Molecular Characterization of the Wild Edible Mushrooms

of the *Pleurotus* species in Kenya

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A Thesis submitted in partial fulfilment for the degree of

Master of Science in Biotechnology in the Jomo Kenyatta University

of Agriculture and Technology

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DECLARATION

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

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Daniel Otieno Ojwang

This thesis has been submitted for examination with our approval as University Supervisors.

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Dr. Lexa Gomezgani Matasyoh

Moi University, Kenya
DEDICATION

This Thesis is dedicated to, first and foremost, my loving mother Hilda Atieno and my wife Berine Awuor whose strength, patience and encouragement enabled me to overcome the many challenges throughout my studies.
ACKNOWLEDGEMENTS

I am grateful to God Almighty who enabled me to conduct and complete the whole research work successfully. My sincere appreciation goes to my supervisors; Dr. Justus M. Onguso, Dr. Lexa G. Matasyoh and Dr. Joel Mutisya for their valuable and constructive suggestions, useful criticism, kind co-operation and inspiration in planning and execution of the research work as well as in editing the thesis.

I express my indebtedness and thanks to Dr. Calvin Onyango, Dr. Harvey Jagger and Dr. Wanjiru Wanyoike for their valuable suggestions, scholarly comments and careful review of the draft. I am also highly grateful to the research team at the ILRI-BecA hub for their technical assistance and friendship during the project period.

Special appreciation also goes to the National Council for Science and Technology and Kenya Industrial Research and Development Institute for financial support. Further appreciation goes to Kenya Forest Service for providing genetic materials.

I would like to thank my family for their patience and love that made this work possible. Finally I would like to remember with tears the departed souls of Professor George M. Siboe and Dr. Joel M. Mutisya for their invaluable contributions in this work. May their souls rest in eternal peace.
TABLE OF CONTENTS

DECLARATION ................................................................. i
DEDICATION ........................................................................ ii
ACKNOWLEDGEMENTS ................................................... iii
TABLE OF CONTENTS ....................................................... iv
LIST OF TABLES ............................................................... vii
LIST OF FIGURES ............................................................. viii
LIST OF APPENDICES ..................................................... ix
ABSTRACT ......................................................................... xi
CHAPTER ONE .................................................................. 1
  1.0 INTRODUCTION ......................................................... 1
    1.1 Background information ........................................... 1
    1.2 Problem statement .................................................. 3
    1.3 Justification of the study .......................................... 4
    1.4 Research hypothesis ................................................ 5
    1.5 Objectives ............................................................... 5
      1.5.1 General objective ............................................... 5
      1.5.2 Specific objectives .............................................. 5

CHAPTER TWO .................................................................... 6
  2.0 LITERATURE REVIEW ............................................... 6
    2.1 Description and classical taxonomy of Pleurotus species .... 6
2.2 World distribution of *Pleurotus* species .......................................................... 7
2.3 Nutrient composition ......................................................................................... 8
2.4 Medicinal value ................................................................................................. 8
2.5 Role in environmental management ................................................................. 9
2.6 Life cycle and growth of *Pleurotus* species ..................................................... 9
2.7 Mating system and gene flow potential ............................................................. 10
2.8 Molecular systematic ....................................................................................... 11
2.9 Biogeography and speciation .......................................................................... 12
  2.10.1 Morphological tools .................................................................................. 12
  2.10.2 Molecular tools ......................................................................................... 13

CHAPTER THREE ....................................................................................................... 15

3.0 MATERIALS AND METHODS ........................................................................... 15

3.1 Sample collection ............................................................................................. 15
3.2 Preparation of tissue cultures ........................................................................... 16
3.3 Isolation and visualization of genomic DNA .................................................... 16
  3.3.1 DNA isolation .............................................................................................. 16
  3.3.2 Gel electrophoresis ..................................................................................... 17
3.4 AFLP analysis .................................................................................................. 18
  3.4.1 Template preparation and adaptor ligation .................................................. 18
  3.4.2 Pre-selective amplification ......................................................................... 19
  3.4.3 Selective amplification ............................................................................... 19
3.5 DNA amplification and sequencing .................................................................... 21
LIST OF TABLES

Table 1: Worldwide distribution of some commonly cultivated *Pleurotus* species....7

Table 2: Nutritional composition of some commonly consumed mushroom species.8

Table 3: Wild *Pleurotus* species used in this study........................................15

Table 4: AFLP primers and polymorphism..........................................................24

Table 5: Genetic diversity estimates among 4 populations of *Pleurotus* species ....26

Table 6: Summary results of AMOVA.................................................................27
LIST OF FIGURES

Figure 1: *Pleurotus* species growing on dead trunk of wood.................................6

Figure 2: A schematic representation of the location of ITS region.......................11

Figure 3: Dendogram of clustering analysis of 84 *Pleurotus* species......................28

Figure 4: Neighbor-joining tree of 12 *Pleurotus* species based on ITS sequences.....29
LIST OF APPENDICES

Appendix 1: Sequence alignments........................................44
Appendix 2: Blast search results for 12 Pleurotus specie..........49
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AMOVA</td>
<td>Analysis of Molecular Variance</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer</td>
</tr>
<tr>
<td>JKUAT</td>
<td>Jomo Kenyatta University of Agriculture and Technology</td>
</tr>
<tr>
<td>KARI</td>
<td>Kenya Agricultural Research Institute</td>
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<td>NCBI</td>
<td>National Centre for Biotechnology Institute</td>
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ABSTRACT

Members of the genus *Pleurotus* are macro fungi belonging to the phylum Basiomycetes. They are important source of food and medicinal compounds among many local communities. Limited studies have been done to identify and characterize *Pleurotus* species based on genetic characteristics in different parts of the world. However, no previous studies have been undertaken to understand the genetic characteristics of the wild species in Kenya. A total of 71 samples of wild *Pleurotus* species were randomly collected from Kakamega Forest, Arabuko Sokoke Forest and Mount Kenya Forest. Thirteen samples of commonly cultivated *Pleurotus* species were obtained from Jomo Kenyatta University of Agriculture and Technology. Genetic variability and phylogenetic relationships were evaluated using amplified fragment length polymorphic markers and ITS sequences of the ribosomal DNA respectively. Five primer combinations used generated 330 polymorphic loci across 84 samples. The mean diversity estimate between the wild (0.27) and cultivated (0.24) species was small and is not statistically significant. However, diversity was great within (89%; P>0.001) than among populations. Phylogenetic analysis revealed *Pleurotus ostreatus, Pleurotus eryngii, Pleurotus* sp. ‘Florida’ and *Pleurotus* sp.YL005 as part of diversity of *Pleurotus* species in Kenya. The broad diversity within populations suggests the possibility of obtaining commercially suitable wild species for cultivation.
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

_Pleurotus_ species commonly known as oyster mushrooms are distributed all over the world and usually grow on hardwood in terrestrial ecosystems (Vilgalys and Sun 1994). Similar to other white-rot fungi, they are important agents of biodeterioration, due to their ability to break down plant materials, especially cellulose and lignin (Carlile and Watkinson, 1994; Buswell _et al._, 1996). They have high commercial value, and thus they have been widely cultivated (Cohen _et al._, 2002; Stamets, 2000). Consumption of wild _Pleurotus_ species is common among the diets of many rural communities during the rainy seasons whereas, urban dwellers have great preference for cultivated species. Consumption of _Pleurotus_ species has increased due to their high nutritional composition, taste and aroma. A recently published FAO study recommends consumption of edible mushrooms to supplement carbohydrate rich diets common among many developing countries (FAO, 2006).

