EVALUATION OF *Ceiba pentandra* FOR STEM DIEBACK DISEASE RESISTANCE AND CHARACTERIZATION BY MOLECULAR MARKERS

BY

COSMAS SORNGMENENYE ABENGMENENG

A THESIS SUBMITTED TO THE DEPARTMENT OF SILVICULTURE AND FOREST MANAGEMENT, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY IN SILVICULTURE AND FOREST MANAGEMENT

FACULTY OF RENEWABLE NATURAL RESOURCES,

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

SEPTEMBER 2013

DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge; it contains neither material previously published by another person nor material which has been accepted for the award of any other degree of the University. References to other peoples' works have been duly acknowledged.

…… …….. ……..

Student Name Signature Date

Certified by:

…… …….. ……..

1. Supervisor's Name Signature Date

…… …….. ……..

2. Supervisors' Name Signature Date

…… …….. ……..

3. Supervisor's Name Signature Date

Certified by:

…… …….. ……..

Head of Department's Name Signature Date

DEDICATION

This project work is dedicated to the Almighty God, my Lord and Saviour Jesus Christ, and the Holy Spirit of God for being the source of my wisdom and the anchor of my life. I also dedicate this work to my parents, Mr. and Mrs. Abengmeneng, and my siblings for their prayers, patience, support and understanding. Last but not the least, I dedicate this work to my children and to all brilliant but needy students as an encouragement to be determined and work hard.

ACKNOWLEDGEMENT

I express my sincere thanks to the Almighty God for His wonderful wisdom, infinite love and the spiritual guidance that enabled me to produce this piece of work. I remain deeply indebted to my supervisors, Dr. Philomena Kumapley (Department of Silviculture and Forest Management, FRNR-KNUST), Dr. Daniel A. Ofori (Forestry Research Institute of Ghana-Kumasi; and currently at World Agroforestry Centre-ICRAF, Nairobi-Kenya) and Prof. Richard Akromah (Department of Crop and Soil Sciences, Faculty of Agriculture-KNUST), for being ready for consultation even outside working days and for their constructive criticisms that have helped shaped this write up. I am very grateful to Dr. Marian D. Quain (Crops Research Institute of Ghana, Fumesua-Kumasi) for guiding me in my practical work. I owe many thanks to the International Foundation for Science (IFS), Kwame Nkrumah University of Science and Technology and Tropenbos International Ghana for their financial support. My appreciation also goes to the Director, Management and Staff of the Forestry Research Institute of Ghana, Fumesua-Kumasi and the Crops Research Institute, Fumesua-Kumasi, for giving me access to their research environment and facilities. A special note of thanks also goes to all the Laboratory Technicians at the Forestry Research Institute of Ghana-Kumasi and Crops Research Institute, Fumesua-Kuamsi, for their assistance during my practical time in the Biotechnology Laboratories of their respective institutions. I am also grateful to all my lecturers, friends and relations whose contributions in diverse ways have led to the successful completion of this thesis.

ABSTRACT

Ceiba pentandra (L.) Gartn is an important multi-purpose tree species in Ghana and demand for it is rising daily. However, the occurrence of stem dieback disease at the nursery and plantations, coupled with the absence of adequate information on resistance levels and the genetic diversity within and among populations, has limited the success of efforts geared at devising efficient strategies for its sustainable management. This study assessed resistance levels through progeny evaluation at both the nursery and in the field. Narrow sense heritability and genetic gain in stem height were also estimated. The diversity among accessions was characterized using five morphological traits. The genetic diversity and gene flow within and among five populations of the species were also studied using Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) markers. The study also looked at markers that are linked to stem dieback resistance. Screening at the nursery from September to November (SNS) showed survival level ranged from 0.20% to 79.20 %. March to May screening (MMS) showed that all the 59 accessions screened had varied levels of survival ranging from 24.4 % to 100 %. Mortality rate was generally higher in the SNS than the MMS. Field study showed that 5 (13.51 %), 9 (24.33%) and 23 (62.16 %) accessions, out of 37 screened, had survival levels between 70.00 and 100 %, 50.00 and 69.90 % and below 50.00 %, respectively. There were significant differences in mean mortality rates among accessions in the nursery and field studies and also in mean stem height and mean diameter growth ($P < 0.001$). Narrow sense heritability was 0.56 and genetic gain in height ranged from -29.58 to 20.89 cm/yr. Mean population diversity index, using the Shannon Information Index (I), was 0.425 ± 0.024, 0.306 ± 0.027 and 0.371 ± 0.018 for the RAPD, ISSR and the combined RAPD and ISSR, respectively. Gene flow estimates

showed low to moderate differences among populations with Gst values at 0.0751, 0.0736 and 0.0799 for the RAPD, ISSR and combined RAPD and ISSR, respectively. Ninety-eight percent of the population differentiation was attributed to within population variation with RAPD polymorphism. ISSR and the combined RAPD and ISSR both showed that a high proportion of genetic diversity resided within populations. Chi-square and G-square tests showed differences among population. Percentage polymorphic loci, Ewens-Watterson Neutrality Test, Nei diversity and Shannon diversity indices, showed Dry Semi-deciduous Forest Inner Zone as the most diverse population. Principal component analysis, defined by axes 1 and 2, accounted for 67.15% of the total variation among accessions. None of the ten markers used was able to differentiate between resistant and susceptible accessions. Nine accessions were identified for conservation as seed trees. Dry Semi-deciduous Forest Inner Zone (DSDFZ-Inner) < Dry Semi-deciduous Forest Outer Zone (DSDFZ-Outlier) < Guinea Savanna Zone (GSZ) < Moist Semi-deciduous Forest Zone (MSDFZ) < Moist/Wet Evergreen Forest Zone (M/WEFZ), in order of decreasing importance, should be preferred in *C. pentandra* seed collection expedition. Decision making for long-term conservation of *C. pentandra* should be made on the basis of both morphological and molecular considerations. Sampling for seed trees should be concentrated on selecting resistant and unrelated accessions within populations rather than collecting bits from the entire range of the species. Known resistant and susceptible lines should be used in further assessment for dieback resistant markers. A large number of dominant markers and the use of co-dominant markers should also be investigated.

TABLE OF CONTENTS

CHAPTER **PAGE**

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

Item	Full meaning
ADB	African Development Bank
ATO	African Timber Organization
C&I	Criteria and Indicators
cm/yr	Centimetres per year
dbh	Diameter at Breast Height
df	Degree of Freedom
DNA	Deoxyribonucleic Acid
EC	European Commission
EDTA	Ethylenediaminetetraacetic Acid
FAO	Food and Agriculture Organization of the United Nations
Fig	Figure
ft	Feet
Ha	Hectare
GHG	Green House Gas
Gt	Gigatone
ITTO	International Tropical Timber Organization
ISSRs	Inter Simple Sequence Repeats
MCPFE	Ministerial Conference on the Protection of Forests in Europe
MTS	Modified Taungya System
M	Molar
µM	Micromolar
µl	Microlitres
ml	Millilitres

mm	Millimetres
m	Metres
m^3	Cubic Metres
mg	Milligrammes
MSS	Mean Sum of Square
%	Percent
NFPDP	National Forest Plantation Development Programme
NTFP	Non-Timber Forest Products
PEFC	Programme for the Endorsement of Forest Certification schemes
PCR	Polymerase Chain Reaction
REDD	Reduced Emissions from Deforestation and forest Degradation
REDD+	Reduced Emissions from Deforestation and forest Degradation with biodiversity conservation and carbon stock components
SFM	Sustainable Forest Management
UNCED	United Nations Conference on Environment and Development
UNEP	United Nations Environment Programme
UNFCCC	United Nations Framework Convention on Climate Change
RAPD	Random Amplified Polymorphic DNA
rpm	Revolution Per Minute
TAE	Tris-acetate Ethlylenediaminetetraacetic Acid
TFPGA	Tools for Population Genetic Analyses ver.1.3
SE	Standard Error
UPGMA	Unweighted Pair Group Method with Arithmetic Averages

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Forests play many significant roles that support the existence of life on earth. They serve as habitats to about two-thirds of all terrestrial species and control a wide variety of ecological processes (Stahl and Christophersen, 2010). Forests also act as sinks for carbon and other biogeochemical substances (Robalino and Herrera, 2009; Madeira, 2008; Vinceti and Petri, 2004) absorbing about 4.8 Gt of carbon in the tropics (Sukhdev, 2010) and as sources of wood for industry and domestic use (Sawyer and Boedhihartono, 2010; Bongers *et al*., 2004; Edmonds *et al*., 2000). In Ghana for instance, the timber industry alone contributes about 6% of the gross domestic product (GDP), gives jobs to over 75,000 people directly, and provides livelihood to about two million people indirectly (Abeney, 1999).

The continued potential of forests to play the above mentioned roles is being threatened by several factors including deforestation (Sukhdev, 2010), invasive species (Jackson and Howard, 2010) and the non-integration of sustainable forestry into the market and its poor access to mainstream private capital (Stevens *et al*., 2009). Among these, deforestation has been the leading factor for discussions in recent times. Edmonds *et al*. (2000) reported that extensive cutting of trees has dramatically changed the natural conditions of forest in the Northern and Southern hemispheres. Several works have revealed that the extent of forest in the tropics is diminishing at a high rate (Bongers *et al.*, 2004; Miyawaki and Abe, 2004; Okali and Eyog-Matig, 2004; Tho, 1990). In Africa for instance, deforestation rate is about 4 million hectares per annum. The causes and timing of deforestation vary with regions

and with forest types. However, factors which are common to most of the regions and forest types attract the most of national concern. Significant among these are; (1) susceptibility of forest to natural factors such as insect pests, diseases, extreme climatic events, fire damage, (2) over exploitation, (3) uncontrolled mining, (4) forest clearing for agricultural purposes and; (5) pressure from population growth (Kelatwang and Garzuglia, 2006). The issues of forest decline have, therefore, become a major concern worldwide in the twenty-first century and stakeholders in forests and forestry are looking at how to manage forests to maximize environmental, economic, social and cultural functions without jeopardizing their extent and quality. This has led to the adoption of the concept of sustainable forest management at the United Nations Conference on Environment and Development's (UNCED) Rio Declaration and Statement in 1992 (Hajjar *et al*., 2009).

Plantations have been considered as critical tool in sustainable forest management and serve as the optimal solution to the perennial problems of over exploitation of the natural forest resources (Chamshama and Nwonwu, 2004). The success of plantation forests in the West, East and Central Africa are, however, limited by several factors. Notable among these are illegal felling, low productivity, low wood quality arising from the neglect, and damaging attacks from pests and diseases (Chamshama and Nwonwu, 2004). According to FAO (2001), susceptibility to pests and diseases in plantations has been shown to increase when species-site matching is poor, planting stock with narrow genetic base are used, optimum stocking levels exceed carrying capacity and through the establishment of large scale monoculture. Several advances have been made to overcome these limitations including the selection of desirable genotypes and the development of vegetative

propagation techniques and establishment of clonal seed orchards for production of high quality seeds (Ofori *et al*., 1996; Lawson, 1994).

Ghana has put in several measures in the fight against forest decline. The most recent is the launching of its National Forest Plantation Development Programme (NFPDP) in September 2001 which, among other things, is aimed at encouraging the development of a sustainable forest resource base to meet future demand for industrial timber and enhance environmental quality. The programme operates under the Modified Taungya System (MTS); a strategy in which farmers are allocated parcels of degraded forest reserve lands to produce food crops for themselves whilst assisting in the establishment and maintenance of trees on the same piece of land. An estimated 6,934.4 kg of seeds of various species have been collected and an area of about 60,000 ha planted since the initiation of the programme. That notwithstanding, wood deficit situation still persists in Ghana at about 4-5 million m^3/yr and 14 million m^3/yr for timber and fuel wood respectively (Ghana Forest Service Division, 2005). Hence, efforts are being stepped up to rapidly rectify the situation, one of which is the establishment of plantations. FAO (2007) reports that the area of forest plantation in Ghana is increasing steadily from the year 2000. This area includes that established under the NFPDP. *C. pentandra* is one of the species that has been selected and is being planted under the NFPDP (Ghana Forest Service Division, 2005). This species has several desirable silvicultural traits, which qualified it for inclusion in the NFPDP. Cobbinah *et al.* (2001) reported some of these qualities to include fast growth, quality of wood, short rotation period and the ability to grow under diverse site conditions.

1.2 Statement of the problem

The success of *C. pentandra* under the NFPDP has been hindered by the presence of stem dieback disease, which kills *C. pentandra* seedlings both in nursery and in the field. The disease has been associated with several species of fungi including *Fusarium solani, Lasiodiplodia theobromae, Fusarium oxysporum, F. pallidoroseum, Aspergillus flavus, Macrophomina* species and *Collettotrichum capsici* (Apetorbor *et al.,* 2003). Attempts have been made to control the disease with fungicides, such as kocide (77 % Copper hydroxide) and aliette (80 % fosetylaluminium). These, however, were found to have a suppressive effect on the growth of *C. pentandra* (Apetorgor *et al*., 2003) and, hence, are deficient in solving the problem. Chemical control, in general, is considered an unattractive control option in forestry. Not only is it expensive but also some of the chemicals are extremely toxic to mammals and other components of the ecosystem. Also, other chemicals disturb the ecological balance of non-pathogenic organisms, whilst many of them are stable and pollute the environment. Furthermore, some chemicals are severely phytotoxic under certain environmental conditions and pests and pathogens can develop resistance to the chemicals making them ineffective (Saxena, 2004; Russell, 1978). Besides, chemical control approaches are rarely successfully used for trees, except for orchard trees (Garbelotto *et al*., 2001). Also, the cost of materials, equipment and labour to control diseases using chemicals are considerable (Lamb *et al*., 1978). The rise in public reluctance to allow chemical use in forest environments renders the development of cost-effective selection and development of genetic resistance a preferred alternative (Wu and Ying, 1997). Biological control, on the other hand, has been given very low priority as its effectiveness is difficult to monitor in the forest environment. The use of resistant genotypes is, therefore, seen as the optimal choice for disease control.

Conventional breeding for resistance, though achievable, is time consuming coupled with the long term nature of tree life cycles (Paolella, 1998; Biggs *et al.*, 1992; Russel, 1978). The use genetically of resistant planting stock in arresting forest decline has, therefore, been recommended in several studies (Cleary *et al*., 2008; Michler *et al*., 2005; Garbelotto *et al*., 2001; Wu and Ying, 1997; Yang *et al*., 1997; Namkoong, 1991; Carson and Carson, 1989; Bingham *et al*., 1971). According to Brown (1996), the origin and maintenance of genetic resistance becomes important in addressing issues of ineffectiveness of varietal resistance or fungicide application in controlling plant diseases. Unlike other control methods, genetic resistance cannot be affected by weather, mechanical or other failures and improper planning that may result in a delay in the application of control measures. This is because it is a basic component of the plant system (Gilbert, 2002). Ofori (2001) notes that the increasing demand for wood can be met by the use of ecologically adapted and genetically improved trees with known genetic variability. He argues further that, notwithstanding this, there is still limited knowledge on the population genetics of most tropical forest trees. *C. pentandra* is, therefore, not an exception to this observation despite its recognition as one of the principal tree species in Ghana's forest cover (FAO, 2007; Okali and Eyog-Matig, 2004). According to Senbeta and Keketay (2001), the remedy expected from plantations can only be met if the right species are planted at the right sites, their proper management practices put in place and reliable seed sources in the vicinity identified. Meanwhile little is known about the ecological and genetic features of *C. pentandra* that could help in devising efficient strategies for its conservation. Furthermore *C. pentandra* has not yet been well studied for geographic and genetic variation in stem dieback resistance. Thus, information on other adaptive traits which are important pre-requisites for studying *C.*

pentandra and stem dieback co-evolution needs to be gathered. Also, few attempts have been made to develop informative molecular markers for *C. pentandra*. The application of molecular marker analysis will, therefore, be useful in defining the genetic structure and enhance marker-assisted selection. This study, therefore, was designed to investigate genetic resistance in *C. pentandra* to stem dieback disease through progeny and provenance evaluation. This was complemented with the use of DNA marker analysis to identify DNA markers linked with dieback disease resistance.

1.3 Research objectives

The overall objective of the research was to increase the number of plantation species in Ghana by identifying dieback resistant genotypes and nursery seasons that reduce the prevalence of stem die-back disease in *C. pentandra*.

The specific objectives were to:

1. identify resistant mother trees to dieback and /or provenances that have individual trees resistant to the stem dieback disease,
2. determine the genetic diversity within and among *C. pentandra* populations in Ghana , and
3. identify molecular markers linked with dieback disease resistance.

1.4 Key questions of the research

1. Which mother trees are more tolerant to the stem dieback disease?
2. Is there any provenance that has more tolerant genotypes?
3. What is the level of genetic diversity within and among *C. pentandra* populations in Ghana?

4. Which molecular markers are linked with dieback disease resistance?

CHAPTER 2

LITERATURE REVIEW

2.1 Characteristics of *Ceiba pentandra* (L.) Gaertn

Ceiba pentandra (L.) Gaertn, of the family Bombacaceae, is a native species of the natural forests of tropical America and tropical Asia (Siepel *et al*., 2004; Burkill, 1985; Irvine, 1961). The genus *Ceiba* has 17 species and close relatives of *C. pentandra* such as *C. aesculifolia* and *C. grandiflora* occur in Jalisco, Mexico (Quesada *et al.,* 2004). *C. pentandra* is commonly called the silk or kapok tree and is known to have a pantropical distribution (Dick *et al.,* 2007; Lobo *et al*., 2005) with populations in the Peruvian and Brazilian Amazon being endangered due to over exploitation (Brondani *et al*., 2003). Dick *et al*. (2007) identified three forms of *C. pentandra* namely the rainforest form, the cultivated form and the savanna form (Table 2.1.1). The natural and exotic ranges of *C. pentandra* are shown in figure 2.1.1.

The wood is used for making stools in the Ashanti Region of Ghana, domestic utensils, boxes, wooden figures, quivers, drums, dugout canoes, plates, trays and coffins, especially, in the Ada area of Ghana (Burkill, 1985; Irvine, 1961). It is also suitable for wooden sandals, heels, rafts, floats, lifeboats, models, particle board and for papermaking. The seeds are very rich in unsaturated fatty acids such as palmitic, linoleic, oleic, and stearic acids (Burkill, 1985). The fibre, commonly called kapok, is used for stuffing cushions, pillows and mattresses, insulation, as an absorbent material and tinder. The gum is eaten to relieve stomach upset, whereas the leaves and fruits are used as a laxative and infusion from the leaf is used for colic treatment in human and livestock (Irvine, 1961).

C. pentandra can be found in various types of forests ranging from the moist evergreen and deciduous forests, to the dry and gallery forests. As a pioneer species in Ghana, it mostly occurs in secondary forests, in gaps and along roadsides (Hall and Swaine, 1981). The species is a fast growing pioneer and tolerates low fertility soils. This makes it a potentially important species for forest regeneration and timber production on disturbed lands. It is particularly successful at colonizing highly disturbed areas. It grows well on light to medium (texture) and acid to neutral soils with rainfall ranging between 750 mm and 3000 mm (Cobbinah *et al*., 2001). Figure 2.1 shows some of the planted natural and exotic ranges of *C. pentandra*. It can grow up to a height of 60 m (200 ft) with a diameter of 2 m or more (6 ft or more) and its buttresses can extend to 8 m (25 ft) up the bole (Irvine, 1961). The buttresses are often developed partly in response to wind and crown symmetry, mostly on the windward side of the bole. The bark and stem of the young trees of some varieties of *C. pentandra* are armed with spines. The leaves are alternate, palmately compound and are obovate to elliptic and have fringe of hair at the top when young with about 5-9 leaflets (Holmgren *et al*., 2004).

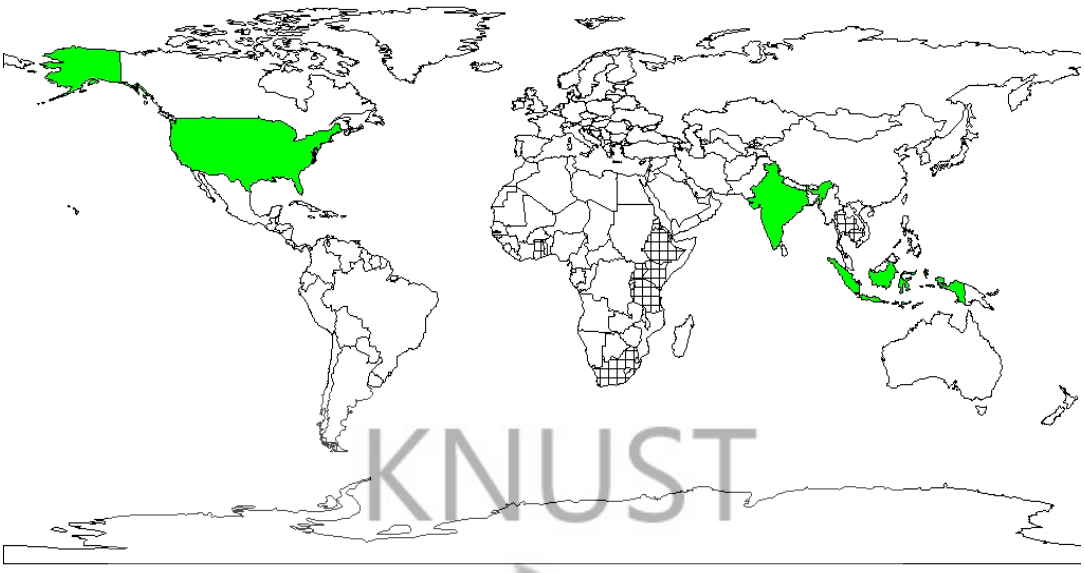
C. pentandra is propagated by seed or cuttings with a planting space of 3 x 3 m for 8 thinning years and 8 x 8 m for 12 thinning years. It begins fruit bearing at the age of 6 - 8 years (Burkil, 1985). It flowers from November to February and the fruits mature between March and April (Cobbinah *et al*., 2001). Flowering is more frequent on forest edges or in drier forests. Flowering and fruiting take place when the tree is leafless and this is believed to be an adaptation that facilitates both mammalian-pollination and wind-dispersal. *C. pentandra* trees lose their leaves in the dry season as a result of drought-deciduousness (Lobo *et al*., 2005). It has a rotation period of 30 - 45 years and an exploitable diameter of 50 cm (Cobbinah *et al*., 2001). Its greatest

density of regeneration is about 1000 stems/km² at 5-30 cm breast height diameters (dbh) in burnt forests (Hawthorne *et al.*, 1995). The seeds are often dispersed by wind or water (Dick *et al*., 2007).

Table 2.1.1 Morphological and cytological differences between three forms of *C. pentandra*.

Character	Rainforest form	Cultivated form	Savanna form
Trunk	Spiny (prickles) and straight	Usually spineless and straight	Spineless and often forked
Buttress	Large	Small	None
Branches	Horizontal	Ascending	Strongly ascending
Reproduction	Super annual	Annual	Annual
Leaves	Narrow	Broader	Broadest
Fruit	Capsule dehiscent	Capsule indehiscent	Capsule dehiscent
Chromosomes 2*n*	80, 88	72 – 80 (variable)	72

Source: Modified from Dick et al. (2007).



Key: Exotic range Natural range

Fig.2.1.1 Planted natural and exotic ranges of *C. pentandra*.
Source: Orwa et al. (2009).

Note: The map shows countries where the species has been planted and it neither suggests that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted.

2.2 Sustainable forest management (SFM)

Sustainable forest management (SFM), according to ITTO (2005), is the process of managing permanent forest land to achieve one or more clearly defined management objectives with regard to the continuous flow of desired forest products and services without undue reduction in its inherent values and future productivity and without undue undesirable effects on the physical and social environment. In the view of Islam *et al*. (2010), SFM is the way of managing forests so that growth exceeds timber harvest, whilst ensuring the availability of economic, environmental and social qualities for the sustainability of forest dependent communities and ecosystems and the forest itself. SFM has, therefore, become a leading issue of discussion worldwide (Obster, 1998) and broad consensus has been reached on the principles, guidelines,

criteria and indicators for it at the international level to ensure uniformity among all participating nations. Some of these include the Montreal Process (Criteria and Indicators for the Conservation and Sustainable Management of Temperate and Boreal Forests), ITTO Process (for tropical forests) or ATO/ITTO Process (for tropical African forests Near East) and the Criteria and Indicators for the Sustainable Management in Dry-zone Africa) (PEFC Council, 2010).

The criteria and indicators (C & I) define objectives and priorities for national forest policies and strategies and monitor implementation progress, in compliance with performance-based certification standards. Whilst the criteria describe a desired state or dynamics of the biological or social and allow a verdict on the degree of achievement of an objective in a specified case, the indicators allow for objectivity in verifying whether the specified state called for by a criterion is reached or not (Poshen, 2000; Prabhu *et al.*, 1998). In Africa, several steps have been taken towards implementing these criteria and indicators. The African Timber Organization, for instance, is testing C & I for the rainforest zones of Central and West Africa with about twenty-one countries in Sub-Saharan Africa working on C & I for dry zones (Prabhu *et al.*, 1998). They are also promoting policies on sustainable forest management, whilst encouraging the domestication of medicinal plants, participatory forest management and delegation of forest management to private agencies (Kelatwang and Garzuglia, 2006). The measures for SFM call for the preservation of the remaining natural forests, the establishment of many more plantations and the rehabilitation of degraded lands. A number of techniques have been suggested in this regard, including natural regeneration, assisted natural regeneration, fire, enrichment planting, plantations, agroforestry and soil and water conservation (Blay *et al.*, 2004).

2.3 Plantations as tools for sustainable forest management

The increased demand for wood for domestic and export industries, fuel-wood and charcoal, and for various non-wood forest products is adding up to the factors exerting pressure on natural forests in Africa. This pressure is being speeded up by urbanization, industrialization and human population growth making the natural forest a less reliable option for attaining sustainable forest management (SFM) and, at the same time, increasing the need for plantations. Several factors are responsible for the shift to plantations in Africa. These include the poorly understood techniques of natural forest management (Lawson, 1994), rapid rate of exploitation of the natural forests, increasing demand for wood and wood products, increased rate of industrialization, slow growth and low yields of the indigenous natural forests, and the increasing export demand for timber and other forest products, which cannot be met from the natural forests (Chamshama and Nwonwu, 2004). Plantations also enhance natural regeneration of native woody species as well as the process of native forest succession over time by attracting seed dispersal agents and providing a net effect for colonizing native species. Other factors are: the concern for meeting environmental services by reserving natural forests as protected areas, technological changes in wood processing, scientific advances that improve productivity while reducing cost through bulk production, and the significant advances that have been made in plantation technology. This has led to a substantial expansion in forest plantations in the past 20 years in Africa (Senbeta and Keketay, 2001). Returns from plantations in Africa have also been encouraging and Lawson (1994) reported estimated internal rates of 18.6 % for short-rotation system, 15.5 % and 13.6 % for Taungya and Line Planting Systems, respectively.

2.4 Dieback disease: a threat to sustainable forest management

Diseases are an important factor in the dynamics of forest populations, though many of their effects usually go unnoticed (Byrne, 2000). Forest diseases cause drastic reduction in forest productivity. Some of these losses include, loss of income to investors, increased cost of products to consumers, loss of natural resources and the restricted production of certain plants (Maloy, 1993; Agrios, 1988). Other disease-related factors are the reduction in current market value, time used in growing the species, the money spent, the potential future losses and the amenity value (Peace, 1962). These losses vary with the plant or plant product, pathogen, locality, environment, control methods practiced and the combination of these factors (Agrios, 1988). Therefore, more efforts have been put in place to protect trees against diseases (Bandara, 1990).

Forest dieback is a condition in trees or woody plants in which peripheral parts are killed, either by parasites, such as fungi and beetles, or due to conditions like acid rain and drought (Allen, 2009). It is part of a complex set of diseases which are similar in symptoms but the exact causes are mysterious, difficult to identify and require detailed long term studies (Girsh and Shankara, 2008; Boa, 1995). The effects of these diseases begin when healthy tissues are weakened by stress and culminate upon attack by weak facultative parasites (saprogens that attack weakened tissues). The existence, origin and causes of these diseases still remain a controversy (Dhakal *et al*., 2004; Houston, 1997; Boa, 1995). Dieback disease kills tree species that are susceptible to it mostly starting from the tip. There are two basic phases of dieback. First, the dieback phase, where there is profuse dying back of tree buds, twigs, branches and rootlets, resulting from stress with the possibility of trees recovering after the stress has abated. Second, the decline phase, where the entire vitality of the

tree lessens, usually caused by secondary-action organism and result in tree death (Houston, 1997).

Different fungal species cause dieback in different tree species. For instance, *Phomopsis azadirachtae* of the Phomopsis genus has been identified as the fungus responsible for dieback of *Azadirachta indica* in India (Girsh and Shankara, 2008). Furthermore, *Heavea brasiliensis* in three rubber estates in Bangladesh attacked by dieback was associated with *Phemopsis* species. The disease begins with the death of immature leaves, young twigs and progressed to larger branches with tree death occurring in some cases (Rahman, 1990). In Bangladesh, *Cytospora* species also causes severe dieback in *S. apetala* (Rahman *et al*., 1990). In plantations, fungi of the genus *Fusiform* and some insect species of the insect order *Lepidoptera* were associated with the dieback disease syndrome of *Dalbergia sissoo* in its natural ranges of South Asia (Boa, 1995). There are several reports on the devastating effects of dieback disease on forest and its ecosystems. In Bagachattra of Chittagong in Bangladesh, dieback in the Keora species (*Sonneratia apetala*) cause thin crowns and heavy death of side branches leaving only few healthy ones close to the apex (Rahman, 1990). In the jackfruit tree (*Artocarpus heterophyllus*) dieback disease causes symptoms ranging from change in leaf colour from green to light green, then to reddish yellow and finally abscission with brown colouration in the transition zone between the healthy and the dead wood (Rahman, 1990). Economic loss from stem dieback is usually caused by repeated annual defoliation and the dieback of twigs and branches, which weaken the trees and make them more susceptible to other diseases, physical injuries and insect damage (Boa, 1995). There are different modes of transmission of dieback. For instance, *Fusarium* dieback in *Acer negundo* and *Persea Americana* is reported to be transmitted by an ambrosia species called *Euwallacea*

fornicates. The beetles carry the fungus from tree to tree or within the same tree and use their mandibles to deposit the fungal spores on the walls of the tunnels they create. The dieback fungus then attacks the vascular tissue, disrupting water, carbohydrate, and mineral flow within the tree and eventually causing branch die back or death of the entire tree (Hodel *et al*., 2012).

In Ghana, dieback in *C. pentandra* was first observed in a mixed plantation in the moist semi-deciduous forest and later in the nursery. The study showed that damping-off fungi caused serious damage to *C. pentandra* seedlings in germination beds before and after emergence. The study isolated *Fusarium solani* and *Lasiodiplodia theobromae* from the seeds and dieback stems of *C. pentandra*. Also, *Fusarium oxysporum, F. pallidoroseum, Aspergillus flavus* and *Macrophomina* species were isolated only from the seeds. Further, *Collettotrichum capsici* was isolated from leaves of diseased seedlings in the study (Apetorgbor *et al*., 2003). The authors achieved some amount of success in controlling the disease using fungicides, such as kocide and aliette. They, however, noted that these fungicides retarded the growth of *C. pentandra* and recommended further research for more efficient means of controlling the disease.

2.5 Disease control, resistance and susceptibility in plants

Resistance is any inherited characteristic of a host plant which lessens the effects of parasitism (Russell, 1978) or the inherent ability of plants to remain relatively unaffected by diseases. Resistance in trees varies from extreme resistance to extreme susceptibility with intermediates or low resistance lying in between. Resistance to plant diseases covers a lot of biological phenomena including genetic, physiological, anatomical or mechanical resistance, tolerance and escape (Maloy, 1993). Resistance

can be classified in several ways. Using the mode of inheritance, resistance can be monogenic, oligogenic or polygenic when it is controlled by one, a few or many genes, respectively (Maloy, 1993; Russell, 1978). In epidemiological terms, resistance can either be horizontal or vertical depending on whether the resistance is effective against all or certain genetic variants of a particular pathogen (Van Der Plank, 1963). Resistance can also be durable, when it is long lasting, or transient when lasting for a short time (Johnson and Law, 1975). Based on the kinds of resistance mechanism involved, resistance can be classified as active (resistance developed by a host in response to pathogen attack) and passive (where resistance mechanism exists before a pathogen attacks). Seedling and mature or adult tree resistance may be classified according to whether seedlings or mature plants are attacked, accordingly. Qualitative resistance is one in which the frequency of distribution of resistant and susceptible plants is discontinuous, while quantitative resistance has a continuous gradation between resistance and susceptibility within plant populations. Immunity is sometimes used in place of resistance. However, whereas immunity is absolute, resistance is relative (Russell, 1978).

The tremendous losses from tree diseases means that only limited gains will be obtained from tree establishment unless trees are grown without much harm or death caused by pest, diseases or severe environment (Zobel and Talbert, 1984). The control of forest diseases is, therefore, of paramount importance. Disease control is defined as the application of practices devised to reduce the damage or loss from forest diseases. There are different degrees of disease control including complete control (total elimination of disease but which is rarely achieved), partial control (the most common type and which depends on factors of the disease triangle), and profitable or economic control (where efforts are geared towards the greatest reduction in disease loss at the

lowest cost). There are also different methods of disease control. Some of these are avoidance of the pathogen, exclusion of inoculum, eradication of inoculum, protection and the use of resistant plants (Infantino *et al*., 2006; Maloy, 1993; Bilgrami and Dube, 1976).

As destructive agents, plant pathogens can cause mortality, reduce fitness of individual plants resulting in the rapid decline of the populations of host species, or dramatic shifts in the structure or composition of plant communities (Gilbert, 2002). Several defense types of resistant mechanisms have been evolved and exhibited by plants in response to pathogen invasion including the constitutive defence. The first line of defence, made up of a number of physical and chemical barriers, is present in tissues before the colonization of pathogens (Bonello *et al*., 2006). This phase of resistance to diseases in trees could be due to hypersensitive reactions, phytoalexins, host-pathogen specific toxins, barriers to pathogen invasion and others (Michler *et al*., 2005; Subramania *et al*., 2005; Carson and Carson, 1989). Antimicrobial phyloalexins are produced by the parenchyma cells and provide a chemical boundary that retards the spread of invading microorganisms. These defence systems of trees depend on their genetic constituents. Hence, differences in genetic variability results in differences in degree of defence (Shigo, 1984). The second defence line is the existence of localized or induced resistance where the plants protect themselves by mobilizing chemical defenses shortly after attack (Bonello *et al*., 2006). This is sometimes referred to as active response (Jones and Takemoto, 2004). During this process, local changes in cell metabolism occur within minutes after attack to respond to the initial invasion, but those involving cell division and differentiation are slower and may take days to months to complete (Bonello *et al*., 2006). Cell metabolism usually targets containing the invasion and enhancing the defence of the plant against

further attack. The third line of defence is the systematic or induced resistance, which is mediated by the accumulation of hydroxybenzoic acid derivative salicylic acid, jasmonic acid, and ethylene (Durrant and Dong, 2004). In part, the systemic effects of pathogenic infection on phenylpropanoid and terpenoid metabolisms, as well as the de-differentiation of phloem and xylem tissues to form traumatic resin ducts causing stronger resin flow, also enhance the effectiveness of systematic or induced resistance. The fungal cell walls themselves are also degraded by pathogenesis-related proteins including lytic enzymes and accumulate following infection to form another defence system. Plants deploy all these localized defence systems to do away with the pathogen initially. However, when the inducing pathogen defies these systems and grows, the infection progresses, and the plant becomes increasingly stressed due to reduction in nutrient and water absorption capacity especially when the roots are first affected (Bonello *et al*., 2006). Eventually, the infected tree becomes susceptible to subsequent attacks by pathogens and dies as a result of mechanical disruption, dysfunction and infection (Shigo, 1984).

Natural defence has been responsible for the long life of most forest trees. For instance, many healthy individual elm trees, representing different varieties, forms and ecotypes, of 300 to 700 years were still alive in the natural forests in the Izvoarele Nerei-Caras Severin and Pojorata-Suceava districts in Rome after having resisted successive Dutch elm disease attacks due to their natural genetic variability (Borlea, 2004). Also, healthy butternut trees were found growing adjacent to trees infected and killed by butternut canker in areas throughout the butternut range (Michler *et al*., 2005). Liberty, a new disease resistance apple cultivar, has also been shown to be resistant to fire blight under natural conditions (Lamb *et al*., 1978). Several studies have looked at natural resistance. Some of these included the study of natural tree

resistance to insect pest (Kay and Wratten, 2003), resistance of indigenous elm species to Dutch elm disease (Borlea, 2003) and forest tree resistance to damage by elephants (Sheil and Salim, 2004). Research has shown that, younger forests have lesser natural resistance than older ones. For instance, susceptibility to Dutch elm disease forest was found to be 50% in forest less than 40 years and 22% in those over 100 years (Borlea, 2004).

