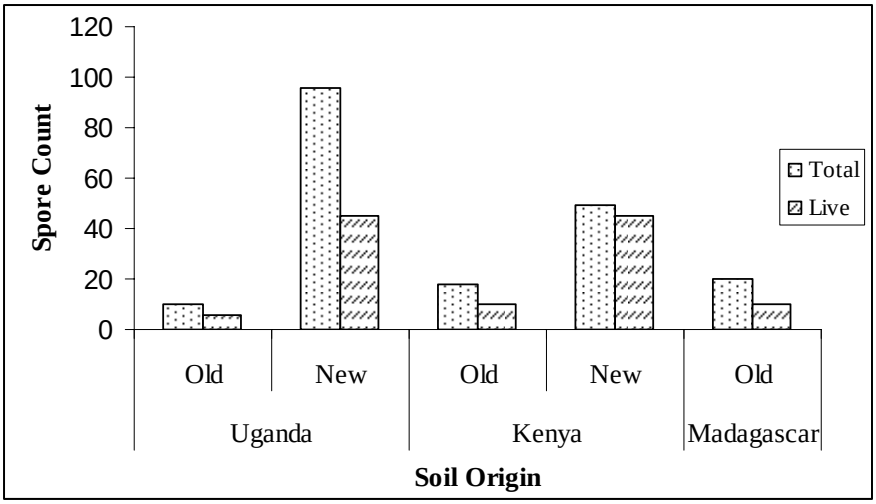

Figure 8: Spore counting in new and old soils

4.2.2 Species abundance and diversity

A total of 22 species (Table 2), representing five genera (Table 3) of arbuscular mycorrhiza were isolated and identified. Three other species from genus *Acaulospora, Glomus* and *Gigaspora* could not be well identified.

Table 5: Species abundance and distribution in the different soil samples

Site	Uganda				Kenya				Madagascar		Total isolates
Collection	Old		New		Old		New			Old	
Replicate	i	ii	i	Ii	i	ii	i	ii	i	ii	
Acaulospora denticulate	0	0	0	0	0	0	0	0	1	0	1
Acaulospora koskei	0	0	0	0	3	0	0	0	0	0	3
Acaulospora mellea	1	4	31	7	0	0	42	15	0	0	100
Acaulospora morrowiae	3	2	0	0	3	2	0	0	0	0	10
Acaulospora paulinae	0	0	6	0	0	0	3	0	0	0	9
Acaulospora scrobiculata	0	0	2	0	0	0	5	0	3	0	10
Acaulospora sp.	0	0	0	0	0	0	0	0	0	4	4
Acaulospora spinosa	0	0	2	2	0	0	0	0	0	0	4
Entrophospora infrequens	0	0	0	5	0	0	0	0	0	0	5
E-strain chlamydospore	0	0	0	5	0	0	0	4	0	0	9
Gigaspora albida	0	0	0	0	0	1	0	0	0	0	1
Gigaspora gigantean	0	0	3	1	1	0	3	1	0	0	9
Gigaspora sp	0	1	0	0	0	0	0	0	0	0	9
Glomus aggregatum	0	0	8	3	0	0	6	0	0	0	17
Glomus claroideum	0	0	0	0	0	3	0	4	0	0	7
Glomus constrictum	0	0	0	5	0	0	0	5	0	0	10
Glomus etunicatum	0	0	0	0	1	1	0	0	0	0	2
Glomus fasciculatum	0	0	0	0	0	0	0	0	2	2	4
Glomus sinuosum	0	0	0	0	0	0	0	1	0	0	1
Glomus sp.	0	0	0	6	0	0	0	0	0	3	9
Scutellospora calospora	0	0	0	0	0	4	0	0	2	0	6
Scutellospora pellucida	0	0	0	3	1	0	0	0	0	0	4
Total Identified spores	4	7	52	37	9	11	59	30	8	11	
Unidentified	5	3	37	66	5	11	0	8	3	17	

Among the five soil samples new and old Ugandan soils showed the highest (1.67) and lowest (0.76) species diversity respectively and similarly species richness (Table 6). In terms of evenness, Madagascar population scored higher than the rest with a value of 0.96, and least was new soil from Kenya with 0.7.

Table 6: Shannon-Weiner's diversity, abundance, richness and spore density estimates

Site		Shannon-Weiner's Diversity Index	Species Richness (S)	Total Abundance	D:	1-D:	1/D:	Evenness	Spore density
Uganda	Old	0.56	2.00	4.00	0.63	0.38	1.60	0.81	
	Old	0.96	3.00	7.00	0.43	0.57	2.33	0.87	3.8
	New	1.26	6.00	52.00	0.40	0.60	2.51	0.70	
	New	2.08	9.00	37.00	0.13	0.87	7.48	0.95	38.4
Kenya	Old	1.46	5.00	9.00	0.26	0.74	3.86	0.91	
	Old	1.47	5.00	11.00	0.26	0.74	3.90	0.91	7.2
	New	0.99	5.00	59.00	0.53	0.47	1.89	0.61	
	New	1.41	6.00	30.00	0.32	0.68	3.17	0.79	19.4
Madagascar	Old	1.32	4.00	8.00	0.28	0.72	3.56	0.95	
	Old	1.34	4.00	11.00	0.27	0.73	3.67	0.97	7.8

4.2.4 Representation of Genera

Arbuscular mycorrhizas are known to have seven genera, but of these five were represented in all the three sites (Table 7).