Many members of the genus _Pleurotus_ are found distributed worldwide in nature. A few of them have been domesticated and are under commercial production. The commonly cultivated species include _Pleurotus sajor-caju, Pleurotus cystidiosus, Pleurotus eryngii_ and _Pleurotus tuberregium_ (Chang and Miles, 1989a). Production of _Pleurotus_ species is increasing due to their ability to grow fast on a wide range of agro-
wastes. Production of *Pleurotus* species is gaining popularity and it is the second most produced mushroom in the world market after *Agaricus* species (Chang, 1999).

Growth of mushroom industry requires new strains with better characteristics. Farmers require mushroom varieties with fast maturity period, increased resistance to both pests and diseases and high yield. Kenya’s rich mushroom biodiversity has great potential to provide new mushroom strains with desirable characteristics for commercial cultivation. The exact characterization and identification at the species level is thus an important step in systematically utilizing the full potential of fungi in specific applications (Lieckfeldt *et al.*, 2001). Many *Pleurotus* species have been identified and characterized in the past using morphological features. The previous studies on *Pleurotus* species in Kenya have also been based on morphological characteristics. However, morphological features in Basidiomycetes fungi are influenced by environmental factors and often fail to detect variations among species and strains that are closely related.

Molecular markers have been used to discriminate mushroom lineages at the species level. They are more stable, reproducible and are not affected by environmental factors hence provide more information on genetic characteristics of any species. Identification and characterization of local strains of *Pleurotus* species using molecular
tools is necessary in selecting new strains for commercial cultivation. Morphological characters alone used in the past are often inadequate for exact strain identification and in resolving the systematics and evolutionary relationships within Basidiomycetes fungi. Molecular genetic data is therefore useful for establishing a reliable taxonomic scheme for *Pleurotus* taxa. The aim of this study was to evaluate variability and relatedness of the wild *Pleurotus* species collected from different parts of Kenya.

1.2 Problem statement

In recent times, edible mushrooms have assumed greater importance in the diets of both rural and urban dwellers in Kenya, unlike previously when consumption was confined to rural communities (Wambua, 2004). Increase in demand for edible mushrooms has resulted in setting up of several mushroom units in different parts of the country. Currently the mushroom production stands at slightly over 500 tons per annum with the production of *Pleurotus* species being the second most produced after *Agaricus* (Concern/GTZ/ MOA., 2005). The total annual mushroom production in Kenya is low and hardly enough to meet the local demand. Rural communities therefore, rely on the collection and consumption of wild species during the rainy seasons. Unfortunately, the seasonality of wild edible mushrooms makes them unreliable source of nutrition. Similarly, lack of clear-cut identification and limited information on their genetic diversity limit their exploitation for commercial production and breeding purposes.
1.3 Justification of the study

Increased productivity of mushroom industry in Kenya requires new mushroom species with improved characteristics such as high yields and increased resistance to pests and diseases. Characterization and identification of wild species is likely to provide strains with desired characteristics. Accurate taxonomic identification and phylogenetic classification is therefore necessary for selecting strains with potential for commercial production and breeding purposes. Molecular markers including rapid amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphic markers (AFLP), restriction fragment length polymorphic (RFLP) markers and microsatellite have all been employed to discriminate different kinds of organisms including mushrooms (Barroso et al., 2000; Vos et al., 1995). AFLP markers have proved to be more reliable compared to other molecular tools for genotyping mushroom lineages. AFLP technique has been successfully applied to discriminate the genomes of Pleurotus ostreatus (Meng et al., 2003), Tricholoma matsutake (Chen et al., 2003), Lentinula edodes (Zhuo et al., 2006), Agaricus bisporus (Gu et al., 2003) G. lucidum (Zheng et al., 2007). Similarly, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has also been widely used for the phylogenetic identification of mushrooms at both the species and genus level (Sanchez-Ballesteros et al., 2000). Different regions of rDNA also evolve at variable rates and this makes them suitable for investigating fungal relationships at different taxonomic levels (Bruns et al., 1991).
1.4 Research hypothesis

Wild *Pleurotus* species in Kenya have broad genetic diversity suitable for commercial cultivation.

1.5 Objectives

1.5.1 General objective

To determine the genetic potential of wild *Pleurotus* species in Kenya for commercial cultivation.

1.5.2 Specific objectives

To examine genetic variability and phylogenetic relationships of the wild *Pleurotus* species collected from different parts of Kenya using AFLP markers and ITS sequences of the ribosomal DNA.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description and classical taxonomy of *Pleurotus* species

*Pleurotus* is a genus of gilled mushroom with wide cap shaped like an oyster shell. Members of the genus *Pleurotus* form basidia and they usually have a mycelial thallus (Hawksworth *et al.*, 1995). The systematic position of *Pleurotus* has been much debated with several species being placed in the former Polyporaceae, tribus Lentineae and the latter in the Tricholomataceae, tribus Resupinateae (Singer, 1951; Corner, 1981). Some mycologists have also placed *Pleurotus* in the family Pleurotaceae, irrespective of sporeprint color and other micro-morphological characters. Current taxonomic classification places *Pleurotus* species in the phylum Basiomycetes, order Agaricales and family Tricholomataceae (Bernardo, 2004; Hawksworth *et al.*, 1995).

![Figure 1. Pleurotus species growing on dead trunk of wood](image)

Figure 1. *Pleurotus* species growing on dead trunk of wood
2.2 World distribution of *Pleurotus* species

*Pleurotus* species are distributed throughout the world as shown in Table I. To date, approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognized species (Singer, 1986). *Pleurotus pulmonarius* and *Pleurotus cystidiosus* are known to be distributed in tropical and subtropical region, while *Pleurotus eryngii* are collected in Europe, Africa and most of Asia except Korea and Japan, where the mushroom is commercially cultivated (Walser *et al.*, 2003; Zervakis *et al.*, 1994; Zervakis and Balis, 1996; Vilgalys and Sun, 1994; Lindequist *et al.*, 2005; Lieckfeldt *et al.*, 2001; Kües, 2000; Kües and Liu, 2000; Kalac and Svobod, 2005; Cohen *et al.*, 2002; Borchers *et al.*, 1999). *Pleurotus ostreatus* is the most important commercial mushroom species within the genus *Pleurotus* and it is widespread in temperate areas (Chang, 1999). The species is quite adaptable to a range of climates and substrate materials, making it one of the most preferred edible mushrooms to many farmers.