2.6 Genetic variation in forest trees

Genetic recombination and mutation are the main sources of genetic variation. Meiosis recombines alleles of genes on the same chromosome through chiasmata formation and those from different chromosomes via random segregation at anaphase I. Meiosis process sometimes leads to errors, which when inherited are termed mutations. When mutation occurs in genes and results in the replacement of one or more nucleotides, it alters the triplet code leading to the production of different or no proteins and sometimes a new phenotype (Murray *et al.*, 2000). According to these authors, this phenotypic variation in turn is partitioned into genetic and environmental variation of which the genetic component is subdivided into additive and non-additive variances. Frequently, phenotypic and genotypic variations tend to be higher in severe physically and biologically stressed species, especially, for quantitative traits used in estimating survival (Geburek, 2000).

Genetic diversity is usually expressed in terms of richness or evenness, with the former taking precedence over the latter in its estimation. Richness refers to the number of different types that can be observed, whereas evenness refers to the equality in the frequencies of the different types (Kindt *et al.*, 2008). Genetic diversity occurs at different levels: among different species, within a single species, among

populations, families and individuals (clones) (Guberek, 2000). In every population, the level of genetic diversity is affected by life history, genetic and ecological characteristics of the organisms in it (Yeh, 2000). Genetic diversity is a basic component of the long term stability of forest trees, because it is the determinant of the adaptive potential. Hence, a good knowledge of the genetic mechanisms affecting tree adaptation and productivity for proper forest management is a pre-requisite (Byrne, 2000).

Conservation of genetic diversity, on the other hand, is essential for the adaptation of populations to changing environments, the reduction of risks due to short-term seed and population viability from inbreeding depression and for the maintenance of genetic resources for future uses (Boshier and Amaral, 2004). Processes, such as gene loss, horizontal gene transfer and rapid rates of sequence divergence, within a lineage resulting in vast variation within and among species and population have resulted in the evolution of specific genes over the past 400 million years (Kirst *et al*., 2003). This variability created by nature is what the tree improver needs to know, isolate and package into desirable trees and multiply them (Zobel and Talbert, 1984). Intraspecific diversity, for instance, is found as an essential parameter for the management of species for maintenance of their evolutionary potential (Rajagodal *et al*., 2000). The basic aim of gene conservation is to maintain the genetic diversity needed to allow the population to adapt to future environmental conditions and to ensure that populations are well adapted to current conditions for adequate members of individuals to survive and reproduce (Namkoong *et al*., 2000). The maintenance of genetic variation is therefore seen as a key component of conservation strategies for several reasons: it guides against the overall vulnerability to diseases in both plantation and natural populations of tree species, its breeding value is relevant

for both commercial plantings and natural populations and its application in native populations enables resistant individuals to be naturally selected when disease pressure is applied to the population (Byrne, 2000). Other reasons for the conservation of genetic diversity, include the adaptation of populations to changing environmental conditions, and the need to maintain genetic resources for possible future uses (Boshier *et al.*, 2004).

Previously, genetic variation was mainly assessed using morphological traits, as this approach is easier and cheaper. It, however, has its own limitations: while only few morphological characters can be utilized for characterization, many of them are very unreliable due to their environmental dependency and sometimes result in misleading conclusion (Islam and Shepherd, 1992). As a result, the phyto-geographical patterns of variation in forest trees are now being studied on a wider scale using molecular markers (Boshier *et al.*, 2004). Molecular markers have several advantages over the morphological characters: whilst most of the morphological characters are sensitive to environmental conditions and growth stages the species, molecular markers are insensitive to such factors and are abundantly present (Bhattacharya *et al.*, 2010). Plant molecular techniques are used to identify and or confirm the identity of plants or plant products and to develop new plant varieties. They can also be used to study the contribution of major genes to speciation in wild plant population, and in the management of plant populations composed of endangered or unwanted species (Henry, 1997). According to this author, plant breeders also use them to analyze genetic resources for the selection of parents for use in crosses, to determine the relationship between breeding lines and to compare pedigree-based assessment of relationships. Molecular marker analysis is also useful

for identifying genomic segments that are responsible for genetic variance of a trait, and for the selection of superior genotypes (Selvaraj *et al*., 2009).

Genetic marker analysis and mapping of forest trees have progressed from isozymes, through restriction fragment length polymorphism analysis, Polymerase Chain Reaction (PCR) and now to genomics (Adams 1983). Though hybridization based techniques may be cheaper to conduct, they require a large quantity of Deoxyribonucleic Acid (DNA) (10 µl or more) and the availability of a suitable DNA probe from the plant being studied (Henry, 1997). The introduction of PCR base techniques (Mullis and Faloona, 1987) has led to the development of new high-throughput marker systems with a large number of protocols being developed for the construction of high-density linkage maps of individual forest trees (Tulsieram *et al.*, 1992). The frequently used marker systems include random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), inter simple sequence repeats (ISSRs) (Zietkiewicz *et al.*, 1994), microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994) and amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995). The PCR based-techniques have advantages in terms of speed and sensitivity. The choice of a technique depends on the objective of the investigation, the output required, the genetic material, the facilities and funds available, restricted public availability and intellectual property rights (Kirst *et al*., 2003; Henry, 1997). Markers with low cost, low labour requirements, high reliability are mostly used for genome mapping, marker-assisted selection, phylogenic studies and conservation (Bornet and Branchard, 2001).

Random Amplified Polymorphic DNA (RAPD) analysis (Williams *et al.*, 1990) is based on the principle of polymerase chain reaction (PCR). RAPD markers

provide information for the understanding of genome structure and evolution (Neale and Williams, 1991). The advantages of RAPD technology include the rapidity of screening for polymorphisms, the identification of a large number of markers and its potential automation (Sobral and Honeycutt, 1993). Also, it can be applied to un-studied taxa due to its non-requirement of DNA sequence information. RAPD technology enables complex genetic variation to be resolved due to the large number of markers the technique produces (Williams *et al*., 1990). RAPD markers are very quick and easy to develop due to their requirement of arbitrary primer sequences (Karp *et al.,* 1997). They are also highly sensitive to experimental conditions such as the genomic DNA concentration and the conditions of the reaction assay (Raflaski *et al.,* 1996). Many RAPD markers are reliable for mapping purposes and are reproducible within and between laboratories with proper reaction conditions (Kirst *et al*., 2003). The use of RAPD makers involves the analysis of a large number of loci, thus providing a more complete evaluation of the genome as compared to other biochemical and molecular markers. Other advantages of RAPD included its low cost and requirement of small amount of DNA. More successes of the RAPD marker system have been reported in plant molecular biology, where it has been used in the detection of genetic variation, the construction of linkage maps and in the identification of markers linked with genes of interest in bulk segregation. RAPD markers have also been used in quantitative traits dissection experiments, germplasm evaluation, genetic fingerprinting and manipulation of genes as well as in the identification of good seed sources, genetic diversity and mating system (Plomio *et al*., 1995). The RAPD technology, however, cannot be used to estimate heterozygosity directly due to the dominant nature of its marker and it has the tendency of producing non-homologous co-migrating bands when comparing species

(Rieseberg, 1996). These different marker systems vary greatly with regard to the inherent information they provide, mode of inheritance, accessibility and reliability. RAPD markers have been successfully used to study the population genetic structure in many forest tree species (Deng *et al*., 2006; Ofori *et al*., 2006; Archak *et al*, 2000; Ofori *et al*., 2001; Cardoso *et al.*, 1998) in the certification of trees and their putative hybrids (Nkongolo *et al.*, 2005). RAPD and ISSR markers have many things in common: they are simple, provide a quick way to screen for DNA polymorphism, require very small amounts of DNA and do not need prior information on template DNA sequence (Jasieniuk and Maxwell, 2001). ISSR markers, however, reveal much higher number of polymorphic fragments and also have more specific reaction than RAPD markers (Williams et al. 1990)

ISSR is based on inter tandem repeats of short DNA sequences. These sequences are highly polymorphic in their sizes even among closely related genotypes due to the lack of evolutionary functional constraints in the non-functioning regions (Aida *et al*., 2012). The primers use in ISSR technique are long (16 - 25 bp) resulting in high stringency with long (200 - 2000 bp) amplification products which are amenable to detection by agarose and polyacrylamide gel electrophoresis (Reddy *et al.*, 2002). The commonly used molecular techniques have some major limitation including the low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to design specific primers for SSR makers (Aida *et al*., 2012). ISSR markers, however, have relatively low cost, high polymorphism and good reproducibility and therefore overcome most of these limitations (Reddy *et al*., 2002). Again, the target sequences for ISSR markers are abundant throughout the eukaryotic genome (Ansari *et al*., 2012). The evolutionary rate of change in ISSR markers is also more linked to the trait of interest than many other types of DNA markers

(Bhattacharya *et al.*, 2010). ISSR marker technique has therefore been recommended as a useful and reliable method for determining genetic polymorphism (Zietkiewicz *et al.*, 1994). ISSR makers are used as highly informative markers for genome mapping, gene tagging, the study of genetic diversity and phylogenetics and evolutionary biology in a wide range of species (Reddy *et al.*, 2002). For instance, ISSR markers were used in the analysis of genetic diversity in wheat (Aida *et al*., 2012), *Vigna umbellate* (Muthusamy *et al*., 2008), cattle, goat and sheep (Askari *et al.*, 2011), water melon cultivars (Amnon *et al*., 2004), *Cymbopogon winterianus* (Bhattacharya *et al*., 2010), *Tectona grandis* (Ansari *et al*., 2012), bluegrass species (Arslan and Tamkoc 2011) and Grevillea (Pharmawati *et al*., 2004). ISSR markers, however, have some shortfalls. For instance, fragments with the same mobility originating from non-homologous regions could cause distortions in the estimates of genetic similarities using ISSR polymorphism (Sanchez *et al*., 1996).

2.7 Screening for disease resistance

Screening for disease resistance can be done using *in-vitro* and *ex vitro* methods. *In vitro* or laboratory selected resistance occurs when exposure to a toxin in the laboratory results in a heritable decrease in susceptibility of a laboratory strain (Bruce *et al.*, 2009). The *in vitro* techniques have precise control of the physical and chemical environmental conditions, ability to rapidly screen a large number of genotypes in a small space, and the exclusion of other microorganisms. The techniques also have less time and cost requirements and take advantage of simplified experimental host-parasite systems in which 1 or a few host cell types can be uniformly challenged by a pathogen or host-specific toxin (Ostry *et al*., 1988). *In vitro* techniques, however, have some shortfalls: resistance is determined by multiple factors, and may be

governed by a series of biochemical reactions influenced by many host, pathogen, and environmental factors which might not be present in *in vitro* testing. Also, the general health of the host and accompanying stress factors may differ from those of intact plants in the field. Again, the potential absence of preformed defensive barriers, induced inhibitory compounds, and organized tissues may limit the usefulness of *in vitro* screening techniques, especially if cell and tissue culture systems are used. Further, pathogenic organisms can occur in cultures and regenerated plants and *in vitro* selection or a specific trait may result in the selection undesirable traits (Cousin *et al.,* 1990).

Ex vitro or field-evolved (or field-selected) resistance, on the other hand, is a genetically based decrease in susceptibility of a population to a toxin due to the exposure of the population to the toxin in the field (Tabashnik, 1994). *Ex vitro* screening for disease resistance could be time-consuming, costly, and dependent upon natural fluctuations in inoculum abundance and weather factors which influence pathogen spread, infection, disease development and expression. Besides, trees in field tests are interact with many pathogens and insect pests and this can affect the test results. Disease resistance or tolerance under field conditions can also be affected by the many environmental variables and the developmental stage and general health of the host which in turn are influence by the complex and dynamic host-pathogen interactions (Ostry *et al*., 1988).

2.8 Assessment of disease resistance

Direct assessment of disease on the plant is usually done by evaluating two parameters: incidence and severity. Incidence is expressed as the percentage of infected/dead plants within a sampling unit whereas severity is expressed as the

percentage of diseased plant tissue in a given area (Infantino *et al*., 2006). Incidence assessment is most suitable for infections leading to the death of the plants whilst severity assessment is necessary when resistance is inherited quantitatively with a continuous gradient of symptom severity in a host plant population (Russell, 1978). Disease severity scales, mostly in the range of 0 - 5 or 1 - 9, have been developed for many pathosystems, depending on the extent of the damage in the infected plant (James, 1974). For instance, a scale of 1 - 5 has been used to rate *Aphanomyces* root rot symptoms on above ground organs as: 1 = healthy plants, 2 = slight yellowing of lower leaves, 3= necrosis of the lower leaves up to the 3rd or 4th node with some stunting, 4 = necrosis of at least half or more of the plants with stunting, more than half of plants in a row dead and 5 = all plants dead or nearly so (Pilet-Nayel *et al.*, 2002). Again, a 0 - 5 scale was used to rate *Fusarium solani* resistance in pea roots where: 0 = no symptoms, 1 = slight hypocotyl lesions, 2 = lesions coalescing around epicotyls and hypocotyls, 3 = lesions starting to spread into the root system with root tips starting to be infected, 4 = epicotyl, hypocotyl and root system almost completely infected and only slight amount of white, uninfected tissue left, 5 = completely infected root (Grunwald *et al*., 2003). Among these, percentage scales accommodate the full range of expression of symptoms and are more preferable. Scoring on severity scales, is usually done visually using the above ground organs, on principal roots or on other plant tissues, based on the type of disease and the accuracy required (James, 1974). Visual scoring, however, has some challenges. For instance, visual acuity depends on the logarithm of the intensity of the stimulus, so the eye can accurately assess only very low or very high levels of the disease and hence making symptom standardization difficult (James, 1974). The challenges presented by visual

scoring could be overcome through the combination of both direct and indirect assessment methods such as molecular marker techniques (Infantino *et al*., 2006).

CHAPTER 3

GERMPLASM COLLECTION AND SEED GERMINATION

3.1 Introduction

Germplasm is the genetic material, seed or vegetative, which is used in plant propagation. The concept behind germplasm collection is based on the Weismannism Theory as proposed by August Weismann in 1886. The theory stipulates that the contents of the reproductive cells (sperms or ova) are passed on unchanged from generation to generation regardless of changes in the rest of the body (Oxford Dictionary of Biology, 2000). Germplasm is the source of genetic potential of living organisms. It is maintained in living tissues, such as the embryo of seed, and gets lost when the seed dies. The total genetic potential (gene pool), of a species is represented by many individuals in a population rather than a single individual necessitating the collection of germplasm. Germplasm collection is also done to identify needed resistant genes when a new crop-threatening hazard, such as a disease, appears. An effective germplasm collection provides broad genetic variation useful for crop improvement, botanical research and conservation of plant biodiversity (Wilkes, 1992). This, in turn, provides flexibility to changing demands and environmental conditions as well as protection against a future loss in performance through inbreeding depression (Jaenicke, 1999). Hence, Wright (1976) recommended that seed for progeny and provenance tests be collected from a number of widely distributed stands, mostly natural stands, across the entire range of the species concerned and the seedlings raised under similar conditions. The germplasm in the current study is seed of *C. pentandra*.

Seed is the structure in plants that develops from the ovule after or without fertilization (Oxford Dictionary of Biology, 2000). Seeds are a convenient form of germplasm use and exchange, and the most common mode of genebank preservation (Wilkes, 1992). As a result, seeds are still the preferred starting materials for propagation of many vital tree species through mass multiplication and selection for tree improvement (Mng'omba *et al*., 2007). Seeds for plantation establishment can be obtained directly from native or naturalized stands, stands established specifically for seed production and from a commercial or non-commercial seed supplier (Jaenicke, 1999). The maintenance of seed viability during seed collection, processing, storage and germination has, therefore, become prime to species conservation strategies (Engelmann *et al.*, 2007). Seed processing is the preparation of seed samples for further use. It is make up of several processes including seed cleaning and drying. Seed cleaning involves the removal of debris, physical contaminants, inert materials, damaged and infected seeds and seeds of other species. Seeds drying, on the other hand, involves the reduction of seeds moisture content to recommended levels for storage using techniques that are not detrimental to seed viability (ISTA 2005). Seed moisture content is the amount of water in a seed sample and is expressed as a percentage of the weight of water contained in a seed sample to the total weight of the seed sample (ISTA, 2005). Seed viability is the percentage of germinating seed in a seedlot and is measured by germinating seed under conditions that would be applied during normal germination. It provides reference level of germination for users (Jaenicke, 1999). Seed storage is both efficient and reproducible; allowing good germination after storage, conservation of a wide range of genetic diversity and requires only minimal maintenance and monitoring (Engelmann *et al.*, 2007; Jaenicke, 1999).

Germination is the initial stage in the growth of a seed into a seedling. The knowledge of germination is known to enhance propagation techniques and forms an essential part of planning species rehabilitation and conservation works. It also enhances plant community processes like recruitment and succession as well as the management of plant population. (Randriatafika *et al*., 2008; Khurana and Singh, 2001). The need to germinate seeds of tropical trees increases each year due to the growing demand from silviculture and forest restoration efforts (Nansen *et al.*, 2001). Moisture content (MC) and percentage germination (PG) of several tropical trees species have therefore been studied (Espahbodi *et al.*, 2007; Baskin and Baskin, 2005). However, little information is available on these parameters in individual trees of *C. pentandra* across the species range in Ghana. The current study, therefore, looked at the MC and PG of *C. pentandra* seeds before and after storage of seeds from five ecological zones of Ghana. The range-wide collection of seeds was necessary, because it increases the chances of including genes good for reliable conservation of the species. This knowledge will help promote seedling establishment for afforestation and forest regeneration.

According to Randriatafika *et al*. (2008), raising seedlings from various seed sources under relatively uniform conditions as in growth chambers, greenhouses, nurseries or field tests, enables the evaluation of the relative contribution of genetics and environment to variation. The high variability in undomesticated germplasm can be attributed clearly to the genetic differences of the seeds, only if seedlings are produced under uniform and optimal conditions. Hence, knowing the full potential of a new species or provenance is prerequisite for assessing how well it might develop under adverse conditions (Jaenicke, 1999). However, knowledge on progeny and provenance variability in initial resistance to stem dieback disease in *C. pentandra* is

still inadequate. The competitive performance of individual trees of *C. pentandra* from the five of its distinctive natural ranges in Ghana was, therefore, evaluated at different seasons (wet and dry seasons). The characteristics of these two seasons are shown in Table 3.3.4. Testing in the different seasons was necessary for the identification of true genetically resistant accessions and the best season for nursing *C. pentandra* seedlings.

3.2 Objectives

The objectives of this study were to:

1. identify accessions of *C. pentandra* with high viability for further screening,
2. identify the level of stem dieback resistance among accessions and populations through nursery screening, and
3. identify a favourable season for raising *C. pentandra* seedlings at the nursery nursery.

3.3 Materials and methods

3.3.1 Germplasm collection

Matured fruits were collected from half-sib families (seeds from trees fertilized by open-pollination in which only the female parent is known) in five ecological zones of Ghana as described by Hawthorne (1995). These included the Dry Semi-Deciduous Forest Zone-Outlier (DSDFZ-Outlier), Dry Semi-Deciduous Forest Inner Zone (DSDFZ-Inner), Moist Semi-deciduous Forest Zone (MSDFZ), Moist/Wet Evergreen Forest Zone (M/WEFZ) and the Guinea Savannah Zone (GSZ). The composition and detailed characteristics of these zones are illustrated in Tables 3.3.1 and 3.3.2, respectively. These individual trees were referred to as accessions/mother trees in the

study. A total of 98 accessions were collected from the five zones. Nine of these accessions were collected from the DSDFZ-Outlier, 24 from the DSDFZ-Inner, 22 from the MSDFZ, 17 from the M/WEFZ and 26 from the GSZ (Table 3.3.3 and Appendix 3.3.1). These zones constitute the natural range of *C. pentandra* in Ghana. Stratified random sampling was used to ensure that genes controlling all traits were included in the samples (Hawkes, 1985). The ecological zones were taken as the strata and a simple random sampling was then used to select individual trees in each stratum. An isolation distance of at least 100m was allowed between sampled trees to ensure that trees with the same pedigrees were avoided following the minimum sampling distance of 100m between trees recommended by Schmidt (2000).

The fruits were harvested from the top of standing trees and kept in nylo-woven sacks with labels to identify them. Trees from which seed samples were collected were given unique labels and the positions of some of them recorded with the aid of a Geographical Positioning System (etrex, 12 channel GPS, software version 3.30, Garmin Ltd, Taiwan). This was done to ensure easy identification and accurate back-sampling. The samples were labelled using the name of the location (first three letters) and the serial number of the tree in the location. For instance, the first two trees sampled from Kwaso were given the codes KWA 1 and KWA 2, respectively.

3.3.2 Seed processing

The seed cleaning and drying were carried out at the Seed Processing Centre of the Forestry Research Institute of Ghana of the Council for Scientific and Industrial Research (FORIG-CSIR), Fumesua-Kumasi. The fruits were air-dried in light shade for one week before seeds were extracted and then further dried for two weeks under

room temperature. Seed moisture content was then determined and the seeds placed in a deep freezer at -13 to -20 ºC, until required.

3.3.3 Determination of moisture content (MC)

This experiment was performed at the Seed Technology Laboratory of CSIR-FORIG, Fumesua-Kumasi. MC test was done using the oven dry method as described by ISTA (2005). Empty drying containers and lids were first cleaned, dried for 1 hour at 130 ^{0}C and weighed prior to withdrawing of the working sample. Four replicates of about 5 g of each pure seed sample (seed sample containing both viable and non-viable seed) were evenly distributed over the surface of the containers. The containers, their lids, and the seed samples were reweighed and placed into the oven. Samples were dried at 103 °C for 17 hours and cooled in a desiccator for 30 to 45 minutes. After cooling, the containers, their lids and the seed were re-weighed. The weight loss was determined and the amount of moisture calculated as a percentage of the lost weight to the fresh weight as stated below:

$$\text{Moisture content}\,(\%) = \frac{w_2 - w_3}{w_2 - w_1} \times 100;$$

where:

W_1 = Weight of empty container with lid;

W_2 = Weight of container with lid and seed sample before drying; and

W_3 = Weight of container with lid and seed sample after drying.

Table 3.3.1 Composition of major forests of Ghana

Major Zones	Forest types	Rainfall (mm)
High forest zone	Wet Evergreen Forest	1750-2000
	Moist Evergreen Forest	1500-1800
	Moist Semi-deciduous Forest	1200-1800
	Dry Semi-deciduous Forest	1250-1500
Savannah zone	Guinea Savannah	900-1500
	Sundan savannah	600-900

Source: Modified from Wagner et al. (2008).

3.3.4 Seeds germination

Germination test was done at the Nursery site of FORIG-CSIR. The test was done in sand before and after storage to determine the viabilities of the accessions. The seeds were sampled just after cleaning as that provided an ideal way to get good representative samples. In each of these tests, 100 seeds per accession were sown in 4 replications with 25 seeds per replicate at a depth of 1-2 cm. The number of germinated seeds was counted at 2-day intervals for 6 weeks and the total germination determined as a percentage following Randriatafika *et al*. (2008) as stated below:

$$GR(\%) = \frac{n}{N} \times 100$$

where:

GR = Germination rate (Percentage Germination)

n = Number of germinated seeds and

N = Number of seeds planted

Table 3.3.2 Characteristic of closed forest types in Ghana

Forest Type	Mean tree height (m)	Commercial timber value	Floristic composition	Degree of deciduous habitat
Wet Evergreen (WE)	32	Lowest	Highest diversity with most characteristic species	Lowest
Moist Evergreen (ME)	43	Second to MS	Lower diversity than WE and more characteristic species than ME	Intermediate synchronous
Moist Semi-deciduous (MS)	50-60	Highest	Many species common to all other forest types	Intermediate synchronous
Dry Semi-deciduous	30-45	Intermediate	Many common species	highest synchronous

Source: Modified from Wagner et al. (2008).

3.3.5 Nursery screening

The experiment was performed at the nursery of FORIG-CSIR, Fumesua-Kumasi. The nursery screening was done in two phases: September-November Screening (SNS) and March-May Screening (MMS). In the first phase, 80 accessions out of the 98 collected and tested as described in section 3.3.1, previously, were used. In addition to the fourteen accessions which did not germinate at all (Table 3.4.1), four others could not also germinate satisfactorily and were excluded from further screening. Five hundred seedlings of each of the remaining 80 accessions, giving a

Table 3.3.3 Ecological zones sampled and number of accessions collected.

Population	Codes	No. of accessions
Dry Semi-deciduous Forest Zone-Outlier	DSDFZ-Outlier	9
Dry Semi-deciduous Forest Inner Zone	DSDFZ-Inner	24
Moist Semi-deciduous Forest Zone	MSDFZ	22
Moist/Wet Evergreen Forest Zone	M/WEFZ	17
Guinea Savanna Zone	GSZ	26
Total		98

total of 40,000 seedlings, were screened. In the second phase, screening was done with 59 accessions using varied numbers of seedlings up to a maximum of 250 seedlings, with a total of 8,701 seedlings for the entire experiment. Seeds from 21 of the accessions were exhausted and were, therefore, excluded in the second phase. The seeds were sown for one week in germination bowls half-filled with river sand levelled uniformly. The bowls were perforated at the bottom to allow drainage of excess water. Seeds were broadcast evenly and carefully covered with a thin layer of sand just enough to ensure that they were not exposed during watering. The seedlings were transplanted one week after germination into black polyethylene bags (size 16.5 x 10.5 cm) filled with top soil and arranged under a shed constructed from palm frond. Watering was done daily to field capacity, except on rainy days when they seedlings were not watered at all. Screening for dieback infection at the nursery was started one week after transplanting; that is, two weeks after germination. The two trials were run for a period of three months as recommended by Wright (1976).

Weekly assessments for the incidence of dieback were done over a period of

12 weeks. The seedlings were counted weekly and dead seedlings removed and recorded. Mortality rates were calculated following the formula proposed by Randriatafika *et al*. (2008) as follows:

$$MR(\%) = \frac{n}{N} \times 100$$

where:

MR = Mortality rate

n = Number of dead seedlings

N = Total number of seedlings planted

The survival rates (SR) were then calculated as;

$$SR = 100 - MR$$

where:

SR = Survival rate

MR = Mortality rate

The accessions were categorized into classes of resistance based on their survival ratings namely, highly resistant (70.00 to 100% high survival), moderately resistant (50.00 to 69.9% moderate survival) and fairly resistant (<50% fair survival). Those with 100% mortality were considered as being susceptible to stem dieback. The resistant accessions were partitioned into their respective ecological zones which were then ranked according to their resistance to stem dieback disease. Relative resistance within and among ecological zones were calculated as follows:

$$\text{Resistance within pops} = \frac{\text{No. of resistant accessions within pop}}{\text{No. of accessions transplanted from pop}} \times 100\%$$

$$\text{Resistance among pops} = \frac{\text{No. of resistant accessions within pop}}{\text{Total no. of accessions for all pops}} \times 100\%$$

Where:

Pops = Populations, Pop = Population, No = Number

Arcsine transformation, following Snedecor and Cochran (1980), was performed on the percentage mortality data to conform to normal distribution and the resultant data used in analysis of variance test using Microsoft Excel 2007 and the level of significance tested at P = 0.05.

Table 3.3.4 Rainfall figures and number of rainy days at Forest Research Institute of Ghana-Femesua.

Year	Month	Total rainfall (mm)	No. of rainy days	Average rainfall (mm)
2007	September	227.9	17	13.4
	October	152.9	16	9.6
	November	61.9	3	20.6
	Average	**442.7**	**36**	**12.3**
2008	March	70.4	4	17.6
	April	45.3	17	2.7
	May	219.5	11	20
	Average	**335.2**	**32**	**10.5**

Source: 2011 database of the Ghana Metrological Service, Ashanti Region, Ghana.

3.4 Results

3.4.1 Seed moisture content and germination tests

Moisture content (MC) before storage ranged from 2.60 ± 0.12 % (accession KON 6) to 23.84 ± 0.42 % (accession KYE 1) whilst that after storage ranged from 2.63 ± 0.37 % to 23.86 ± 0.12 % in these accessions, respectively (Table 3.4.1). Mean MC before and after storage for all accessions was 10.92 % and 11.09 %, respectively. There was an increase in mean MC for all seed lots ranging from 0.01 % in 16 accessions (16.33

%) to 3.35 % in 1 accession (1.02 %). Most of the accessions had a rise in MC of between 0.1 to 0.8 %. The number of accessions and the corresponding range of rise in MC are shown in Figure 3.4.1. Fourteen accessions (14.29 %) of the 98 did not germinate at all before and after storage. Accession ABB1 had the highest percentage germination (PG) before and after storage of 93.00 % and 89.00 % respectively (Table 3.4.1). Mean germination before and after storage for all accessions, which germinated, was 43.38 % and 40.37 %, respectively. A majority of the accessions have PG in the range of 50 - 100 % (Fig 3.4.2). The DSDFZ-Outlier had the highest mean germination before and after storage of 65.11 % and 62.33 %, respectively (Fig 3.4.3). The detailed results of the percentage germination of all accessions are shown in Table 3.4.1.

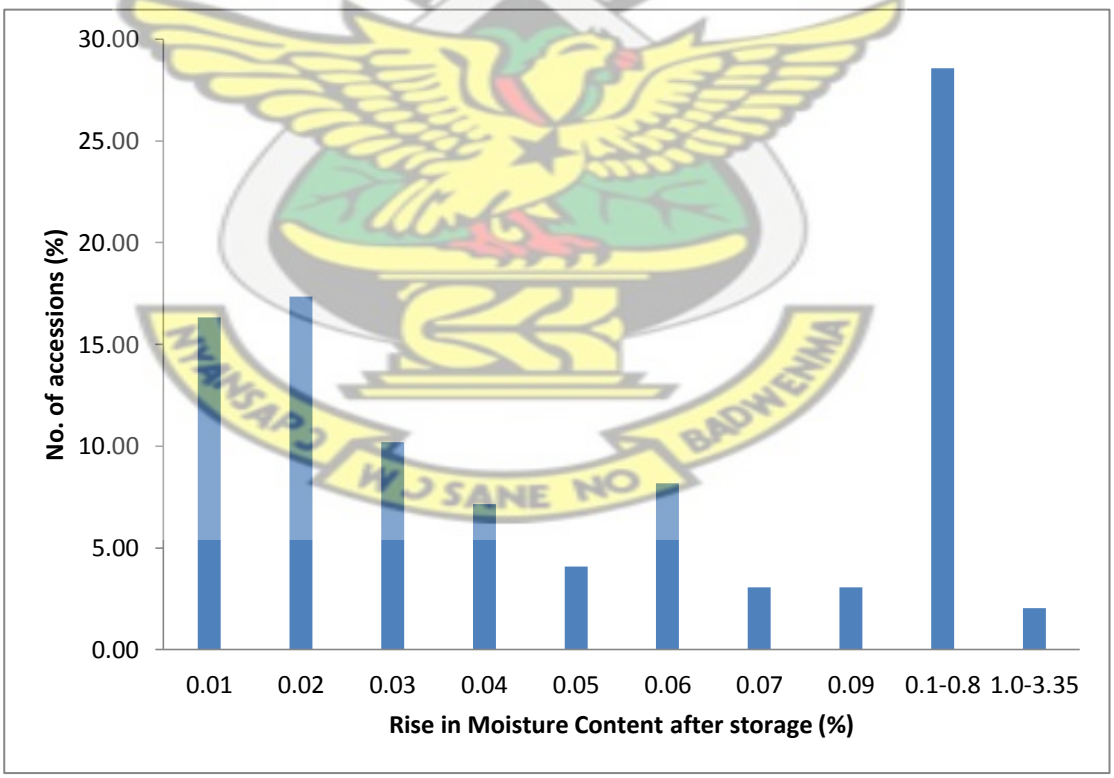


Fig 3.4.1 Rise in Seed Moisture Content (MC) of 98 accessions of *C. pentandra* after seeds were stored for five months at -13 ^{o}C to -20 ^{o}C.

Table 3.4.1 Moisture content and percentage germination of C. pentandra accessions before and after five months storage. (Refer to appendix 3.3.1 for names of ecological zones and localities of accessions).