Table 7: Genera of the identified Species

Genus	Species
Acaulospora	*Acaulospora morrowiae, A. mellea, A.paulinae, A.scribiculata, A. spinosa,A. Koskei, A. denticulata, A. spp*
Glomus	*Glomus aggregatum, G.spp, G. constricum, G.. etunicatum, G. claroidem, G. sinuosum, G.fascitulatum,*
Gigaspora	*Gigaspora sp, G. gigantea, G. Albida*
Scutellospora	*Scutellospora pellucida, S. calospora*
Entrophospora	*E. strain chlamydospore, E. infrequescence*

The five genera that were represented were *Acaulospora, Glomus, Gigaspora, Scutellospora* and *Entrophosphora*. These were represented as follows

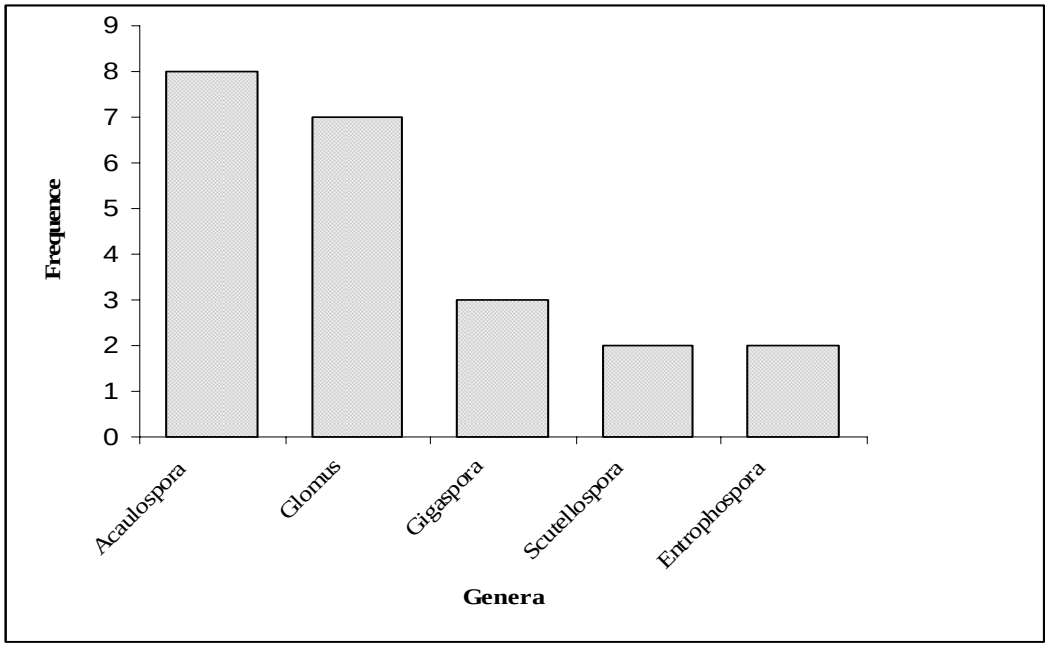

Figure 9: Representation of fungal genera in all the samples

Whereas all the five genera were represented in Uganda and Kenya, Madagascan soil represented only three genera (Figure 10)

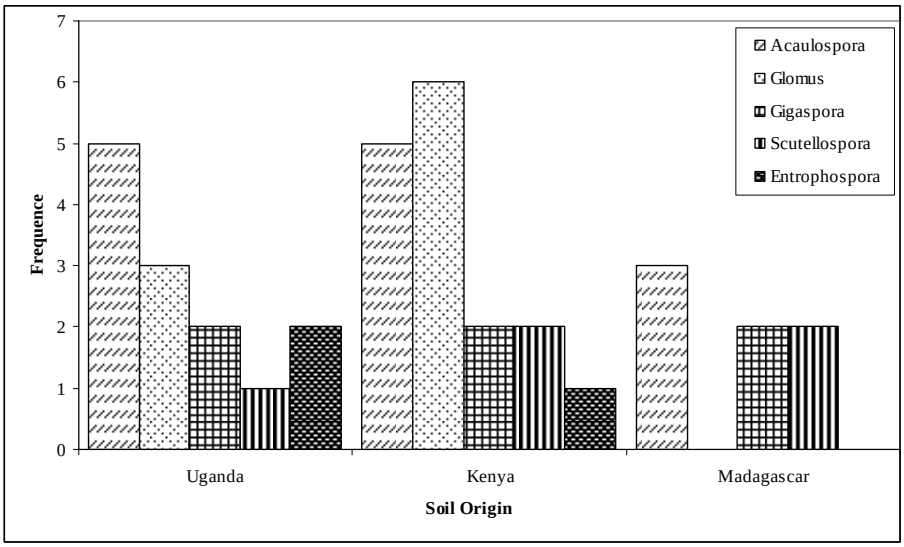

Figure 10: Representation of genera per soil sample

4.3 Dependency of host species on AM

4.3.1 Results of height measurement

The inocula and seed had significant effects on the heights of seedlings ($0.001 \leq p \leq 0.002$) over the first five weeks of height measurement. Despite the observed reduction in spore count, there were no observable effects on seedling performance of soil storage. But in general, non-mycorrhizal plants had lesser growth rate compared with those with the inoculated ones.