<table>
<thead>
<tr>
<th></th>
<th>Europe</th>
<th>Asia</th>
<th>N.America</th>
<th>S.America</th>
<th>Africa</th>
<th>Australasia</th>
</tr>
</thead>
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<tr>
<td><em>P. ostreatus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. pulmonarius</em></td>
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<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td><em>P. populinus</em></td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><em>P. djamor</em></td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. eryngii</em></td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td><em>P. tuber-regium</em></td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Source: Zervakis and Balis, (1996); +present; - absent
2.3 Nutrient composition

*Pleurotus* species have been used as human food for centuries due to the variety of flavours and textures they can provide. Nutritional composition of *Pleurotus* species compared to other commonly consumed mushroom species is illustrated in Table 2. *Pleurotus* species are rich in protein and low in fat, and carbohydrates. They also contain vitamins like riboflavin and thiamine that are necessary for good health.

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th>Auricularia species</th>
<th>Lentinus species</th>
<th>Volvariela species</th>
<th>Pleurotus species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%w/w)</td>
<td>7.7</td>
<td>12.7</td>
<td>21.2</td>
<td>30.4</td>
</tr>
<tr>
<td>Fat (%w/w)</td>
<td>0.8</td>
<td>2</td>
<td>10.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Carbohydrate (%w/w)</td>
<td>87.6</td>
<td>79.6</td>
<td>58.6</td>
<td>57.6</td>
</tr>
<tr>
<td>Thiamine (mg/100g of d.wb)</td>
<td>0.2</td>
<td>7.8</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Riboflavin (mg/100g of d.wb)</td>
<td>0.9</td>
<td>4.9</td>
<td>3.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Source: Crisan and Sands (1978)

2.4 Medicinal value

The consumption of *Pleurotus* species has several positive effects on the general human health because of a number of health promoting substances they possess (Kües and Liu, 2000). Many *Pleurotus* species have yielded potential biologically active compounds that exhibit anticancer activity *in vitro* or in animal models (Borchers *et al.*, 1999). These compounds include hemicellulose, polysaccharides, lipopolysaccharides, peptides, proteins, glycoproteins, nucleosides, triterpenoids, complex starches, lectins, lipid derivatives and other metabolites (Kalac and Svobod, 2005; Lindequist *et al.*, 2005).
2.5 Role in environmental management

Many basidiomycetes have the capability to produce simultaneously the hydrolytic and oxidative enzymes which are needed to degrade complex lignocellulosic substrates (Kirk et al., 2008; Buswell et al., 1996). Great diversity within Pleurotus species suggests variability in terms of yield and Biological Efficiency (BE) (Buswell et al., 1996). Pleurotus species can therefore be used to profitably manage the agricultural waste materials left after harvesting and at the same time used as important source of food.

2.6 Life cycle and growth of Pleurotus species

The development of fruiting bodies is a highly organized process, which requires the coordination between genetic, environmental and physiological factors (Kües, 2000). Formation of various tissues within the developing primordium alternate between light and dark phases (Boulianne et al., 2000; Walser et al., 2003) This promotes the elongation of the stripe and the expansion of the cap, giving rise to a fully developed fruiting body (Moore et al., 1979; Kües and Liu, 2000).

During fruit body formation, nuclear fusion and meiosis occur only in the specialized basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each Basidium has commonly four monokaryotic basidiospores. These spores germinate into homokaryotic hyphae (Stamets, 1993; Kang, 2004). A single basidiospore germinates to be a mass of homokaryotic mycelium, each cell of which contains a single haploid nucleus (Chang and Miles, 1989b). The homokaryotic mycelia
continue to grow until the hypha fuse with the other hyphae which have compatible mating type. After fusion between compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed.

2.7 Mating system and gene flow potential

Members of the genus *Pleurotus* are heterothallic (self-sterile) and sexual reproduction is governed by the mating type genes (Eugenio and Anderson, 1968b). The spore gets off the gill and away from the mushroom cap. Once the spores have cleared the bottom of the cap, air currents carry them away. When the spores are a few millimetres away from the cap they can be picked up by the faster winds and carried considerable distances thus enabling them to cross with the same species (Perberdy et al., 1993; Terakawa, 1957).

Mating type genes prevent mating between genetically identical cells. They have a bifactorial tetrapolar incompatibility mating systems which has two unlinked mating type factors designated as A and B (Eugenio and Anderson, 1968a). Factor A controls nuclear pairing, clamp cell formation, coordinate cell division and clamp cell septation whereas factor B is responsible for the control of nuclear migration, septa dissolution and clamp cell fusion. Two monokaryotic mycelia are compatible if they have different alleles at both loci. Multiple allelism for mating type genes was also reported by Terakawa (1957). Because of this multiple allelism of mating type, the out breeding potential is estimated close to 100% in nature and the inbreeding potential can be as low as 25% (Eugenio and Anderson, 1968a)
2.8 Molecular systematic

Most taxonomic and phylogenetic studies of Basidiomycota have been based on the analysis of morphological characters. Recently, relationships among species in several genera of Basidiomycota have often been established by amplification of nuclear sequences by Polymerase Change Reaction (Pringle et al., 2000; Bos, 1996). Investigations have mainly focused on nucleotide sequences of the internal transcribed spacer (ITS) located between the nuclear rDNA 18S and 28S subunit genes, and made it possible to determine the relationships between fungal species from the genus *Pleurotus* (Molcalvo et al., 1995). Ribosomal RNA genes exist in genomes as multiple copies arranged in tandem repeats along one or more chromosomes (Figure 2).

![Figure 2](image.png)

**Figure 2.** A schematic representation of the location of ITS region.

Several features of rDNA make it appropriate for systematic and phylogenetic studies. First, this region of the genome is well characterized and conserved. Many primers already are available to amplify regions of the rDNA repeat that would supply sequence data for a wide range of taxa (White et al., 1990). Second, substantial research has been done on rDNA from many fungi, so ample datasets are available for reference. Additionally, different regions of rDNA evolve at variable rates, which can be used to investigate fungal relationships at different taxonomic levels (Bruns et al., 1991).
2.9 Biogeography and speciation

The information on phylogeny and biogeography provides a framework for understanding the relationship among different components of evolution at the species level, including geographic variation, genetic isolation mechanisms, and morphological evolution (Avise, 1989). Speciation in many mushroom groups is often associated with tremendous levels of genetic divergence that suggest an ancient origin for some species. Because of their ephemeral fruiting patterns, the ranges and distributions of most mushroom species are poorly known (Vilgalys and Sun, 1994).