Accession	Moisture content before storage			Moisture content after storage			Percentage germination	
	Mean	Min	Max	Mean	Min	Max	Before storage	After storage
ABB 1	11.73 ± 0.07	11.60	11.86	11.74 ± 0.08	11.90	12.54	93	89
ABF 1	17.24 ± 0.14	17.10	17.36	17.26 ± 0.24	17.24	17.61	53	52
ACH 1	13.61 ± 0.27	12.93	14.17	13.63 ± 0.28	12.70	13.47	45	41
ADI 1	12.20 ± 0.34	11.98	12.52	12.31 ± 0.36	11.72	12.81	30	27
ADU 1	10.10 ± 0.20	10.10	11.32	10.12 ± 0.24	9.68	10.18	82	79
ABF 1	13.00 ± 0.38	12.09	13.90	13.09 ± 0.40	14.19	15.32	47	46
AFR 1	13.24 ± 0.14	12.82	13.42	13.25 ± 0.16	14.67	15.35	40	39
AKD 1	10.20 ± 0.21	14.26	15.21	10.22 ± 0.25	9.60	14.30	52	50
AKD 2	11.23 ± 0.12	11.21	11.27	11.24 ± 0.14	10.38	11.24	43	43
AKK 1	12.10 ± 0.44	13.27	15.14	12.70 ± 0.47	12.53	13.03	42	40
AKO 1	17.04 ± 0.45	15.73	17.84	17.11 ± 0.48	14.93	16.29	3	2
AOF 1	12.25 ± 0.05	12.12	12.30	12.27 ± 0.11	12.95	13.68	31	28
ASA 1	15.50 ± 0.30	15.38	15.66	15.52 ± 0.40	15.44	18.26	0	0
UST 3	15.30 ± 0.31	15.22	15.42	15.50 ± 0.34	15.12	18.15	0	0
ASE 1	13.77 ± 0.16	13.29	14.00	13.87 ± 0.19	12.28	14.22	85	81
AYE 1	10.44 ± 0.32	9.83	11.13	11.00 ± 0.36	10.33	12.33	70	66
AYE 2	11.81 ± 0.11	11.42	11.92	11.82 ± 0.14	11.31	12.41	65	63
BAW 1	2.94 ± 0.45	1.73	3.72	2.98 ± 0.47	1.73	3.72	4	3
BAW 10	3.63 ± 0.18	6.08	6.86	3.68 ± 0.20	6.08	6.86	70	60
BAW 11	3.91 ± 0.15	3.48	4.15	3.94 ± 0.18	3.48	4.15	65	50
BAW 12	4.80 ± 0.09	4.67	5.04	5.00 ± 0.12	4.67	5.04	15	11
BAW 13	5.71 ± 0.30	4.98	6.24	6.00 ± 0.35	4.98	6.24	68	66
BAW 2	3.25 ± 0.27	2.46	3.64	3.27 ± 0.32	2.46	3.64	4	3
BAW 3	2.68 ± 0.23	2.24	3.28	3.10 ± 0.26	2.24	3.28	33	28
BAW 4	4.23 ± 0.21	3.95	4.85	4.68 ± 0.25	3.95	4.85	83	79
BAW 5	3.88 ± 0.23	3.53	4.53	4.00 ± 0.28	3.53	4.53	31	28
BAW 6	3.50 ± 0.15	3.28	3.92	3.56 ± 0.17	3.28	3.92	31	28
BAW 7	5.29 ± 0.15	5.02	5.67	5.30 ± 0.19	5.02	5.67	3	1
BAW 8	3.14 ± 0.30	2.71	4.01	3.20 ± 0.32	2.71	4.01	3	2
BAW 9	5.61 ± 0.21	3.13	3.95	5.62 ± 0.25	3.13	3.95	2	1
BIB 1	12.19 ± 0.14	11.94	12.49	13.24 ± 0.22	12.54	13.54	25	20
BUF 1	12.00 ± 0.13	12.01	12.32	12.32 ± 0.16	11.22	12.25	65	61
BUS 1	9.00 ± 0.20	8.98	9.40	9.41 ± 0.21	8.03	9.12	65	64
BUS 2	14.10 ± 0.12	14.10	14.22	14.16 ± 0.13	12.97	13.82	62	61
BUS 3	18.00 ± 0.22	18.01	18.32	18.19 ± 0.11	17.28	19.22	0	0

BUS 4	17.98 ± 0.12	17.02	19.98	18.00 ± 0.41	18.00	20.11	0	0
DAN 1	13.03 ± 0.11	12.73	13.26	13.06 ± 0.13	12.86	13.50	52	49
DMF 1	10.36 ± 0.41	11.77	13.56	10.38 ± 0.06	11.80	12.50	0	0
DMF 2	16.40 ± 0.13	16.02	16.64	16.46 ± 0.10	11.89	12.73	2	1
DNY 1	11.94 ± 0.06	11.82	12.09	11.95 ± 0.19	13.26	14.19	50	47
EDW 1	12.09 ± 0.10	11.86	12.33	12.10 ± 0.31	12.86	14.05	70	62
EDW 2	12.35 ± 0.19	11.87	12.75	12.38 ± 0.15	13.60	14.20	26	24
GYE 1	12.47 ± 0.31	11.93	13.11	13.11± 0.07	12.56	13.79	21	18
KEC 10	8.82 ± 0.15	8.51	9.16	9 .00 ± 0.08	8.51	9.16	71	69
KEC 9	10.71 ± 0.07	10.57	10.87	10.88 ± 0.18	10.57	10.87	58	54
KOK 1	12.19 ± 0.80	9.91	13.69	12.29 ± 0.27	14.45	14.92	44	38
KON 1	5.25 ± 0.18	4.78	5.53	5.29 ± 0.33	4.78	5.53	20	19
KON 10	3.03 ± 0.27	2.56	3.71	3.12 ± 0.18	2.56	3.71	2	1
KON 11	6.23 ± 0.33	5.64	7.11	6.26 ± 0.23	5.64	7.11	35	30
KON 12	5.11 ± 0.17	4.65	5.37	5.12 ± 0.57	4.65	5.37	52	50
KON 13	5.73 ± 0.33	5.18	6.61	5.74 ± 0.25	5.18	6.61	22	19
KON 2	4.25 ± 0.18	3.71	4.54	4.28 ± 0.12	3.71	4.54	1	1
KON 3	5.50 ± 0.23	5.16	6.19	5.54 ± 0.04	5.16	6.19	11	10
KON 4	4.00 ± 0.57	2.33	4.92	4.22 ± 0.25	2.33	4.92	2	1
KON 5	4.25 ± 0.25	3.50	4.66	4.28 ± 0.19	3.50	4.66	2	2
KON 6	2.60 ± 0.12	2.62	3.06	2.63 ± 0.37	2.62	3.06	0	0
KON 7	3.09 ± 0.64	1.77	4.48	3.12 ± 0.13	1.77	4.48	10	7
KON 8	7.54 ± 0.25	7.11	8.17	7.60 ± 0.21	7.11	8.17	30	28
KON 9	5.69 ± 0.19	5.23	6.11	5.70 ± 0.41	5.23	6.11	40	38
KUE 1	8.13 ± 0.37	7.16	8.78	8.16 ± 0.05	7.16	8.78	55	54
KUE 2	6.65 ± 0.13	0.32	9.51	6.70 ± 0.42	0.32	9.51	71	69
KUE 3	7.94 ± 0.21	7.47	8.45	8.00 ± 0.24	7.47	8.45	66	65
KWA 1	14.60 ± 0.41	10.80	12.54	14.64 ± 0.11	12.17	13.80	68	60
KWA 2	13.55 ± 0.25	12.50	16.09	13.60 ± 0.13	13.44	13.83	0	0
KYE 1	23.84 ± 0.42	23.01	23.90	23.86 ± 0.12	22.66	25.38	52	47
MAM 1	12.15 ± 0.24	11.56	12.72	12.95 ± 0.34	12.38	13.53	35	30
MSH 1	13.50 ± 0.11	13.02	13.65	13.54 ± 0.03	13.57	14.24	45	24
MSH 2	16.74 ± 0.13	16.14	16.75	16.76 ± 0.21	11.69	13.49	0	0
NKA 1	15.34 ± 0.12	15.03	15.44	15.53 ± 0.29	14.45	17.08	91	88
NKA 2	21.00 ± 0.34	21.02	21.50	21.01 ± 0.04	19.35	21.35	89	87
NKA 3	12.00 ± 0.03	12.01	12.34	12.20 ± 0.15	11.65	11.75	10	9
NKIN 1	18.00 ± 0.21	18.04	18.33	18.11 ± 0.16	10.15	11.27	40	38
NKIN 2	11.01 ± 0.29	10.36	11.50	11.12 ± 0.28	10.77	11.54	7	6
NKW 1	13.00 ± 0.04	13.30	13.32	13.01 ± 0.31	12.69	13.19	62	60
NKW 2	13.23 ± 0.15	13.20	13.66	13.25 ± 0.14	13.11	13.64	50	49
NKW 3	15.00 ± 0.08	15.06	15.64	15.02 ± 0.25	14.62	15.05	34	32
NKW 7	9.54 ± 0.11	9.37	9.87	9.60 ± 0.13	9.37	9.87	77	68
ODO 4	9.82 ± 0.16	9.37	10.14	9.85 ± 0.09	9.37	10.14	82	79
ODO 6	8.38 ± 0.28	7.74	9.10	8.40 ± 0.06	7.74	9.10	74	73
PAK 1	16.93 ± 0.31	16.62	16.93	17.00 ± 0.15	17.36	19.18	0	0
PAK 2	17.00 ± 0.14	17.01	17.40	17.20 ± 0.12	19.20	20.31	0	0

PAW 5	9.04 ± 0.25	8.63	9.78	9.10 ± 0.12	8.63	9.78	32	30
PEA 1	13.73 ± 0.13	13.37	13.99	13.74 ± 0.09	15.27	17.54	46	47
PIN 1	14.38 ± 0.09	14.32	14.67	14.42 ± 0.14	12.66	14.29	72	69
POK 1	10.64 ± 0.06	10.42	10.74	10.65 ± 0.13	10.13	11.83	70	66
PRE 1	16.98 ± 0.15	16.22	16.24	17.00 ± 0.08	16.85	19.20	0	0
SKD 1	11.70 ± 0.12	11.43	11.99	11.74 ± 0.31	13.43	14.92	33	32
SOF 1	13.69 ± 0.12	13.62	16.78	13.71 ± 0.16	13.30	14.29	30	29
SPY 1	19.42 ± 0.09	11.81	12.22	20.00 ± 0.32	21.05	22.19	88	85
SPY 2	12.91 ± 0.14	12.56	13.23	13.00 ± 0.32	14.05	15.67	54	51
SPY 3	10.94 ± 0.13	10.65	11.27	10.95 ± 0.09	9.26	10.79	74	70
TAC 1	13.52 ± 0.08	13.31	13.56	14.20 ± 0.13	14.40	16.84	0	0
TAN 1	13.65 ± 0.31	13.03	14.52	13.70 ± 0.21	4.94	15.45	27	26
TNK 1	9.78 ± 0.16	9.47	10.20	9.80 ± 0.11	9.50	9.95	17	16
TWE 1	16.88 ± 0.24	16.74	16.92	16.95 ± 0.26	16.26	16.98	45	40
UST 1	19.53 ± 0.32	19.23	19.89	20.30 ± 0.45	16.64	20.28	0	0
UST 2	16.75 ± 0.31	16.45	16.88	20.00 ± 0.32	18.85	21.00	0	0
YAM 1	10.50 ± 0.09	10.30	10.62	10.75 ± 0.41	9.94	11.15	35	34

Note: 1. Percentage germination values were rounded to whole numbers
2. Min = Minimum value, Max = Maximum value

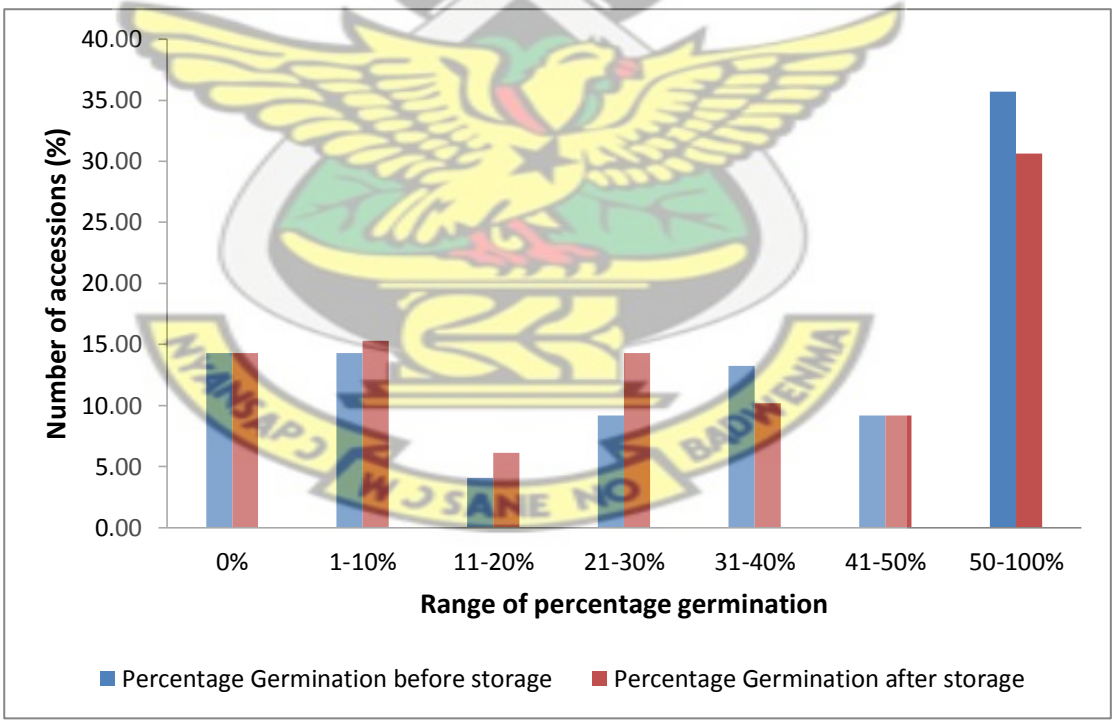


Fig 3.4.2 Percentage germination in 98 accessions of *C. pentandra* before and after seeds were stored for five months at -13 °C to -20 °C.

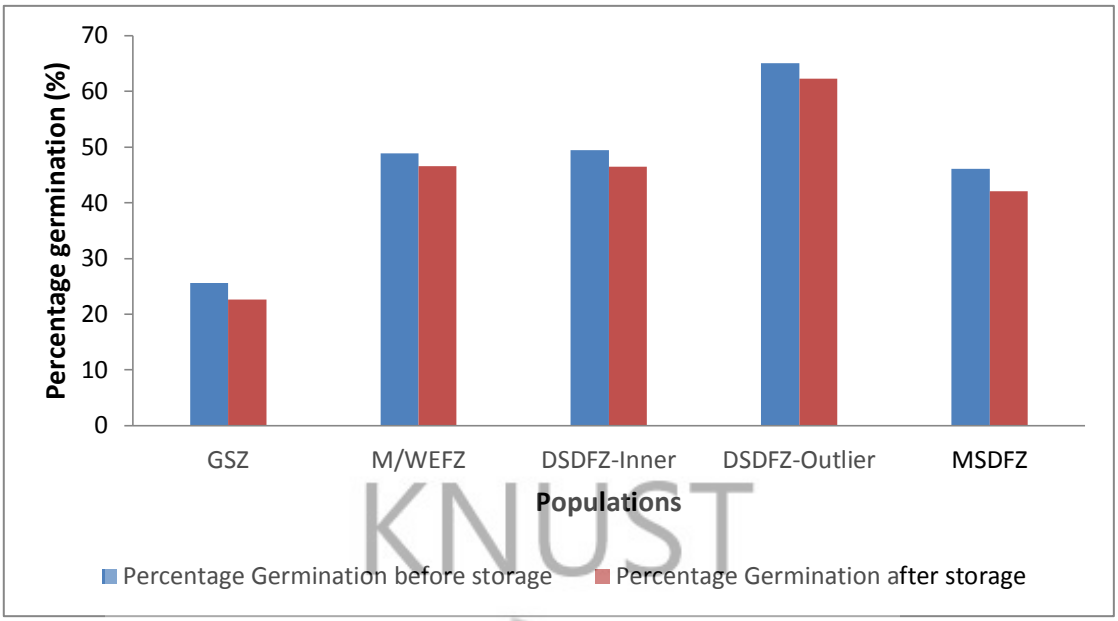


Fig. 3.4.3 Percentage germination of *C. pentandra* seedlots from five populations before and after seeds were stored for five months at -13 ^{o}C to -20 ^{o}C.

3.4.2 Nursery screening of *C. pentandra* seedlings for dieback resistance

In the phase one (SNS) screening, 44 (55 %) out of the 80 accessions had varied levels of survival rates ranging from 0.2 to 79.20 % (Table 3.4.2). The remaining 36 had 100 % mortality. In terms of individual seedling mortality, 3,736 (9.34 %) seedlings of the total 40,000 tested survived and the rest, 36,264 (90.66 %) died. Over 85 % of the seedling mortality occurred within the first five weeks after transplanting, between September and the first week of November (Fig 3.4.4). None of the accessions was without susceptibility to dieback disease, 1 (1.25 %) had survival level of 79.20 %, 4 (5.00 %) between 50 % and 69.9 % and 39 (48.75 %) less than 50 % (Table 3.4.2). Sixteen accessions (36.36 %) out of the 44 surviving accessions belonged to the DSDFZ-Inner, 5 (11.36 %) each to the DSDFZ-Outlier and the GSZ, 10 (22.72 %) to the MSDFZ and 8 (18.18 %) to the M/WEFZ (Table 3.4.3).

The phase two screening (MMS) on the other hand, showed that all the 59

(100 %) accessions screened had varied levels of survival rates ranging from 24.50 to 100 % (Table 3.4.2). A total of 6,915 (79.47 %) seedlings survived whilst the rest 1,786 (20.53 %) died. A majority of the seedlings (73.02 %) died between the 8th and the 12th weeks (Fig. 3.4.4). Forty accessions (67.80 %) had survival levels in the range of 70.00 to 100 %, 9 (15.25%) between 50.00 to 69.90 % and 10 (16.95 %) of less than 50.00 % (Table 3.4.2). Fifteen accessions (25.42 %) out of the 59 belonged to the DSDFZ-Inner, 7 (11.86 %) to the DSDFZ-Outlier, 6 (10.17 %) to the MSDFZ, 9 (15.26 %) to the M/WEFZ, and 22 (37.29 %) to GSZ (Table 3.4.4). Analysis of variance showed significant differences in mortality rates ($P < 0.001$) among accessions for both the SNS (Appendix 3.4.1) and the MMS (Appendix 3.4.2). Thirteen accessions (36.11 %) of the 36, which had 100 % mortality during the SNS season, had varied survival rates ranging from 78.33 % to 100 % during the MMS (Table 3.4.5).

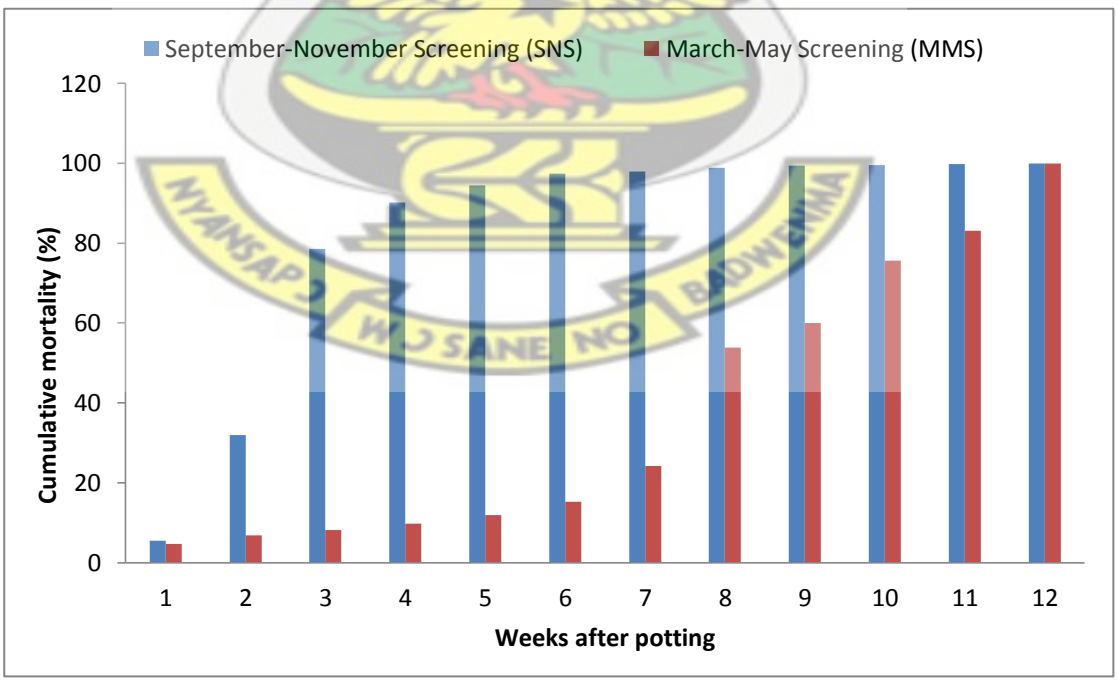


Fig 3.4.4 Weekly mortality of *C. pentandra* accessions at the nursery.

Table 3.4.2 Survival levels of C. pentandra accessions screened from September-November (SNS) and March-May (MMS) at the nursery.

Survival levels (%)	Accession	Ecological zone	Survival (%)		Total	
			SNS	MMS	SNS	MMS
	MAA 1	M/WEFZ		100		
High survival	BAW 1	GSZ		100	1	40
(70-100)	BAW 6	GSZ		100	(1.25 %)	(67.80 %)
	TAN 1	DSDFZ-Inner		99.29		
	GYE 1	MSDFZ		99.26		
	NKIN 1	DSDFZ-Inner		98.80		
	OFA 1	M/WEFZ		98.57		
	BUF 1	DSDFZ-Inner		98.40		
	ASE 1	DSDFZ-Inner		98.40		
	KEC 9	DSDFZ-Outlier		96.67		
	KUE 2	DSDFZ-Outlier		96.46		
	ABF 1	DSDFZ-Inner		94.80		
	KWA 1	MSDFZ		94.8		
	DAN 1	MSDFZ		94.74		
	NKIN 2	DSDFZ-Inner		94.71		
	EDW 1	MSDFZ		94.00		
	PAW 5	DSDFZ-Outlier		94.00		
	WOA 1	MSDFZ		93.57		
	ACH 1	MSDFZ		93.33		
	SOF 1	M/WEFZ		92.00		
	KYE 1	M/WEFZ		91.67		
	KON 13	GSZ		91.60		
	BAW 4	GSZ		91.49		
	SKD 1	DSDFZ-Inner		91.20		
	NKW 1	DSDFZ-Inner		91.11		
	KUE 1	DSDFZ-Outlier		91.00		
	TNK 1	DSDFZ-Inner	79.20	90.00		
	ADU 1	DSDFZ-Inner		90.00		
	KON 9	GSZ		88.80		
	KEC 10	DSDFZ-Outlier		88.33		
	BAW 5	GSZ		85.60		
	MAM 1	M/WEFZ		84.80		
	DNY 1	DSDFZ-Inner		84.80		
	AYE 2	DSDFZ-Inner		82.80		
	BAW 8	GSZ		81.82		
	NKA 1	M/WEFZ		80.40		
	AYE 1	DSDFZ-Inner		79.20		

	BAW 12	GSZ		78.57		
	KON 5	GSZ		78.33		
	KUE 3	DSDFZ-Outlier		70.00		
Moderate survival (50-69.9)	KWA 1	MSDFZ	69.40		4 (5.00 %)	9 (15.25 %)
	BUF 1	DSDFZ-Inner	62.80			
	MSH 1	DSDFZ-Inner	53.60			
	BUS 1	M/WEFZ	50.80			
	BAW 2	GSZ		65.00		
	KON 1	GSZ		62.50		
	KON 8	GSZ		60.40		
	KON 11	GSZ		60.00		
	SPY 3	DSDFZ-Inner		60.00		
	KON 6	GSZ		58.82		
	KON 2	GSZ		57.50		
	BAW 7	GSZ		53.33		
	ODO 6	DSDFZ-Outlier		52.80		
Fair survival (<50)	EDW 1	MSDFZ	48.00		39 (48.75 %)	10 (16.95 %)
	KEC 9	DSDFZ-Outlier	44.40			
	GYE 1	MSDFZ	44.00			
	AYE 2	DSDFZ-Inner	41.60			
	TWE 1	MSDFZ	38.80			
	MAM 1	M/WEFZ	27.00			
	AYE 1	DSDFZ-Inner	21.20			
	AOF 1	MSDFZ	18.20			
	NKW 2	DSDFZ-Inner	14.20			
	KUE 3	DSDFZ-Outlier	13.00			
	BAW 4	GSZ	10.60			
	KON 12	GSZ	9.40			
	AKD 2	DSDFZ-Inner	9.40			
	DYN 1	DSDFZ-Inner	8.60			
	EDW 2	MSDFZ	7.60			
	NKA 1	M/WEFZ	7.00			
	SOF 1	M/WEFZ	6.40			
	ABB 1	MSDFZ	6.00			
	POK 1	M/WEFZ	5.40	35.60		
	NKA 3	M/WEFZ	4.80	35.71		
	BAW 3	GSZ	4.60			
	KON 9	GSZ	4.40			
	KUE 1	DSDFZ-Outlier	4.20			
	KWA 2	MSDFZ	4.00			
	TAN 1	DSDFZ-Inner	3.80			
	NKA 2	M/WEFZ	3.40	39.60		

AFE 1	DSDFZ-Inner	3.00			
KON 1	GSZ	2.80			
KEC 10	DSDFZ-Outlier	2.60			
NKIN 1	DSDFZ-Inner	2.40			
AKD 1	DSDFZ-Inner	2.20			
MSH 2	DSDFZ-Inner	1.80			
BIB 1	MSDFZ	1.60			
DAN 1	MSDFZ	1.40			
BUS 2	M/WEFZ	1.40			
PIN 1	DSDFZ-Inner	1.00			
SPY 2	DSDFZ-Inner	0.60			
ABF 1	DSDFZ-Inner	0.40			
ODO 6	DSDFZ-Outlier	0.20			
KON 7	GSZ		42.50		
KON 4	GSZ		38.33		
BAW 9	GSZ		36.00		
AKD 1	DSDFZ-Inner		36.00		
BAW 11	GSZ		32.40		
KON 3	GSZ		25.00		
BAW 10	GSZ		24.40		
TOTAL		80.00	59.00	100	100

Note: In the SNS, 36 (45 %) accessions had 100 % mortality and were eliminated from the list.

Table 3.4.3 Distribution of surviving accessions screened from September-November within and among populations at the nursery.

Population	Number of accessions screened	Number of resistant accessions	Within zones (%)	Among Zones (%)
DSDFZ-Inner	22	16	72.72	36.36
DSDFZ-Outlier	9	5	55.56	11.36
MSDFZ	17	10	58.82	22.73
M/WEFZ	10	8	80	18.18
GSZ	22	5	22.72	11.36
Total	80	44		100%

Table 3.4.4 Distribution of surviving accessions screened from March-May within and among populations at the nursery.

Population	Number of accessions screened	Number of resistant accessions	Within pops (%)	Among pops (%)
DSDFZ-Inner	15	15	100	25.42
DSDFZ-Outlier	7	7	100	11.86
MSDFZ	6	6	100	10.17
M/WEFZ	9	9	100	15.26
GSZ	22	22	100	37.29
Total	59	59		100%

Note: Pops = Populations

Table 3.4.5 Seasonal variation in resistance and susceptibility of *C. pentandra* accessions to stem dieback disease at the nursery.

Population	Progenies	SNS		MMS	
		No. of seedlings planted	Survival (%)	No. of seedlings planted	Survival (%)
M/WEFZ	NKA 1	500	0.00	250	80.40
GSZ	BAW 1	500	0.00	30	100.00
	BAW 12	500	0.00	70	78.57
	BAW 5	500	0.00	250	85.60
	BAW 6	500	0.00	8	100.00
	BAW 8	500	0.00	11	81.82
	KON 13	500	0.00	250	91.60
	KON 5	500	0.00	60	78.33
DSDFZ-Outlier	KUE 2	500	0.00	113	96.46
	PAW 5	500	0.00	250	94.00
DSDFZ-Inner	NKIN 2	500	0.00	170	94.71
	NKW 1	500	0.00	45	91.11
	SKD 1	500	0.00	250	91.20
Total	13				

Note: These accessions were rated susceptible in the SNS and resistant in the MMS seasons.

3.5 Discussion

The results of the study showed that there was a gain in moisture content in all the accessions after the storage. The slight gain in MC in all the accessions after the storage could be attributed to the effect of condensed air trapped in the seed storage bags during packaging as well as improper zip-locking. It could also have been caused by the influx of moisture from the storage medium as a result the periodic de-freezing

arising from power fluctuations and the microscopic pores in the containers. A rise in moisture content by 1% is known to halve the storage life of orthodox seeds (Gosling, 2007). Seeds lose viability during storage when seed moisture content rises. This is because moisture increases the respiration rate of seed, which in turn raises seed temperature and thereby killing the seed (Mulawarman *et al*., 2003). The authors note that rise in moisture content also causes mould growth leading to further damage to refrigerated seeds. The gain and/or loss in moisture content was minimal in most of the accessions and suggest that the deep-freeze storage and air-drying methods could be used for the processing of *C. pentandra* seeds. Air drying is noted to be an adequate method for seed drying in the tropics (Evans, 1982) and the most common mode of drying in the world (Miller and Copeland, 1997).

Generally, variability in percentage germination before and after storage was very small and similar in all the accessions (Table 3.4.1). The inability of the fourteen accessions to germinate and the low percentage germination of the fifteen accessions after storage (accessions with PG between 1.00 % and 10.00%) could be due to factors relating to the seed themselves as well as the drying, storage and nursery conditions. According to Randriatafika *et al*. (2008), external factors, such as high relative humidity and temperatures, influence seed quality and viability resulting in low germination rates. Linington and Pritchard (2001) note that longevity of orthodox seeds increases with reduced MC. Tietema *et al*. (1992) have attributed poor germination of tree seeds to several reasons, including morphologically matured seeds which have physiologically dormant or rudimentary embryos, mechanically resistant seeds and/or impermeable coats, seeds with embryo eaten by predators and seed dormancy. Also, deterioration of mature seeds from the mother plant can result in reduced rates of germination (Stubsgaard, 1992) as well as when there are inhibition

problems (Randriatafika *et al*., 2008). Besides these, processes such as rough handling, excessive drying, exposure to extreme temperatures and moulding can affect the viability of freshly harvested seeds. The highest percentage germination before storage was 93.00 % while that after storage was 89.00 % (Table 3.4.1). Again, the majority of the accessions had above 50 % germination (Fig 3.4.2) in the first four weeks after sowing. These results conformed to the findings of Cobbinah *et al*. (2001), who reported percentage germination in *C. pentandra* to be in the range of between 80.00 to 90.00 %. Kyereh *et al*. (1999) reported 56.00 % in light and 53.00 % in dark germination of *C. pentandra* seed under shade-house conditions over a period of 25 and 19 days, respectively. The current findings also compared favourably with the 80.00 % and 68.00 % germination in *Leucaena leucocephala* and *Acacia auroculiformis*, respectively, over 30 days as reported by Mahmud *et al*. (2005). According to these authors, synchronous germination in trees is genetically controlled and an indication of minimal genetic diversity. The synchronous germination pattern observed in the study could therefore probably be indicative of minimal genetic variability. This, however, needs to be investigated further. Seed polymorphism exists within species resulting in variability in seed size, shape, colour, germination behaviour and dormancy. For instance, seed size, among other things, depends on the ovary form, size of embryo and amount of endosperm present which, in turn, are determined by the genetic potentials of the individual trees (Mayer and Poljakoff-Mayer, 1982). Generally, thin seed coat makes it easier for the seed to rupture or soften paving the way for water and oxygen to enter into the embryos thereby mobilizing the food reserves in the embryo and leading to the germination of the seed. Hence, the synchronous germination could also imply uniformity in the seed coat thickness and serves as another indicator of minimal genetic diversity among the

accessions screened. The variability in percentage germination observed could, however, suggests differences in the genetic makeup of the accessions studied. These assertions, however, need to be established in further studies. Based on the PG results obtained, all the accessions were recommended for further screening, except the fourteen which did not germinate at all.

Percentage mortality was higher in the September-November Screening (SNS) (90.66 %) than in the March-May Screening (MMS) (20.53 %). The SNS had more frequent rainfalls than the MMS and that might have accelerated the seedling mortality in the former (Table 3.3.4). The differences in rainfall amounts could probably also be the reason why the 13 accessions, which were found to have 100 % mortality in the SNS screening, had above 50.00 % survival rates in the MMS (Table 3.4.5). These findings are supported by reports from earlier studies. For instance, Kozlowski, (1971) found that environmental stress, such as high rainfall, triggered seedling death by reducing seedlings vigour. Also, Charkrabarty *et al.* (1993) observed that dieback in *C. pentandra* was severer with increase in relative humidity. Further, seedling mortality in *Pongamia pinnata* (L.) caused by *Fusicladium pongamia* was reported to be higher in a nursery with higher rainfall than that with a low rainfall (Krishnambika *et al*., 2011). This probably could be due to the fact that increased rainfall amounts cause increase in relative humidity which, in turn, influenced dieback development by facilitating the rapid movement of the spores of the fungi that caused the disease. The change in status of the 13 accessions from susceptible in the SNS to resistant in the MMS could also be due to disease escape. This occurs when susceptible plants do not become diseased for some season due to anatomical or physical characters, such as the occurrence of leaf hairs, thick cuticle, or modified stomata, or due to environmental factors which are not conducive for

disease development (Maloy, 2005). The proportion of death attributable to the dieback disease and environmental stress was, however, difficult to quantify due to the unevenness of environmental factors. It is therefore recommended that future studies be done under green house or other methods with more uniform and controlled conditions.

The accessions MAA 1, BAW 1 and BAW 6 had 100 % survival and were rated non-susceptible in the MMS stage as illustrated in Table 3.4.2. This may not mean they are really non-susceptible to stem dieback. It could, however, be that the number of progenies that were tested per these accessions was just too small and included individuals with high resistance. Some of these accessions, especially BAW 1 and BAW 6, were rated susceptible in the SNS experiment when 500 seedlings of them were screened. This strongly attests to the fact that they could not be totally non-susceptible but could probably be an instance of disease escape. Again, Bingham *et al.* (1971) argue that the rating of an individual as susceptible does not mean that the individual does not have the potential resistance mechanisms and genes, but, rather, that these may be rare and of such a low frequency that only few or none of them survived in the presence of the pathogen. TNK 1, KWA 1, BUF 1, MSH 1, BUS A, EDW 1, KEC 9, GYE 1, AYE 2, TWE 1 and MAM 1, keeping the order, were more preferable for conservation as mother trees from the nursery screening.

Generally, survival levels were higher within populations than among them during both seasons (Tables 3.4.3 and 3.4.4). This means there is a higher possibility of finding several resistant accessions within a population than sampling the entire range of the species. In the SNS, the Dry Semi-Deciduous Forest Inner Zone (DSDFZ-Inner) had the highest number of accessions with high survival levels followed by the Moist Semi-Deciduous Forest Zone (MSDFZ), the Moist/Wet

Evergreen Forest Zone (M/WEFZ) and the least were the Dry Semi-Deciduous Forest Zones-Outlier (DSDFZ-Outlier) and the Guinea Savanna Zone (GSZ) (Table 3.4.3). The MMS on the other hand, showed a decreasing order of GSZ > DSDFZ-Inner > M/WEFZ > DSDFZ-Outlier > MSDFZ in terms of the number of accessions in the three survival classes (Table 3.4.2). The causes of the variability in seedling survival, besides the differences among populations and the varying number of accessions used per population, were not immediately known. Again, results from the study did not clearly follow any predictable ecological pattern for the five zones studied. Thus, the variability in resistance and susceptibility among the ecological zones were neither floristically nor edaphically structured. The variations in results could be due to the differences in the development of resistance mechanisms. According to Apetorgbor *et al.* (2003), dieback was first detected in the Bobiri Forest Reserve located in Moist Semi-deciduous Forest zone (MSDFZ), which might have resulted in individuals from this zone developing resistance to the disease and thereby making the pathogens to lose their virulence in the zone. Besides, this forest zone has a bimodal rainfall system with intermittent dryness. It therefore stands to reason that the dryness might have had an adverse effect on the proliferation of the disease in the zone probably by interrupting with the life cycle of the causative agents. The dryness might either be timely causing the drying up of the fungal spores or greatly affecting their transmission from one plant to the other. This may be the reason why the Moist/Wet evergreen forest zone (M/WEFZ) which has high amounts of rainfall with its accompanying high relative humidity, is acting as the sink for the disease. High amounts of rainfalls are known to have caused high rates of dieback in India (Charkrabarty *et al*., 1993). Also, differences in the genetic integrity of the populations studied could be the reason for the variation in dieback resistance.

Espahbodi *et al*. (2007) attributed the significant differences between *Sorbus torminalis* seedling survival in nursery beds to differences in seed sources. According to these authors, seeds from different latitudes could alter seedling performance even within the same species. It is therefore suggested that further studies be conducted with seeds collected from different latitudes to substantiate the effects of seed sources on the resistance of *C. pentandra* to stem dieback disease. The polyethylene bags used for the experiment might have also contributed to the ambiguity of the trend observed. Research has shown that these bags can cause root curling and spiral growth resulting in dieback and other seedling disorders (Jaenicke, 1999).

3.6 Conclusion

The results of this study showed that individual trees of *C. pentandra* from the five populations in Ghana have synchronous but varied percentage germination rates. This variability did not, however, follow any known pattern. The study also showed that maximum percentage germination in *C. pentandra* could be achieved in a period of three to four weeks when viable seeds are sown. Resistance to stem dieback was higher within ecological zones than among them. Again, susceptibility of *C. pentandra* to stem dieback disease seemed to increase with increase in the amount of rainfall. This knowledge, as part of the *C. pentandra* phenology, could be used for monitoring biodiversity and to assist in the planning of future ecological rehabilitation works.

3.7 Recommendations

It is recommended that:

1. individual tree selection, based on genetic worth, be preferred in *C. pentandra* seed collection for resistance to stem dieback disease.
2. the peak of the rainy season of the planting site be preferred as the best time for screening *C. pentandra* for stem dieback disease resistance so as to obtain genuinely resistant individuals. However, when resistant individuals are already found and the desire is to raise enough seedlings for large scale planting, it is advisable to do the nursery work during the minor rainy season. The latter will ensure that environmental stress resulting from increased relative humidity does not affect the seedling growth.
3. efforts geared at sampling for stem dieback resistance in *C. pentandra* be focused on selecting accessions within populations with high stem dieback resistance, when resources are limited.
4. larger quantities of seed be taken from accessions with low percentage germination rates and smaller quantities from those with high rates, in order to optimize the number of seedlings needed.
5. further screening of seedlings in the field be done to enable a more reliable assessment of stem dieback disease to be made.

CHAPTER 4

PROGENY VARIATION IN RESPONSE TO STEM DIEBACK ATTACK IN *C. pentandra*

4.1 Introduction

Dieback is a common disease causing widespread death in several species from widely varied causative agents. Damage to leaves and stems occurs on affected trees sometimes causing disfiguration from the dieback of twigs and branches (Boa, 1995). The author notes that economic loss from stem dieback is usually caused by the repeated annual defoliation and the dieback of twigs and branches, which weakens the trees and makes them more susceptible to other diseases, physical injuries and insect damage. The concept of forest decline as a distinct syndrome has received intense debate and research (Beever *et al*., 1996). For instance, Gilbert and Steven, (1996) note that 31 % of multi-aged seedling and sapling populations of *Tetragastis panamensis* were attached by stem dieback with 13 (72 %) stems suffering dieback out of every 18 canker stems. The authors note that the infection sometimes results in rapid plant death with no other external symptoms with individual trees tolerating the infection to varying degrees. Sudden oak dieback is also reported to have affected between 40 % and 80 % of trees in any given stand of the oak tree (Garbelotto *et al.*, 2001). Further, canker and dieback were responsible for the death of 36 % of 17 994 trees of *Grevillea robusta* in small-scale farms in Kenya (Njuguna *et al*., 2011).

The rise in demand for *C. pentandra* seedlings for large scale commercial plantation establishment under the NFPDP has become inevitable. Hence seeds of *C. pentandra* are being collected from the wild to be used to raise seedlings to meet the demands of the NFPDP. However, there is relatively insufficient information on

genetic and morphological traits, based on which selection and subsequent breeding could be made. Also, dieback of *C. pentandra* seedlings and saplings has been observed in both the nursery and plantation (Apetorbor *et al.*, 2003) and it poses a great threat to large scale commercial establishment of this species and puts high investment in this direction at risk. There is therefore the need for the identification and use of resistant stock. The estimation of genetic parameters, such as prediction of breeding values, is also essential for the selection of genetic resistant genotypes.