Seedlings growing in Madagascan and Kenyan soils showed highest and lowest performance respectively all through the period of height measurement. Whereas the mean height of the inoculated plants was in real terms higher than the control experiment, the significance of the differences varied over the course of growth. In the first six weeks, the mean height of seedlings growing in Madagascan soils was significantly higher than that for Kenya, Uganda and control, the latter three not being different. For instance in the first week of measurement (3rd week of growth), the mean height of seedlings in Madagascan soil (7.56cm) was significantly higher than the mean heights for Uganda (6.11cm), Kenya (6.32cm) and control (6.79) which were not significantly different from each other (L.S.D, 5%=0.533). At the fourth measurement, the mean

height in Madagascan soils (9.1cm) was higher than the mean of seedlings in Ugandan, Kenyan and control soils that is 7.56cm, 7.29cm and 7.48cm respectively (5%, LSD=0.908).

The mean height of Tavete (one site in Kenya) seedlings was however, consistently significantly higher than the seedlings from the other site (Kedowa) (figure 11). In the first week of measurement, the former had a mean height of 7.5cm while the latter had a mean height 5.57cm. The difference between Tavete (M=9.04cm) and Kedowa (M=6.39cm) seedlings was still prominent in the fourth week (5% LSD=0.605). The blocks had no significant effect on heights.

Figure 11: Effect of provenance on response to inocula.
Tavete seedlings (circled green) were more vigorous that Kedowa seedlings (red circle)

Considering the mean increments, the effect of seed and soil was evident ($p<0.001$), while the effects of soil were inconsistent. However, the mean increment for Tavete seedlings remained consistently higher than of Kedowa. For example considering the first mean increment (MI), that for seedlings growing in Madagascan (MI=0.781) and Ugandan (MI=0.859) soils were not significantly different. Both were however higher and different from those raised on Kenya soils (MI=0.325) and control plots (MI=0.250) which did not significantly differ from each other (L.S.D =0.1663). The Tavete seed lot also showed a significantly higher overall increment (MI=0.704) than the Kedowa seed lot (MI=0.429), the differences specifically evident in Uganda and Madagascan soils (L.S.D, 5%=0.1663). The effect of seed source on height for both seed

lots is well illustrated in figure 12. Storage did not have any significant effect on mean height or increment of the seedlings.