2.10 Characterization techniques

2.10.1 Morphological tools

Macrofungi have been traditionally characterized based on their micro and macroscopic features. Macroscopic descriptions are based on the size, shape, color and texture of the pileu. Other descriptors include the size of lamellae (height, thickness, breadth, and width), shape (attachment), color and texture; Stipe size including length, width, texture, color and cuticle (aculopellis) feel Color (Kirk et al., 2008). Unfortunately, the phenotypic approach has been largely criticized for its lack of standardized and stable terminology and for its high subjectivity to environmental conditions (Brasier, 1997).
2.10.2 Molecular tools

Perspectives for fingerprinting the genomes of mushrooms have recently arisen from molecular markers based on the polymerase chain reaction. These procedures have provided novel and very powerful reproducible and reliable DNA fingerprinting methods, (Vos et al., 1995). Molecular markers such as rapid amplified polymorphic DNA (RAPD) markers, restriction fragment length polymorphic (RFLP) markers, microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species (Barroso et al., 2000).

2.10.2.1 Random amplified polymorphic DNA markers

Random amplified polymorphic DNA (RAPD) has been successfully applied in the determination of genetic diversity in several mushroom breeding materials intended for crossing (Khush et al., 1991). This is because RAPD technique is simple and efficient, and it requires no prior sequence knowledge (Karp, 1997a). However, the RAPD technique has proved not to be reproducible especially between laboratories as it is highly influenced by experimental conditions (Jones et al., 1997b; Virk et al., 2000; Staub and Serquen, 1996). The preferential amplification of DNA fragments also masks relatedness between taxa or populations and limit reproducibility (Mueller and Wolfenbarger, 1999).
2.10.2.2 Restriction fragment length polymorphic markers

Restriction fragment length polymorphic markers (RFLPs) have been used for analysis of genetic diversity of fungal species because of their specificity and codominant nature (Chyi et al., 1992). However, the RFLP analysis generates relatively small numbers of polymorphisms and is therefore not suitable for studying new or alternative crops such as wild mushrooms where little prior data is available (Pradhan et al., 1992; Lanner et al., 1997).

2.10.2.3 Amplified fragment length polymorphic markers

Amplified fragment length polymorphic (AFLP) is a highly accurate method to detect polymorphisms among individuals, populations, and independently evolving mushroom lineages (Mueller and Wolfenbarger, 1999). The visible polymorphism of AFLP fragments is primarily generated through variations in restriction enzymes sites, and the incorporation of PCR allows for rapid and efficient marker generation. AFLP technique has widely been used to study many mushroom lineages including *Pleurotus ostreatus* (Zhuo et al., 2006 Zheng et al., 2007).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection

Samples of *Pleurotus* species growing on either tree barks or other substrates (wood, soil or leaf litters) were randomly collected from Arabuko Sokoke Forest, Kakamega Forest and Mt. Kenya Forest in Kenya (Table 3). Each collection site constituted a population. Individual sample in each population was collected 10-20 m apart to avoid sampling the same individual several times. Similarly, populations were over 300km apart. Thirteen samples were obtained from Jomo Kenyatta University of Agriculture and Technology (JLUAT).

| Table 3. Wild and cultivated of *Pleurotus* species used in this study |

<table>
<thead>
<tr>
<th>Sample identification codes</th>
<th>AS01</th>
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<th>AS66</th>
<th>AS70</th>
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<td>AS39</td>
<td>KK05</td>
<td>KK47</td>
<td>KK91</td>
<td>MK71</td>
<td>MK16</td>
<td></td>
</tr>
<tr>
<td>AS29</td>
<td>AS55</td>
<td>AS40</td>
<td>KK07</td>
<td>KK50</td>
<td>KK53</td>
<td>MK88</td>
<td>MK20</td>
<td></td>
</tr>
<tr>
<td>AS41</td>
<td>AS41</td>
<td>KK12</td>
<td>93JK</td>
<td>75JK</td>
<td>74JK</td>
<td>48JK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72JK</td>
<td>30JK</td>
<td>92JK</td>
<td>19JK</td>
<td>22JK</td>
<td>25JK</td>
<td>04JK</td>
<td>46JK</td>
<td></td>
</tr>
</tbody>
</table>

AS, MK, KK represent species from Arabuko Sokoke, Mt. Kenya and Kakamega Forests. JK represents cultivated species obtained from JLUAT.
3.2 Preparation of tissue cultures

The young and healthy fruit bodies of *Pleurotus* species were prepared by breaking either the cap or stem to expose the interior tissue, followed by excising and inoculating small tissue fragments using a sterile scalpel in petri dishes containing potato dextrose agar as described by Stamets (2000). A total of 84 samples formed mycelium after incubation at 25-28°C for 5 days. Mycelia were sub cultured after every 10 days until pure cultures were obtained. The cultures were then preserved at 4°C as stock cultures.

3.3 Isolation and visualization of genomic DNA

3.3.1 DNA isolation

Total genomic DNA was extracted following the cetyltrimethyl ammonium bromide method (Doyle and Doyle, 1988). Mycelium (0.1g) of each sample was collected using sterile scalpel from the agar medium and put into 1.2 ml tubes and then ground into fine powder for 5 min using a 2000 Geno/Grinder (Troemner, Inc., Beirut, Lebanon). The crushed mycelia were resuspended in 0.5 ml extraction buffer (100 mM Tris-HCl [pH 8], 2% [wt/vol] CTAB, 50 mM EDTA, 0.7 M Nacl, 1% [vol/vol] β-mercaptoethanol and 1% [w/v] PVP) and incubated for 1 hr at 65°C. Solution (0.5ml) of chloroform-isoamyl alcohol (24:1 vol/vol) was added into the mixture of extraction buffer and the two phases were mixed several times by inverting tubes gently. The resulting emulsion was centrifuged at 4500 x g, 20°C, for 5 min using Beckman
Coulter, Allegra™ 25R Centrifuge (Beckman Coulter, Inc., CA, USA). The upper aqueous phase was mixed with 50µL of NaAC and 400µL of isopropanol in 1.2 ml tubes. Samples of DNA were left to precipitate for 12h at 4°C and centrifuged at 3500 x g, 20°C for 5 min. The supernatant was discarded and pellets air-dried on a clean paper towel in the hood for 1 h before washing two times with an equal volume of 70% ethanol. Pellets of DNA were then resuspended in a low-salt TE buffer (Tris-HCl pH 8.0, EDTA 0.5M) and incubated at 37°C for 30 min with 2µL of DNAse-free RNAsA (10 mg/ml). Purified DNA was then stored at 4°C.