The current study looked at the estimation of narrow sense heritability (h^2) and genetic gain in height growth and survival rates in *C. pentandra* using 37 accessions from five populations of Ghana. Height is a key economic trait in plantation development of this species since growth in height depends on the level of resistance to dieback or recuperative ability after fungal infection. The rationale of the study was that wild, undomesticated, out-bred forest trees are highly variable and their natural populations retain a wide genetic diversity that helps them resist pests and diseases. Initial selection of the most desirable trees from the natural stands could improve the genetic quality of the tree species (Frampton, 1996). The null hypothesis to this study was that all the *C. pentandra* in Ghana are genetically similar and that no gain can be achieved through selection. This was based on the preposition that the long-distance seed dispersal biology of *C. pentandra* by animals, wind and water in Africa and the Neotropics, as reported by Dick *et al*. (2007), has made all populations of the species in Ghana homogenous.

4.2 Objectives

The objectives of this chapter were to:

1. estimate the level of narrow sense heritability and genetic gain in height growth of C. *pentandra*.
2. identify the level of stem dieback resistance in accessions of *C. pentandra* in the field.

4.3 Materials and methods

4.3.1 Study site

The field trial was established at Abofour, one of the research plots of the Forestry Research Institute of Ghana of the Council for Scientific and Industrial Research. Abofour is located between latitude 6.15 º N and longitude 1.10 º W in the Afram Headwaters Forest Reserve. It falls within the Dry Semi-Deciduous Forest Zone with mean annual rainfall in the range of 1250-1500 mm (Hall and Swaine, 1981). The reserve was created in 1908 and it is about 123.3 sq-km (47.6 sq-miles). The major factors that influenced the choice of this site are that it is one of the sites which is most likely to be reforested with the end products of the planting stock that is being assessed and that it is neither too dry (compared to the Savanna Zone) nor too wet (compared to the Moist/Wet Evergreen Zones).

4.3.2 Progeny trials

Seeds from 37 half-sib families (open-pollinated trees in which only one parent; the female, is known) were used in this study (Appendix 4.3.1). The seeds were collected, processed, germinated and screened under shed as described in sections 3.3.1, 3.3.2, 3.3.4 and 3.3.5, respectively. The seedlings were transplanted to the field

at the age of five months. The design for field testing was done using 30 seedlings per accession in a randomized complete block design (RCBD) with three replications. Ten seedlings were planted in each replicate in two rows with five seedlings per row. The planting distance was 2.5 m x 2.5 m within and between rows. A total of 1110 plants were planted. Trees that previously occupied the plot were all felled. Weed control was done by manual weeding at least once every month after planting. Data on growth in height and diameter were taken at the time of planting using tape measure and callipers, respectively. Mortality was assessed at 12 weeks after planting and beating-up carried out to ensure that all mortalities due to planting shock were nullified. Mortality was again assessed at 12 months after planting.

4.3.3. Data collection and analysis

Growth was estimated as y_2-y_1, where y_1 and y_2 are height or diameter at time of planting and 12 months, respectively. Data were subjected to analysis of variance using Microsoft Excel 2007 at a P-value of 0.05. Mortality data were arc-sine transformed (Snedecor and Cochran, 1980) before subjecting the data to analysis of variance. Standard errors of mean height, diameter increments and survival rates were also computed to depict the variation about the means. Narrow sense heritability (h^2) was estimated following Zobel and Talbert (1991) as described below:

$h^2 = V_G/V_P$ . . . . . (Equation 1)

$V_P = V_G+V_E$ . . . . . (Equation 2)

where:

h^2 = Narrow sense heritability

V_G = Variations arising from differences in genetic constitution (between accessions variation)

V_P = Variations in phenotypic characters

V_E = Variation due to environment (within accessions variation)

From the analysis of variance table (Table 4.3.1),

$V_G = (MS_1-MS_3)/r$ . . . . . (Equation 3)

where:

MS_1 = Mean square between accessions = $V_E + rV_G$ . . . . . (Equation 4)

MS_2 = Mean square of blocks

MS_3 = Mean square among accessions = V_E

r = Number of replications = number of blocks

Table 4.3.1 Analysis of variance

Source of variation	Df	MS	F
Between accessions	f-1	MS_1	MS_1/MS_3
Blocks	r-1	MS_2	
Within accessions	f (r-1)	MS_3	

The selection differential (S) was estimated as shown below.

$S = Xs - X\mu$ . . . . . (Equation 5)

where:

S = Selection differential (difference between mean of selected individual and the population mean (Jansson, 2005)).

$X\mu$ = Mean of population

Xs = Mean phenotypic value after selection (sample mean)

Genetic gain (G) was estimated as:

G = Percentage gain *h^2 . . . . (Equation 6)

where:

h^2 = Narrow sense heritability and

The illustrations of the estimation of selection differential and genetic gain are shown in Appendices 4.3.2 and 4.3.3, respectively.

Dieback severity was assessed using the number of dead plants, number of attacked leaves, number of progenies with visible dead dieback stems and length of dead dieback stems at 12 months after planting. Mortality and survival rates were computed as described in section 3.3.5. The average number of progenies with dieback stems and the number of attacked leaves for each accession were estimated in percentage and graded on a 10-point scale following Potts *et al*. (2004) as: 0 - 3 % = 1, 4 - 6 % = 2, 7 - 12 % = 3, 13 - 17 % = 4, 18 - 25 % = 5, 26 - 38 % = 6, 39 -50 % = 7, 51 - 63 % = 8, 64 -75 % = 9, and 76 - 100 % =10. Percentage survival and length of dieback stem were, however, graded on 4-point and 7-point scales respectively. Survival rate was graded as follows: 70 - 100 % = 1, 50 - 69.9 % = 2, 1 - 49.9 % = 3, 0 % = 4 and length of stem dieback as <1 cm = 1, 1 - 2 cm = 2, 3-4 cm = 3, 5 - 6 cm = 4, 7 - 8 cm = 5, 9 - 10 cm = 6 and ≥ 11 cm = 7. Thus accessions with smaller values performed better. All the points were added up to represent the severity score of the individual accession. Selection of accessions for resistance was based on the performance of their progenies. Accessions were ranked based on (1) survival rates alone and (2) on the total severity score from all the parameters measured. Accessions were further categorized into their respective populations and the populations ranked according to their resistance levels to stem dieback disease.

4.4 Results

4.4.1 Heritability and genetic gain

Mean height increment was significantly different ($P < 0.001$) among the 37 accessions studied (Appendix 4.4.1). Height increment, selection differential, narrow sense heritability and genetic gain varied among the accessions (Table 4.4.1). Narrow sense heritability (h^2) in height growth was 0.56 indicating that 56 % of the observed variation in height growth was genetically controlled, whereas 44 % was due to environmental factors. Genetic gain varied from 20.89 % (DNY 1) above mean performance to as low as -29.58 % (TAN 1) below mean performance. Twenty two accessions (59.46 %) performed above average (Table 4.4.1).

4.4.2 Progeny evaluation for height and diameter growth

The mean stem height and diameter growth were both significantly different as shown in appendices 4.4.2 and 4.4.3 respectively. Mean height increment ranged between 6.03 cm (TAN 1) and 177.00 cm (DNY 1) (Fig 4.4.1). Mean diameter growth also ranged from 0.9 mm for KON 13 to 41.77 mm for AYE1 (Fig. 4.4.2). Twenty-two accessions (59.46 %), out of the 37 accessions screened, had mean height increment above the overall mean height growth of 106.24 cm/yr. Selection of these 22 accessions resulted in a selection differential of 26.36 cm/yr (S = 132.6 - 106.24). Genetic gain in height was then estimated to be 14.76 cm/yr and this represents a gain of 11.13 % in height growth per year. The genetic gain in height growth for selecting a particular accession is shown in Table 4.4.1.

4.4.3 Survival rates

Mortality rate was significantly different among accessions (P= 0.001) as illustrated in appendix 4.4.3. Survival rate among accessions ranged from 13.33 % to 90.00 %. Fourteen accessions (37.84 %) out of the 37 accessions had survival rates of 50 – 90 %, and thus falling in the moderate to high survival classes (Table 4.4.2). Six of these accessions (16.22 %) belonged to the DSDFZ-Inner, 2 (5.41 %) to the DSDFZ-Outlier, 3 (8.10 %) to the MSDFZ, 2 (5.41 %) to the M/WEFZ and one (2.70 %) to GSZ. Twelve (85.71 %) of these fourteen accessions were among those ranked between 1 and 10 after the relative levels of dieback resistance were accessed based on (1) survival, (2) number of attacked leaves, (3) number of progenies with dieback stems and (4) length of dieback stems (Table 4.4.3). Appendix 4.4.4 shows some distinct variation in resistance levels among individuals of the same accession.

Table 4.4.1 Height increment and genetic gain in height growth among 37 accessions of *C. pentandra*.

Accession	Mean height (cm/yr)	Deviation	Percentage deviation	Percentage gain	Genetic gain	Rank
DNY 1	177.00	70.76	66.61	37.30	20.89	1
KON 8	163.27	57.03	53.68	30.06	16.83	2
AYE 1	157.62	51.38	48.36	27.08	15.16	3
SOF 1	157.39	51.15	48.15	26.96	15.10	4
ODO 6	150.82	44.58	41.96	23.50	13.16	5
SKD 1	148.87	42.63	40.13	22.47	12.58	6
BUF 1	140.73	34.49	32.46	18.18	10.18	7
NKIN 2	138.65	32.41	30.50	17.08	9.56	8
POK 1	131.78	25.54	24.04	13.46	7.54	9
KWA 1	129.61	23.37	22.00	12.32	6.90	10
MAM 1	129.61	23.37	22.00	12.32	6.90	11
KUE 2	127.33	21.09	19.85	11.12	6.23	12
NKA 2	124.92	18.68	17.58	9.84	5.51	13
ABF 1	123.14	16.90	15.91	8.91	4.99	14
ACH 1	118.48	12.24	11.53	6.46	3.62	15
EDW 1	117.79	11.55	10.87	6.09	3.41	16
GYE 1	116.80	10.56	9.94	5.57	3.12	17
NKA 1	115.98	9.74	9.16	5.13	2.87	18
DAN 1	115.30	9.06	8.53	4.78	2.68	19
NKIN 1	113.07	6.83	6.43	3.60	2.02	20
KEC 10	109.59	3.35	3.15	1.76	0.99	21
KUE 1	109.38	3.14	2.96	1.66	0.93	22
ASE 1	104.38	-1.86	-1.75	-0.98	-0.55	23
AYE 2	96.48	-9.76	-9.18	-5.14	-2.88	24
KUE 3	91.47	-14.77	-13.91	-7.79	-4.36	25
KEC 9	88.97	-17.27	-16.26	-9.11	-5.10	26
BAW 10	83.20	-23.04	-21.69	-12.15	-6.80	27
KON 9	82.37	-23.87	-22.47	-12.58	-7.04	28
MSH 1	75.17	-31.07	-29.25	-16.38	-9.17	29
BAW 1	73.49	-32.75	-30.83	-17.26	-9.67	30
MAA 1	59.38	-46.86	-44.11	-24.70	-13.83	31
BAW 4	55.28	-50.96	-47.96	-26.86	-15.04	32
PAW 5	52.09	-54.15	-50.97	-28.54	-15.98	33
KON 13	49.30	-56.94	-53.59	-30.01	-16.81	34
WOA 1	48.92	-57.32	-53.96	-30.22	-16.92	35
BAW 5	47.15	-59.09	-55.62	-31.15	-17.44	36
TAN1	6.03	-100.21	-94.33	-52.82	-29.58	37
Mean	**106.24**					

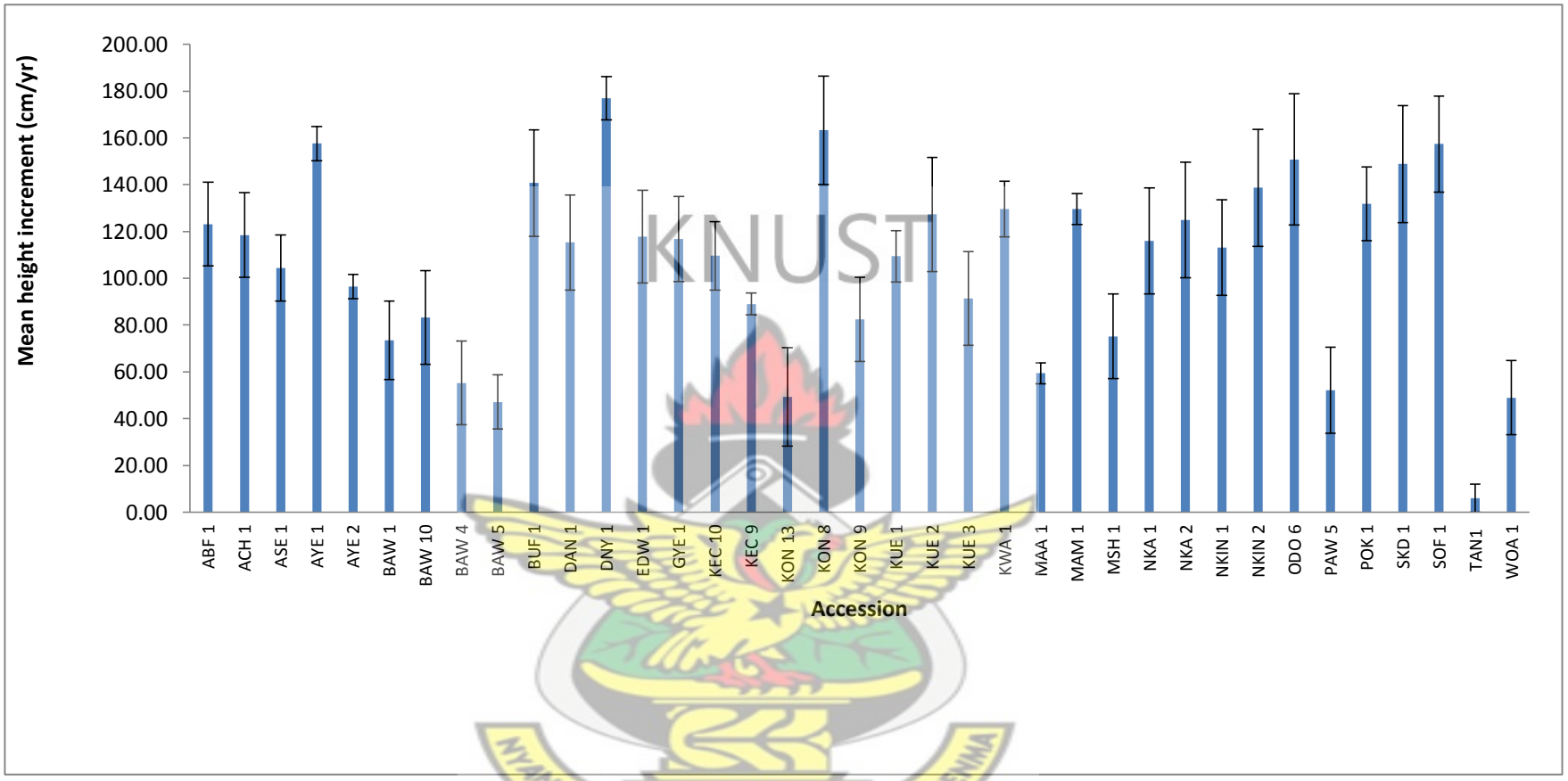


Fig 4.4.1 Height increment among 37 accessions of *C. pentandra* in response to stem dieback infection. Bars = ± standard error of means.

(See Appendix 4.3.1 for names of populations and localities of accessions).

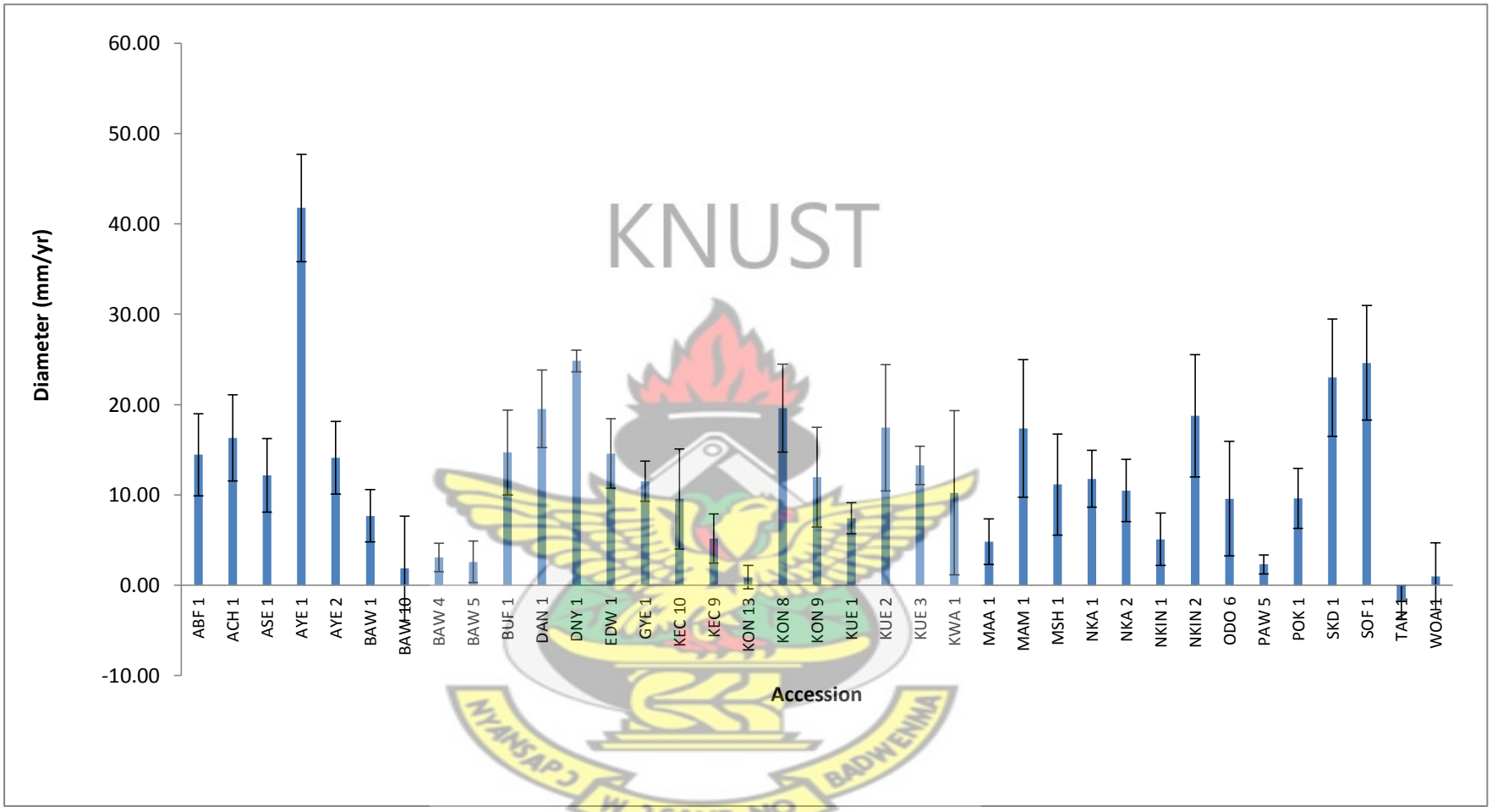


Fig 4.4.2. Diameter increment among 37 accessions of *C. pentandra* in response to stem dieback infection. Bars = ± standard error of means. (See Appendix 4.3.1 for names of populations and localities of accessions).

Table 4.4.2 Survival levels of *C. pentandra* accessions screened in the field

Survival levels (%)	Accession	Ecological zone	Survival (%)	Total
	MSH 1	MSDFZ	90.00	
Highly	MAM 1	M/WEFZ	80.00	5
resistant	EDW 1	MSDFZ	73.33	(13.51 %)
(70-100 survival)	KWA 1	MSDFZ	73.33	
	KUE 2	DSDFZ-Outlier	70.00	
Moderately	DAN 1	DSDFZ-Inner	63.33	
resistant	SKD 1	DSDFZ-Inner	63.33	
(50-69.9 survival)	BUF 1	DSDFZ-Inner	63.33	9
	ABF 1	DSDFZ-Inner	60.00	(24.33 %)
	NKIN 1	DSDFZ-Inner	60.00	
	ASE 1	DSDFZ-Inner	60.00	
	BAW 5	GSZ	56.67	
	NKA 1	M/WEFZ	53.33	
	KUE 1	DSDFZ-Outlier	50.00	
Fairly resistant	BAW 1	GSZ	46.67	
(<50 survival)	KUE 3	DSDFZ-Outlier	46.67	
	ODO 6	DSDFZ-Outlier	46.67	23
	DNY 1	DSDFZ-Inner	46.67	(62.16 %)
	KON 13	GSZ	43.33	
	POK 1	M/WEFZ	43.33	
	KEC 9	DSDFZ-Outlier	43.33	
	GYE 1	MSDFZ	43.33	
	BAW 10	GSZ	40.00	
	NKA 2	M/WEFZ	40.00	
	AYE 2	DSDFZ-Inner	40.00	
	ACH 1	MSDFZ	36.67	
	AYE 1	DSDFZ-Inner	36.67	
	KEC 10	DSDFZ-Outlier	36.67	
	NKIN 2	DSDFZ-Inner	33.33	
	SOF 1	M/WEFZ	33.33	
	BAW 4	GSZ	33.33	
	PAW 5	DSDFZ-Outlier	30.00	
	KON 9	DSDFZ-Outlier	30.00	
	WOA 1	MSDFZ	26.67	
	KON 8	GSZ	26.67	
	MAA 1	M/WEFZ	23.33	
	TAN 1	DSDFZ-Inner	13.33	
Total				37(100 %)

Table 4.4.3 Ranking of accessions based on total stem dieback disease severity score using survival rates, mean number of attacked leaves, mean length of dieback stem and mean number of trees with dieback.

Accession	Survival (%)	Score 1	Attacked leaves (%)	Score 2	Length of dieback stem(cm)	Score 3	Mean no. of trees with dieback (%)	Score 4	Total score	Ranking
DAN 1	63.33	2	0.00	1	0.00	1	0.00	1	5	1
BAW 10	40.00	3	0.00	1	0.00	1	0.00	1	6	2
ABF 1	60.00	2	14.55	4	0.00	1	0.00	1	8	3
EDW 1	73.33	1	23.94	5	0.00	1	0.00	1	8	3
MAM 1	80.00	1	19.87	5	0.08	1	0.00	1	8	3
BAW 1	46.67	3	0.00	1	1.06	2	10.00	3	9	4
NKIN 1	60.00	2	25.81	5	0.00	1	0.00	1	9	4
NKA 2	40.00	3	32.94	6	0.00	1	0.00	1	11	5
BAW 4	50.00	2	45.35	7	0.00	1	0.00	1	11	5
KUE 1	50.00	2	42.29	7	0.00	1	0.00	1	11	5
ASE 1	60.00	2	22.01	5	0.99	1	10.00	3	11	5
KEC 9	43.33	3	0.00	1	26.11	7	3.33	1	12	6
KWA 1	73.33	1	42.30	7	4.11	3	0.00	1	12	6
MSH 1	40.00	3	17.13	4	3.33	3	6.67	2	12	6
TAN 1	30.00	3	60.91	8	0.00	1	0.00	1	13	7
MAA 1	23.33	3	41.98	7	0.07	1	6.67	2	13	7
ODO 6	46.67	3	39.72	7	1.00	2	3.33	1	13	7
POK 1	43.33	3	43.15	7	0.67	1	6.67	2	13	7
BUF 1	63.33	2	35.41	6	1.33	2	10.00	3	13	7
DNY 1	46.67	3	30.76	6	4.89	3	3.33	1	13	7
GYE 1	43.33	3	33.25	6	4.56	3	6.67	2	14	8
WOA 1	26.67	3	48.67	7	1.89	2	6.67	2	14	8
ACH 1	36.67	3	52.40	8	1.22	2	3.33	1	14	8
NKIN 2	33.33	3	31.29	6	6.67	4	3.33	1	14	8
KUE 3	46.67	3	65.67	9	2.00	2	3.33	1	15	9
KON 8	26.67	3	28.53	6	7.56	5	3.33	1	15	9
SOF 1	33.33	3	37.18	6	5.33	4	6.67	2	15	9
PAW 5	30.00	3	48.77	7	2.03	1	16.67	4	15	9
SKD 1	63.33	2	20.84	7	6.32	4	10.00	3	16	10
NKA 1	53.33	2	52.95	8	6.22	4	6.67	2	16	10
KON 13	43.33	3	38.27	6	13.33	7	3.33	1	17	11
KUE 2	70.00	1	95.16	10	6.22	4	6.67	2	17	11

AYE 1	36.67	3	33.29	6	9.94	6	10.00	3	18	12
KON 9	30.00	3	26.06	6	6.00	4	20.00	5	18	12
AYE 2	40.00	3	54.89	8	9.73	6	6.67	2	19	13
BAW 5	56.67	2	69.32	9	14.70	7	3.33	1	19	13
KEC 10	36.67	3	68.49	9	5.72	4	13.33	4	20	14

4.5 Discussion

The narrow sense heritability estimated was 0.56. Since heritability is a measure of the degree to which parents transfer heritable traits to their offspring (Jansson, 2005; Zobel and Talbert, 1991), the genetic effect on height growth is 56 %. This value is similar to results from other studies in forest trees. For instance, heritability values of 0.74 and 0.51 were recorded in the total height growth of two-year old *Khaya anthotheca* and *Khaya ivorensis* respectively (Ofori *et al*., 2007). McKeand *et al.* (2008) also reported narrow sense heritability in height growth of Loblolly pine to range from 0 to 0.62 at different planting sites. A positive correlation is known to exist between phenotype and breeding value, increased potential for natural selection and high heritability (Jansson, 2005). The high heritability obtained in the current study therefore suggests that selection of individuals based on height increment has the potential to retain high productivity in future generation of the species. Heritability estimates are known to be population, trait and test environment specific (Goncalves *et al*., 2009; Jansson, 2005). Also, tree characteristics vary in the degree of genetic versus environmental influence and that genetically inferior trees may appear phenotypically desirable if planted in an unusually favourable micro-environment. Likewise, genetically superior trees may appear phenotypically undesirable due to poor environmental conditions (Frampton, 1996). Accessions which performed below average could possibly do well if planted in a different test

environment and those which performed above the mean could possibly perform poorly if planted in another environment. It is, therefore, suggested that this study be repeated in the remaining four populations and the differences in genetic gain be established.

The values of the genetic gain (-29.58 to 20.89 cm/yr) obtained in the study indicate the presence of different levels of resistance to dieback and, hence, a high potential for selection of dieback tolerant individuals. Selection becomes a more effective tool in genetic improvement when all traits of economic importance are evaluated (Silva *et al*., 2008). Therefore, this study should be repeated and heritability estimated based on other economic traits, such as seed size, seed weight, seed colour, number of seeds per pod, leaf size, leaf length, leaf width, petiole length, petiole colour, degree of spines, stem colour and stem diameter. This will enable the accurate prediction of genetic gain for efficient selection in *C. pentandra* and also enhance its use as a multipurpose species. The twenty-two accessions that had mean height above the mean performance (Table 4.4.1) were, therefore, recommended for selection, protection and use as seed trees. This followed the recommendations of Namkoong *et al*. (2000) that only genotypes whose phenotypes approximate the population mean are good for selection while those below should be considered as selection disadvantage. *C. pentandra* is known to have a pan tropical distribution (Lobo *et al.*, 2005) and to be a widespread rainforest tree species (Dick *et al*., 2007). In Ghana, it is found in all the forest types (Hall and Swaine, 1981). These suggest that the species has a wide environmental adaptability. However, the 44 % variability attributable to the growth environment suggests that some environments may be more suitable for the growth of the species than others.

The range of survival levels, 23.33 % to 90.00 %, indicates the existence of varied degrees of resistance classes among the accessions. A majority of the accessions, 14 (37.84 %), had survival rates between 50-90 %. Again, differences between accessions accounted for 49.72 % of the total variation in mortality rates. The large variation in stem dieback resistance within and among the accessions, as shown in the study, suggests that selection for resistance can be efficient. Therefore, further analysis should be made at age two and four to estimate juvenile-mature correlation to ascertain the best age to undertake selection in *C. pentandra*. When this is done, a range wide seed collection mission could be undertaken to allow for screening at different sites in order to capture the total genetic variation as well as estimation of genotype by environment interactions.

The results further showed that there was variation in levels of resistance among populations. Most of the accessions with good resistance levels came from the Dry Semi-Deciduous Forest Zone-Inner (DSDFZ-Inner). This is very similar to the order observed in the nursery screening as described in Chapter 3, thus making the population a more preferred site for *C. pentandra* seed collection than the others. The degree to which trees were affected and killed by *Armillaria ostoyae* varies with geo-climatic zones, subzones and site series, whereas host resistance to invasion varies with host genetics, age, and vigour (Cleary *et al*., 2008). In another study, source regions of pest and diseases were identified to have high resistance to due to many years of exposure (Adams *et al*., 2002). On the contrary, the authors note that, trees in regions into which pests and diseases have been newly introduced were heavily killed as a result of the absence of selection for resistance. Based on these findings, it is therefore possible that stem dieback in *C. pentandra* existed earlier in the DSDFZ-

Inner than all the other populations although it was first detected in the MSDFZ (Apetorgbor *et al*., 2003).

Mean height growth was at a low of 6.03 cm in TAN 1 and a high at 177.00 cm in DNY 1 (Fig 4.4.1). A greater proportion of the variation observed in stem height was attributed to differences between accessions. One accession, TAN 1, had mean diameter growth at 12 months lower than its mean height at planting (Fig 4.4.2). The negative growth shows the extent of dieback of seedlings. The variation in growth performance was mainly as a result of the stem dieback disease. Accessions with reduced growth were heavily defoliated due to heavy leaf infection. According to Boa (1995), defoliation depletes the energy reserves of the tree and increases its susceptibility to other pests and diseases. Resistance to disease in trees is also known to be affected by tree vigour (Cleary *et al.*, 2008). In Kenya, poor growth in *Grevillea robusta* was attributed to canker and dieback disease (Njuguna *et al*., 2011). According to Zobel and Talbert (1984), variability within a species, detectable by observations and experimentations, are usually genetically controlled with the individuals evolving through natural selection, and are adapted to a well defined environment with the ability to survive and reproduce. Also, differences in growth and development among species, populations within species and individual plants of woody species are known due to variation in their genetic constituents (Kozlowski and Pallardy, 1997). Thus, the growth differences observed in the current study might be due to some genetic differences in the accessions of *C. pentandra*. It was also noted that individual progenies of accessions had different reactions to stem dieback disease. Some individual progenies performed better than others within accessions (Appendix 4.4.4). For instance, 11 out of the 22 best performing accessions had survival rates of less than 50 %. The high increment recorded was, therefore, due to

the individual progenies' growth potentials. Therefore, further studies should be narrowed down to the individual progeny level for the best ones to be selected and conserved as seed trees. The selection of accessions in this study was based on their growth rates (Table 4.4.1) and resistance to stem dieback (Table 4.4.3). Based on these, 22 accessions (59.46 %), out of the 37 accessions tested: DNY 1, KON 8, AYE 1, SOF 1, ODO 6, SKD 1, BUF 1, NKIN 2, POK 1, KWA 1, MAM 1, KUE 2, NKA 2, ABF 1, ACH 1, EDW 1, GYE 1, NKA 1, DAN 1, NKIN 1, KEC 10 and KUE 1, were suitable for conservation as mother trees. Selection using these criteria has been shown to yield good results in previous studies.

4.6 Conclusion

At 12 months, there was significant variation in tree height among accessions across the five populations. This is important for tree breeders, who would like to carry out selection as early as possible. It could, therefore, be concluded that greater gains in plantation yield of *C. pentandra* could be realized by selecting fast growing accessions. Twenty-two accessions, based on these results, were selected from the 37 accessions to advance their generations. This is the first study in Ghana to demonstrate the existence of variability in narrow sense heritability and genetic gain in *C. pentandra* which has important practical implications for genetic resource management and tree improvement programmes in Ghana. The detection of genetic differences at an early age would therefore be very useful for agroforestry tree improvement programmes in Ghana.

The results showed that accessions differed in their response to stem dieback disease with much of the variation occurring within populations. This indicates that, with careful selection, sufficient resistant accessions could be found in each of the

natural ranges of the species. The differences in variation within accessions were larger and similar for height growth and mortality rate. This implies that adequate amounts of materials with good levels of stem dieback resistance can be found within the progenies of accessions with high resistance levels. This, by critical selection, could be exploited for the plantation establishment of *C. pentandra*. There is, therefore, the need to further investigate the genetic relatedness of the variability observed within and among accessions using more reliable methods, such as molecular techniques. Research on stem dieback resistance should be done as part of a package of studies on other potential dieback disease control methods so that the identified resistant varieties can be deployed in an integrated management strategy. Again, though the ecological significance of dieback in the population dynamics of *C. pentandra* is not yet known, the substantial variation in mortality rates and the strong reduction in growth of infected plants suggest that this disease has the potential to be an important factor in regulating populations of *C. pentadra* in Ghana's forests. These findings are important; first because they indicate that all the accessions of *C. pentandra* in all populations are potentially at risk. Further, they may be used to guide future resistance screening efforts, for example, by placing more emphasis on sampling more trees within populations rather than on expanding the number of populations, given the same amount of sampling effort. Finally, they may be used to generate testable models of disease dynamics based on the small but significant differences observed among populations.

4.7 Recommendations

1. Since the study was done at only one site, it is suggested that it be repeated in more than one test sites at different ages of the species to fine-tune the species-site matching for *C. pentandra*.
2. A breeding programme for *C. pentandra* should be initiated to enhance the profitability of genetic gains in the future.
3. The variation in resistance and susceptibility at different populations and the mechanisms involved in the host-pathogen interaction that lead to stem dieback should further be studied.
4. Also, an integrated approach, including the use of resistant varieties, practices that enhance tree health, early disease detection, mixed species plantations and targeted chemical treatment, may hold some promise for the control of stem dieback in *C. pentandra* and should be investigated.

CHAPTER 5

MORPHOLOGICAL AND GENETIC RELATEDNESS AMONG *C. pentandra* POPULATIONS

5.1 Introduction

Plants are at the mercy of the environment as they cannot move and, therefore, rely on genetic variation for continual survival (Lark, 2008). Genetic diversity is of prime importance for the survival and adaptation to future climatic changes (Henry, 1997). This is because high levels of genetic variation provide the ability for tree species to adjust to new environments, allowing local adaptation and the migration of better-suited provenances along ecological gradients (Williams *et al*., 2007). Analysis of species population genetic diversity is seen as an essential tool for its conservation and management (Vendramin and Hansen, 2005; Archak *et al*., 2003; Ofori, 2001). Several studies have looked at the identification and estimation of genetic variation in forest trees based on morphological traits (Ofori *et al*., 2007; Ofori *et al*., 2001; Monteleone *et al*., 1996). Morphological variation is sometimes constrained by the environment since its variation is dependent on the environment unlike molecular variation which is independent on the environment. As a result, genetic markers are increasingly being used for the study of genetic diversity in recent times. The polymorphism determined by these markers is one of the valuable parameters for study of populations and understanding of their genetic differences (Askari *et al*., 2011). Some of the molecular techniques used, particularly, when no genetic information is available, include RAPD (Deng *et al*., 2006; Ofori, 2001; Brown, 1996) and ISSR (Xia *et al*., 2008; Archak *et al*., 2003; Lee *et al*., 2003; Ofori, 2001) and the combined analysis of RAPD and ISSR polymorphisms (Gupta *et al*., 2008;

Ofori, 2001).

C. pentandra is an important timber species in Ghana, but little is known about the pattern and extent of genetic variability in the species that exist in the different ecological zones of Ghana. The amount of variation within the species was, therefore, studied using both morphological and genetic markers. This will ensure the right measures are taken for the sustainable management of *C. pentandra*. It would also allow for the comparison of phenotypic characters with genetic markers to better explain the variations in the accessions and provides sufficient information for the identification of superior genotypes. A better understanding of the genetic relationship among different accessions of *C. pentandra* could also contribute to the development of *in situ* and ex-situ conservation strategies. Furthermore, analysis of the distribution patterns of genetic variation of this species would be useful for the restoration of its degraded populations through the designation of appropriate seed zones.

5.2 Objectives

The objectives of this study were to:

1. estimate the diversity within and among five populations of *C. pentandra* in Ghana using both morphological and genetic characterization, and
2. identify genetically unrelated accessions for conservation as seed trees.

5.3 Materials and Methods

5.3.1 Morphological characterization

The progeny trial plot described in sections 4.3.1 and 4.3.2 were used for this study. Data were collected on five morphological characters namely stem height, diameter, stem colour, leaf stalk colour and amount of spines for 37 accessions at 12 months

after transplanting in the field. Data on average stem height and diameter increment as obtained in section 4.3.3 were used. Stem colour was characterized as deep-green or light-green, while petiole colour was characterized as deep-purple or light-purple. The amount of spines was estimated as a proportion of the total area of stem covered with spines expressed in percentage. The morphological data were transformed into binary numerals; 1 for presence and 0 for absence, and used to study the diversity and relatedness among accessions. Stem and petiole colours were preferred, because they were easily observable, could be characterized with high accuracy and could be used in rapid prediction of stem dieback resistance in *C. pentandra* without detailed field and molecular study.