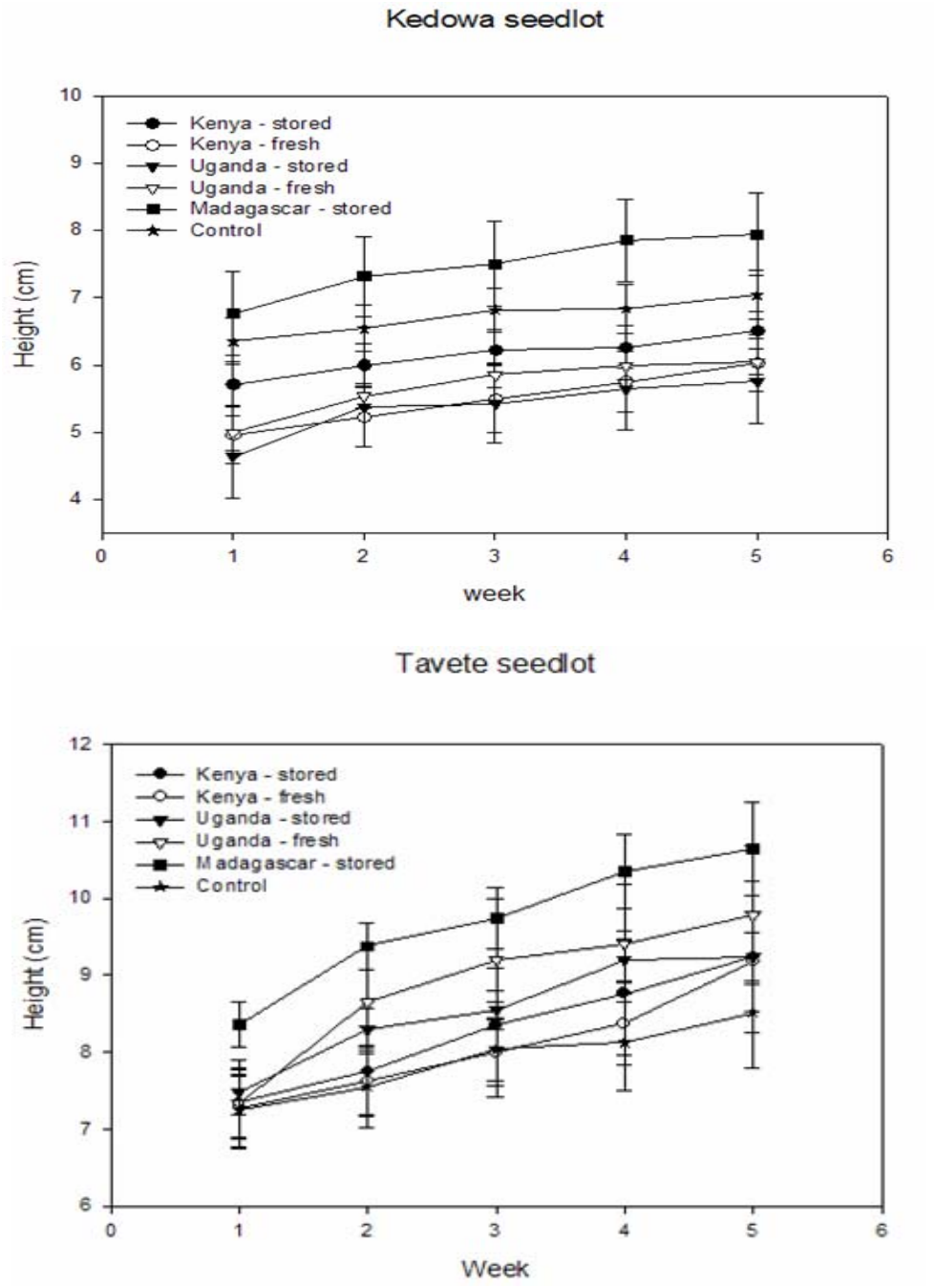

Figure 12: Mean height of Tavete and Kedowa seed-lots

4.3.2 Correlations

There were no correlations between the number of spores or species diversity with either height or diameter (Table 8).

Table 8: Correlation coefficients and corresponding P-values between different parameters, h is mean height in each week of measurement

Variables	Coefficient of correlation	P-Value
Spore diversity *h_1	0.615	0.193
Spore diversity *h_2	0.500	0.313
Spore diversity *h_3	0.595	0.213
Spore diversity *h_4	0.477	0.338
Spore diversity *h_5	0.519	0.291
Spore diversity *h_6	0.477	0.338
Total number of spores*h_1	0.449	0.372
Total number of spores *h_2	0.191	0.718
Total number of spores *h_3	0.558	0.250
Total number of spores *h_4	0.409	0.421
Total number of spores *h_5	0.505	0.307
Total number of spores *h_6	0.409	0.421
Total number of live spores *h_1	0.620	0.189
Total number of live spores *h_2	0.403	0.428
Total number of live spores *h_3	0.613	0.195
Total number of live spores *h_4	0.477	0.339
Total number of live spores *h_5	0.538	0.270
Total number of live spores *h_6	0.477	0.339

CHAPTER FIVE

DISCUSSION

5.1 Genetic structure of *A. gummifera*

5.1.1 Levels of genetic variation within and between *A. gummifera* populations

The amount of variation detected in this study (14 haplotypes) demonstrates the effectiveness of the PCR-RFLP methodology in detecting polymorphism in the chloroplast genome of *Albizia gummifera*. Most of the observed polymorphism seemed to be due to small insertions or deletions (indels) of between 5 and 20 base pairs. Only nine mutations were detected partly because of the higher frequency of indels as compared with point mutations in the chloroplast genome and partly because this method is more likely to detect indels than point mutations (Raspé *et al.* 2000). Indeed the only point mutations that may be detected are those that lead to the appearance or the disappearance of a restriction site.

Of the three populations, Ugandan samples exhibited the highest genetic diversity, followed by Madagascar and then Kenya. The observed values can be well explained by the degradation histories of the study sites. Empirical evidence suggests that population changes associated with habitat degradation lead to an erosion of genetic variation due to increased random genetic drift, elevated inbreeding, and reduced gene flow, where fragment/population size and isolation fall below critical levels (Young *et al.*, 1996; Cavers, 2003; Davies, 2006).

However, the severity of genetic bottleneck is determined by the severity of habitat loss, that is to say, the severity and duration of a reduction in population size. Facts from this study are; first of all, the study sites were significantly degraded. While in Uganda the forest from which the samples were collected may seem to be disturbed, there were still a number of mature trees (trees in the upper stratum) from which leaves were picked. These, though few, probably have

persistently maintained a larger proportion of genetic variation of the founding populations to explain the highest genetic diversity.

Paradoxically, the site in Madagascar was degraded by slash and burn agriculture. The relatively lower diversity suggests that the trees from which the leaves were collected were re-colonizing. As theory predicts (Mckay *et al.*, 2005), these colonizers may have suffered different kinds of bottlenecks and founder effects with a consequent reduction in genetic diversity. Similarly, the degradation in Kedowa Forest site gives a plausible explanation for the observed values.