3.3.2 Gel electrophoresis

Quality and quantity of DNA was confirmed using agarose gel electrophoresis. Solution of 1% agarose was prepared by melting 1.0 g agarose in 100 ml of 1x TBE (0.1M Tris-HCl pH 8.0; 0.1M Boric acid; 0.5M EDTA) buffer in a microwave for 2 min. The solution was allowed to cool for 5 min minutes then 1 µl of ethidium bromide was added and stirred to mix. The gel was cast using a supplied tray and comb and allowed to set for of 30 min at 25°C on a flat surface. DNA sample was mixed with 2 µl 1x loading buffer and loaded alongside 5 µl of 1kb ladder into the separate wells. DNA samples were run in the gel for 1hr at 80V after which the gel was photographed using UVP Bioimaging Camera (SFC Inc., CA, USA). Purified DNA was diluted to 200µm/µL using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, USA).
3.4 AFLP analysis

3.4.1 Template preparation and adaptor ligation

AFLP analysis was carried out following the standard procedure described by Vos et al. (1995) and adapted in the AFLP® Plant Mapping protocol of the Applied Biosystems Inc. (Forster City, CA, USA). The suitability of the restriction enzymes used to cut the genomic DNA was initially tested. The genomic DNA was digested with MseI (frequent-4-base cutter-TAA) and EcoRI (rare-6-base cutter-AATTC) restriction enzymes supplied by Applied Biosystems (Forster City, CA, USA) separately and then in combination. A restriction-ligation enzyme master mix was prepared by combining 0.5 μl of T4 DNA ligase (1 U/μl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% (v/v) glycerol) with 4.5 μl adapter/ligation solution (EcoRI/MseI adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 Mm Mg-acetate, 50 mM K-acetate). The genomic DNA (5.5μl) was incubated for 2.5 hr at 37°C with 0.5 μl of EcoRI/MseI (1.25 U/μl each in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA, 50% glycerol (v/v), 0.1% Triton® X-100), and 5 μl of 5× reaction buffer (50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate). The adapter pairs were first annealed to make them double stranded by heating the tubes in a water bath at 95°C for 5 min. The tubes were then left to stand at 25°C for 10 min. This reaction mixture was incubated at room temperature overnight. The restriction-ligation products were diluted by adding 189 μL of low TE buffer (0.1 mM EDTA, 15mM Tris-HCl) to 11 μL of the reaction mixture in a 1.5 ml micro-centrifuge tube to give the appropriate
concentration for subsequent PCR. An aliquot (5 μl) of each digested products was run on 1.5% agarose gel in 1× TBE buffer to check for complete digestion of DNA samples. A 1kb DNA size marker was used to check the size of the DNA.

### 3.4.2 Pre-selective amplification

Amplification of the adapter-ligation restriction products was performed for subsequent selective amplification using pre-selective primers provided by Applied Biosystem, USA. Diluted restriction-igation reaction product (4.0 μl) was mixed with 1.0 μl AFLP pre-selective primer pairs and 15 μl core mix from AFLP ligation and preselective amplification module P/N 402004. PCR amplification was carried out at initial hold-time of 2 min at 72 °C followed by 20 cycles of 20 s at 94 °C, 20 cycles of 30 s at 56 °C and 20 cycles of 2 min at 72 °C and a further hold time of 30 min at 60°C using Applied Biosystem GeneAmp 9700 thermo cycle machine (Applied Biosystem, CA, USA). The pre-selective amplification reaction products were verified by mixing 10 μl of each pre-selective amplification product with 2 μl of 1× loading dye and run on 1.5% agarose gel in 1× TBE buffer at 90 V/cm for 45 min. The gel was stained with 1 μg1/100ml ethidium bromide and photographed using UVP Bioimaging Camera (SFC Inc., Osaka, Japan).

### 3.4.3 Selective amplification

Selective AFLP amplification was performed following the method described by Vos et al. (1995). Pre-selective amplification product (10 μl) was diluted with 190 μl
low salt TE (Tris-EDTA) buffer. The selective PCR amplification was performed using various combinations of two AFLP primers specific for *Eco*RI and *Msp*I primer adapters. A total of 14 primer pairs; *Eco*R1-AAC/*Msp*I-CTC, *Eco*R1-ACA/*Msp*I-CAT, *Eco*R1-AT/*Msp*I-CTG, *Eco*R1-AGG/*Msp*I-CTG, *Eco*R1-AGG/*Msp*I-CAT, *Eco*R1-AG/*Msp*-C, *Eco*R1-AT/*Msp*-CTA, *Eco*R1-AG/*Msp*-CAT, *Eco*R1-TA/*Msp*-C, *Eco*R1-ACA/*Msp*-CTC, *Eco*R1-ACA/*Msp*-CTG, *Eco*R1-ACA/*Msp*-CTA, *Eco*R1-AGG/*Msp*-CTC and *Eco*R1-AGG/*Msp*-CTA were screened on eight samples for protocol optimization and to identify the primer pairs that produced the most polymorphic fragments. The diluted pre-selective amplification product (3 μl) was mixed with 1 μl fluorescently labeled *Eco*RI primer, 1 μl of *Msp*I primer and 15 μl of AFLP core mix. Amplification of selective reaction product was performed on a Applied Biosystems GeneAmp 9700 thermocycler (ABI, Forster City, CA, USA) with the following parameters; an initial two minutes at 94 ºC followed by one cycle of 94 ºC for 20 s, 66 ºC for 30 s and 72 ºC for 2 min. This cycle was repeated eight times with a lowering of the annealing temperature of 1 ºC per cycle. This was followed by 20 cycles of 94 ºC for 20 s, 56 ºC for 30 s and 72 ºC for 2 min and a further hold time of 30 min at 60 ºC. The selective PCR product was prepared by adding 12 μl of Gene Scan 500 LIZ internal size standard supplied by Applied Biosystems Inc. (Forster City, CA, USA) to 1 ml deionised formamide HiDi. Loading buffer (9 lµ) was added to 1 μl of the selective amplification products in a MicroAmp PCR Plate and resolved in ABI capillary electrophoresis system (ABI Inc., Forster City, CA, USA) and analyzed on ABI 3130 genetic analyzer (Forster City, CA, USA).
3.5 DNA amplification and sequencing

DNA (2 µl) isolated from 12 strains of *Pleurotus* species were mixed with 18 µl Accupower PCR Premix cat. #K-2016 (Bioneer Inc. Daejeon, South Korea) and amplified using forward ITS-1 (5’-TCCGTAGGTGAACCTGCGG-3’) primers and reverse ITS-4 (5’–TCCTCGCTTATTGATATGC-3’) primers. PCR was performed using GeneAmp 9700 Eppendorf thermocycler (Applied Biosystem Inc., CA, USA) with the following program: 95°C for 1 min, 35 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 2 min, and a final elongation at 72°C for 10 min. PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and examined on a 1.6% (w/v) agarose gel stained with ethidium bromide. For sequencing, an ABI PRISM 3.1 BigDye Terminator kit (Perkin Elmer, USA) was used and the electrophoresis was carried out on an ABI PRISM 3700 Genetic Analyzer. Sequencing was carried out for both strands using the forward and reverse primers used for initial amplification.