5.3.2 Genetic characterization

5.3.2.1 DNA Sample collection

Fresh leaves were collected from three individuals for each of the 37 accessions that were transplanted in the field as described in sections 4.3.1 and 4.3.2 at the age of 12 months. The leaves were thoroughly washed with distilled water and dried with tissue paper to get rid of any possible contaminants. Fresh leaf tissue (200 mg), between 12-16 discs, was taken using a cork borer. The discs were placed in labelled 1.5 ml eppendoff tubes, frozen in liquid nitrogen and transported to the Biotechnology Laboratory for DNA extraction.

5.3.2.2 Genomic DNA extraction

Two methods of DNA extraction were used: the modified Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) adopted by the Cocoa Research Institute of Ghana, Tafo-Ghana for the extraction of DNA from Cocoa leaves and the

modified DNA isolation method described by Egnin *et al.* (1998). The modified CTAB method yielded DNA which was very difficult to pick with the micropipette. The extraction was repeated twice, each time with the same sample types but with different sample sizes so as to circumvent the problem but the results were not different. These genomic DNA sets failed to amplify efficiently with all the primers used and hence were discarded. The modified Egnin *et al.* (1998) extraction method, on the other hand, yielded high quality DNA which amplified with the primers used. DNA from this method was used for further analysis in this study.

The CTAB extraction method was done at the Biotechnology Laboratory of FORIG, Fumesua-Kumasi. Twelve to sixteen discs of fresh leaf samples were ground to fine powder in 2 ml liquid nitrogen. The powdered samples were topped up with 750 µl of CTAB buffer. The CTAB buffer used contained 2% CTAB, Tris HCl-1M, NaCl -5 M, EDTA-0.5 M, 2% PVP and 2-Mecaptoethanol/DTT- 0.1%). The mixture of the powdered sample and CTAB buffer was incubated with intermittent vortexing at 65°C. The samples were cooled at room temperature and 750 µl chloroform-isoamyl alcohol added to separate other proteins from the DNA and RNA. Samples were then mixed by several inversions of the tube and centrifuged at 14000 rpm for 15 minutes. The aqueous phases of the samples were then transferred into a clean 1.5 ml microfuge tube and the chloroform-isoamyl-alcohol cleansing step repeated to obtain the aqueous phase. Nucleic acids (DNA/RNA) were precipitated by adding ice cold isopropanol (two third of the sample volume) and kept overnight. The isopropanol in the mixture was decanted and the pellets were washed with washing buffer (76 % Ethanol, 10 mM Ammonium acetate) on a rocking surface for 15 minutes and centrifuged at 6000 rpm for 4 minutes. The washing buffer was decanted and pellet washed in 80 % ethanol and then centrifuged at 6000 rpm for 4 minutes.

The RNA was digested with RNAse. Thereafter, the pellets were dried in a vacuum for the ethanol to evaporate. The DNA was then dissolved in 100 µl Tris EDTA.

The second extraction was done at the Crops Research Institute of the Council for Scientific and Industrial Research, Fumesua-Kumasi. The DNA extraction buffer contained 1 M of Tris-HCl (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0), (10000 mwt) poly-vinyl-pirrolidone, 20 % sarkosine, sodium metabisulphite, 10 % sodium ascorbate. A total of 66 DNA samples were extracted from 36 accessions. Leaf tissue (200 mg) was weighed into 2 ml eppendorf tubes and ground to fine powder in liquid nitrogen. Eight hundred micro-litres of Buffer A (extraction buffer) were added and incubated at 90 ^{o}C for 10 minutes. The mixture was vortexed every 5 minutes and allowed to cool at room temperature for 2 minutes. Four hundred micro-litres of 5 M potassium acetate (Egnin *et al.*, 1998 used 800 µl of phenol chloroform isoamyl alcohol) were added and mixed gently by inversion 5 to 6 times and incubated on ice for 30 minutes with shaking and centrifuged for 10 minutes at 13000 rpm. The supernatant was transferred to another eppendorf tube. Ice cold isopropanol (1% of the sample volume) and 3 M sodium acetate (10 % of the sample volume) were added, mixed by inversion about 10 times. The DNA was precipitated at -20 ^{o}C for one hour and centrifuged at 13000 rpm for 10 minutes to pellet the DNA. The supernatant was poured and pellets washed with 800 µl of 80 % ethanol and centrifuged at 14000 rpm for 5 minutes. The alcohol was discarded and DNA pellets air-dried. DNA was re-dissolved in 500 µl of 1X TE Buffer and treated with 4 µl of RNAse at 37 ^{o}C for 30 minutes to remove the RNA component. Two hundred and fifty micro-litres of 7.5 M ammonium acetate were added and incubated on ice for 3 min and centrifuged at 13000 rpm for 5 minutes. The supernatant was transferred into fresh 1.5 ml tube and 700 µl of isopropanol added, mixed by inversion and

centrifuged at 13000 rpm for 15 minutes. The supernatant was discarded and pellets washed with 1 ml of 80 % ethanol, centrifuged at 14000 rpm for 5 minutes and dried at room temperature. DNA pellets were dissolved in 200 µl 1X TE Buffer and stored at -13 ^{o}C to -20 ^{o}C until required.

5.3.2.3 Determination of DNA quality and quantity

The quality and quantity of the DNA were determined using gel electrophoresis and spectrophotometer. With the gel electrophoresis, a mixture of 1 µl of genomic DNA and 2 µl of 6X loading dye were run on 0.8 % agarose gel. The intensities of DNA band florescence were compared with 100 bp standard DNA ladder and the DNA quality checked and the quantity estimated. The DNA quality was again verified with spectrophotometer (UV/Vis Spectrophotometer U-2001, Hitachi Instrument Inc.). Prior to the actual test with the genomic DNA sample, a reference point was run in a 1 ml quartz cuvette containing 500 µl of TE and the equipment zeroed. The quartz cuvette was cleansed and 5 µl of genomic DNA and 495 µl of TE put in and the UV absorbance at λ260 (A_{260}) and λ280 (A_{280}) and the ratio A_{260}/A_{280} determined. According to Henry (1997), an A_{260}/A_{280} of 1.8 indicates pure DNA.

5.3.2.4 RAPD and ISSR amplification

Several optimization experiments were conducted with DNA samples taken from each of the accessions in which different concentrations of template DNA, dNTPs, and *Taq* polymerase were used and the strongest and most reproducible pattern determined. A total number of 45 RAPD and 10 ISSR primers (Invitrogen, UK) were screened using randomly selected DNA samples and the primers that worked well (quality of amplification products and reproducibility) with the species were selected. From the

preliminary screening, 5 of the RAPD and 5 of the ISSR primers were reproducible and amplified with distinct and visible bands. These were selected for further analysis of all the DNA samples used. In performing the PCR, premix for genomic DNA was prepared using 1 µl 10x Buffer (this provided the right medium for the reaction to take place), 0.2 µl Taq polymerase (Industricod, South Africa), 0.9 µl of 25 mM $MgCl_2$, 0.4 µl 10 mM dNTPs, 0.5 µl of each primer, 6 µl nuclease free water, 1 µl DNA (10 ng/µl) which added up to a total reaction volume of 10 µl. Ten micro-litres of nuclease free water were used as a control. The thermal cycler (GenAmp PCR System 9700, version 3.09, Applied Biosystems), was programmed for 30 cycles and was run for 2 hours, 27 minutes and 2 seconds. Initial denaturation was 3 minutes at 94 ^{o}C, followed by 30 cycles of 1 minute at 94 ^{o}C, 1 minute at 48 ^{o}C, 1 minute at 72 ^{o}C and 7 minutes final extension step at 72 ^{o}C. The hold temperature was set at 4 ^{o}C.

5.3.2.5. Detection of variations in PCR products

The amplified PCR products were analyzed using electrophoresis on 2 % agarose gels stained with ethidium bromide. An agarose-TAE mixture was made and heated for 5 minutes in a microwave oven until the gel was completely melted. The mixture was partly cooled and 7 µl of ethidium bromide added and allowed to cool further. Ethidium bromide (5 µl) was also added to the running buffer in the tank. The cooled liquid gel mixture was poured into a horizontal gel casting tray (BioRad, UK Ltd) with combs inserted and allowed to solidify. The gel slab was then placed in the electrophoresis tank which was two-thirds filled with TAE buffer with no air bubbles trapped underneath. The combs were gently removed and additional buffer added until it reached the fill line. Loading dye (3 µl) was added to each amplified DNA sample before loading into the wells. The samples were electrophoresed at 120 V,

143 mA and 17 W for 45 minutes. The gel was visualized under a UV trans-illuminator (Alpha Imager) and banding patterns recorded by a camera linked to a computer. One accession, SOF 1, did not have any amplification bands and was excluded from further evaluation.

In the diversity study, DNA samples, from all the 36 accessions that amplified, were loaded in separate lanes on the gel and repeated for all the 5 RAPD and 5 ISSR reproducible primers. The assay also included a sample without genomic DNA, as a negative control to rule out the possibility for self amplification of the primers or the contamination of genomic DNA. Differences between accessions were detected based on the relative movement of their DNA fragments in the electric field provided by the process. Variability among accessions was determined by the positions of their respective DNA fragments on the gel in comparison with a standard 100 bp DNA size maker. Amplified DNA loci were manually scored as present (1) or absent (0) for each primer and a bi-variate 0-1 data matrix generated. Every scorable band was considered as a single locus/allele. Variations in band presence were recorded as polymorphisms.

5.3.2.6 Analysis of RAPD and ISSR polymorphism

The similarity and dissimilarity matrices were computed using the bi-variate 0 - 1 data matrix generated. The phenotypic data, the RAPD, the ISSR and the combined RAPD and ISSR polymorphism were analyzed separately. Variables were analyzed on the basis of several indices of population genetics, such as observed number of alleles, effective number of alleles, Nei's genetic distance, Shannon diversity index, expected heterozygosity, percentage polymorphic loci and number of neutral loci using Popgene version 1.32 (Yeh *et al.*, 1999). Principal component analysis was done

using GenALEx version 6.1 (Peakall and Smouse, 2006). The pair-wise genetic distances were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure described by Nei and Li (1979) with GenStat Discovery (Edition 3) (VSN International, 2003) as GD=1-dxy/dx+dy-dxy.

Where:

GD = Genetic distance between two accessions,

dxy = total number of common loci (bands) in two accessions,

dx = total number of loci (bands) in accession 1 and

dy = total numbers of loci (bands) in accession 2.

Dendrograms were constructed for the genetic similarities based on Nei's standard genetic distance (Nei, 1972) among populations and accessions generated by GenStat Discovery (Edition 3) (VSN International, 2003) using UPGMA (Sneath and Sokal, 1973). The strength of the dendrograms nodes were estimated with a bootstrap analysis using 10000 permutations. This was done to enable the global genetic relationships among the populations and accessions to be observed. Allele frequencies were computed based on Lynch and Milligan's (1994) Taylor expansion estimate. Hardy-Weinberg Equilibrium was assumed in all the estimations. The variance components and their significance levels for variation within and among population were analyzed using Analysis of Molecular Variance (AMOVA) at 999 permutations. The AMOVA was conducted in GenALEx (version 6.1) using PhiPT (analogue of F_{ST} fixation index). Also, the Wright's F_{ST} statistic ($F_{ST} = \theta$) measure was also applied to determine genetic heterogeneity among the populations using the Weir and Cockerham (1984) procedure. Jackknife over loci was applied to obtain a variance estimate of θ and bootstrap over loci was applied to generate 95 % confidence intervals with 10000 replications using TFPGA (version 1.3) (Miller, 1997). F_{ST}

coefficient was used in estimating relative population differentiation (Hu *et al.*, 1999). The null distribution of pairwise F_{ST} values on the hypothesis of no differences between the populations was tested by a permutation test with 10 000 replicates. Percentage of polymorphic loci, genetic diversity at the locus level, gene flow parameters: Chi-square, G-tests and Nm, were estimated using Popgene version 1.32 (Yeh *et al.,* 1999).

Table 5.3.1 Nucleotide sequences of the primers used to detect randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers by amplification of *C. pentandra* DNA in a PCR.

Primer identification	Nucleotide sequence (5′→3′)
RAPD Primers	
GEN60-3C	CGAAGCGATC
GEN60-3J	GAGCAGGCTG
OperonA-13	CAGCACCCAC
OPA 1	GAACAGCCTG
OPA 9	CAACAGCACC
ISSR Primers	
C3101E08	TCTCTCTCTCTCTCTCAT
C3101E09	AGAGAGAGAGAGAGAGCT
C3101E10	GAGAGAGAGAGAGAGACT
C3101E11	GAGAGAGAGAGAGAGATT
C3101E12	CACACACACACACACACT

5.4 Results

5.4.1 Morphological diversity

Generally, accessions were clustered into two groups in the dendrogram generated at a similarity coefficient of 0.92: a very diverse group A with five subclusters (A_1 - A_5) and a less diverse group (B) (Fig 5.4.1). Both groups contained accessions drawn from all the five populations with varied morphological traits. Accessions KEC 10,

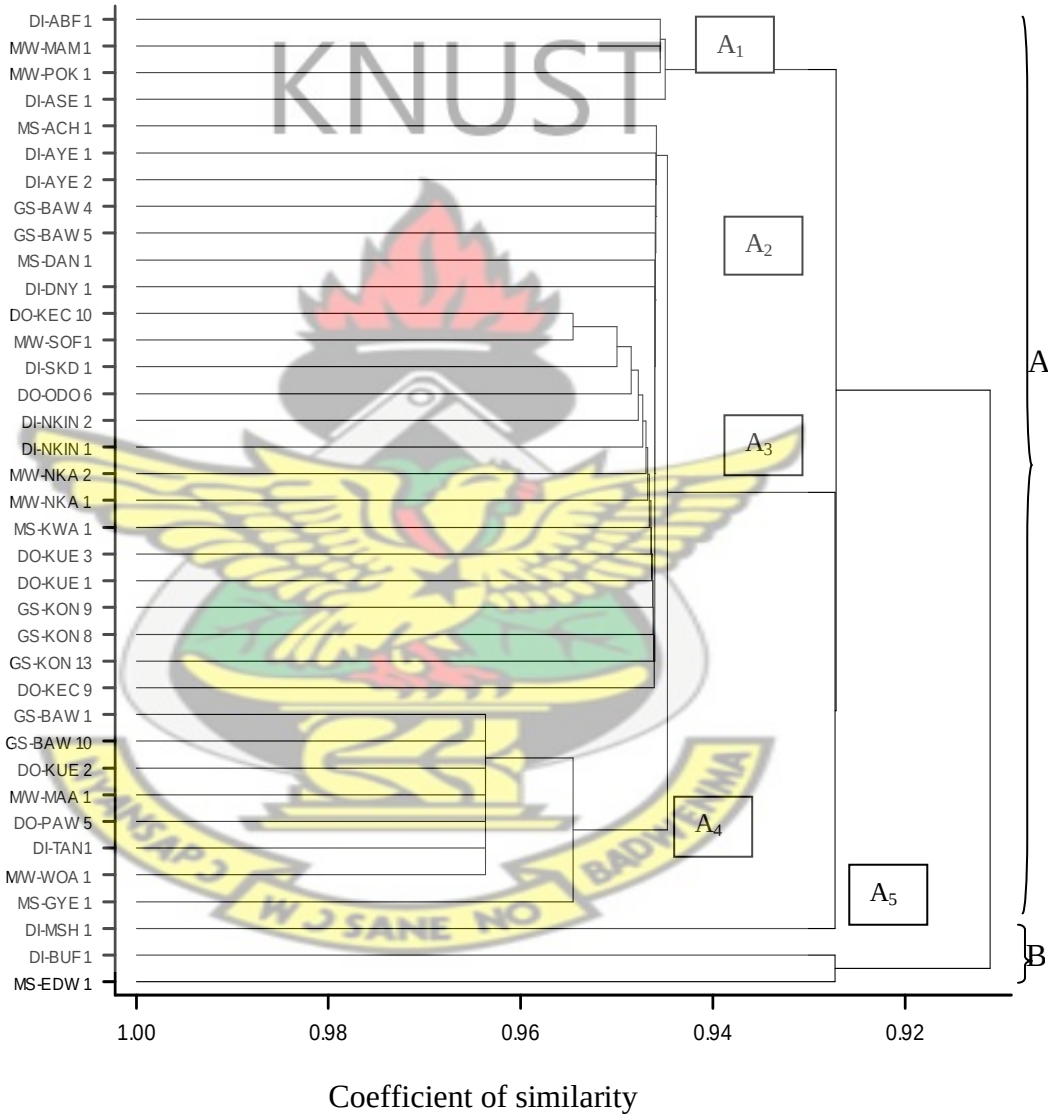


Fig 5.4.1 Relationships among 37 accessions of *C. pentandra* based on five morphological traits. The dendrogram was generated based on UPGMA cluster analysis using similarity matrix of relativized Euclidean distances.

Key: DI = Dry Semi-deciduous Forest Zone-Inner, DO = Dry Semi-deciduous Forest Zone-Outlier, MS = Moist Semi-deciduous Forest Zone, M/W = Moist/Wet Evergreen Forest Zone, GSZ = Guinea Savanna Zone.

SOF 1, GYE 1 and MSH 1 from group (A) and BUF 1 and EDW 1 from group (B) were highly variable and unique from all the others. The genetic similarity revealed among the accessions based on all the five morphological traits ranged from 0.91 to 0.96 (Fig 5.4.1 and Appendix 5.4.13).

5.4.2. RAPD diversity

After screening a series of primers, a total of five primers that produced strongly amplified polymorphic bands were selected for RAPD-PCR analysis (Table 5.3.1). Analysis of genetic diversity at the locus level showed that DSDFZ-Inner was the most diverse ecological zone based on Nei and Shannon diversity indices (Table 5.4.1). Sixteen (94.12 %) out of the 17 loci studied showed that there were differences among populations in the Chi-Square (χ^2) (Appendix 5.4.1), G-Square (Appendix 5.4.2) and Ewens-Watterson Test for Neutrality (Appendix 5.4.3) tests. The average Ewens-Watterson Test for Neutality for all populations was 65.88 % with DSDFZ-Inner having the highest number of neutral loci (Table 5.4.2). Percentage polymorphic loci (PPL) ranged from 70.59 % in M/WEFZ to 100 % in the DSDFZ-Inner (Table 5.4.3). Mean percentage polymorphic loci (PPL) for all populations was 87.06 %. Two private bands were found in the GSZ population. The mean population diversity using the Shannon information index (I) was 0.425 ± 0.024. The GSZ population was the most diverse (I = 0.557) with DSDZ-Outlier (I = 0.342) being the least (Table 5.4.4). The average number of effective alleles over all the populations was 1.449 ± 0.034. The highest was in GSZ population (1.654 ± 0.071) and the lowest in DSDFZ-Outlier (1.297 ± 0.060). Mean expected heterozygosity (H_e) for all populations was 0.277 ± 0.017. The analysis of molecular variance (AMOVA) among populations showed that 98 % of the differentiation was

attributed to within population variation and 2 % to among population variation. Indices of population differentiation, PhiPT (0.020) (Table 5.4.5) and Gst (0.0751) (Appendix 5.4.4) and gene flow, Nm (6.15599) (Appendix 5.4.4) are indications of significant genetic similarity among the five populations. The UPGMA cluster produced showed that the five populations were clustered into three groups (Fig 5.4.2). The DSDFZ-Inner and GSZ were more related. Though the DSDFZ-Outlier, MSDFZ and M/WEFZ were also clustered together, the M/WEFZ was quite different compared to the other two. The pair-wise genetic distance ranged from 0.067 to 0.166 (Table 5.4.6) indicating that MSDFZ is more related to the DSDFZ-Outlier than the rest. Association among the 36 accessions revealed by UPGMA cluster analysis is shown in Figure 5.4.3. The accessions were clustered into two major groups, C and D at a similarity coefficient of 0.5. Cluster C had two sub-clusters (C_1 and C_2) likewise cluster D (D_1 and D_2) at a similarity coefficient of 0.64 and 0.65, respectively. All groups contained accessions from the entire range of the species. The genetic similarity revealed among the accessions ranged from 0.06 to 1 (Appendix 5.4.14). Fourteen (38.89 %) of the accessions, representing all the five ecological zones, had a coefficient of similarity of 1 (Fig 5.4.3). The most diverged accessions were BAW 1 and KON 8 with 94 % dissimilarity. This was followed by 88% dissimilarity between accessions NKA 1 and KON 8, ACH 1 and KON 8, MAA 1 and KON 8, KEC 1 and BAW 5, and between BAW 4 and BAW 5 (Appendix 5.4.14). Two accessions, KUE 3 and BAW 5, were unique among the 36 screened.

Table 5.4.1 Genetic diversity at the locus level among five populations of *C. pentandra* revealed by RAPD polymorphism.

Population	Most diverse loci	Measure of diversity		Genetic variation statistics over all loci		Ranking
		Nei 1973	Shan-Non	Nei 1973	Shannon	
DSDFZ-Inner	RL4,5,6,7,9	0.4976	0.6908	0.376±0.116	0.557± 0.132	1
DSDF-Outlier	RL9	0.4976	0.6908	0.206±0.130	0.342± 0.182	5
GSZ	R6,7,12,13	0.4984	0.6915	0.331±0.174	0.489± 0.232	2
M/WEFZ	RL4,5,6,7	0.4649	0.6576	0.236±0.185	0.360± 0.265	4
MSDFZ	RL4,5,6,7,8,10,16	0.4142	0.6047	0.270±0.149	0.420± 0.206	3

Note: The loci shown in the table were the most diverse based on the overall Nei and Shannon diversity indices in their respective populations.

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

Table 5.4.2 Ewens-Watterson Test for neutrality among five populations of *C. pentandra* using RAPD polymorphism.

Population	No. of loci	No. of neutral loci	% neutral loci	Ranking
DSDFZ-Inner	17	16	94.12	1
DSDFZ-Outlier	17	8	47.06	4
GSZ	17	14	82.35	2
M/WEFZ	17	8	47.06	4
MSDFZ	17	10	58.82	3

Key: 1 = DSDFZ-Inner, 2 = DSDFZ-Outlier, 3 = GSZ, 4 = M/WEFZ, 5 = MSDFZ.
Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone

Table 5.4.3 Percentage polymorphism among five populations of *C. pentandra* revealed by RAPD polymorphism.

Population	No. of loci	No. of polymorphic loci (PL)	Percentage PL	Ranking
DSDFZ-Inner	17	17	100	1
DSDF-Outlier	17	15	88.24	2
GSZ	17	15	88.24	2
M/WEFZ	17	12	70.59	3
MSDFZ	17	15	88.24	2

Key: 1 = DSDFZ-Inner, 2 = DSDFZ-Outlier, 3 = GSZ, 4 = M/WEFZ, 5 = MSDFZ.
Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone

Table 5.4.4 Genetic diversity estimates among five populations of *C. pentandra* based on RAPD polymorphism.

Population	N_a	N_e	I	H_e	UH_e
DSDFZ-Inner	1.765(0.161)	1.558(0.082)	0.482(0.055)	0.325(0.040)	0.339(0.042)
DSDFZ-Outlier	1.765(0.161)	1.297(0.060)	0.342(0.044)	0.206(0.032)	0.222(0.034)
GSZ	2.000(0.000)	1.654(0.071)	0.557(0.032)	0.376(0.028)	0.405(0.030)
M/WEFZ	1.412(0.228)	1.385(0.083)	0.360(0.064)	0.236(0.045)	0.262(0.050)
MSDFZ	1.647(0.191)	1.353(0.052)	0.382(0.049)	0.240(0.033)	0.267(0.036)

Note: Mean values over loci are outside brackets and standard error *(SE)* inside the brackets.

Key: N_a = No. of different Alleles, N_e = No. of effective alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q)), H_e = Expected Heterozygosity = 2 * p * q , UH_e = Unbiased Expected Heterozygosity = (2N / (2N-1)) * H_e , Assumed Hardy-Weinberg Equilibrium, q = (1 - Band Frequency)$^{1/2}$ and p = 1 – q, Where:q = frequency of the recessive allele in the population , p = frequency of the dominant allele in the population.

Table 5.4.5 Summary of the analysis of molecular variance (AMOVA) within and among five *C. pentandra* populations based on RAPD polymorphism.

Source	df	SS	MS	Est. Var.	%
Among Populations	4	15.471	3.868	0.070	2%
Within Populations	31	104.695	3.377	3.377	98%
Total	35	120.167		3.448	100%

Stat	Value	
	Among populations	Within populations
PhiPT	0.020	0.336

Note: PhiPT = AP / (WP + AP), AP = Estimation of variation among population, WP = Estimation of variation within populations, Est. Var = Estimated variance. Levels of significance are based on 10000 iterations.

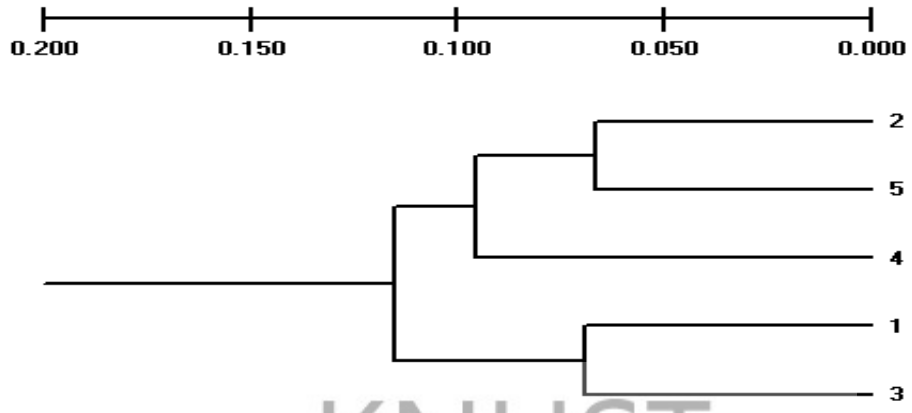


Fig 5.4.2 Dendrogram showing relationships among five populations of *C. pentandra* based on RAPD polymorphism.

Key: 1 = DSDFZ-Inner, 2 = DSDFZ-Outlier, 3 = GSZ, 4 = M/WEFZ, 5 = MSDFZ.

Table 5.4.6 Genetic distances among five *C. pentandra* populations based on RAPD Polymorphism.

Population	DSDFZ-Inner	DSDFZ-Outlier	M/WEFZ	MSDFZ
DSDFZ-Outlier	0.097			
GSZ	0.069	0.128		
M/WEFZ	0.087	0.109	0.166	
MSDFZ	0.079	0.067	0.133	0.082

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone

5.4.3 ISSR diversity

The Nei and Shannon diversity indices showed that population DSDFZ-Inner was the most diverse at the locus level (Table 5.4.7). Percentage polymorphic loci (PPL) ranged from 71.43 % in DSDFZ-Outlier, GSZ and M/WEFZ to 85.71 % in DSDFZ-

Inner and MSDFZ (Table 5.4.8). Average PPL for all populations was 77.14 %. No unique bands were found in all the five populations. The mean population diversity using the Shannon information index (I) for all the populations was 0.306 ± 0.027. The DSDFZ-Inner population was the most diverse (I = 0.413) and the least diverse population was the M/WEFZ (I = 0.249). The average number of effective alleles over all the populations was 1.280 ± 0.031. The highest was in DSDFZ-Inner population (1.433 ± 0.087) and the lowest in DSDFZ-Outlier (1.202 ± 0.053) (Table 5.4.9). The mean expected heterozygosity (He) for all the populations was 0.190 ± 0.018. The analysis of molecular variance (AMOVA) showed that among population diversity was very low and that most of the genetic variation resided within populations (Table 5.4.10). This is supported by the high index of gene flow (Nm = 6.2890) (Appendix 5.4.8). The PhiPT and Gst estimates were equally low at 0.035 (Table 5.4.10) and 0.0736 (Appendix 5.4.8), respectively, indicating moderate genetic differentiation among the populations. The Chi-Square (χ^2) and G-Square analysis also showed that, out of the 14 loci, there were differences among populations at 10 loci (71.43 %), each, (Appendix 5.4.5) and (Appendix 5.4.6), respectively. Again, this was reflected in the Ewen-Watterson's tests which showed majority, 12 (85.71 %), of the sites to be neutral (Appendix 5.4.7). The average Ewens-Watterson Test for Neutality for all populations was 38.54 % with DSDFZ-Inner having the highest number of neutral loci (Table 5.4.11).

The five populations were clustered into two main groups based on the UPGMA tree. Whereas GSZ, MSDFZ, DSDFZ-Outlier and DSDFZ-Inner were clustered together, the GSZ and MSDFZ were more related. The M/WEFZ was unique from the rest (Fig 5.4.4). The pair-wise genetic distances among populations showed that the MSDFZ and the GSZ were most related (Table 5.4.12). The

association among accessions based on UPGMA cluster analysis is shown in Figure 5.4.5. The dendrogram contained two major clusters, E and F, at a

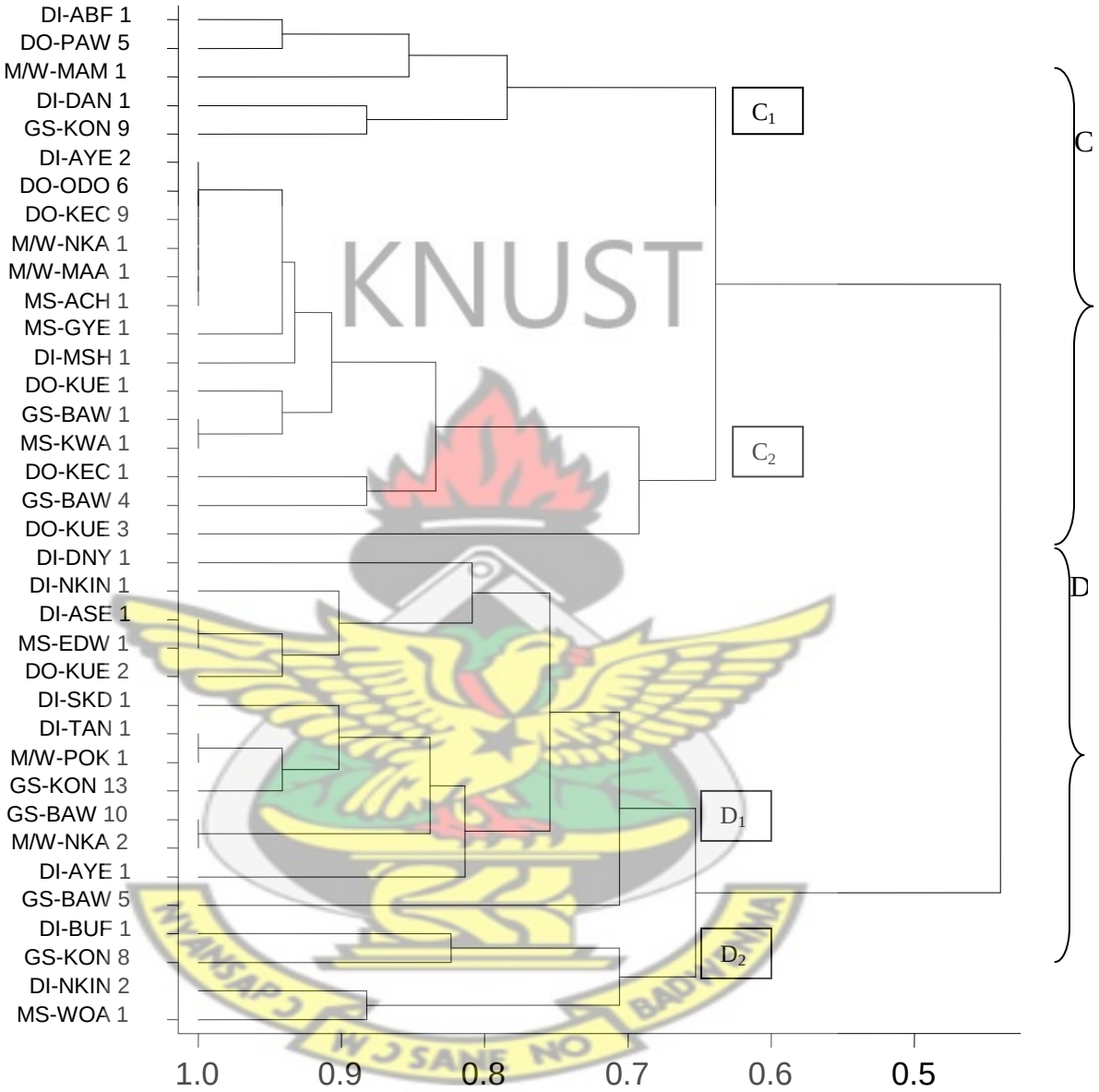


Fig 5.4.3 Dendrogram showing relationships among 36 accessions of *C. pentandra* based on RAPD polymorphism. The dendrogram was generated based on UPGMA Cluster analysis.

Key: DI = Dry Semi-deciduous Forest Zone-Inner, DO = Dry Semi-deciduous Forest Zone-Outlier, MS = Moist Semi-deciduous Forest Zone, M/W = Moist/Wet Evergreen Forest Zone, GSZ = Guinea Savanna Zone.

similarity coefficient of 0.5 with accessions drawn from all the five populations with all forms of morphological traits. Cluster E had four sub-clusters (E_1- E_4), at a similarity coefficient of 0.74. The similarity coefficient ranged from 0.21 to 1 (Appendix 5.4.15) with 21 (58.33 %) of the accessions having a coefficient of similarity of 1. Four accessions, BAW 10, SKD 1, DAN 1 and NKA 2, were unique among the 36. Accessions BAW 10 and ASE 1 were the most unrelated among the 36 with about 79% dissimilarity between them.

Table 5.4.7 Genetic diversity at the locus level among five populations of *C. pentandra* as revealed by ISSR polymorphism.

Population	Most diversed loci	Measure of diversity		Genetic variation statistics over all loci		Ranking
		Nei 1973	Shannon	Nei 1973	Shannon	
DSDFZ-Inner	IL6	0.499	0.692	0.267±0.163	0.411±0.229	1
DSDF-Outlier	IL14	0.369	0.556	0.149±0.128	0.254±0.197	4
GSZ	IL6, 7	0.494	0.688	0.202±0.171	0.319±0.244	3
M/WEFZ	IL9, 10	0.369	0.556	0.149±0.128	0.254±0.197	4
MSDFZ	IL7,10	0.414	0.605	0.193±0.125	0.324±0.180	2

Table 5.4.8 Percentage polymorphism among five populations of *C. pentandra* as revealed by ISSR polymorphism.

Population	No. of loci	No. of polymorphic loci (PL)	Percentage PL	Ranking
DSDFZ-Inner	14	12	85.71	1
DSDF-Outlier	14	10	71.43	2
GSZ	14	10	71.43	2
M/WEFZ	14	10	71.43	2
MSDFZ	14	12	85.71	1

Note: PL= Polymorphic Loci

Table 5.4.9 Genetic diversity estimates among five populations of C. pentandra based on ISSR polymorphism. Average and standard error (SE) over loci in brackets.

Population	N_a	N_e	I	H_e	UH_e
DSDFZ-Inner	1.714(0.194)	1.433(0.087)	0.413(0.061)	0.268(0.044)	0.280(0.046)
DSDFZ-Outlier	1.429(0.251)	1.202(0.053)	0.254(0.053)	0.149(0.034)	0.160(0.037)
GSZ	1.429(0.251)	1.293(0.073)	0.315(0.062)	0.196(0.042)	0.211(0.045)
M/WEFZ	1.143(0.275)	1.220(0.062)	0.249(0.063)	0.154(0.041)	0.171(0.045)
MSDFZ	1.429(0.251)	1.253(0.056)	0.297(0.057)	0.181(0.037)	0.201(0.041)

Note: 1) Refer to Table 5.4.4 for meaning and formulae for N_a, N_e, I, H_e and UH_e.
2) DSDFZ-Inner, DSDFZ-Outlier, GSZ, M/WEFZ and MSDFZ have meaning as explained in Table 5.4.1.

Table 5.4.10 Summary of the analysis of molecular variance (AMOVA) within and among five *C. pentandra* populations based on ISSR polymorphism.

Source	df	SS	MS	Est. Var.	%
Among populations	4	7.09	1.773	0	0%
Within populations	31	71.743	2.314	2.314	100%
Total	35	78.833		2.314	100%

Stat	Value	
	Among popns	Within Popns
PhiPT	0.035	0.59

Note: PhiPT = AP / (WP + AP), AP = Estimation of variation among population, WP = Estimation of variation within populations, Est. Var = Estimated variance, Popns = Populations, Levels of significance are based on 10000 iterations.