Despite the differences in diversity, Kenyan and Ugandan populations are very similar, although Kenyan population had lower diversity. This indicates that Kenyan population in contrast to Madagascan population has had a very recent degradation that has been detrimental to its genetic diversity. According to Dawson and Powell (1999), afromontane habitats-typical of Kedowa forest are supposed to have high diversity since they act as island of genetic diversity. A report by UNEP (2006) shows an extensive recent degradation in Mau forests. According to UNEP (2006), that recent 2001 forest excision, as well as the illegal, irregular and unplanned settlements affected 54.3 % and 27.3 % of the total area of the Eastern Mau Forest Reserve and South West Mau Forest Reserve (where Kedowa site is located) respectively. This is in line with our observations as the population from which our sample is derived represents a heavily modified forest edge community, and as such it is likely that the *A. gummifera* population here has experienced substantial impact from human activity.

As reported by Green and Sussman (1990) documented that the fiercest degradation that ever hit the Vohimana site was in 1985 where the rate of degradation of the rain forests was estimated by Landsat images to have been more than 66%. It could probably be due to the recent conservation that the forest has had chance to regenerate some of their diversity to account for

the relatively higher genetic diversity than Kenya. Besides, these forests have inherently less in common with Uganda or Kenya, such that degradation does not congruently affect much of the former's diversity.

Nonetheless, the total variation (h_{TOT} =0.886) in the species is high in relation to those observed in other out crossing species (Cavers *et al.*, 2003; Navarro *et al.*, 2005) and in some species in the same region (Dawson and Powell, 1999). And just as many studies have revealed in several tree species (Cavers *et al.*, 2003; Trindade, 2001), variation within populations is greater than among populations. The high values of variability within populations and the low levels of genetic variation among populations suggest the existence of reproduction model without inbreeding and panmixia. Such a model shows high heterozygosity within a population and low genetic variability between populations because of a high gene flow due to lack of geographical barriers just as the results of the research (Φ_{PT} = 0.249, $p<0.01$). Although cpDNA data may not directly show the level of heterozygosity or inbreeding, the PhiPT value reveals that the overall differentiation between the populations is relatively low given the substantial distances between populations.

5.1.2 Levels of gene flow and genetic differentiation

In theory, widely-distributed, extensively out-crossing species with effective pollen or seed dispersal mechanisms should have higher genetic similarity and low differentiation (Cavers *et al.*, 2005). Although the overall differentiation is quite low (Φ_{PT} = 0.249, $p<0.01$), there is substantial haplotypic differentiation between the pairs of populations. Gene flow and differentiation are lowest and highest respectively between Kenya or Uganda and Madagascan populations. Like many studies have verified the isolation by distance theory (Wright, 1931; Cavers *et al.*, 2005), populations that are neighboring each other are expected to have higher gene flow and hence less differentiation than populations which are distant. In addition, the magnitude and pattern of

genetic subdivision that was found among populations provides compelling evidence for the significance of barriers to gene flow. The Eastern arm of the great East African rift is likely to act as a barrier between Uganda and Kenya, whilst the ocean separation between Madagascar and continental Africa is likely to form a substantial barrier for gene flow.

According to Wolf *et al.* (1997), the chloroplast genome is more likely to exhibit substantial geographic structuring of populations due to its maternal inheritance (and hence limitation of gene flow to seed dispersal) and an inherently lower mutation rate in the chloroplast molecule. The results of this study verify this expectation. Certainly, the potential for contemporary gene flow between populations is highly restricted, given the fact that all the forests where the individuals were collected were degraded and the monitoring for seeds, which has been going on for more than a year, showed that most of the fruits aborted and hence not enough seed is produced to aid sufficient gene flow. Furthermore, the seeds themselves are orthodox and hence even when they disperse, they may easily die before having suitable conditions for germination. Actually many studies have revealed more gene flow by pollen than by seed with implications that cpDNA data will almost certainly show increased levels of population differentiation and diversity due to more limited dispersal ability.

Since chloroplast DNA has slow rate of differentiation, geographic structuring of chloroplast variation is more likely to reflect very long term processes. Hence, the observed differences may not only be attributed to gene flow, selection or mutation but to long term migration differences. The differences in migration may be the most plausible explanation for populations like Uganda and Madagascar that have the highest gene flow, yet with the highest number of private haplotypes. Geological studies show that Madagascar was separated from mainland Africa roughly 100 Ma (Raven & Axelrod, 1974) or even earlier, but during the process of continental drift, a considerable number of families, genera and species dispersed intercontinentally.

However, in the process of Madagascar's drift to the east, there must have been a considerable number of genera and genetic distributions that were split (Leroy, 1978) providing both Madagascar and Africa with a common stock of taxa, yet with some unique haplotypes.

Though not many studies have compared distinctiveness of various species at molecular level, a number have illustrated a reduction in species diversity from particular genera between mainland Africa and Madagascar. For example, Meve and Liede (2002) showed that in genus Asclepiadoideae the total number of species in the old world were 1900, of which apparently 1250 are in Africa and 125 in Madagascar. This implies that early earth movements must have had substantial effects on all diversity. Despite the high gene flow between Uganda and Madagascar there are still few haplotypes that are shared between the two populations. As documented by Wright (1959), the gene flow between those populations is still very low to maintain a panmictic population.