3.6 Sequence editing and alignment

Resulting sequences with readable chromatograms were assembled and edited using DNA Baser Version 3 (DNASTar, Inc. Madison, USA). However, unreadable chromatograms characterized by a more or less sudden overlapping of sequence peaks starting at certain given positions in the sequence could not be assembled together in the DNA Baser program. It was therefore not possible to edit these chromatograms manually and to reconstruct complete sequence hence they were removed from the
analyses. Each unique ITS sequences were first used as a query to retrieve closely related sequences from the GeneBank.

The unique ITS and the retrieved sequences were aligned using CLUSTALW multiple alignment program available at http://www.ebi.ac.uk/Tools/msa/clustalw2. The aligned sequences were visually checked, adjusted and then analyzed using MEGA v5.05. In this analysis, because of the inclusion of divergent sequences and the differences in length among the aligned DNA sequences, a large number of gaps (i.e. insertions and deletions) were introduced in the aligned dataset. As a result, the gaps were treated as missing data.

3.7 Data analysis

Genotypes were scored for presence (1) and absence (0) of AFLP bands using GeneMapper Software version 4.0 (Applied Biosystem Inc., Forster City, CA, USA). Only sharp and precise bands were scored to generate a data matrix. Category bins were created to group peaks based on the sizes of the allele. A threshold peak height was set at 50 -500 relative fluorescent units (Palsson et al., 1999). Bands present in all accessions were not scored. Distance matrices for all pairs of genotypes were constructed from the AFLP data matrix using the Euclidean distance method (Kaufman and Rousseeuw, 1990).

The AFLP data set was subjected to Nei’s gene diversity index (H) to quantify variability within the population and to investigate genetically close populations. Allele frequency-based Nei’s genetic distance and unweighted pair group method of arithmetic
averages (UPGMA) clustering methods were employed using tools for population genetic analysis (TFPGA) software version 1.3 (North Arizona University, Arizona, USA) (Miller, 1997). Cluster analysis was performed using the genetic distance matrices generated by the Euclidean distance method to reveal the patterns of genetic relationships among genotypes. The results of cluster analysis were presented in the form of dendrograms to infer relationships among genotypes.

Neighbor-Joining (NJ) and Maximum Parsimony (MP) analyses were performed as described by Tamura et al. (2011). NJ and MP analyses were performed using MEGA software version 5.05 developed by Arizona State University, USA (Tamura et al., 2011). Support for phylogenetic groupings was assessed by bootstrap analysis (1,000 replicates) with random addition of sequences during each heuristic search (Felsenstein, 1985). Only significant bootstrap replication frequencies above 50% were indicated. Other indices for the generated topology, including tree length, a consistency index as well as retention index were calculated. The database search of sequences for a possible match to the ITS sequences was performed using the basic sequence alignment Basic Local Alignment Search Tool (BLAST) program run against the GenBank database (http://www.ncbi.nlm.nih.gov/blastn) on 5th October, 2011. Additional four sequences were included as reference ITS sequences. The four reference sequences were chosen based on their comparable sequence lengths to the 12 sequences of ITS and in several cases the availability of two or more strains for the same species.
CHAPTER FOUR

4.0 RESULTS

4.1 AFLP polymorphism

A total of 643 AFLP loci were generated from 84 samples of *Pleurotus* species using five primer combinations (Table 4). The primer combinations used produced 330 polymorphic loci across all the species accounting for 51% of the total scorable loci (Table 4). The number of scorable loci generated by each AFLP primer pair varied from 20 to 228. The number of polymorphic loci for each primer pair varied from 16 to 116. The loci ranged in size from 51 to 497 bp as generated by GeneMapper version 4.1. The number of loci varied for different primer combinations. The primer combination of 5’*Eco* + AGG- *Mse* + CTC 3’ gave the smallest number of both scorable (20) and polymorphic (16) loci, respectively while 5’*Eco* + AAC- *Mse* + CTG 3’ gave the highest number of both scorable (228) and polymorphic (116) loci. Five primer pairs used generated an average of 7 scorable and 4 polymorphic loci across all the 84 species studied.

Table 4. AFLP primers and polymorphism

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Total number of loci</th>
<th>Polymorphic loci</th>
<th>Polymorphism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-AAC/M-CTC</td>
<td>228</td>
<td>116</td>
<td>52</td>
</tr>
<tr>
<td>E-ACA/M-CAT</td>
<td>115</td>
<td>102</td>
<td>89</td>
</tr>
<tr>
<td>E-AT/M-CTG</td>
<td>160</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>E-AGG/M-CTG</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>E-AGG/CAT</td>
<td>120</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>643</td>
<td>330</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Genetic diversity of Kenyan *Pleurotus* species

The genetic diversity among the studied *Pleurotus* species was very small as revealed by the estimates of Nei’s unbiased genetic diversity in Table 5. The genetic diversity values ranged from 0.27 to 0.24 between species obtained from Arabuko Sokoke and Mt. Kenya. Cultivated species had similar levels of genetic diversity with the wild species from Mt. Kenya. The same order of gene diversity was revealed by Shannon’s information index (I) and heterozygosity values in which wild species from Arabuko Sokoke was the most heterozygous (H=0.26) while both the JKUAT and Mt. Kenya population had the least heterozygosity values (H=0.23) each. The percentage polymorphic loci were also in close agreement with the diversity estimates. Wild species from Arabuko Sokoke had high diversity estimates and percentage polymorphic loci. The observed number of alleles (na) and the effective number of alleles (ne) were also high in populations with high diversity (Arabuko Sokoke) and low in those with low diversity (JKUAT and Mt. Kenya). Nei’s unbiased diversity values among the studied species were very small. This was the case with Shannon Information Index, percentage polymorphic loci and heterozygosity values.
Table 5. Genetic diversity estimates among 4 populations of *Pleurotus* species