Table 5.4.11 Ewens-Watterson Test for Neutrality among five populations of *C. pentandra* using ISSR polymorphism.

Population	No. of loci	No. of neutral loci	% neutral loci	Ranking
DSDFZ-Inner	14	11	78.57	1
DSDFZ-Outlier	14	4	28.53	2
GSZ	14	4	28.53	2
M/WEFZ	14	4	28.53	2
MSDFZ	14	4	28.53	2

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

Nei's genetic distance

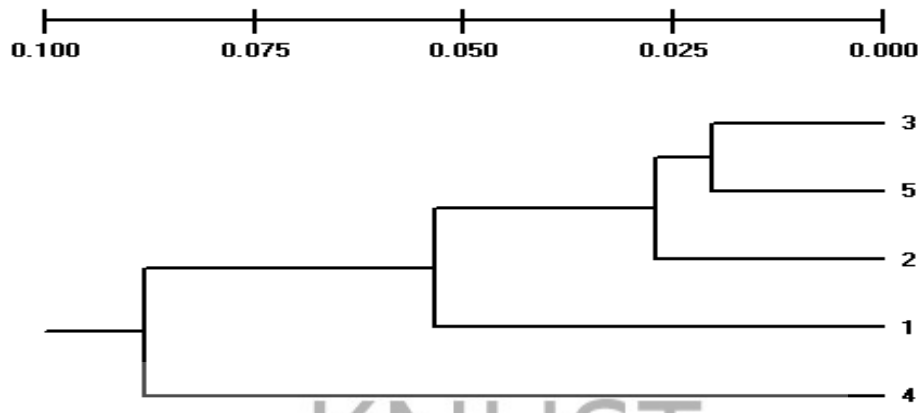


Fig 5.4.4 Dendrogram showing relationships among five populations of *C. pentandra* based on ISSR polymorphism.

Key: 1 = DSDFZ-Inner, 2 = DSDFZ-Outlier, 3 = GSZ, 4 = M/WEFZ, 5 = MSDFZ.

Table 5.4.12 Genetic distances among five *C. pentandra* populations based on ISSR polymorphism.

Population	DSDFZ-Inner	DSDFZ-Outlier	GSZ	M/WEFZ
DSDFZ-Outlier	0.084			
GSZ	0.073	0.074		
M/WEFZ	0.179	0.163	0.072	
MSDFZ	0.113	0.071	0.07	0.106

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

5.4.4 RAPD and ISSR diversity

Genetic diversity at the locus level showed that DSDFZ-Inner was the most diverse population (Table 5.4.13). Percentage polymorphic loci (PPL) ranged from 61.29 % in MSDFZ to 87.10 % in DSDFZ-Inner (Table 5.4.14). Average PPL for all the five populations was 78.06 %. The mean population diversity for all populations, using the Shannon Information Index (I), was 0.371 ± 0.018. The GSZ had the highest Shannon Information Index (I) (0.465 ± 0.040) and expected heterozygosity (He) (0.310 ± 0.029) whereas DSDFZ-Outlier had the least I (0.287 ± 0.033) and H_e (0.169 ± 0.030) (Table 5.4.15). AMOVA for populations showed that 100 % of the differentiation was attributed to within population variations and 0 % attributed to among populations. The PhiPT value as well was low at 0.001 (Table 5.4.16). Also, the Chi-Square (χ^2) (Appendix 5.4.9) and G-Square (Appendix 5.4.10) tests all showed that there were differences among populations at 27 (87.10 %) loci out of the 31 loci studied. The indices of population differentiation, Gst, was estimated to be 0.0799 indicating moderate genetic differentiation among the populations. The Ewen-Watterson's tests showed that majority, 27 (85.10 %), of the sites were neutral indicating differences among the populations (Appendix 5.4.11). The average Ewens-Watterson Test for Neutality for all populations was 57.42 % with DSDFZ-Inner having the highest number of neutral loci (Table 5.4.17). Also, Gene flow, Nm, was estimated to be 5.7544 indicating significant amount of gene flow among populations (Appendix 5.4.12). Figure 5.4.6 shows the genetic differentiation among *C. pentandra* populations in Ghana. There were three clusters with the M/WEFZ being unique from the others. While the DSDFZ-Inner and the GSZ clustered together, the DSDFZ-Outlier and the MSDFZ were more related. Nei's genetic distances among the five populations ranged from 0.011 between MSDFZ and M/WEFZ to 0.069 between

DSDFZ-Inner and DSDFZ-Outlier (Table 5.4.18). The ranking of population based on RAPD only, ISSR only and the combined RAPD and ISSR polymorphism showed that the GSZ population was the most diverse (Table5.4.19). Two unique bands were

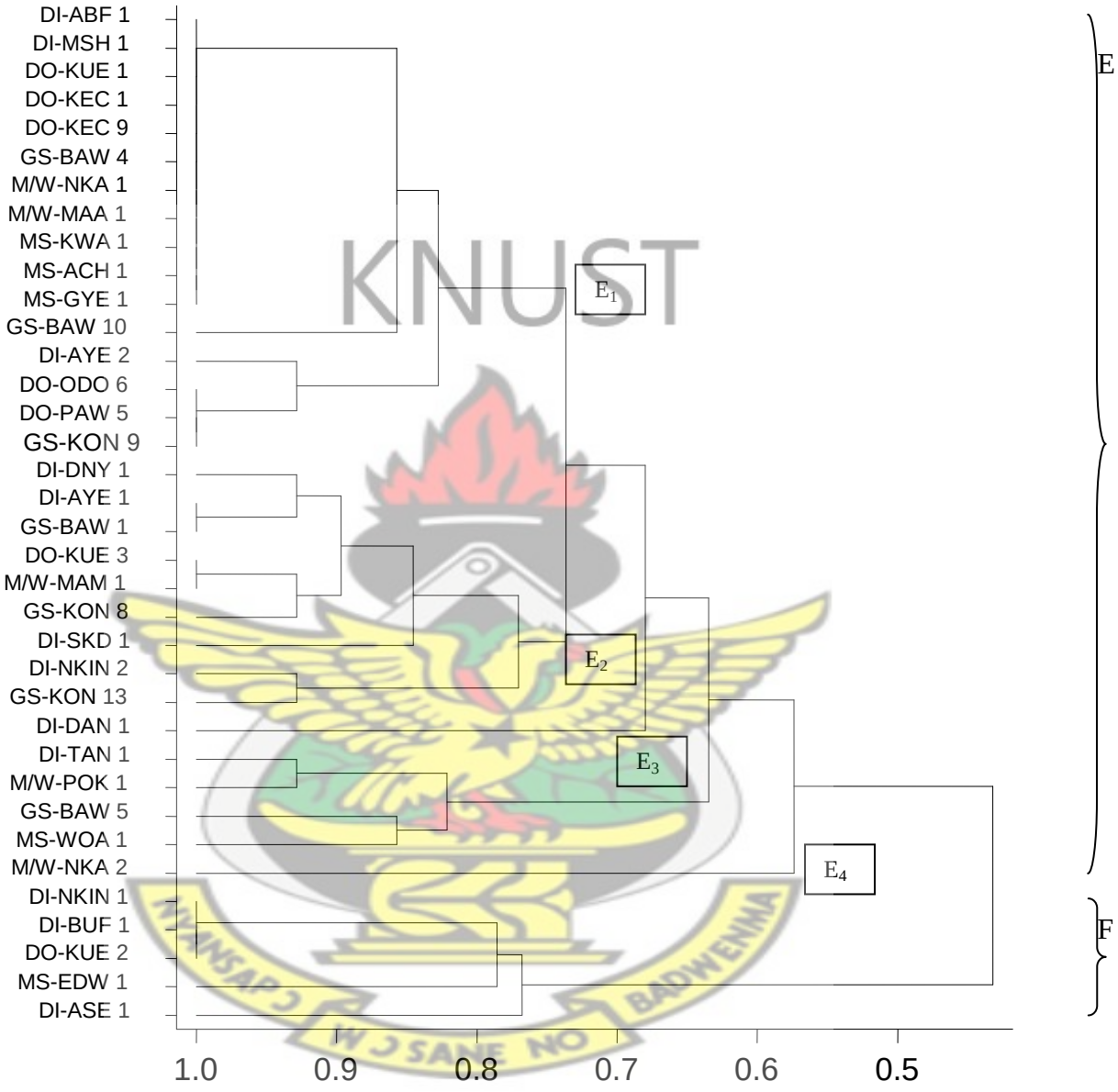


Fig 5.4.5 Relationships among 36 accessions of *C. pentandra* based on ISSR polymorphism. The dendrogram was generated based on UPGMA cluster analsysis.

Key: DI = Dry Semi-deciduous Forest Zone-Inner, DO = Dry Semi-deciduous Forest Zone-Outlier, MS = Moist Semi-deciduous Forest Zone, M/W = Moist/Wet Evergreen Forest Zone, GSZ = Guinea Savanna Zone.

found in the GSZ population. Five selected RAPD and five ISSR primers produced 123 reproducible and clear amplification bands among the 36 individuals of which 4 (3.25 %) were polymorphic indicating a low level of genetic diversity between the different zones. Principal Coordinates Analysis (PCA) also partitioned the accessions into three main groups (Fig 5.4.7).

The principal coordinates analysis (PCA) defined by axis 1 and 2 (Fig 5.4.7), accounting for 67.15 % of the total variation, provided another view of the relationships among the accessions. The dendrogram showed two clusters at a coefficient of similarity of 0.6: the more diverse group G and the less diverse Group H (Fig 5.4.8). Cluster G had two sub-clusters, G_1 and G_2, at a similarity coefficient of 0.62. Similarity coefficient ranged from 1 to 0.23 (Appendix 5.4.16) with 4 (11.11 %) of the accessions having a similarity coefficient of 1 and are duplicates. Accessions BUF 1 and KUE 1 had the highest degree of dissimilarity of 77 %. Four accessions, BAW 1, KUE 3, DAN 1 and BAW 5, were very unique among the 36. Like the results from the RAPD and ISSR analysis, variation among accessions as revealed by the combined data of RAPD and ISSR was neither population- structured nor followed any known pattern as illustrated by the PCA (Fig 5.4.7) and the dendrogram (Fig 5.4.8).

Table 5.4.13 Genetic diversity at the locus level among five populations of *C. pentandra* as revealed by combined RAPD and ISSR polymorphism.

Population	Most diverse loci	Measure of diversity		Genetic variation statistics over all loci		Ranking
		Nei 1973	Shannon	Nei 1973	Shannon	
DSDFZ-Inner	RIL12,13	0.499	0.692	0.296±0.170	0.447±0.229	1
DSDF-Outlier	RIL9	0.498	0.691	0.180±0.130	0.302±0.191	4
GSZ	RIL4,5,6,7	0.498	0.691	0.295±0.161	0.448±0.218	2
M/WEFZ	RIL4,5,6,7	0.488	0.681	0.250±0.180	0.380±0.256	3
MSDFZ	RIL16	0.465	0.658	0.171±0.156	0.274±0.237	5

Table 5.4.14 Percentage polymorphism among five populations of *C. pentandra* as revealed by combined RAPD and ISSR polymorphism.

Population	No. of loci	No. of polymorphic loci (PL)	Percentage PL	Ranking
DSDFZ-Inner	31	27	87.1	1
DSDF-Outlier	31	25	80.65	2
GSZ	31	27	87.1	1
M/WEFZ	31	23	74.19	3
MSDFZ	31	19	61.29	4

Note: PL=Polymorphic Loci

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

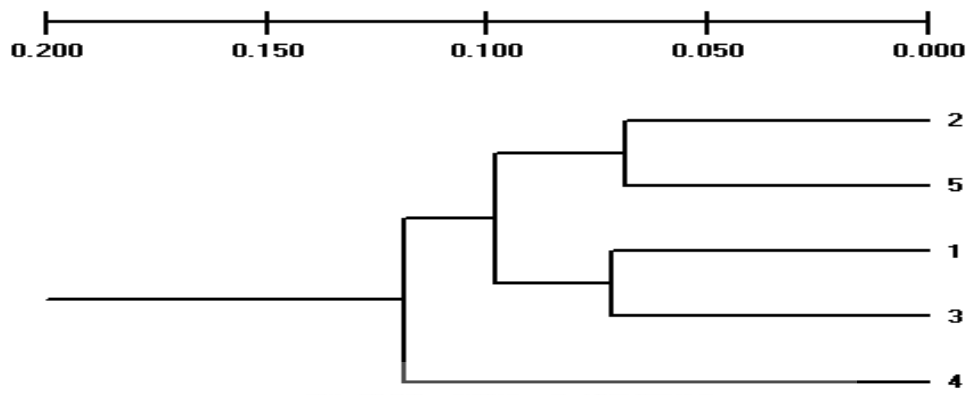


Fig 5.4.6 Dendrogram showing relationships among five populations of *C. pentandra* based on RAPD and ISSR polymorphism.

Key:
1 = DSDFZ-Inner, 2 = DSDFZ-Outlier, 3 = GSZ, 4 = M/WEFZ, 5 = MSDFZ.

Table 5.4.15 Genetic diversity estimates among five populations of *C. pentandra* based on RAPD and ISSR polymorphism. Mean and standard error (SE) over loci in brackets.

Population	N_a	N_e	I	H_e	UH_e
DSDFZ-Inner	1.742(0.122)	1.502(0.060)	0.451(0.041)	0.299(0.030)	0.312 (0.031)
DSDFZ-Outlier	1.613(0.144)	1.233(0.039)	0.287(0.033)	0.169(0.022)	0.180 (0.024)
GSZ	1.742(0.122)	1.527(0.061)	0.465(0.040)	0.310(0.029)	0.339 (0.032)
M/WEFZ	1.290(0.175)	1.311(0.055)	0.310(0.046)	0.199(0.031)	0.221 (0.035)
MSDFZ	1.548(0.153)	1.308(0.039)	0.343(0.037)	0.213(0.025)	0.237 (0.027)

Note: 1) N_a = No. of different Alleles, N_e = No. of effective alleles = 1 / (p^2 + q^2), I = Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q)), H_e = Expected Heterozygosity = 2 * p * q , UH_e = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He. Assumed Hardy-Weinberg Equilibrium q = (1 - Band Frequency)$^{1/2}$ and p = 1 – q. Where: q = frequency of the recessive allele in the population, p = frequency of the dominant allele in the population. 2) DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

Table 5.4.16 Summary of the analysis of molecular variance (AMOVA) within and among five *C. pentandra* populations based on RAPD and ISSR polymorphism.

Source	df	SS	MS	Est. Var.	%
Among Populations	4	22.562	5.640	0.000	0%
Within Populations	31	176.438	5.692	5.692	100%
Total	35	199.000		5.692	100%

Stat	Value	
	Among populations	Within populations
PhiPT	0.001	0.410

Note: PhiPT = AP / (WP + AP), AP = Estimation of variation among population, WP = Estimation of variation within populations, Est. Var = Estimated variance. Levels of significance are based on 10000 iterations

Table 5.4.17 Ewens-Watterson Test for Neutrality among five populations of *C. pentandra* using RAPD and ISSR polymorphism.

Population	No. of loci	No. of neutral loci	Percentage neutral loci	Ranking
DSDFZ-Inner	31	25	80.65	1
DSDFZ-Outlier	31	12	38.71	4
GSZ	31	24	77.42	2
M/WEFZ	31	18	58.06	3
MSDFZ	31	10	32.26	5

Key: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone.

Table 5.4.18 Nei genetic distances among five *C. pentandra* populations based on RAPD and ISSR polymorphism.

Population	DSDFZ-Inner	DSDFZ- Outlier	GSZ	M/WEFZ
DSDFZ-Outlier	0.069			
GSZ	0.015	0.062		
M/WEFZ	0.034	0.023	0.029	
MSDFZ	0.049	0.015	0.049	0.011

Note: DSDFZ-Inner = Dry Semi-deciduous Forest Inner Zone, DSDFZ-Outlier = Dry Semi-deciduous Forest Zone-Outlier, GSZ = Guinea Savanna Zone, M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, MSDFZ = Moist Semi-deciduous Forest Zone

Table 5.4.19 Summary of the measure of genetic diversity in five populations of *C. pentandra* using the Shannon Information Index (I) based on RAPD, ISSR and combined RAPD and ISSR polymorphism.

	Shannon Information Index (I)							
Population	RAPD	Rating	ISSR	Rating	RAPD and ISSR	Rating	Total Rating	Ranking
1	0.482(0.055)	2	0.413(0.061)	1	0.451(0.041)	2	5	2
2	0.342(0.044)	5	0.254(0.053)	4	0.287(0.033)	5	14	5
3	0.557(0.032)	1	0.315(0.062)	2	0.465(0.040)	1	4	1
4	0.360(0.064)	4	0.249(0.063)	5	0.310(0.046)	4	13	4
5	0.382(0.049)	3	0.297(0.057)	3	0.343(0.037)	3	9	3

Note: 1) Mean values of Shannon Information Index (I) over loci are outside brackets and standard error *(SE)* inside the brackets

Key: 1= Dry Semi-deciduous Forest Inner Zone (DSDFZ-Inner), 2=Dry Semi-deciduous Forest Zone-Outlier (DSDFZ-Outlier), 3=GSZ = Guinea Savanna Zone, 4=M/WEFZ = Dry Moist/Wet Evergreen Forest Zone, 5=MSDFZ = Moist Semi-deciduous Forest Zone.

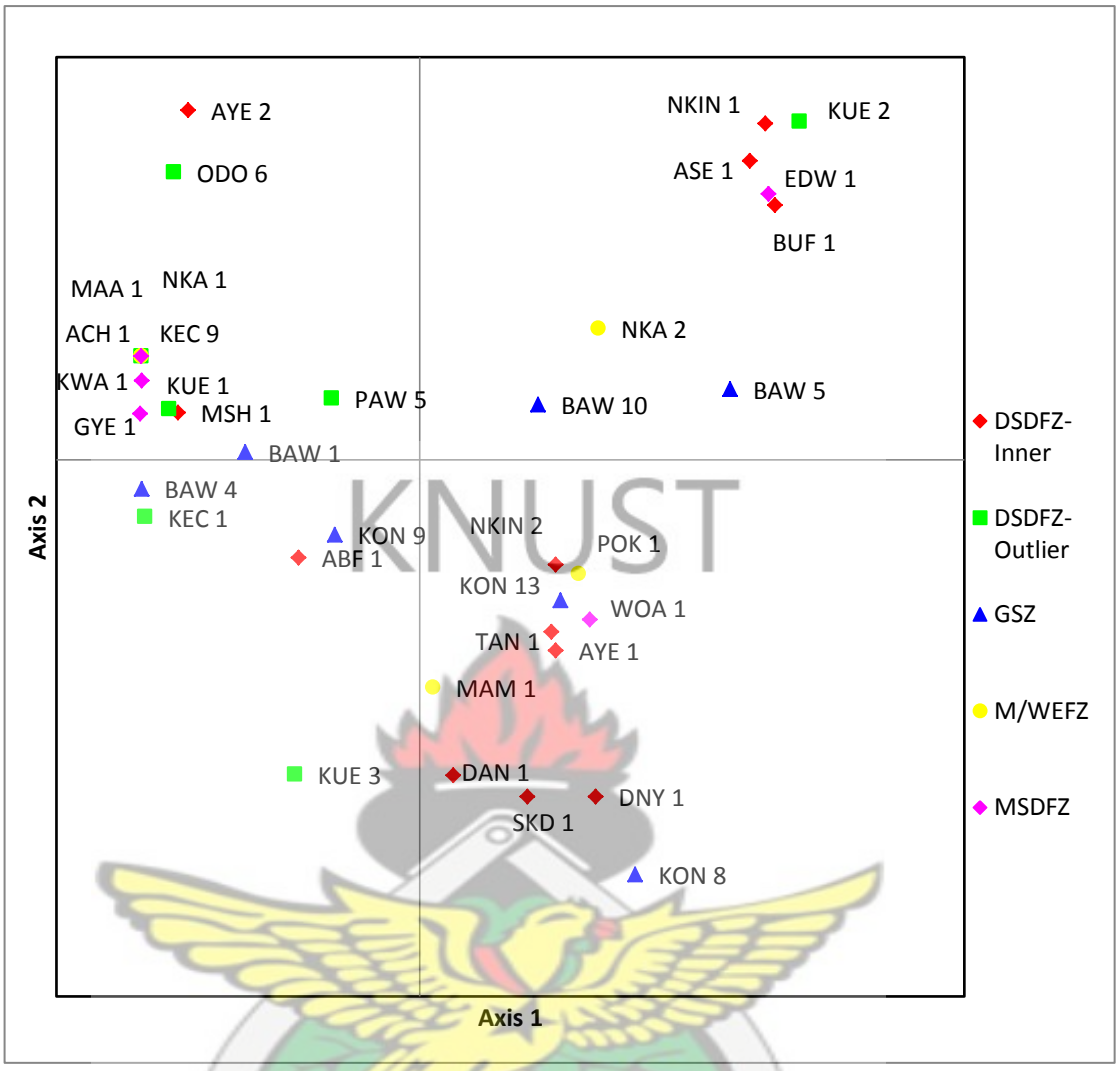


Fig 5.4.7 Principal coordinates analysis of five *C. pentandra* populations based on RAPD and ISSR polymorphism analysis.

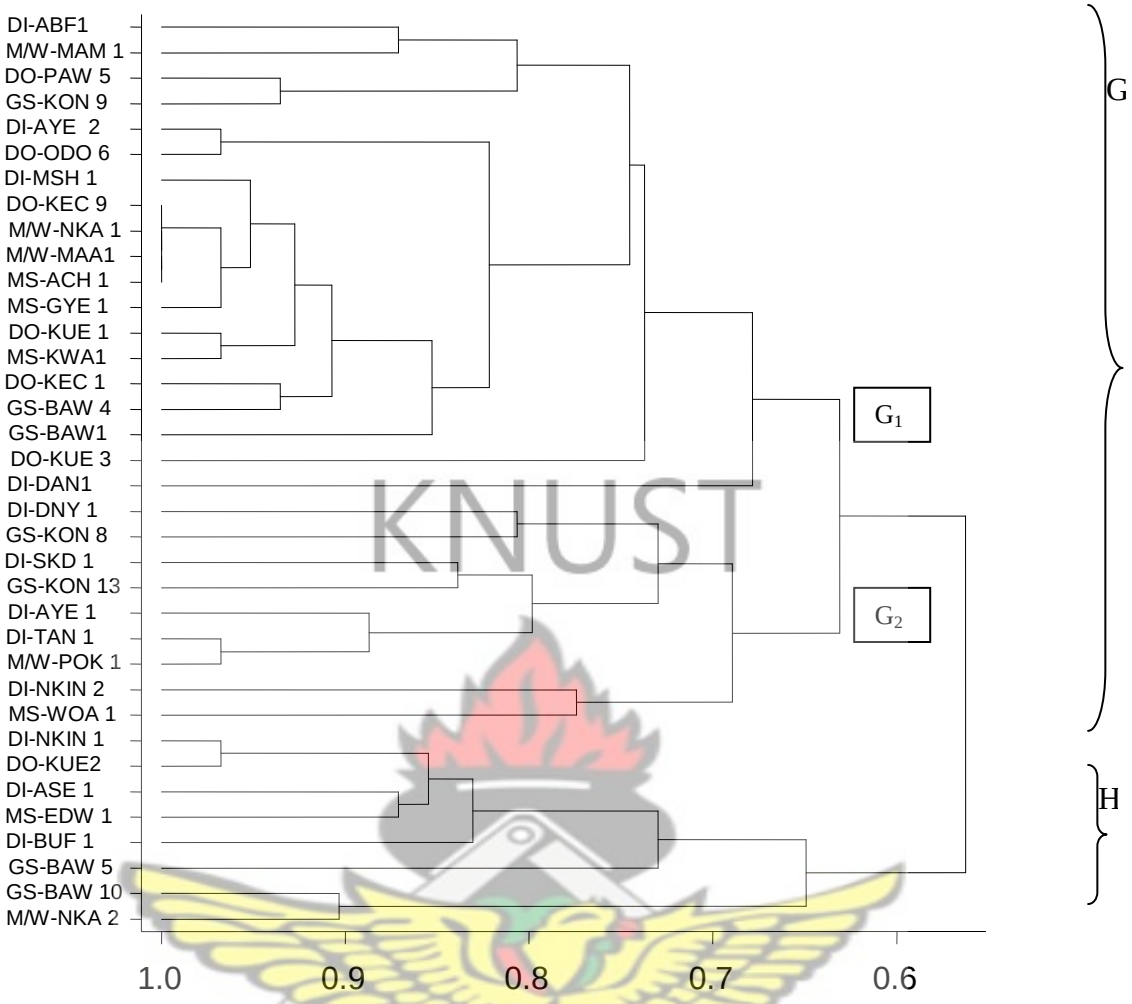


Fig 5.4.8 Relationships among 36 accessions of *C. pentandra* based on RAPD and ISSR polymorphism. The dendrogram was generated using UPGMA Cluster analysis.

Key: DI = Dry Semi-deciduous Forest Zone-Inner, DO = Dry Semi-deciduous Forest Zone-Outlier, MS = Moist Semi-deciduous Forest Zone, M/W = Moist/Wet Evergreen Forest Zone, GSZ = Guinea Savanna Zone.

5.5 Discussion

5.5.1 Morphological variation

Dick *et al*. (2007) identified three forms of *C. pentandra*; the rainforest form which is mostly spiny, the cultivated form and the savannah form both of which are spineless. These zonal distinctions were, however, not recognized in this study though the various forms were observed. A majority of the accessions, 91 - 96 %, shared common morphological traits across all the five populations (Fig 5.4.1). The relatively high coherency in percentage spines, deep-green stem colour and light-purple petiole colour could probably explain the strong resemblance between the five accessions ABF 1, MAM 1, POK 1, ASE 1 and ACH 1. Also, the accessions MSH1, BUF1 and EDW 1 had deep-purple petiole, hence were clustered together (Figs 5.4.1). Percentage spines in these eight accessions were generally high, ranging from 32.00 % to 91.00 %, with an average of 63.45 %. Similarly, stem height increment was high among them ranging between 104.38 cm/yr and 161.06 cm/yr with an average of 128.70 cm/yr (Fig 4.4.1). Likewise, their survival rates were high and ranged between 43.33 % and 80 %. Three of these accessions; MAM 1, MSH 1 and EDW 1 were rated as having high potential of survival and establishment, and three others (ABF 1, ASE 1 and BUF 1), had moderate survival rates (Table 3.4.2). Hence, the amount of spines, deep-green stem colour and light-purple or deep-purple petiole colour could, therefore, be used together with other recommendations, as indicators in selection for stem dieback resistance in *C. pentandra*. Besides these, there was no clear trend that could adequately account for the differences between the two clusters formed from the morphological studies. Thus, accessions were neither structured by morphological traits nor populations. This implies that the different populations in the natural range of C. *pentandra* in Ghana contain the various morphotypes of the

species which are comparatively similar characteristically. This, however, needs further verification due to the nature and limited number of traits used in this study. Several studies have also acknowledged the difficulties in characterizing morphological variation in tree species (Brodie *et al*., 1997; Edwards and Schreckenberg, 1997). Muthusamy *et al*. (2008) have noted the inadequacy of morphological characters in explaining relationship between individuals due to their non-significant distinction and the requirement of plants to grow to maturity prior to identification and their instability due to environmental influences. For instance, morphological analysis of cones proved to be insufficient for detecting genetic relationship between different forms of *Pinus mugo* (Monteleone *et al.*, 1998). Since, the current study was done on one year-old plants, these colour distinctions could vary with age. It is therefore suggested that accessions be monitored in plants older than one year to certify these findings in future studies since morphological descriptors are plastic or vary with the environment. The dendrograph, considering all indicators, presents a variety of ways in which accessions could be selected. For instance, using the degree of dissimilarity as a guide, the following selection could be made: ASE 1 from A_1, DNY 1 or any other member from A_2, KEC 10 or SOF 1, SKD 1, ODO 6, NKIN 2 and NKIN 1 from A_3, GYE 1 and any other member from A_4, MSH 1 from A_5 and BUF 1 or EDW 1 from cluster B.

5.5.2 Molecular diversity

At the locus level, 21 loci were observed to be the most diverse based on the Nei and Shannon indices (Table 5.4.1). Thus, differences in the 36 accessions screened could be seen more at these loci than the rest. Analysis based on the combined RAPD and ISSR data as well as the separate data on RAPD and ISSR polymorphism revealed

that diversity was higher within populations than among them (Tables 5.4.5, 5.4.10 and 5.4.16). Diversity within populations was high, but the diversity among population was very low as shown by the PhiPT values for RAPD (PhiPT = 0.02), ISSR (PhiPT = 0.035) and the combined RAPD and ISSR (PhiPT = 0.001), hence, suggest little or no structuring among the populations. This observation was supported by the AMOVA analysis which showed 2 % differentiation among populations for RAPD polymorphism (Table 5.4.5) and 0 %, each, for ISSR (Table 5.4.10) and the combined RAPD and ISSR polymorphism (Table 5.4.16). The theta θ (= F_{ST}) statistic showed similar results to those of PhiPT and AMOVA. According to Potter (1990), Nm value above 1 is an indication of significant gene flow among populations whilst Nm < 0.5 is an indication of population isolation. Hence, the observed Nm values above 5 are indications of low genetic differentiation among the five *C. pentandra* populations. Even though populations were found to have high genetic similarity, large differences were observed among individual accessions. The high diversity within populations observed in the current study may probably be caused by both insect pollinators and human intervention through the repeated transfer of materials within the populations as well as the breeding system of *C. pentandra* as it is predominantly an out-crossing species. The protection given to *C. pentandra* trees during traditional long-term management in farms would be expected to allow diversity to increase, especially, if it is coupled with gene flow by transportation of fruits from village to village by humans or other animals.

Boshier and Amaral (2004) reported that the combination of inbreeding frequency reduction and the maintenance of breeding system flexibility in naturally outcrossing tree species is efficient in maximizing the conservation of genetic variation in species which exhibit both out-crossing and inbreeding, including *C.*

pentandra. None of the populations studied seemed to be heavily affected by genetic drift, founder effects or bottleneck events and gene flow seems to be the major factor homogenizing all the populations. The activities of animals and human beings are known to have increased diversity of two populations of *Prosopis juliflora* species though they were 53.8 km apart (Hamza, 2010). Lobo *et al*. (2005) reported that monkeys, elephants, birds, bats or primates, bees and wasps are the main agents of pollination for *C. pentandra*. All the parameters used for the diversity study, percentage polymorphic loci, Ewens-Watterson Neutrality Test, Nei diversity index, Shannon diversity index and H_e, using the three techniques, all showed that DSDFZ-Inner was the most diverse population. This was also supported by the results of screening for dieback resistance at the nursery (Chapter 3), in the field and in the heritability and genetic gain estimates (Chapter 4). This is not surprising because the DSDFZ-Inner zone is surrounded by the rest of the populations and could possibly share genetic materials with them. According to Taylor (2000), colonies of straw-coloured flying fox bat from the Kumasi Zoo cover about 43-mile radius (69 km) in search for food every night and are the main dispersers of *Milicia excelsa* seeds in the Dry Semi-deciduous Forest Inner Zone. The high bats activity in the DSDFZ-Inner and GSZ could, therefore, have contributed to the high diversity observed in these populations. Another reason could be due to the high level of interaction of people in these populations. People who move from Northern Ghana, in the GSZ, to Southern Ghana to farm mostly settle in DSDFZ-Inner and the MSDFZ. They usually carry with them various seed germplasm mixed with all kinds of seeds probably including those of *C. pentandra*. Also, their beddings, especially the pillows, are mostly stuffed with *C. pentandra* fluff containing seed remains. These could have served as fertile sources of gene migration, thereby reducing inbreeding in these southern zones. A

greater number of these settler farmers dwell in the DSDFZ-Inner than in the other zones due to its close proximity to the GSZ than the others. The DSDFZ-Outlier, by its physical location, seems to be isolated from the rest the zones probably due to the partitioning offered by the Volta Lake. This might have served as a barrier to gene flow leading to an increased within-population homozygosity resulting in the low diversity observed (Tables 5.4.4 and 5.4.13). Ohnishi *et al*. (2007) also found genetic diversity to be least in three isolated populations of *Ursus thibetanus* in Japan compared to the continuous ones.

Ranking of populations based on only the combined RAPD and ISSR polymorphism was the same as that based on the three sets of data, RAPD, ISSR and combined RAPD and ISSR. Again, the average unbiased genetic diversities were high with RAPD (0.299 ± 0.019) followed by the combined RAPD and ISSR (0.256 ± 0.014) and ISSR (0.205 ± 0.019). This further confirms the usefulness of combining the two techniques compared to when they are used separately. According to Ofori (2001), combining RAPD and ISSR data allows a larger portion of the genome to be sampled resulting in more robust conclusion than the individual techniques.

The three dendrographs present several ways in which selection of genetically unrelated accessions could be made. Accessions with coefficient of similarity of one are duplicates and only one should be preferred at a time. For instance, the accessions AYE 2, ODO 6, KEC 9, NKA 1, MAA 1, ACH 1, BAW 1, KWA 1, ASE 1, EDW 1, TAN 1, POK 1, BAW 10 and NKA 2, are all duplicates based on the RAPD polymorphism and only one should be taken (Fig 5.4.3). Likewise, the 21 accessions, ABF 1, MSH 1, KUE 1, KEC 1, KEC 9, BAW 4, NKA 1, MAA1, KWA 1, ACH 1, GYE 1, ODO 6, PAW 5, KON 9, AYE 1, BAW 1, KUE 3, MAM 1, NKIN 1, BUF 1, and KUE 2 are duplicates, based on the ISSR polymorphism (Fig 5.4.5). Again, the

four accessions, KEC 9, NKA 1, MAA1 and ACH 1 are duplicates, based on the combined RAPD and ISSR polymorphism (Fig 5.4.8). The accessions were drawn from all the five ecological zones and were neither population or morphologically structured nor followed any known pattern.

The PCA which accounted for 67.17 % of the total variation in axis 1 and axis 2 showed some uniqueness in 13 accessions (36.11 %). These included AYE 2, ODO 6, PAW5 and BAW 1(first quadrant; top left on PCA chart), NKA 2, BAW 10 and BAW 5 (second quadrant; top right on PCA chart), DAN 1, MAM 1, DNY 1, KON 8 and SKD 1 (third quadrant; bottom right on PCA chart) and KUE 3 (forth quadrant; bottom left on PCA chart) as shown in Figure 5.4.7.

Out of the six most dissimilar accessions revealed by the three marker techniques, two accessions were from the DSDFZ-Inner (BUF1 and ASE 1), two were from the GSZ (BAW 1 and KON 8) and one was from the DSDFZ-Outlier (KUE 1). Also, four out of the 13 unique accessions shown by the PCoA originated from the GSZ, three each from the DSDFZ-Inner and the DSDFZ-Outlier, two from M/WEFZ and one from the MSDFZ. The exact causes of this uniqueness were not clear in the study. Here again, the GSZ and the DSDFZ-Inner topped the list in terms of rarity. This same trend was observed regarding the distribution of genetic diversity within and among populations as explained in section 5.5.2. Also, only the GSZ produced a private band. These two populations may therefore contain good repositories of rare genes. Hence, further studies should be done on these populations when searching for rare but desirable genes. Only three of these accessions, KON 8, BUF 1 and KUE 1, from the three dendrograms and seven from the PCA: KON8, ODO 6, MAM 1, DNY 1, SKD 1, NKA 2 and DAN 1, were previously identified to have the potential of giving a future gain when selected (Table 4.4.1). This means only nine accessions

(24.32 %) out of the 37 tested were useful in the sustainable management of *C. pentandra*. This number is woefully inadequate and calls for the need for further studies in this subject matter.

5.6 Conclusion

C. pentandra in Ghana constitutes a rich source of biodiversity and its conservation and utilization require a good knowledge of its genetics. This could assist in the understanding of its evolutionary history and response to environmental changes, the mechanisms of its local spread and adaptation and the prediction of the potential for populations of *C. pentandra* to evolve in response to evolution of resistance to stem dieback disease. To contribute to this knowledge, five morphological traits and three molecular marker techniques, RAPD and ISSR, and the combined RAPD and ISSR, were used to characterize *C. pentandra* from five ecological zones of Ghana. The morphological traits did not give any clarity in classifying accessions by either ancestry origins, populations or resistance to stem dieback disease. Though these characters did not give any strong correlation, accessions with high percentage spines, deep-green stem and deep-purple leaf stock colours seemed to have higher levels of stem dieback disease resistance and better growth. The study also showed that genetic diversity in *C. pentandra* is independent of population type but depends on the degree of germplasm exchange. So, considering the non-population and non-morphological structuring of accessions and the high within population diversity, it is advisable that sampling for conservation and plantation be concentrated on selecting resistant and unrelated accessions within populations rather than collecting bits from the entire range of the species.