The presence of unique haplotypes yet in presence of gene flow may indicate that reproductive isolation has occurred between the populations given the different selection pressures that are available in the different sites. From a neo-Darwinian perspective, new species arise when natural selection acts on genetic variation at the population level, eventually leading to reproductive isolation because of alternative accumulated mutations (Mayr, 1982). Although it is not clear how much time '*eventually*' represents whether speciation requires a complete shut-off of genetic exchange among diversifying lineages (Lawton-Rauh *et al.*, 2007), the high genetic distance indicates that these populations are not acting as a single interbreeding population and as, such some haplotypes have remained distinct. Many studies have illustrated that speciation can readily occur in less than 150ya (Thoday and Gibson 1962; Dobzhansky and Pavlovsky, 1971). The genetic distances between Uganda and Kenya may even tend to grow wider given the low gene exchange that is currently being experienced. However, verification of the reproductive isolation

between our populations will require analysis using markers that sample the nuclear genome as well, since pollen may well be capable of dispersing trans-oceanically.

Despite the long distance, the high gene flow between Uganda and Madagascan populations may have been as a result of one episodic gene transfer, for example, through human transfer of seed. However looking at Asclepiadoideae flora (not at molecular level though) Meve and Liede (2002), concluded that, the Africa- Malagasy distributions are only inconspicuously influenced by these presumable introductions but most likely by some long distance dispersal events effected by anemochorous seeds, besides other historical geological forces.

5.2 Diversity of mycorrhiza

The diversity of AMF depends on the type of ecosystem, agricultural practices and soil conditions. Helgason *et al.* (1998) found higher AMF diversity in a woodland ecosystem in comparison to an arable land ecosystem. Soil disturbance can reduce the density of spores, length of mycelium and species richness of AMF and no tillage conditions stimulate the mycorrhizal activity (Boddington and Dodd, 2000; Dodd, 2000). A higher diversity of spores in Ugandan new soils than Kenya new soil implies better conditions for spore development in the former. Just like the trend is for observed genetic diversity of *A. gummifera*, the degradation in Kenya must have had an impact on the spore diversity. The higher diversity of spores in old Kenya soil than the others must be relating to the conditions of the soil, for instance, the level of moisture, the handling process among others that the soils were exposed to before storage.

Although analysis of spores may give a general view of diversity, it may not be very conclusive since not all AMF colonizing the plant roots are found as spores. Besides, not all spores are easy to identify when sieved directly from field soil, for instance some of the spores are ruptured. In addition, the variations in spore development may also sometimes be misleading.

5.2.1 Activity of mycorrhizal inoculum

The differences in diversity between the stored and fresh soil is indicative of the effect of storage on the survival of microorganisms. Microbes are easily affected by aeration as well as storage conditions. Ugandan soils lost over 90% of the total spores, while Kenyan soils lost approximately 30% of the spores. This was probably due to the fact that the former contained more moisture than the latter. As reported by Mondal and Hyakumachi (2000), soil moisture accelerates the loss of microbes in stored soils. Although the loss may seem substantial, there was no evidence to support Mondal and Hyakumachi (2000)'s conclusion that fungal propagules exposed to non-sterile soil for an extended period become exhausted, and lose viability and virulence since the results did not show any significant differences between seedlings grown in the stored and fresh soils.

5.2.2 Dependency and responses of *A. gummifera* to inoculum

The role of mycorrhiza plays in performance of the seedlings has been illustrated in Figure 12. Even though the performance of Kenyan seedlings would be expected to be highest in its own soils, the seedlings were seen to perform best in Madagascan soils, followed by Ugandan and finally Kenyan soils. The response to inoculum especially in the seedlings growing in Kenyan soils may be attributed to many factors discussed in the subsequent sections.

5.2.2.1. Fungal species differences

Functional diversity tests on different AMF have shown that different AM fungal species have different effects on plant performance and nutrient cycling. Particular AM fungal species, and not all the components of any AM fungal population, appear to play a role at a given stage of plant performance (Jeffries and Barea, 2001). This implies that whereas a soil may seem more diverse, not all those fungi may colonise in the host's initial growing stages. This explains why

diversity may not correlate with growth. Even then, a host having the same species of fungi may also exhibit contrasting colonization behavior that is either Arum or Paris type.

5.2.2.2 Arum versus Paris colonisation

Another proposition that may have brought about the differences in plant responses to growth is the difference in colonisation pattern of the mycorrhiza in the different soil treatments. Based on pattern of colonization, arbuscular mycorrhizas are divided into two types; the *Arum* type and *Paris* type. In the *Arum* type, intercellular hyphae grow in a longitudinal manner along the root and penetrate the cortical cells to form arbuscules. Arbuscules arise from these intercellular hyphae on short side branches, typically at right angles to the main root axis (Smith and Smith, 1997). In the *Paris* type, intercellular hyphae are absent and the hyphae are entirely intracellular and irregularly coiled, some of them forming arbuscules that are not terminal but are localised in definite layers. The arbuscules are formed as intercalary structures and called arbusculate coils (Smith and Smith, 1997). Whereas the *Arum*-forming mycorrhizas are typical of fast-growing plants, they have been commonly isolated in other slower growing plants like the trees. Nevertheless, the *Paris* type morphology is the one more often seen in plants in natural ecosystems

These colonization patterns may cause a difference in plant responses in that, in the Arum type, the fungus colonizes the plant rapidly implying faster mineral acquisition by the plants. This contrasts with the colonization in the Paris which is relatively slow as hyphae have to pass from cell to cell within the plant cortex. Although slower nutrient acquisition may be reflected in lower growth rates, this is true only in the early stages of plant growth. This is because whereas the arum type colonises very fast, the life span of the arbuscules which are the active sites of nutrient exchange are shorter as opposed to that in the Paris type.