<table>
<thead>
<tr>
<th>Population ID</th>
<th>Sample size</th>
<th>na</th>
<th>ne</th>
<th>h</th>
<th>I</th>
<th>% loci</th>
<th>H</th>
</tr>
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<tbody>
<tr>
<td>AS</td>
<td>34</td>
<td>1.98</td>
<td>1.42</td>
<td>0.27</td>
<td>0.41</td>
<td>99.1</td>
<td>0.26</td>
</tr>
<tr>
<td>KK</td>
<td>25</td>
<td>1.88</td>
<td>1.39</td>
<td>0.25</td>
<td>0.39</td>
<td>92.1</td>
<td>0.25</td>
</tr>
<tr>
<td>MK</td>
<td>11</td>
<td>1.73</td>
<td>1.37</td>
<td>0.24</td>
<td>0.37</td>
<td>83.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean</td>
<td>23</td>
<td>1.86</td>
<td>1.39</td>
<td>0.25</td>
<td>0.39</td>
<td>91.43</td>
<td>0.25</td>
</tr>
<tr>
<td>JKUAT</td>
<td>13</td>
<td>1.72</td>
<td>1.37</td>
<td>0.24</td>
<td>0.35</td>
<td>82.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

na= Observed number of alleles; ne= Effective number of alleles; h = Nei's unbiased measure of genetic diversity; I = Shannon's Information index; % loci = Percentage polymorphic loci; H=mean heterozygosity. JKUAT represents population of cultivated species. Populations of wild species are represented by AS- Arabuko Sokoke, KK-Kakamega, MK-Mt. Kenya

4.3 Analysis of Molecular Variance

Summaries of analyses of molecular variance (AMOVA) are represented in Table 6. Of the total observed allele frequency variations, the majority was found from within populations (89 %). The remaining 11% could be attributed to frequency variations among populations. The contributions from each of these sources were significantly greater than 0, indicating statistically significant (P<0.001) genetic differentiations within rather than among populations. The degree of gene differentiation among populations in terms of allele frequency (F_{ST}) was also moderately low (0.125).
Table 6. Summary results of AMOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Variance components</th>
<th>% variation</th>
<th>P-values</th>
<th>F&lt;sub&gt;st&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among population</td>
<td>6.611</td>
<td>11%</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Within Population</td>
<td>52.848</td>
<td>89%</td>
<td>&lt;0.001</td>
<td>0.125</td>
</tr>
<tr>
<td>Total</td>
<td>59.459</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.4 Cluster analysis

A dendrogram based on Nei’s genetic distance (D) is illustrated in Figure 4. The dendrogram clustered 71 wild and 13 cultivated *Pleurotus* species into 3 major clades. Clade I and II consisted mainly of wild species (KK, MK, AS) with bootstrap values of 38% and 67% respectively. The cultivated species (JK) formed a distinct cluster with a bootstrap support of 66%. Distribution of the wild species within each cluster did not correspond to their geographical origin.
Figure 3. Dendogram of clustering analysis of 84 Pleurotus species. KK (blue) AS (red) MK (pink) and JK (green) represents species from Kakamega, Arabuko Sokoke, Mt. Kenya and cultivated species from JKUAT respectively.
4.5 Internal transcribed spacer sequence data

The phylogenetic relationships based on the ITS sequences was obtained by the neighbor joining (NJ) tree (Figure 4).

![Phylogenetic tree of 12 Pleurotus species based on ITS sequences. AS, KK, and MK represent species collected from Arabuko Sokoke, Kakamega, and Mt. Kenya respectively. JK are cultivated species obtained from JKUAT.](image)

Phylogenetic tree revealed little genetic differences within the species studied; indicating that these species were very similar. Two clades were identified within 12 Pleurotus species with clade I consisting of 50KK, 28AS, 20MK, 31MK, 72JK whereas clade II consisted of 5KK, 7KK, 39AS, 52AS, 71MK, 31MK and 92JK, 30JK. Species
in clade I grouped with the reference strains with well supported bootstrap value (100%).

Sequences (Appendix 1) for a possible match to the rDNA ITS sequence of 12 strains of *Pleurotus* species yielded 1098 hits on the query sequence in the nucleotide databases at the NCBI. The highest match was *Pleurotus* sp. ‘Florida’ (AY368662), ITS-1, 5.8 S, and ITS-2 nuclear rDNA sequence. The score for this match is 1110 bits with an E value of zero. The alignment (Appendix 2) of 604 total nucleotides showed 88% minimum and 100% maximum identities. The identified sequences had associated species identification in the GenBank database, belonging to *Pleurotus ostreatus* (JF75887), *Pleurotus eryngii* (EU233964), and *Pleurotus* sp. YL005 (FJ687276).
CHAPTER FIVE

5.0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussions

The five AFLP primer pairs (E + ACA/M + CTC, E + AT/M + CAT, E + ACA/M + CTA, E + ACC/M + CTC, and E + AT/M + CTC) used revealed 330 polymorphic loci. This confirmed the suitability of AFLP markers to evaluate genetic variability in mushroom lineages at species level (Terefework et al., 2001). The use of AFLP markers in assaying genetic variations among Basidiomycetes fungi has been widely reported in many mushroom lineages (Mueller and Wolfenbarger, 1999). The used primer pairs can be good candidate primer combinations to verify genetic diversity of other Pleurotus species.

Closely related or similar Pleurotus species were distributed across Kakamega (KK), Mt. Kenya (MK) and Arabuko Sokoke (AS) Forests (Figure 3). Close similarity within and among the populations (Table 5) suggested the likelihood of a small degree of variability in terms of growth characteristics, colour, size, Biological efficiency (BE) and susceptibility to both pests and diseases. Minimal gene flow events could be responsible for the observed low genetic variations within the wild species. Similar studies reported low levels of variations in species with a restricted distribution, or those that have long been cultivated for commercial purposes, or in populations with just a
few individuals, or in species that reproduce exclusively asexually (Old et al., 1984; Burdon and Roelfs, 1985).

The cultivated species (JK) formed a distinct cluster with a few species distributed within the wild populations. Production of many cultivars from limited number of elite lines could be responsible for low diversity observed within the cultivated species. Distribution of a few cultivated species within the populations of wild species could be due to human-mediated spore dispersal following the increased setting up of several mushroom units in many parts of the country.

The identification of Pleurotus ostreatus, Pleurotus eryngii, Pleurotus sp. ‘Florida’ and Pleurotus sp. YL005 as part of the genetic diversity of Pleurotus species in Kenya suggests that efforts to domesticate wild genotypes could save local farmers from the burden of importing similar species. Close similarity between the wild and cultivated species also indicated close phylogenetic relationships between the wild and cultivated species. It therefore implies that the wild species have great potential for commercial cultivation.