5.7 Recommendations

1. Resistant accessions with larger genetic distances between them should be preferred in selection for conservation.
2. Nine accessions, DNY 1, KON 8, ODO 6, SKD 1, BUF 1, MAM 1, NKA 2, DAN 1 and KUE 1, keeping the order, are recommended for conservation as mother trees after considering both the heritability and genetic relatedness.
3. Future studies should employ more specific and high throughput PCR techniques such as Simple Sequence Repeats (SSR) or Microsatellites for better discrimination between accessions.

CHAPTER 6

IDENTIFICATION OF GENETIC MARKERS LINKED TO STEM DIEBACK DISEASE RESISTANCE IN *C. pentandra*

6.1 Introduction

Breeding for resistance in trees, though successful in many cases, is often hampered by factors, such as the long term nature of their life cycles, labor intensive methods of data collection, and the intensive land requirements for progeny testing (Biggs *et al*., 1992). As part of the attempts to tackle these challenges, the scope of forest genetics has been expanded to include biotechnology; and molecular markers are now being used in the identification of resistant genes (FAO, 2003). These attempts have resulted in the availability of several plant resistance genes which are being increasingly preferred by tree breeders in disease control (Meyer *et al*., 2003; Jones, 2001; Rommens and Kishore, 2000). For instance, disease-resistant genes have been identified for several diseases of plant species including the white pine blister rust in *Pinus lambertiana* (Devey *et al*., 1995), and *Melanospora medusa* resistance in poplar hybrid (Newcombe *et al.*, 1996). The success of this rests on the fact that these genes are very polymorphic and have varied recognition specificities (Pryor and Ellis, 1993).

The long generation times of trees, together with poor juvenile-mature trait correlations, have also promoted interest in marker-assisted selection (MAS) to accelerate breeding through early selection. The approach relies on the identification of DNA markers which explain a high proportion of variation in phenotypic traits (Butcher and Southerton, 2007). The approaches to marker-assisted selection in forest trees largely depends on comprehensive dissection of complex traits to their

individual genes and the identification of marker-trait associations that account for a sizable portion of the segregating variation in the breeding population (Neale and Kremer, 2011). Marker-assisted selection in forest trees is very essential, particularly, in situations where trait heritability is low and selection occurs at the level of the individual tree (Grattapaglia, 2007). It also allows the application of intensive selection without the need for land, labour and time costs associated with conventional selection after the markers linked to traits of interest have been identified (Tommasini *et al*., 2009).

Chapters 3 and 4 of this study showed the existence of stem dieback resistance in the populations of *C. pentandra* in Ghana. It was therefore necessary to further investigate the possibility of identifying markers that are linked to stem dieback disease using ten highly polymorphic and reproducible RAPD and ISSR primers identified in this study. The rationale behind the study was that resistant individuals have unique bands which are also present in their bulk samples but which are absent in susceptible individuals and their bulk samples and/or susceptible individuals have unique bands which are also present in their bulk samples but which are absent in resistant individuals and their bulk samples.

6.2 Objectives

The main objective was to identify markers that are linked to stem dieback resistance in *C. pentandra*.

6.3 Materials and methods

Fresh leaves were obtained from five accessions, in the progeny trial described in section 4.3.2, whose progenies segregated into resistant and susceptible individuals.

For each accession, leaves were sampled from three individuals marked as either resistant or susceptible making a total of 30 samples. Leaf sample collection, DNA extraction, testing and quantification were done as described in sections 5.3.2.1, 5.3.2.2, 5.3.2.3 and 5.3.2.4, respectively. For each accession, equal quantities of DNA were combined from the 3 resistant progenies to form 3-plant bulk resistant sample and also from the 3 susceptible progenies to form a 3-plant bulk susceptible sample making up a total of 10 bulk DNA samples. Together, 40 DNA samples (15 resistant samples, 15 susceptible samples, five 3-plant bulk resistant samples and five 3-plant bulk susceptible samples) were obtained and used in this study. PCR amplification was done with the 5 RAPD and 5 ISSR polymorphic primers that were chosen in the previous study in a 10 µl reaction volume as described in section 5.3.2.4. The amplified PCR products were run on 2 % agarose gel using the principle of electrophoresis as stated in section 5.3.2.5. The order of accessions on the gel was: one 3-plant half-sib bulk resistant sample followed by three resistant progenies followed by one 3-plant half-sib bulk susceptible plant and then the three susceptible samples. This pattern was repeated for all the five accessions and the loading order maintained for all the 10 primers.

6.4 Results

All the 10 primers produced amplification products in almost all the 40 samples. However, none of them gave clear differentiation between the resistant and susceptible accessions, either by presence or absence of distinctive bands. Each primer was reproducible, thus consistently produced the same segregation patterns on two repetitions. The figures below showed examples of the lack of differences in banding patterns produced by primer GEN60-3J (Fig 6.4.1) and Aperon A-13 (Fig

6.4.2) with 40 DNA samples from five accessions which showed symptoms of stem dieback.

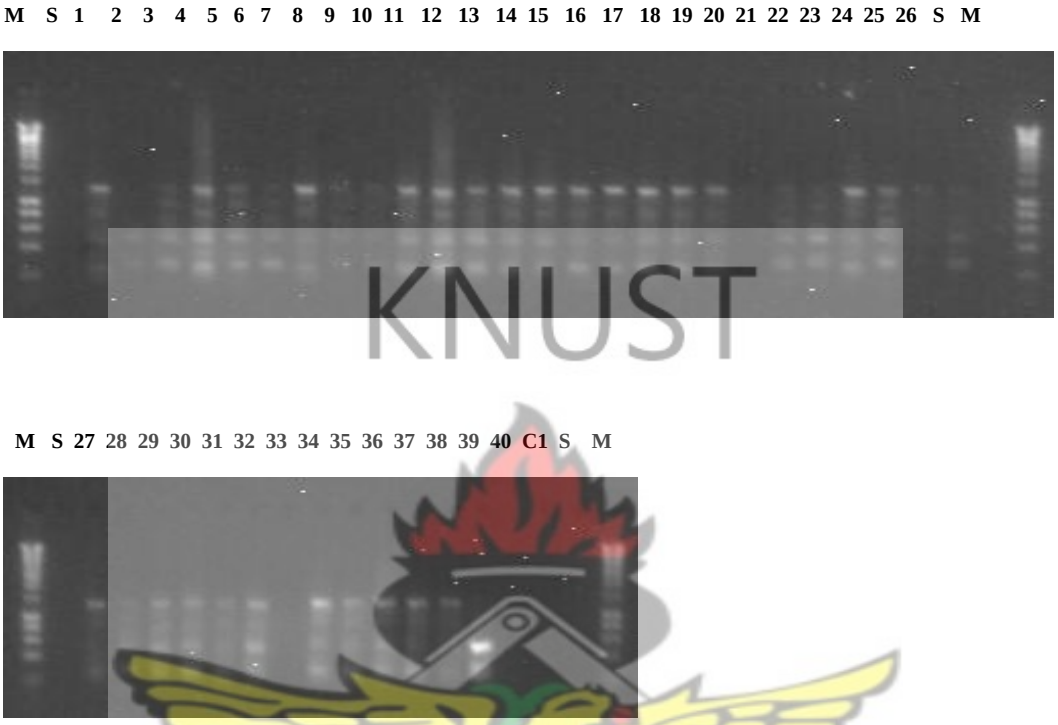


Fig 6.4.1 RAPD profiles amplified from DNA from 40 accessions of *C. pentandra* using primer GEN60-3J in an agarose gel stained with ethidium bromide.

Key:
M = Standard DNA marker (100bp), S = Empty lane, ABF 1 (Lane 1 = 3-plant bulk resistant sample, Lanes 2-4 = resistant progenies, Lane 5 = 3-plant bulk susceptible sample, Lanes 6-8 = susceptible progenies); MAM 1 (Lane 9 = 3-plant bulk resistant sample, Lanes 10-12 = resistant progenies, Lane 13 = 3-plant bulk susceptible sample, Lanes 14-16 = susceptible progenies); DNY 1 (Lane 17 = 3-plant bulk resistant sample, Lanes 18-20 = resistant progenies, Lane 21 = 3-plant bulk susceptible sample, Lanes 22-24 = susceptible progenies); DAN 1 (Lane 25 = 3-plant bulk resistant sample, Lanes 26-28 = resistant progenies, Lane 29 = 3-plant bulk susceptible sample, Lanes 30-32 = susceptible progenies); KUE 2 (Lane 33 = 3-plant bulk resistant sample, Lanes 34-36 = resistant progenies, Lane 37 = 3-plant bulk susceptible sample, Lanes 38-40 = susceptible progenies), C1 = Negative control (PCR mixture and water). (See appendix 3.1 for ecological zones and locations of accessions).

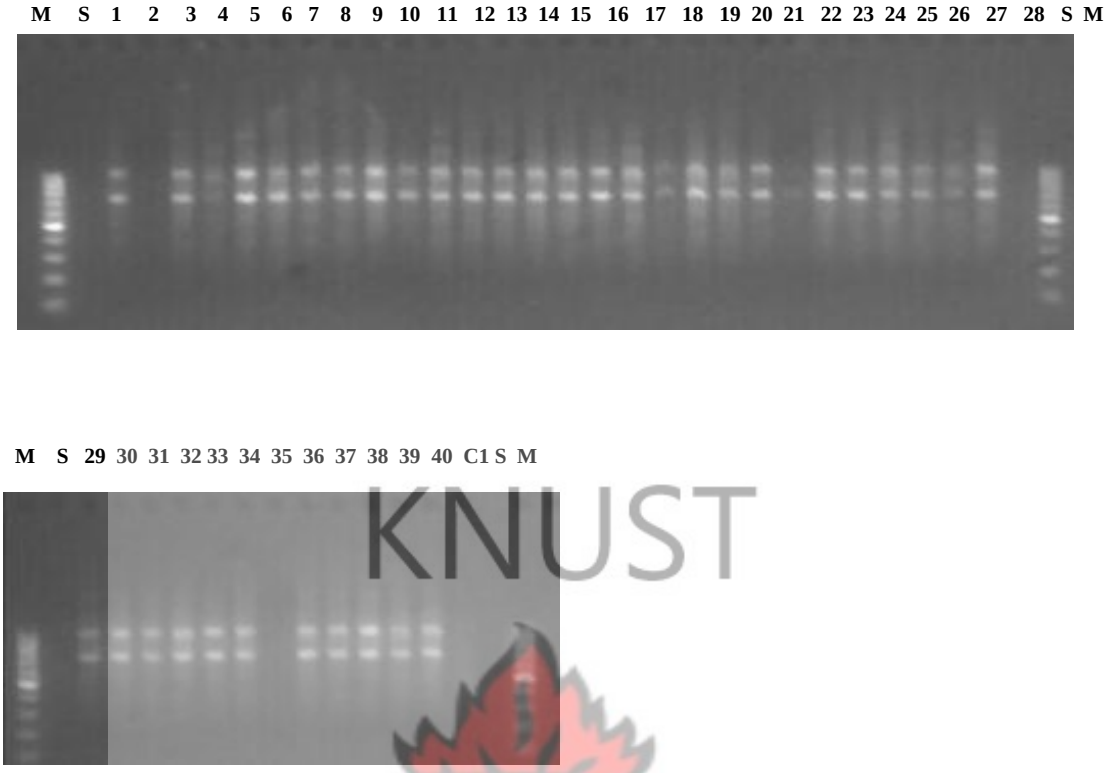


Fig 6.4.2 ISSR profiles amplified from DNA from 40 accessions of *C. pentandra* using primer C3101E10 in an agarose gel stained with ethidium bromide.

Key: See key under figure 6.4.1. C1 = Negative control (PCR mixture and water).

6.5 Discussion

A genetic marker is described as a gene which is inherited successfully, assigned unambiguously to the phenotype or to a set of one or more loci and which, mostly, has a 1:1 relationship between phenotype and genotype (Farooq and Azam, 2002). The identification of DNA markers linked to disease resistance is a logical starting point for molecular cloning of genes for which there is little information on specific biochemical functions. Several studies have illustrated the utility of RAPD in the identification of markers linked to pest and disease resistance in a wide variety of organisms including the yellow genes (B) from yellow squash (Brown and Myers, 2001), leaf rust resistance gene *Lr19* in wheat (Gupta *et al*., 2006) and cassava anthracnose in cassava (Akinbo *et al*., 2007). ISSR marker techniques, on the other

hand, have been used to identify the fertility restoration gene, Rf-1, in rice (Akagi *et al*., 1996*)* as well as the gene which confers resistance to race 4 of *Fusarium* wilt (Ratnaparkhe *et al.*, 1998).

The 5 RAPD and 5 ISSR used in the current study, however, failed to distinguish between resistant and susceptible accessions. Possible explanations of this could be: first primer limitations: RAPD and ISSR markers are probably not tightly linked with the gene responsible for stem dieback resistance in *C. pentandra.* RAPD, in particular, is preferred as most useful for marker-assisted selection only when they are tightly linked to the disease resistance gene (Brown and Myers, 2001). Secondly, the limited number and types of markers evaluated could have also reduced the possibility of finding dieback resistant associated markers. For instance, in a bulk segregant analysis to find rust resistance genes in wheat, Gupta *et al.*, (2006) found out that only 16 of the 700 RAPD primers screened were associated with the resistant locus. Yin *et al.* (2004) reported the necessity for large number of markers in finding association with a trait in open-pollinated forest trees with long evolutionary history due to the higher possibility of linkage disequilibrium constraining to extremely short genomic regions. *C. pentandra* is not an exception to this rule. RAPD markers are known to have relatively low level of polymorphism in forest tree mapping due to their dominant mode of inheritance (Nkongolo *et al.*, 2005). Thirdly, the absence of a molecular-based genetic-linkage map coupled with the inadequate knowledge on stem dieback quantitative trait loci (QTL) in *C. pentandra* might have greatly hindered the functionality of the markers used. Schnell *et al*. (2007) recommended, among others, the production of linkage maps and quantitative trait loci (QTL) mapping as prerequisite for locating QTL for disease resistance. A successful mapping of QTL for a trait requires the selection of inbred homogenous parents with contrasting response

to the trait and crossing these to produce homozygous F_1 plants which, in turn, are crossed to produce F_2 plants and the latter populations screened for resistant markers (Akinbo *et al*., 2007; Howarth and Yadav, 2002). This is, however, not feasible for long term timber tree species like *C. pentandra,* like other tree species, it was not possible to do this in the short duration of the research. The success of marker-assisted selection is also known to be limited by other factors including the low resolution of marker-trait associations, the small proportion of phenotypic variation explained by QTL and the low success rate in validating QTL in different genetic backgrounds and environments (Butcher and Southerton, 2007). Further, there is a high possibility that the accessions selected for the study were not really genetically resistant due to the short period of observation.

6.6 Conclusion

The establishment of DNA marker diagnosis for stem dieback resistance represents an important step toward accelerated identification and multiplication of *C. pentandra* plants. Though the markers used could not differentiate between the resistant and susceptible genotypes, the high levels of genetic variability in *C. pentandra* as revealed in Chapters 3, 4 and 5 of the study, presents an important source of valuable information for developing plants with improved economic characteristics.

6.7 Recommendations

1. It is advisable that future studies use samples that have been screened for over twelve months in the field and the resistance levels verified through clonal trials.
2. All efforts must be put in place to identify specific markers linked to dieback resistance in *C. pentandra* to enhance marker-assisted selection and conservation of this species.
3. It is also recommended that future studies use plants that have been proven to be resistant or susceptible.
4. Further, a large number of markers known to be highly polymorphic (eg. RFLP and AFLP) and co-dominant markers (eg. Microsatellites), should be used in further assessment of dieback-resistant markers.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 Conservation considerations of *C. pentandra*

C. pentandra is an important timber species in Ghana and has become one of the preferred choices for timber. The species is, however, facing increasingly great pressure of over-exploitation because of the increase in demand for its products. The species has, therefore, been incorporated into the National Forest Plantation Development Programme of Ghana to ensure its sustainable management for the continual production of the benefits obtained from it. Unfortunately, the existence of stem dieback disease and the unknown extent and pattern of distribution of genetic variation within and among populations have posed a great threat to the success of initiatives using *C. pentandra* in plantation/reforestation programmes. Globally, *C. pentandra* is gradually being endangered. Notably, *C. pentandra* is one of the endangered species in the Peruvian and Brazilian Amazon forests (Brondani *et al*., 2003). There is, therefore, a need for genetic information on the species in order to design proper management conservation strategies for it (Deng *et al*., 2006).

7.2 Phenotypic (morphological) characterization of stem dieback resistance

The phenotype of a tree is the product of its genetic constitution and its growth environment (Jansson, 2005). Over the years, natural conditions and human interventions have imposed greater fitness on some genotypes resulting in the evolution of superior phenotypes and variability among genotypes (Namkoong *et al*., 2000). The target of tree breeders, therefore, is to maintain sufficient quantities of these superior phenotypes (Apiolaza, 2009). The aim of this is to maintain as much

genetic diversity as possible to ensure that the resultant populations have sufficient adaptation to current environmental conditions for adequate numbers of individuals to survive and reproduce (Namkoong *et al*., 2000). This is because, the knowledge of genetic parameters is essential in understanding the strength of genetic control of traits (Vasudeva *et al*., 2004). Seed collection from open-pollinated trees widely spaced in natural stands or unimproved plantation is essential for advanced-generation selection with little fears of finding genetic relatedness among them. Also, the use of open- or wind-pollinated offspring is the easiest, simplest, and a cost effective approach of creating a progeny population. Furthermore, the use of open-pollinated seed is advantageous for the provision of the estimates of parental general combining ability as well as the additive genetic variance and heritability values for the test population (Zobel and Talbert, 1984).

In the current study, seed from open-pollinated *C. pentandra* trees were collected, nursed and the seedlings screened for resistance to stem dieback disease in both the nursery and the field through progeny and provenance tests. At the nursery, resistance to stem dieback was assessed, based on survival rates, in two phases: September-November Screening (SNS) and March-May Screening (MMS) (Chapter 3). Survival rate was generally higher in the MMS. The SNS had higher amounts of rainfall than the MMS (Table 3.3.4) and this could partly be responsible for the severe mortality observed. This suggests that screening of *C. pentandra* for stem dieback resistance will yield good results when done during the peaks of the rainy season, whereas the raising of seedlings from already screened resistant planting stock will best be done during the dry season. The number of accessions with resistant progenies was higher within populations than among them (Tables 3.4.3 and 3.4.4). This is an indication that the repository of the resistant genes is at the individual tree level rather

than the population level. This implies that within a single population, many accessions with good resistance to stem dieback can be identified and conserved for seed collection.

Results from the progeny and provenance trials showed that a majority of the accessions, 23 (62.16 %), were rated as fairly resistant (Table 4.4.2). This again confirmed that stem dieback disease load is high in *C. pentandra.* Analysis of variance showed significant differences in height growth (Appendix 4.4.1), diameter (Appendix 4.4.2) and mortality rate (Appendix 4.4.3). Fourteen (37.84 %), of out of the 37 accessions screened, had survival rates greater than 50 %. Six of these came from the Dry Semi-deciduous Forest Inner Zone (DSDFZ-Inner) making it the population with the highest number of accessions with high survival rates. This was followed by the Moist Semi-deciduous Forest Zone (MSDFZ), Dry Semi-deciduous Forest Outlier Zone (DSDFZ-Outlier) and the Moist/Wet Evergreen Forest Zone (M/WEFZ) and the Guinea Savanna Zone (GSZ). This suggests the order of preference in selecting populations with tolerance to *C. pentandra* stem dieback disease. A greater proportion of the variation in height growth and mortality rates was attributed to the differences that exist between accessions and suggests the existence of the potential for improvement by selecting the best performed accessions. The multiple-parameter assessment for dieback further serves as a good source of information for selecting accessions to meet the multi-purpose role of *C. pentandra* (Table 4.4.3). The narrow sense heritability estimated was high, 0.56 and similar to results from other earlier studies and further suggests that improvement could be attained by selecting the best accessions. The environmental influence shown in this study was high, 44 % and indicative that, species-site matching is important for

maximum productivity of the species, even though *C. pentandra* is known for its existence in all forest types in Ghana (Hall *et al*., 1981).

7.3 Phenotypic (morphological) and genetic characterization of variability

Characterization of *C. pentandra* was done using morphological characters and RAPD and ISSR polymorphism in the current study. RAPD and ISSR techniques were preferred because they are recommended for species with no prior availability of molecular genetic information (Ofori, 2001). This study pioneers the molecular works of *C. pentandra* in Ghana. The five morphological traits used in the study grouped accessions into two main clusters. Though there was no known trend for the distinction, accessions with high percentage of spines, deep-green stem colour and light-purple or deep-purple petiole colour seemed to be linked with high growth in height, diameter, large genetic gain and high survival rates. These associations were rather weak and applied to only a few accessions. They could, however, be used together with other considerations, as indicators for selecting for stem dieback disease resistance and good performance in *C. pentandra*.

RAPD, ISSR and the combined RAPD and ISSR polymorphism revealed higher diversity within populations than among them. This fact was supported by all the three statistics used in the study, AMOVA, PhiPT and F_{ST}. These were adjudged, following Wright (1978), to be commensurate with little or very little genetic differentiation within the ecological zones. This was supported by the low gene flow estimates which indicated low to moderate differences among populations. Gene flow was identified as the key factor homogenizing all the populations. The basis for the cluster patterns in all the dendrograms, produced by RAPD, ISSR and the combined

RAPD and ISSR polymorphism as well as the Principal Component Analysis (PCoA), was however not clear in the study.

7.4 Marker-assisted selection

DNA markers provide a powerful tool to quantify existing levels of genetic variation in breeding and production populations of forest trees (Grattapaglia, 2007). This is made possible by the fact that resistance, like other traits, is expressed at the cell, tissue, whole-tree, and or stand levels (Namkoong, 1991). The response of plants to disease at the cellular and molecular levels allows the discovery of changes in gene expression in the tissues attacked by pathogens, and the identification of genetic components involved in the interaction between host and pathogens (Huang *et al*., 2003). For instance, markers linked to resistance genes of a disease caused by green spruce aphid have been identified in spruce clones (Day, 2002). The current study did not find any unique bands which were either present in the resistant progenies but not in the susceptible ones or vice versa (Fig 6.4.1 and 6.4.2). This could be due to several factors. For instance, the selected accessions might not have been really genetically resistant or susceptible as early sampling might have introduced errors, since the accessions used for the study showed high performance in growth and survival with age in the field. These problems could be overcome if accessions are manually infected with the right causative agent and true resistant and susceptible individuals screened. Again, the absence of adequate information on the linkage mapping and quantitative trait linkage (QTL) in *C. pentandra* was also a barrier. This is because the successful selection of individuals based on a known marker requires that its linkage disequilibrium with the traits of interest first be identified. According

to Mackay (2001), low linkage disequilibrium can cause inconsistent associations in families and populations.

7.5 Limitations of the study

A number of shortcomings were encountered and worth noting. These included the limited number of accessions used. Though many attempts were made to collect as many accessions as possible, the number of accessions per population that germinated and provided adequate number of seedlings, was small. As such, this might not have well represented the populations from which they were sampled. Also, variations in the number of accessions per populations could be a source of bias though none was detected in the study. The dominance nature of the RAPD and ISSR may render the estimates of population parameters less accurate due to the lack of complete genotypic information. Furthermore, the number of RAPD and ISSR markers used in the study was small and could have limited the chances of finding markers linked to the disease. This is the first progeny and provenance work on *C. pentandra* and the results cannot be extrapolated to the entire country since the study was done at only one site. Inferences about variation in genetic gain are therefore limited to the site on which the accessions were tested.

7.6 Conclusion

C. pentandra, an ecologically and economically important multipurpose forest tree species in Ghana, faces danger as a result of the recent rapid forest depletion and advancement of infection from stem dieback disease caused predominantly by *Fusarium solani* and *Lasiodiplodia theobromae.* Evidence of disease resistance within natural *C. pentandra* populations suggest that the species could be conserved

through the use of resistant planting stock through single tree selection and breeding. Development of a formal stem dieback resistance programme to breed selected resistant trees will allow the multiplication of resistant genotypes and help maintain genetic diversity in *C. pentandra* populations. The continued refinement of nursery production systems and silvicultural guidelines will also help ensure the successful restoration of the next generation of stem dieback-resistant *C. pentandra* individuals. Based on the current findings, it is advisable that selection of *C. pentandra* accessions for conservation be based on both morphological and molecular considerations. The large proportion of genetic variation in *C. pentandra* found to be among individuals within populations, implies that considerable amount of genetic variation of the species can be obtained by sampling a larger number of plants from one or two populations, rather than smaller collections from many different populations, and the same would be true for the development of any conservation plan for this species.

The lack of differences in genetic resistance or susceptibility among the accessions used in the study could be resolved by using manual infection. The efficiency in differentiating populations using only five RAPD and five ISSR proved the usefulness of the combined power of these PCR-based markers. With molecular markers, the plant breeder will be able to select plants resistant to stem dieback disease.

There was significant variation in tree growth among accessions in this study, indicating that there are genetic differences among accessions in the natural populations of *C. pentandra*. Therefore, tree improvement programmes can select genetically more productive accessions for reforestation. The study also suggested that natural populations of *C. pentandra* from the GSZ and DSDFZ-Inner of Ghana are genetically more diverse compared to the other populations. This is a hypothesis,

however, and there is no direct evidence to confirm that the variation patterns are primarily the result of natural selection. However, assuming this reflects adaptive variation, it would be prudent for tree improvement and conservation programs to collect *C. pentandra* seed from these populations of the country for planting and conservation in Ghana. Findings from the study of the genetic diversity and relatedness among *C. pentandra* populations significantly increased the possibilities of in-depth investigation of the genetic status of *C. pentandra* in the current forest fragments as well as remnant populations in preserved areas. The results from this study also provided baseline information for the species restoration and conservation programmes in Ghana. This will also ensure that restored populations are sufficiently diverse genetically to maintain themselves in the long term.

7.7 Recommendations

1. Further studies should be done in all potential growing sites of *C. pentandra* for site-specific accessions and progenies to be selected.
2. The study should also be repeated with other molecular techniques such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Single Sequence Repeats (SSRs) or micro-satellites, Internal Transcribed Spacer (ITS) and cDNA markers. This should also include the estimation of out-breeding and cross breeding paternity, cyoplasmic gene transfer through pollen, inter-population gene transfer and levels of interspecific hybridization coefficients.
3. Further studies should be done with accessions infected with the right causative agent and true resistant and susceptible individuals screened for resistant markers.

4. More genetics research is needed in order to develop a comprehensive tree improvement and conservation programmes for *C. pentandra* in Ghana. For instance, linkage mapping and quantitative trait linkage (QTL) in *C. pentandra* should be developed and linkage equilibrium with traits of interest be identified.

REFERENCES

Abeney, E. A. (1999). The worldwide interest in forests and forestry. In: Abeney, E. A., Owusu, J. G. K. and Frimpong, E. A. (editors). Workshop for Media Personnel on Forestry and Wildlife Reporting Proceedings. Institute of Renewable Natural Resources, KNUST, Ghana. Pp 1-13.

Adams, W. T. (1983). Application of Isozymes in Tree Breeding. In: Tanksley, S. D. and Orton, T. J. (Editors). Isozymes in Plant Genetics and Breeding. Elsevier Science Publishers, Amsterdam. Pp 60-64.

Agrios, G. N. (1988). Plant Pathology. Third edition. Academic Press, Inc., San Diego. Pp 3-38, 265-283.

Aida, A. R., Attia, S. A. A., Elham, A. A., Abd, E., Hanna, N. S. and Nasseef, J. E. (2012). Genetic Diversity Based on ISSR and Protein Markers Associated with Earliness Trait in Wheat. *World Applied Sciences Journal* 20 (1): 23-33.

Akagi, H., Yokozeki, Y., Inagaki, A., Nakamura, A. and Fujimura, T. (1996). A co-dominant DNA marker closely linked to the rice nuclear restorer gene, Rf-1, identified with inter-SSR fingerprinting. *Genome* 39: 1205-1209.

Akinbo, O., Gedil, M., Ekpo, E. J. A., Oladele, J. and Dixon, A. G. O. (2007). Detection of RAPD markers-linked to resistance to cassava anthracnose disease. *African Journal of Biotechnology* 6 (6): 677-682. http://www.academicjournals.org/AJB. [Accessed: 12/01/2011].

Allen, C. D. (2009). Climate-induced forest dieback: an escalating global phenomenon? *Unasylva* 60: 43-48.

Amnon, L., Claude, E. T., Newman, M., Rendy, O. U. K., Zhang, X. and Xu, Y. (2004). ISSR and AFLP Makers differ among American water melon cultivars with limited genetic diversity. *Journal of American Society of Horticultural Science* 129(4): 553-558.

Ansari, S. A., Narayanan, C., Wali, S. A., Kumar, R., Shukla, N., Rahangdale, S. K. (2012). ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* L.f.) populations. *Annals of Forest Research* 55(1): 11-23.

Apetorgbor, M. M., Siaw, D. E. K. A. and Gyimah, A. (2003). Decline of *Ceiba pentandra* seedlings, a tropical timber species in nurseries and plantations. *Ghana Journal of Forestry* 11(2): 51-53.

Apiolaza, L. A. (2009). Very early selection for solid wood quality: screening for early winners. *Annals of Forest Science* 66: 6011-6021.

Archak, S., Gaikwad, A. B., Gautam, D., Rao, E. V. V. B., Swamy, K. R. M. and Karihaloo, J. L. (2003). Comparative assessment of DNA fingerprinting techniques

(RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. *Genome* 46: 362-369.

Arslan E. and Tamkoc, A. (2011). The application of ISSR-PCR to determine the genetic relationship and genetic diversity between narrow leaved bluegrass (*Poa angustifolia*) and rough bluegrass (*Poa trivialis*) accessions. *Turkey Journal of Biology* 35: 415-423.

Askari, N., Abadi, M. M. and Baghizadeh, A. (2011). ISSR markers for assessing DNA polymorphism and genetic characterization of cattle, goat and sheep populations. *Iranian Journal of Biotechnology* 9(3): 222-229.

Bandara, P. D. M. G. D. (1990). Insects and diseases of forest plantations in Sri Lanka. In: Proceedings of the IUFRO Workshop on Pest and Diseases of Forest Plantations in the Asia-Pacific Region. Hutacharern, C., MacDicken, K. G., Ivoiry, M. H. and Nair, K. S. S (editors). Regional Office and Asia and the Pacific (RAPA) and Food and Agricultural Organization of the United Nations, Bangkok. Pp 81-85.

Baskin, C. C. and Baskin, M. J. (2005). Seed dormancy in trees of climax tropical vegetation types. *Tropical Ecology* 46(1): 17-28.

Becker, J. and Heun, M. (1994). Barley microsatellites: allele variation and mapping. *Plant Molecular Biology* 27(4): 835-845.

Beever, R. E. Forster, R. L. S., Rees-George, J., Robertson, G. I., Wood, G. A. and Winks, C. J. (1996). Sudden decline of cabbage tree (*Cordyline australis*): Search for the cause. *New Zealand Journal of Ecology* 20(1): 53-68.

Bhattacharya, S., Bandopadhyay, T. K. and Ghosh, P. D. (2010). Efficiency of RAPD and ISSR markers in assessment of molecular diversity in elite germplasms of *Cymbopogon winterianus* across West Bengal, India. *Emirates Journal of Food and Agriculture* 22 (1): 13-24. **http://ffa.uaeu.ac.ae/ejfa.shtml** [Accessed: 12/11/2013].

Biggs, A. R., Miles, N. W., and Bell, R. L. (1992). Heritability of suberin accumulation in wounded peach bark. *Phytopathology* 82: 83-86.

Bilgrami, K. S. and Dube, H. C. (1976). A Textbook of Modern Plant Pathology. Vikas Publishing House PVT Limited. New Delhi. Pp 326.

Bingham, R. T., Hoff, R. J. and McDonald, G. I. (1971). Disease resistance in forest trees. *Annual Review of Phytopathology* 9: 433-452.

Blay, D., Bonkoungou, E., Chamshama, S. A. O. and Chikamai, B. (2004). Rehabilitation of degraded lands in Sub-Saharan Africa: Lessons Learned from Selected Case Studies. FORNESSA and IUFRO-SPDC. Pp 1-16.

Boa, E. R. (1995). A guide to the identification of diseases and pests of neem (*Azadirachta indica*). Food and Agricultural Organization of the United Nations, Bangkog. Pp 1-71.

Borlea, G. F. (2004). Ecology of elms in Romania. *Invest Agrar: Sist Recur For.* 13 (1): 29-35.

Bonello, P., Gordon, T. R., Herms, D., A., Wood, D. L. and Erbilgin, N. (2006). Nature and ecological implications of pathogen-induced systemic resistance in conifers: A novel hypothesis. *Physiological and Molecular Plant Pathology* 68: 95–104.

Bongers, F., Poorter, L. and Hawthorne, W. D. (2004). The forests of Upper Guinea: Gradients in large species composition. In: An Ecological Atlas of woody plants species. Poorter, L., Bongers, F., Kouale, F. N. and Hawthorne, W. D. (editors). CABI Publishing, Wallingford, UK. Pp 41-52.

Bornet, B. and Branchard, M. (2001). Nonanchored Inter Simple Sequence Repeat (ISSR) Markers: Reproducible and Specific Tools for Genome Fingerprinting. *Plant Molecular Biology Reporter* 19: 209–215.

Boshier, D. and Amaral, W. (2004). Threats to forest ecosystems and challenges for the conservation and sustainable use of forest genetic resources. In: Vinceti, B., W. Amaral and Meilleur, B. (editors). Challenges in managing forest genetic resource for livelihoods: examples from Argentina and Brazil. International Plant Genetic Resources Institute, Rome, Italy. Pp 7-28.

Boshier, D. H. (2000). Mating systems. In: Forest conservation genetics: principles and practice. Young, A., Boshier, D., Boyle, T. (editors). CSIRO Publishing/CABI Publishing, Melbourne/Wallingford. Pp 63-79.

Brodie, A.W., Labarta-Chávarri, R. A., Weber, J. C. (1997). Tree germplasm management and use on-farm in the Peruvian Amazon: a case study from the Ucayali region, Peru. Research Report, Overseas Development Institute (ODI), London and the World Agroforestry Centre (ICRAF), Nairobi. Pg 8.

Brondani, R. P. V., Gaiotto, Missiaggia, A. A., Kirst, M., Gribel, R. and Grattapaglia, D. (2003). Microsatellite markers for *Ceiba pentandra* (Bombacaceae), an endangered tree species of the Amazon forest. *Molecular Ecology Notes* 3: 177-179.

Brown, N. R. and Myers, R. J. (2001). RAPD Markers Linked to Morphological and Disease Resistance Traits in Squash. *Cucurbit Genetics Cooperative Report* 24: 91-93.

Brown, J. K. M. (1996). The choice of molecular marker methods for population genetics studies of plant pathogens. *New Phytopathology* 133: 183-195.
Burdon, J. J. (1987). *Diseases and Plant Population Biology*. Barnes, R.S.K, Birks, H.J.B., Connor, E.F., Harper, J.L., and Paine, R.T. (Editors). Cambridge University Press, Cambridge. Pg. 208.

Bruce, E., Tabashnik, J. B. J., Rensburg, V. and Carriere, Y. (2009). Field-evolved insect resistance to Bt crops: definition, theory, and data. *Journal of Economic Entomology* 102(6): 2011-2025.

Burkill, H. M. (1985). The Useful Plants of West Africa. Second edition. Royal Botanic Gardens, Kew. Pp. 278-283.

Butcher, P. and Southerton, S. (2007). Marker-assisted selection in forestry species. In: Current status and future perspectives in crops, livestock, forestry and fish. Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A. and Dargie, J. D. (editors). Food and Agriculture Organization of the United Nations, Rome. Pp 284- 305.

Byrne, M. (2000). Disease threats and the conservation genetics of forest trees. In: Forest Conservation Genetics: Principles and Practice. Young, A., Boshier, D. and Boyle, T. (editors). CSIRO Publishing. Collingwood, Australia. Pp 159-166.

Cardoso, M. A., Provan, J., Powell, W., Ferreira, P. C. G., de Oliveira, D. E. (1998). High genetic differentiation among remnant populations of endangered *Caesalpinia echianata* Lam. (Leguminoseae, Caesalpinioideae). *Molecular Ecology*. 7: 601-608.

Carson, S. D. and Carson, M. J. (1989). Breeding for resistance in forest trees-A quantitative genetic approach. *Annual Review of Phytopathology*. 27: 373-95.

Chamshama, S. A. O. and Nwonwu, F. O. C. (2004). Forest plantations in Sub-Saharan Africa. A report prepared for the project "*Lessons Learnt on Sustainable Forest Management in Africa"*. Pp 1-20.