Even though and probably, for the above reason the Arum type is more common in short lived plants like grasses, herbs or shrubs, this doesn't rule out the possibility of this type of mycorrhiza being found in forest soils, especially in degraded areas which may be more dominated by herbs and shrubs. Actually, the results do not contradict this because the soils from Mabira Forest were collected from a colonizing forest, with many ground shrubs and herbs. This soil must have had both the Arum type from the shrubs and the Paris type from the trees hence the intermediate growth in the early stages. The soils from Kedowa forest were collected from a strict nature reserve with limited undergrowth thus the seedlings are indicative of a slow colonizing Paris type pattern of mycorrhiza. The Vohimana site has also experienced recent slash and burn agriculture and therefore most likely to have the Arum type of mycorrhiza, explaining the fast growth of seedlings in the Madagascan soils. Even if Ingleby (2007) like many other researchers believes that host identity is the sole determinant of the colonization pattern, with implications that a particular host cannot have two AMF patterns, a number of studies prove otherwise. For example studies carried out by Cavagnaro *et al.* (2001) on tomatoes and Kubota *et al.* (2005) on cucumber have shown that the fungal identity also can determine AM morphology.

In addition to the above Smith and Smith (1997) even speculate that the Paris type of mycorrhiza may not only delay playing their expected role but some may tend be parasites in certain environmental conditions. Though parasitism in mycorrhiza has not been well studied, this speculation arose from the conclusion that some Paris type mycos do not form arbuscules; yet to date these have been the only widely agreed sites for nutrient exchange.

Since little or no specificity has been documented in AM, as also clearly evidenced from our study, a host plant conceivably gradually selects the AMF assemblage that can optimise its growth. This has been supported by various studies that have shown that different AMF species sporulated differentially with different plant species with which they were associated. This

means that for Kenyan seeds growing in their own soil, there was no added fungal choices to select from which would probably enhance their growth like in the other soil types.

5.2.2.3 No colonisation

Seedlings growing in Kenyan soils barely showed an effect of mycorrhiza despite the higher spore abundance and diversity which leads to the speculation that there was little colonisation. Since there are a number of experiments that have suffered lack of colonization despite using spores sourced direct from the ecosystem site, it shows that this form of AM propagule (spores) is not the main inoculum source for that target plant. Besides, a number of plants have been shown to experience a rapid initiation of the AM colonization when more mycelia rather than spores are present in the soil. Therefore spore diversity may not necessarily translate into colonization. Various studies have demonstrated the ecological role of the AM mycelium as inoculum source especially particularly in arid and semiarid ecosystems typical of Kenya.

Adding to the above, there is also amounting evidence that many species of symbiotic fungi especially members from Acualosporaceae and from Glomales have innate dormancy. This implies that when spores are exposed to favorable growth conditions, they have to go throughout a period of up to three months prior to becoming germinable.

5.2.2.4 Bacteria communities in the soil

Theory has it that in the rhizosphere, AMF interact with different kinds of bacteria. Different functional groups of bacteria such as N2-fixing bacteria, plant growth-promoting rhizobacteria, phosphate-solubilising bacteria and antagonists of plant pathogens have been reported to be associated with the rhizosphere of different plants colonised by AMF. These interactions can be found at all stages of the AMF life cycle, from spore formation and germination through root colonisation to external hyphae. The nature of these interactions may however be inhibitory or

stimulatory, competitive or mutualistic to each other or for the plant depending on environmental conditions. While negative interactions include reduced spore germination and hyphal length in the extramatrical stage, decreased root colonisation and a decline in the metabolic activity of the internal mycelium, positive interactions on the other hand include enhanced spore germination, mycorrhizal development and function (Hildebrandt *et al*., 2002). Though few studies have shown the magnitude of the interactions in different plants, synergistic positive interactions have been reported between AMF and plant growth promoting bacteria such as nitrogen fixers, fluorescent pseudomonads and sporulating bacilli. Since bacterial are more prone to disturbance then their reduced activity may also translate in reduced activity of the mycorrhiza, which may explain the limited response of the plants to inoculation in the experiment.

5.2.2.5 Soil fertility

The response of plants to mycorrhiza is also influenced by the fertility of the soil where the plants grow. Where soils are fertile plant response to mycos will not be as visible as where the soils are infertile indicating that probably the loam that was used in the experiment was very fertile, obscuring the effects of mycorrhiza.