5.2 Conclusions

Wild Pleurotus species from different parts of Kenya are similar to the cultivated species. However, diversity within species is high. The high genetic diversity within populations can be used for selection of more commercially suitable Pleurotus species. AFLP markers revealed polymorphism across the 84 samples used. AFLP
markers and rDNA ITS sequence analysis showed that the wild and cultivated *Pleurotus* species were closely related.

### 5.3 Recommendations

More analysis of multiple additional genes from larger collections of wild and cultivated *Pleurotus* species from different locations in Kenya is needed to fully understand their diversity and molecular phylogeny. Conservation of wild *Pleurotus* species is also necessary to maintain the genetic diversity of this species in nature. Selection of commercial mushrooms from the wild population is also possible.
REFERENCES


Terakawa, H. (1957). The nuclear behavior and the morphogenesis in Pleurotus ostreatus

*Science Paper of College of General Education, University of Tokyo* 7: 61-68.


APPENDICES

Appendix 1: Sequence alignment of ITS region from 12 strains of *Pleurotus* species

Sequence alignment from genotypes of *Pleurotus* species. Sequence alignments were performed using the Clustal_W algorithm (Higgins *et al.*, 1991) available at the European Molecular Biology Laboratory (EMBL-EBI). Identical regions that were aligned across all genotypes are indicated with asterisk.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Sequence (alignment)</th>
</tr>
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<tbody>
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<td>Pleurotus_ostreatus_JF70007_</td>
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</tr>
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<td>Pleurotus_sp.YL005_Fv87276_</td>
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</tr>
<tr>
<td>Pleurotus_eryngii_EU233964_</td>
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</tr>
<tr>
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</tr>
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<td>28AS</td>
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</tr>
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Pleurotus_sp.'Florida'_AY368666 AGCCACCTT-TGGGCACCTGTTGCATCGATGAGGATGAGGCCAGGAAATTGA  380
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39AS  A---CACTCCATAGT---GATTCTTAAATGATC  604

*  **  *  *  **  ***  **  ***
Appendix 2: Blast search results for 12 *Pleurotus* species

72JK> gb|JF758887.1| Pleurotus ostreatus strain pl.n0087 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=655

Score = 1110 bits (601), Expect = 0.0  Identities = 601/601 (100%), Gaps = 0/601 (0%)  Strand=Plus/Plus

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25AS> gb:EU238964.1 | Pleurotus eryngii isolate D3808.1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence Length=607

Score = 1099 bits (595), Expect = 0.0 Identities = 595/599 (99%), Gaps = 1/599 (0%) Strand=Plus/Plus

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Query
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Subject
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Query
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Subject
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Query
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Subject
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Query
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Subject
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Sequence comparison:

**Query**

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56
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**Subject**

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56
```

Sequence identity: 100.0%

**Description**

The image contains a sequence alignment between a query and a subject. The alignment shows a high degree of similarity, with 100% identity, indicating a strong match. The sequences are compared using the Needleman-Wunsch algorithm, which is a dynamic programming approach used for finding the optimal global alignment between two sequences.

The alignment includes a score of 1110 bits and an expectation of 0.0, suggesting a statistically significant match. The identities are calculated as 100%, and gaps are minimal, indicating a very precise alignment.

This type of sequence comparison is crucial in bioinformatics for identifying homologous sequences, understanding evolutionary relationships, and validating databases.
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SKKs gb|AY368662.1| Pleurotus sp. 'Florida' strain ASI 2181 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence
Length=638

Score = 1110 bits (601), Expect = 0.0 Identities = 601/601 (100%), Gaps = 0/601 (0%) Stran=Plus/Minus

Query 1  TCAAAATTGTCAATTTGTCTTCCTGGGAGATAGAGACGTTCCTGTCTCTATCCTGGAG  60

Subject 604 TCAAAATTGTCAATTTGTCTTCCTGGGAGATAGAGACGTTCCTGTCTCTATCCTGGAG  549

Query 61  TTGATGAGTGAATTTATACATACATACATGCGGAGAGGACGTTCCTGTCTCTATCCTGGAG  120

Subject 544 TTGATGAGTGAATTTATACATACATACATGCGGAGAGGACGTTCCTGTCTCTATCCTGGAG  485

Query 121  AAAGAAGCAAGCAGCTGTAAGGAGACCATCCACAAATCCAAACACTGCACATGGA  180

Subject 484 AAAGAAGCAAGCAGCTGTAAGGAGACCATCCACAAATCCAAACACTGCACATGGA  425

Query 181  AGAACCACAAAGTGAGTTGAGAATTTAATGACAGTCAACACAGAGATGCGGATGG  240

Subject 424 AGAACCACAAAGTGAGTTGAGAATTTAATGACAGTCAACACAGAGATGCGGATGG  365

Query 241  CAAGGGGCGCAAGGCTGCGCTCAAGAAGATTGATCACGTCAATCTGCAATCATA  300

Subject 364 CAAGGGGCGCAAGGCTGCGCTCAAGAAGATTGATCACGTCAATCTGCAATCATA  305

Query 301  CTTCTGCATTGCCTCGGTTCTCACTCGGGGAGAGGAGATGCGGATGG  360

Subject 304 CTTCTGCATTGCCTCGGTTCTCACTCGGGGAGAGGAGATGCGGATGG  245

Query 361  TTGATGAGTGAATTTATACATACATACATGCGGAGAGGACGTTCCTGTCTCTATCCTGGAG  420

Subject 244 TTGATGAGTGAATTTATACATACATACATGCGGAGAGGACGTTCCTGTCTCTATCCTGGAG  480

Query 421  GTGGAAGAATGAAAAAGAGGCTGAGTGTCAAGCAGCAGCTCTAAATCCAGACAAAGGTC  480

Subject 104 GTGGAAGAATGAAAAAGAGGCTGAGTGTCAAGCAGCAGCTCTAAATCCAGACAAAGGTC  125

Query 481  TGACGCTTTGAGACGCTCTGAGAACTCTAAATCCAGACAAAGGTC  540

Subject 124 TGACGCTTTGAGACGCTCTGAGAACTCTAAATCCAGACAAAGGTC  65

Query 541  GTGGAAGGCTGACATGCCGCTCCTAGGGCCAGCAGAGAATGCTGTAATGATG  600

Subject 64 GTGGAAGGCTGACATGCCGCTCCTAGGGCCAGCAGAGAATGCTGTAATGATG  5

Query 601 C 601

Subject 4 C 4
7KEJ|gb|JF758887.1| Pleurotus ostreatus strain pln0037 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=659

Score = 1110 bits (601), Expect = 0.0  Identities = 601/601 (100%), Gaps = 0/601 (0%) Strand=Plus/Minus

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