5.2.2.6 Plant genotype

Although studies have also shown that the genotype of a plant species appears to determine the AM morphologies. The explanations for this are not yet documented.

The results of no correlations coupled with the least inoculum response plus lowest genetic diversity may imply that, *A. gummifera* in Kedowa exist as very few scattered individuals or at least did at one time. This means that the individuals may have a tendency to lose genetic diversity through inbreeding and genetic drift. In sampling for mycorrhiza, (because a higher density of

other trees may be in the vicinity), it is more likely that other mycos sampled were not those associated with *A. gummifera* and could not colonise on its roots as fast. In Mabira, since *A. gummifera* is found in mixed forest, then its roots and hence the rhizosphere are easy to identify.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Populations of *Albizia gummifera* are characterized by high diversity with chloroplast markers, more variation being attributed to intra-population variation. The populations are also significantly differentiated.
2. The fungal communities in all the study sites were equally diverse.
3. There was no evidence of local adaptation of *A. gummifera* but the mycorrhiza contributes significantly to the growth of seedlings.
4. There was so significant effect of soil storage on mycorrhiza activity though plants benefit from exposure.

6.2 Recommendations

6.2.1 For management

The gene flow between the *A. gummifera* populations is less than 4 migrants these populations may have evolved at the 'major' genes. Breakdown in genetic cohesion implies that introduction of genotypes from outside can easily interfere with the process of evolution of locally adapted races, and so we need to be very cautious about introducing seed. Besides, gene flow, these populations are very distinct given the high number of unique haplotypes there in and may have had different migration routes, which makes them quite different. Therefore, as an obligation to maintain the genetic integrity of the restored populations, interchange of propagules would not be appropriate.

However, if increasing genetic diversity in the ecosystem is one of the ultimate goals of FOREAIM project, then the Madagascan populations can also be used for restoration. Limited introduction of seed from other sources can produce new genotypes to be 'tested' by natural

selection. Consequently and regardless of the provenance of introduced seed, if it hybridizes with the native genotypes there will be an increase in genetic variance that is likely to increase the rate of evolution. However, precaution should be taken as to avoid the negative consequences of introductions such as out breeding depression.

Lack of specificity to mycorrhiza would permit the use of the populations all though the target restoration sites with confidence. Still, because seedlings showed an enhanced growth with mycorrhiza, then it is suggested that seedlings to be used for restoration can be inoculated in nursery to enhance their vigor in the nursery and hence survival in the field. However, the potential negative consequences of long distance transfer of germplasm material like cost, probably outweigh the benefits.

6.2.2 For Research

Populations having sufficient gene flow does not guarantee adaptive similarities since populations in the early stages of speciation may continue to have high levels of gene flow despite adaptive differences (Endler, 1977). Conversely, populations with low levels of gene flow and high genetic differentiation may not have adaptive differences and may never become reproductively isolated (i.e., full species). This implies that an analysis of neutral markers does not aid final decision making. Like Cavers *et al.* (2004) suggested, it is recommended that for more accurate decision making, thorough analysis of quantitative trait loci should be an important aspect.

Even in presence of no adaptation, there are factors that are critical before any introductions, for instance, the influence of numbers of individuals and genetic variation represented in the founding population on colonization, establishment, growth, and evolutionary potential.

Evolution of populations requires genetic variation, and the larger the genetic variance, the greater the potential for adaptive evolutionary change (Falconer, 1981; Hartl and Clark, 1989).

Because restoration usually begins with relatively small populations, the amount of genetic variation represented in the founding population can be critical. In small populations, stochastic changes in size can severely reduce the genetic variation within a population, thus increasing the opportunity for non-adaptive evolution by random genetic drift at the expense of adaptive change by natural selection (Ellstrand and Elam, 1993). Reduction in population size and genetic variance is expected to increase the opportunity for inbreeding and subsequent inbreeding depression. Therefore, there is need to work out suitable founding populations before introduction of *A.gummifera*.

In addition, though the physical and ecological forces that promote genetic differentiation may be understood in theory, the relative importance of contemporary versus historical biogeographical factors remains contentious. For better genetic management, it is worth differentiating between these.

Besides, research into other basic biological and ecological processes that are taking place at the restoration site can enhance the long term viability and credibility of the practice of restoration. In addition to genetic diversity, understanding other factors that influence birth, growth, reproduction and death within plant populations will not only give insights on how genetic diversity changes with time, it will also implicate factors that affect success of organisms. This will take into account the influence of other inter-specific interactions (as well as mycorrhiza) on population establishment, colonization, growth, and community development. Interactions such as competition, herbivory, predation, parasitism, and mutualisms all play roles in the development and fate of restored sites. The ecological effects of non-native species on native

communities should also be looked at in case of introduction of especially the Madagascan populations.

However, because of genetic drift, any one haplotype should not be assumed to be completely representative of variation among the whole genome. It is therefore important that the data are derived from an adequate number of markers or base pairs.

Lastly, as inoculation experiments are followed up, other factors other than numbers of spores can be investigated for example the types of fungi that in reality colonise the roots other than assuming that all spores observed in the soil will colonise. Probably these may be better correlated with growth than total numbers.

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