Charkrabarty, P. K. Meshram, M. K. Sheo, R. and Raj, S. (1993). An Outbreak of dieback and canker in white silk cotton (*Ceiba pentandra*). In: Apetorgbor, M. M., Siaw, D. E. K. A. and Gyimah, A. (2003). Decline of *Ceiba pentandra* seedlings, a tropical timber species in nurseries and plantation. *Ghana Journal of Forestry* 11(2): 51-53.

Cleary, M., van der Kamp, B. and Morrison, D. (2008). British Columbia's southern interior forests: Armillaria root disease stand establishment decision aid. *BC Journal of Ecosystems and Management* 9(2): 60-65.

Cobbinah, R. J., Saiw, D. E. K. A. and Gyimah, A. (2001). Guide to Tree Planting in Ghana. Forestry Research Institute of Ghana (CSIR), Kumasi-Ghana. Pp. 22, 26, 28.

Cousin, M. T., Raux, J., Millet, N. and Michel, M. F. (1990). Maintenance of MLOs (mycoplasma-like organisms) on *Populus alba* micropropagation. *Journal of Phytopathology* 130: 17-23.

Crute, I. R. (1987). The geographical distribution and frequency of virulence determinants in *Bremia lactucae*. Blackwell Science, London. Pp 193-212.

Deng, C., Zhou, J., Gao, W., Sun, F., Qin, R. and Lu, L. (2006). Assessment of genetic diversity of *Lycoris longituba* (Amaryllidaceae) detected by RAPDs. *Journal of Genetics* 85 (3): 205-207.

Devey, M. E., Delfinomix, A., Kinloch, B. B. and D. B. Neale, D. B. (1995). Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in sugar pine. *Proceedings of the National Acadamy of Science, USA* 92: 2066-2070.

Dhakal, L. P., Aryal, H. L., Kjaer, E. D. and Lilleso, J. P. B. (2004). Breeding for die-back resistant *Dalbergia sissoo* in Nepal: Generation turnover plan for *Dalbergia sissoo* BSOs with infusion of die-back disease resistant families. Forest and Landscape Working Papers no. 4. *Forest and Landscape*, Denmark. Pp 1-16.

Diaz-Matallana, M., Schuler-Garcia, I., Ruiz-Garcia, M., de Jaramillo, H. E. (2009). Analysis of diversity among six populations of Colombian mango (*Mangifera indica* L. cvar. Hilacha) using RAPDs markers. *Electronic Journal of Biotechnology* 12(3) http://www.ejbiotechnology.info/content/vol12/issue3/full/10/10.pdf. [Accessed: 7/01/2011].

Dick, C. W., Birmingham, E., Lemes, M. R., and Gribel, R. (2007). Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. *Molecular Ecology* 16: 3039-3049.

Dinoor, A., and Eshed, N. (1987). The analysis of host and pathogen populations in natural ecosystems. Blackwell Science, London. Pp 75-88.

Doyle, J. J., and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Durrant, W. E. and Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*. 42:185–209.

Edmonds, R. L., Agee, J. K. and Gara, R. I. (2000). Forest Health and Protection. McGraw-Hills Companies, Inc. New York. Pp 2-21.

Edwards, D., Schreckenberg, K. (1997). Demand from smallholder farmers for multipurpose tree germplasm: implications for policy, research and development. In: Policy aspects of tree germplasm demand and supply. Simons, A. J., Kindt, R., Place, F. (editors). Proceedings of an international workshop. The World Agroforestry Centre (ICRAF), Nairobi. Pp 9-26.

Egnin, M., Mora A, and Prakash, C. S. (1998). Factors enhancing *Agrobacterium tumefaciens* – Mediated Gene Transfer in peanut (*Arachis hypogeal* L.). *In vitro Cellualar and Developmental Biology of Plants* 34: 310-318.

El-Kassaby, Y. A., Cook, C. (1994). Female reproductive energy and reproductive success in a Douglas-fir seed orchard and its impact on genetic diversity. *Silvae Genetica* 43:243-246.

Engelmann, F., Dulloo, M. E., Astorga, C., Dussert, S. and Anthony, F. (editors) (2007). Complementary strategies for *ex situ* conservation of coffee (*Coffea arabica* L.) genetic ressources. A case study in CATIE, Costa Rica. Topical reviews in Agricultural Biodiverstity. Bioversity International, Rome, Italy. Pg 4.

Espahbodi, K., Nodoushan, M. H., Tabari, M., Akbarinia, M., Jalali, S. G and Hosseini, S. M. (2007). Seed Source Effects on Seed Emergence, Seedling Survival and Growth on Wild Service (*Sorbus torminalis*) Seedlings. *International Journal Of Agriculture & Biology* 9 (3): 426-430.

Evans, J. (1982). Plantation Forestry in the Tropics. Oxford University Press. New York. Pp. 147-163.

FAO (2007). Forest Health and Biosecurity Working Papers. Overview of forest pests, Ghana. *Forest Resources Development Service*. *Forest Management Division, Forestry Department*. *Working Paper FBS/16E*. FAO, Rome, Italy. Pp 1-10.

FAO (2003). Pest Management in Forestry. Forestry department. Food and Agriculture Organization of the United Nations. Rome. Pp 1-16.

FAO (2001). Protecting plantations from pests and diseases. In: Chamshama, S.A.O. and Nwonwu, F. O. C. (2004). Forest plantations in Sub-Saharan Africa. A report prepared for the project "*Lessons Learnt on Sustainable Forest Management in Africa"*. Rome, Italy. Pp 12-15.

Farooq, S. and Azam, F. (2002). Molecular markers in plant breeding-I: Concepts and Characterization. *Pakistan Journal of Biological Sciences* 5 (10): 1135-1140.

Frampton, J. (1996). The tree improvement process. *Limbs and Needles* 23(4):10, 12, 14.

Frisvold, G. B. and Condon, P. T. (1998). The Convention on Biological Diversity and Agriculture: Implications and Unresolved Debates. *World Development* 26 (4). 551-570.

Garbelotto, M., Svihra P, and Rizzo, D. M. (2001). New pests and diseases: Sudden oak death syndrome fells 3 oak species. *California Agriculture* 55(1): 9-19.

Geburek, T. (2000). Effects of environmental pollution on the genetics of forest trees. In: Forest Conservation Genetics: Principles and Practice. Young, A., Boshier, D. and Boyle, T. (editors). CSIRO Publishing. Collingwood, Australia. Pp 135-157.

Ghana Forest Service Division (2005). National Forest Plantation Development Programme: The modified taungya system and private developers. Annual Report, Forest Service Division, Ghana. Pg 3.

Gilbert, G. S. (2002). Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* 40:13-43.

Gilbert, G. S. and Steven, D. (1996). A canker disease of seedlings and saplings of *Tetragastis panamensis* (Burseraceae) caused by *Botryosphaeria dothidea* in a lowland tropical forest. *Plant Disease*. 80: 684-687.

Gillet, E., Gomory, D. and Paule, L. (2005). Measuring genetic variation within and among populations at marker loci. In: Conservation and Management of Forest Genetic Resourses in Europe. Geburek, T. and Turok, J. (editors). Arbora Publishers, Zvolen. Pp 237-273.

Girsh, K. and Shankara, B. S. (2008). *Phomopsis azadirachtae*- The Die-Back of Neem Pathogen. *Electronic Journal of Biology* 4(3): 112-119.

Gosling, P. (2007). Raising trees and shrubs from seed. Forestry Commission Practice Guide. Forestry Commission, Edinburgh. Pp 10-21.

Grattapaglia, D. (2007) Marker-assisted selection in *Eucalyptus*. In: Current status and future perspectives in crops, livestock, forestry and fish. Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A. and Dargie, J. D. (editors). Food and Agriculture Organization of the United Nations, Rome. Pp 252-281.

Grunwald, N. J., Coffman, V. A., and Kraft, J. M. (2003). Sources of partial resistance to *Fusarium* root rot in the *Pisum* core collection. *Plant Disease* 87: 1197-2001.

Gupta, S., Srivastava1, M., Mishra, G. P., Naik, P. K., Chauhan, R. S., Tiwari, S. K., Kumar, M. and Singh, R. (2008). Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *African Journal of Biotechnology* 7 (23): 4230-4243.

Gupta, S. K. and Charpe, A. (2006). Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. *Theoretical and Applied Genetics*. DOI 10.1007/s00122-006-0362-7.

Hajjar, R., Gough, A., Mathey, A., Nitschke, C., Paudel, K. S., Skrivanos, P., Waeber, O. P. and Innes, J. (2009). Criteria and indicators for sustainable forest management in the face of decentralization: Are they still relevant in their current form. XIII World Forestry Congress. Buenos Aires, Argentina. Pp 1-6.

Hall, J. B. and Swaine, M. D. (1981). Distribution and Ecology of Vascular Plants in a Tropical Rain Forest. Forest Vegetation in Ghana. Dr. W. Junk Publishers. The Hague. Pp 145, 383.

Hamza, B. N. (2010). Genetic variation within and among three invasive *Prosopis juliflora* (Leguminosae) populations in the River Nile State, Sudan. *International Journal of Genetics and Molecular Biology* 2(5): 92-100. http://www.academicjournals.org/IJGMB. [Accessed: 5/01/2011].

Hansson, B., Westerberg, L. (2002). On the correlation between heterozygosity and Wtness in natural populations. *Molecular Ecology* 11: 2467-2474.

Hawkes, J. G. (1985). Report on a consultancy Mission to Ethiopia for GTZ to advice PCRC/E on germplasm exploration, conservation, multiplication and evaluation. In Engels, J. M. M, Hawkes, J. G. and Worede, M. (editors). Plant Genetic Resource of Ethiopia. Pp 189-190.

Hawthorne, W. D. (1995). Ecological Profile of Ghanaian Forest Trees. Tropical Forestry Papers No. 29. Pp. 70-72.

Henry, R. J. (1997). Practical Application of Plant Molecular Biology. Chapman and Hall. London. Pp 5-220.

Hodel, D. R., Liu, A., Arakelian, G., Eskalen, A. and Stouthamer, R. (2012). *Fusarium* dieback: A new and serious insect-vectored disease of landscape trees. *Western Aborist:* 58-63.

Holmgren, M., Poorter, L., Siepel, A., Bongers, F., Buitelarr, M. Chatelain, C., Gautier, L., Hawthorne, W. D., Helmink, A. T. F., Jongking, C. C. H., Os-Breijer, H. J., Wieringa, J. J. and van Zoest, A. R. (2004). Ecological profiles of rare and endemic species. In: Biodiversity of West African Forests. Poorter, L, Bongers, F., Kouame, F. N. and Hawthorne, W. D. (editors). CABI Publishing, Wallingford, UK. Pg. 404.

Houston, D. R. (1987). Forest tree declines of the past and present: current understanding. *Canadian Journal of Plant Pathology* 9: 349-360.

Howarth, C. J. and Yadav, R. S. (2002). Successful marker assisted selection drought tolerance and disease resistance in pear Millet. IGER INOVATIONS. http://www.fao.org/teca/system/files/mas-pm.pdf. [Accessed: 7/01/2011].

Huh, K. M., Lee, Y. H and Huh, W. H (1999). Genetic diversity and population structure of *Gleditsia japonica* var. *koraiensis* in Korea. *Botanical Bulletin of Academia Sinica* 40: 159-164.

Infantino, A., Kharrat, M., Riccioni, L., Coyne, C. J., McPhee, K. E. and Grunwald, N. J. (2006). Screening techniques and sources of resistance to root diseases in cool season food legumes. *Euphytica* 147: 201–221.

Irvine, F. R. (1961). Woody Plants of Ghana with Special Reference to their Uses. Oxford University Press. Pg 191.

Islam, R., Siwar, C., Ismail, M. S. and Chamhuri, H. N. (2010). Criteria and Indicators for Sustainable Forest Management inMalaysia. *American Journal of Environmental Sciences* 6 (3): 212-218.

Islam, A. K. M. R. and Shepherd, K. W. (1992). Production of wheat-barley recombinant chromosomes through induced homeologous pairing 1. Isolation of recombinants involving barley arms 3HL and 6HL. *Theoretical and Applied Genetics* 83: 489-494.

ISTA (2005). International Rules for Seed Testing. Edition 2005. International Seed Testing Association, Bassersdorf, Switzerland. ISTA homepage available from: www.seedtest.org/en/home.html. [Accessed: 06/11/2010].

ITTO (2005). Revised ITTO criteria and indicators for sustainable forest management of tropical forests including reporting format. ITTO Policy Development Series No. 15. ITTO, Yakohama, Japan. Pp1- 4.

Jackson, J. W. and Howard, G. W. (2010). Unwelcome guests. The threat of invasive species to tropical forests. *ITTO Tropical Forest Update* 20(1): 22-24.

Jaenicke, H. (1999). Good Tree Nursery Practices. Practical Guidelines for Research Nurseries (editor: Hince). International Centre for Reseach in Agroforestry, Nairobi, Kenya. Pp 4-5.

James, W. C. (1974). Assessment of plant disease and losses. *Annual Review of Phytopathology* 12: 27-48.

Jansson, G. (2005). Quantitative genetics. In: Conservation and Management of Forest Resources in Europe. Geburek, Th. and Turok, J. (editors). Arbora Publishers, Zvolen. Pp 213-235.

Jasieniuk, M., and Maxwell, B. D. (2001). Plant diversity: new insights from molecular biology and genomics technologies. *Weed Science* 49: 257-265.

Johnson, R. (1987). Selected examples of relationships between pathogenicity in cereal rusts and resistance in their hosts. Blackwell Science, London. Pp 181-192.

Johnson, R. and Law, C. N. (1975). Genetic control of durable resistance to yellow rust (*Puccinia striiformis)* in the wheat cultivar Hybride de Bersee. *Annals of Applied Biology* 81: 385.

Jones, D. and Takemoto, D. (2004). Plant innate immunity-direct and indirect recognition of general specific pathogen-associated molecules. *Current opinion in Immunology* 16: 48-62.

Jones, D. G. (2001). Putting the knowledge of plant resistance genes to work. *Current Opinion in Plant Biology* 4: 281-287.

Kang, M. S. (1985). SAS program for calculating stability-variance parameters. *Hereditary* 76:142-43.

Karp, A., Kresovich, S., Bhat, K. V, Ayada, W. G. and Hodgkin, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI technical bulletin no 2. International Plant Genetic Resources Institute, Rome, Italy. Pp 1-6.

Kay, M. K. and Wratten, S. D. (2003). Ecosystem function and the prediction of tree resistance to defoliators. *Proccedings: IUFRO Kanazawa 2003 "Forest Insect Population Dynamics and Host Influences".* Pp 78-80.

Kelatwang, S. and Garzuglia, M. (2006). Changes in forest area in Africa 1990-2005. *International Forestry Review* 8 (1): 23-30.

Khurana, E. and Singh, J. S. (2001). Ecology of tree seed and seedlings: Implications for tropical forest conservation and restoration. *Current Science* 80 (6): 748-757.

Kindt, R., Jamnadass, R., Muchugi, A., Dawson, I., Kipruto, H. and Poole, J. (2008). A Guide for Studying Molecular Genetic Variation in Tropical Trees: common methods for statistical analysis of dominant data. World Agroforestry Centre, Nairobi, Kenya. Pp 28-182.

Kirst, M., Johnson, A. F., Baucom, C., Ulrich, E., Hubbard, K., Staggs, R., Paule, C., Retzel, E., Whetten, R. and Sederoff, R. (2003). Apparent homology of expressed enges from wood-forming tissues of Loblolly pine (*Pinus taeda* L.) with *Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America* 100: 7383-7388.

Kozlowski, T. T. and Pallardy, S. G. (1997). Growth Control in Woody Plants. Academic Press. San Diego. Pp 1-3.

Krishnambika, N., Suryanarayana, V. and Naik, S. T. (2011). Assessment of foliar diseases and its influence on seedlings growth parameters of *Pongamia pinnata (*L.) Pierre and economic loss assessments. *Karnataka Journal of Agricultural Science* 24 (2): 260-261.

Kyereh, B., Swaine, M. D. and Thompson, J. (1999). Effect of light on the germination of forest trees in Ghana. *Journal of Ecology* 87: 772-783.

Lamb, R. G., Aldwinckle,H. S., Way, R. D. and Terry, D. E. (1978). Liberty, a new disease resistant apple. *New York's Food and Life Sciences Bulletin* 73: 1-2.

Lark, K. G. (2008). Interrogating the genetic variation of inbred plants. *Вестник ВОГиС, Том 12,* 150:158.

Lawson, G. J. (1994). Indigenous trees in West African forest plantations: the need for domestication by clonal techniques. In: Leakey, R. R. B., Newton, A. C. (editors) Tropical trees: the potential for domestication and the rebuilding of forest resources. London, HMSO, (ITE Symposium, 29). 112-123. http://nora.nerc.ac.uk/4652/. [Accessed: 05/07/2011].

Lee, S., Kim, Y. and Kim, W. (2003). Lack of allozyme and ISSR variation in the Rare endemic tree species, Berchemia berchemiaefolia (Rhamnaceae) in Korea. *Annals of Forest Science* 60: 357–360.

Linington, S. H. and Pritchard, H. W. (2001). Gene banks. *Encyclopaedia of Biodiversity* 3:165-180.

Lobo, J. A., Quesada, M. and Stoner, K. E. (2005). Effects of pollination by bats on the mating system of *Ceiba pentandra* (Bombacaceae) populations in two tropical life zones in Costa Rica. *American Journal of Botany* 92: 370-376.

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

Mackay, T. F. C. (2001). The genetic architecture of quantitative traits. *Annual Review Genetics.* 35: 303-339.

Madeira, E. C. M. (2008). Policies to Reduce Emissions from Deforestation and Degradation (REDD) in Developing Countries. *An examination of the issues facing the incorporation of REDD into market-based climate policies* Pp 7-82.

Mahmud, S., Hoque, R. A. T. M. and Mohiuddin, M. (2005). Germination Behaviour and Initial Growth Performance of Eight Multipurpose Tree Species. *International Journal of Agriculture and Biology* 7(4): 539-542.

Maloy, O. C. (2005). Plant Disease Management. *The Plant Health Instructor*. http://www.apsnet.org/edcenter/intropp/topics/Pages/PlantDiseaseManagement.aspx. [Accessed: 9/01/2011].

Maloy, O. C. (1993). Plant Disease Control. Principles and Practice. John Wiley & Sons, Inc. New York. Pp 1-22, 279-290.

Mayer, A. M. and Poljakoff-Mayer, A. (1982). The Germination of Seeds. Third edition. Pergamos Press. Oxford. Pp 1-43.

Mckeand, S. E., Li, B., Grissom, J. E., Isik, F. and Jayawickrama, K. J. S. (2008). Genetic parameter estimates for growth traits from diallel tests of Loblolly Pine throughout the Southeastern United States. *Silvae Genetica* 57 (3): 101-110.

Meyer, B. C., Kozik, A., Griego, A., and Kuang, H. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell*. 15:809-834.

Michler, C. H., Pijut, P. M., Jacobs, D. F., Meilan, R., Woeste, K. E. and Ostry, M. E. (2005). Improving disease resistance of butternut (*Juglans cinerea*), a threatened fine hardwood: a case for single-tree selection through genetic improvement and deployment. *Tree Physiology* 26: 121-128.

Miller, B. M. (1997). Tools for polulation genetic analysis (TFPGA) 1.3. A windows program for the analysis of allozyme and molecular population genetic data. Computor software distributed by the author. http://www.marksgeneticsoftware.net/tfpga.htm. [Accessed: 7/01/2011].

Miller, B. M. and Copeland, L. O. (1997). Seed Production. Principles and Practices. Chapman and Hall International Publishing. New York. Pp.34, 108, 127-130.

Miyawaki, A. and Abe, S. (2004). Public awareness generation for the reforestation in Amazon tropical lowland region. *Tropical Ecology* 45(1): 59-65.

Mng'omba, A. S., du Toit, E. S. and Akinnifesi, K. F. (2007). Germination Characteristics of Tree Seeds: Spotlight on Southern African Tree Species. *Tree and Forestry Science and Biotechnology* 1 (1): 1-8.

Mulawarman, J. M. R., Sasongko, S. M and Irianto, D. (2003). Tree Seed Management-Seed Sources, Seed Collection and Seed Handling: A Field Manual for Field Workers and Farmers. International Centre for Research in Agroforestry (ICRAF) and Winrock International. Bogor, Indonesia. Pp 1-67.

Mullis, K. B. and Faloona, F. A. (1987). Specific synthesis of DNA in vitro via a polymerasecatalyzed chain reaction. *Methods in Enzymology* 155: 335-350.

Murray, B. G., Young, A. G. and Boyle, T. J. (2000). Basic genetics. In: Forest Conservation Genetics: Principles and Practice. Young, A., Boshier, D and Boyle, T. (editors). CSIRO Publishing. Collingwood. Australia. Pp 7-19.

Muthusamy, S., Kanagarajan, S. and Ponusamy, S. (2008). Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology*. 11(3) http://www.ejbiotechnology.info/content/vol11/issue3/full/8/index.html. [Assessed: 7/01/2011].

Namkoong, G., Koshy, M. P., and Aitken, S. (2000). Selection. In: Forest Conservation Genetics: Principles and Practice. Young, A., Boshier, D and Boyle, T (Editors). CSIRO Publishing. Collingwood, Australia. Pp 101-144.

Namkoong, G. (1991). Maintaining genetic diversity in breeding for resistance in forest trees. *Annual Review of Phytopathology* 29: 325-342.

Nansen, C., Tchabi, A. and Meikle. W. G. (2001). Successional sequence of forest types in a disturbed dry forest reserve in southern Benin, West Africa. *Journal of Tropical Ecology* 17: 529-539.

Neale, D. B. and Kremer, A. (2011). Forest tree genomics: growing resources and applications. *Nature Review Genetics* 12: 111-122.

Neale, D. B. and Williams, C. G. (1991). Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. *Canadian Journal of Forest Resources* 21: 545-554.

Nei, M. and Li, W. (1979). Mathematical model for studying genetical variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* 76(10): 5269-5273.

Nei, M. (1972). Genetic distance between populations. *American Naturalist* 106: 283-292.

Newcombe, G. and Bradshaw, H. D. (1996). Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. *Canadian Journal of Forest Resources* 26: 1943-1950.

Njuguna, J. W., Barklund, P., Ihrmark, K. and Stenlid, J. (2011). A canker and dieback disease threatening the growth of Grevillea robusta on small-scale farms in Kenya. *African Journal of Agricultural Research* 6(3): 748-756.

Nkongolo, K. K., Michael, P. and Demers, T. (2005). Application of ISSR, RAPD, and cytological markers to the certification of *Picea mariana*, *Picea glauca*, and *Picea engelmannii* trees, and their putative hybrids. *Genome* 48: 302-311.

Obster, A. (1998). Criteria and indicators of sustainable forest management and related certification schemes. Inter-organizational processes of implementation in global forestry. Paper presented to the 39th Annual Convention of the International Studies Association (ISA), Minneapolis, Minnesota, USA. March 17-21. Pp 1-5.

Ofori, D. A., Opuni-Frimpong, E. and Cobbinah, J. R. (2007). Provenance variation in *Khaya* species for growth and resistance to shoot borer *Hypsiphyla robusta*. *Forest Ecology and Management* 242: 438-443.

Ofori, D. A. (2001). Genetic diversity and its implications for the management and conservation of Milicia species. PhD thesis, University of Aberdeen. Pp 47.

Ofori, D., Newton, A. C., Leaky, R. R. B., and Grace, J. (1996). Vegetative propagation of *Milicia excelsa* Welw. by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management* 84: 39-48.

Ohnishi, N., Saitoh, T., Ishibashi, Y. and Oi, T. (2007). Low genetic diversities in isolated populations of the Asian black bear (*Ursus thibetanus*) in Japan, in comparison with large stable populations. *Conservation Genetics* 8: 1331-1337.

Okali, D. and Eyog-Matig, O. (2004). Lessons learnt on sustainable forest management for Africa: Rain forest management for wood production in West and Central Africa. *KSLA/AFORNET/FAO PROJECT REPORT*. Pp 6,14.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A. (2009). *Ceiba pentandra*. Agroforestree Database: A tree reference and selection guide version 4.0. http://www.worldagroforestry.org/af/treedb. [Accessed: 12/06/2010].

Ostry, M. E., McRoberts, R. E., Ward, K. T. and Resendez, R. (1988). Screening hybrid poplars *in vitro* for resistance to leaf spot caused by *Septaria musiva*. *Plant Disease* 72: 497-499.

Oxford Dictionary of Biology (2000). Sixth edition. Oxford University Press Inc. New York. Pp 628-629.

Paolella, P. (1998). Introduction to Molecular Biology. First Edition. WCB/McGraw-Hill Companies, Inc. New York. Pp 12-25.

Peace, T. R. (1962). Pathology of trees and shrubs reference to Britain, Oxford University Press., New York. Pp 13, 17, 143.

Peakall, R. and Smouse, P. E. (2006). Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6: 288-295.

PEFC Council (2010). Requirements for Sustainable Forest Management Standards-Part 1: Temperate, boreal and plantation forests. PEFC ST 1003:200x, Part 1 (ED), version 1.0. PEFC Council. Pp 5, 19.

Pharmawati, M., Yan, G. and McFarlane, I. J. (2004). Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (Proteaceae). *Australian Systematic Botany* 17: 49-61.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. (2002). Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. *Theoretical and Applied Genetics* 106: 28-39.

Plomion, C., Bahrman, N., Durel, C. E. and O'malley, D. M. (1995). Genomic mapping in Pinus pinaster (maritime pine) using RAPD and protein markers. *Hereditary* 74: 661-668.

Poshen, P. (2000). Social Criteria and Indicators for Sustainable Forest Management. A guide to ILO text. Forest Certification Working Paper no. 3. Eschborn. Pp 3-6.

Porter, A. H. (1990) Testing nominal species boundaries using gene flow statistics: the taxonomy of two hybridizing admiral butterflies *(Limenitis:* Nymphalidac). *Systematic Zoology* 39: 131-148.

Potts, M. B., Milgate, A., Joyce, K., Mohammed, C., Vaillancourt, E. R. and Dutkowski, G. (2004). Quantitative genetic control of *Mycosphaerella* resistance in *Eucalyptus globulus* and impact on growth. In: Borralho, N., *et al.* (2004). Eucalyptus in a Changing World. Proceedings of the IUFRO Conference, Aveiro 11-15 October 2004. Pp 1-7 http://eprints.utas.edu.au/7528/1/Potts_et_al_Aveira_IUFRO_myco.pdf. [Accessed: 2/02/2009].

Prabhu, R., Colfer, C. and Shepherd, G. (1998). Criteria and Indicators for Sustainable Forest Management: New Findings from CIFOR's Forest Management Unit Level Research. *Rural Forestry Development Network. Network paper 23a.* Pp 1-6.

Pryor, T. and Ellis, J. (1993). The genetic complexity of fungal resistance genes in plants. *Adv. Plant Pathol*. 10: 281-305.

Quesada, M., Stoner, K. E., Lobo, J. A., Herrerias-Diego, Y., Palacios-Guevara, C., Munguia-Rosas, M. A., Salazar, K. A. O and Rosas-Guerrero, V. (2004). Effects of Forest Fragmentation on Pollinator Activity and Consequences for Plant Reproductive Success and Mating Patterns in Bat-pollinated Bombacaceous Trees. *Biotropica* 36(2): 131-138.

Raflaski, J. A., Vogel, J. M. Morgante, M. Powell, W. Andre, C. (1996). Generating and Using DNA Markers in Plants. In: Birren, B and Lai, E. (Eds.). *Nonmammalian Genomic Analysis*. Academic Press, San Diego. Pp 75-130.

Rahman, M. A. (1990). Diseases in forest of Bangladesh. In: Proceedings of the IUFRO Workshop on Pests and Diseases of Forest Plantations in the Asia-Pacific Region. Hutacharern, C., MacDicken, K. G., Ivoiry, M. H. and Nair, K. S. S. (editors). Regional Office for Asia and the Pacific (RAPA) and Food and Agricultural Organization of the United States. Bangkok. Pp 89-90.

Rahman, M. A., Shayesta, B. and Basak, A. C. (1990). Summary of recent activities on forest diseases in Bangladesh. In: Proceedings of the IUFRO Workshop on Pests and Diseases of Forest Plantations in the Asia-Pacific Region. Hutacharern, C., MacDicken, K. G., Ivoiry, M.H. and Nair, K. S. S. (editors). Regional Office for Asia and the Pacific (RAPA) and Food and Agricultural Organization of the United States. Bangkok. Pp 91-93.

Rajagopal, J., Bashyam L. and Bhatia, S. (2000). Evaluation of genetic diversity in the Himalayan poplar using RAPD markers. *Silvae Genetica* 49: 49-66.

Randriatafika, F., Rabenantoandro, J. and Rajoharison, R. A. (2008). Analyses of Seed Germination of Littoral Forest Native Species in Southeastern Madagascar. http://www.freewebtown.com/nhatquanglan/index.html. [Accessed: 04/04/2011].

Ratnaparkhe, M. B., Tekeoglu, M. and Muehlbauer, F. J. (1998). Inter simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. *Theoretical and Applied Genetic* 97: 515-519.

Reddy, P. M., Sarla, N. and Siddiq, E. A. (2002). Inter simple sequence repeat (SSR) polymorphism and its application in plant breeding. *Euphytica* 128: 9-17.

Robalino, J. and Herrera, L. D. (2009). Trade and Deforestation: A literature review. World Trade Organization Economic Research and Statistics Division. Staff Working Paper ERSD-2010-04. Pg 3.

Rieseberg, L. (1996). Homology among RAPD fragments in interspecific comparison. *Molecular Ecology* 5: 99-105.

Rommens, C. M. and Kishore, G. M. (2000). Exploiting the full potential of disease-resistance genes for agricultural use. *Current Opinion in Biotechnology* 11: 120-125.

Russell, G. E. (1978). Plant breeding for pest and disease resistance. Butterworth & Co (Publishers) Limited. London. Pp 3-138.

Sanchez de la Hoz, M. P., Davila, J. A., Loarce, Y. and Ferrer, E. (1996). Simple sequence repeat primers used in polymerase chain reaction amplifications to study genetic diversity in barley. *Genome* 39: 112-117.

Saxena R. C. (2004). Neem for ecological pest and vector management in Africa: Outlook for the New Millennium: In Neem: Today and in the New Millennium, Koul, O.and Wahab S. (editors). Kluwer academic publishers, Dordrecht. Pp 43-63.

Sayer, J. and Boedhihartono, A. (2010). Sustaining the potential. Applying the ITTO/IUCN Guidelines for the Conservation of Biodiversity in Tropical Production Forests. *ITTO Tropical Forest Update* 20 (1): 11-12.

Schnell, R. J., Kuhn, D. N., Brown, J. S., Olano, C. T., Phillips-Mora, W., Amores, F. M., and Motamayor, J. C. (2007). Development of a marker assisted selection program for cacao. *Phytopathology* 97: 1664-1669.

Schmidt, L. (2000). Guide to the Handling of Tropical and Subtropical Forest Seed. Danida Forest Seed Centre. Pg 6.

Senbeta, F. and Keketay, D. (2001). Regeneration of indigenous woody species under the canopies of tree plantations in Central Ethiopia. *Tropical Ecology* 42(2): 175-185.

Selvaraj, G. M., Narayana,M., Schubert, M. A., Ayers, L. J., Baring, R. M., and Burow, D. M. (2009). Identification of QTLs for pod and kernel traits in cultivated peanut by bulked segregant analysis *Electronic Journal of Biotechnology* [online]. 12(2) http://www.ejbiotechnology.info/content/vol12/issue2/full/8/index.html. [Accessed: 7/01/2011].

Sheil, D. and Salim, A. (2004). Forest Tree Persistence, Elephants, and Stem Scars. *BIOTROPICA* 36(4): 505-521.

Shigo, A. L. (1984). Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* 22: 189-214.

Siepel, A., Poorter, L and Hawthorne, W. D. (2004). Ecological Profiles of large timber species. In: Biodiversity of West African Forests. Poorter, L, Bongers, F., Kouame, F. N. and Hawthorne, W. D (editors). CABI Publishing, Wallingford, UK. Pg 403.

Silva, F. F., Pereira, M. G., Ramos, H. C. C., Junior, P. C. D., Pereira, T. N. S. P., Gabriel, A. P. C., Viana, A. P. and Ferreguetti, G. A. (2008). Selection and estimation of the genetic gain in segregating generations of papaya (*Carica papaya* L.). *Crop Breeding and Applied Biotechnology* 8: 1-8.

Sneath P. H. A. and Sokal R. R. (1973). Numerical taxonomy. Freeman, San Francisco. Pg 359.

Snedecor, G. W. and Cochran, W. G. (1980). Statistical Methods. Seventh edition. Iowa State University Press, Ames, Iowa. Pp 107-180.

Soller, M. and Beckmann, J. S. (1983). Genetic polymorphism in varietal identification and genetic improvement. *Theoretical and Applied Genetics* 67 (1): 25-33.

Stacy, E. A. (2001). Cross-fertility in two tropical tree species: evidence of inbreeding depression within populations and genetic divergence among populations. *American Journal of Botany* 88: 1041-1051.

Stahl, J. and Christophersen, T. (2010). Forests to the fore. Convention on Biological Diversity and its agenda. *ITTO Tropical Forest Udate* 20 (1): 3-7.

Stevens, J., Pedersen, M., Droege, M. K. and Larsen, V. P. (2009). Institutional Investment in Tropical Forests and Plantations in the Developing World:Social Benefits and Investor Returns. *XIII World Forestry Congress* Buenos Aires, Argentina, 18-23 October 2009. Pg 1.

Stubsgaard, F. (1992). Seed storage. Lecture note number C-9. Danida Forest Seed Centre. Krogerupvej 21 DK-3050 Humlebaek. Denmark. Pp 1-4.

Subramania, S., Graham, M. Y., Yu, O., Graham, T. L. (2005). RNA interference of soybean isoflavone synthase genes leads to silencing in tissues distal to the

transformation site and to enhanced susceptibility to Phytophthora sojae. *Plant Physiology* 137: 1345–53.

Sukhdev, P. (2010). Worth more standing? The economics of biodiversity and ecosystem services in tropical forests. *Tropical Forest Update* 20 (1): 8-10.

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis. Annual Review of Entomology* 39: 47-79.

Taylor, D. (2000). Ghana's Treetop Bats. A BCI biologist studies flying foxes along the Gold Coast, documenting their vital roles in Africa's lucrative timber market. *Bats Mangazine* 8 (3). http://www.batcon.org/index.php/media-and-info/bats-archives.html?task=viewArticle&magArticleID=971. [Accessed: 30/12/10].

Tho, T. P. (1990). Forest entomology in reference to plantation forestry in the Asia-Pacific Region; An Overview. In: Proceedings of the IUFRO Workshop on Pests and Diseases of Forest Plantations in the Asia-Pacific Region. Hutacharern, C., MacDicken, K. G., Ivoiry, M. H. and Nair, K. S. S. (editors). *Regional Office for the and the Pacific (RAPA) and Food and Agricultural Organization of the United States.* Bangkok. Pp 2-7.

Tietema, T., Merkesdal, E. and Schroten, J. (1992). Seed Germination of Indigenous Trees in Botswana. Acts Press. Nairobi, Kenya. Pp 7-27.

Tommasini, L., Calderon-Vazquez, C. L., Ashworth, V. E. T. M., Durbin, M. L. and Clegg, M. T. (2009). Towards a Program of Marker-Assisted Selection on Valuable Avocado Traits. *California Avocado Society Yearbook* 92: 137-164.

Tulsieram, L. K., Glaubitz, J. C., Kiss, G. and Carlson, J. E. (1992). Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. *Bio-Technology* 10: 686-690.

Van Der Plank (1963). Plant Diseases: Epidemics and Control. Academic Press. New York. Pp 12-42.

Vasudeva, R., Hanumantha, M. and Gunaga, R. P. (2004). Genetic variation for floral traits among teak (*Tectona grandis* Linn. f.) clones: Implications to seed orchard fertility. *Current science* 87 (3): 358-362

Vendramin, G. G. and Hansen, O. K. (2005). Molecular markers for characterizing diversity in forest trees. In Conservation and Management of Forest Genetic Resourses in Europe. Geburek, T. and Turok, J. (editors). Arbora Publishers, Zvolen. Pp 337-368.

Vinceti, B. and Petri, L. (2004). Introduction to IPGRI's role and *modus operandi*, with special reference to the BMZ project. In: In: Vinceti, B., Amaral, W. and Meilleur, B. (editors), Challenges in managing forest genetic resource for livelihoods: examples from Argentina and Brazil. International Plant Genetic Resources Institute, Rome, Italy. Pp 93-94.

Vos, P. R., Hogers, R., Bleeker, M., Reijans, M., Lee, T. Van De., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP; a new technique for fingerprinting. *Nucleic Acids Research* 23 (21): 4407-4414.

VSN International (2003). GenStat Discovery. Edition 3. http://wwww.vsi.co.uk/downloads/genestat-discovery/. [Accessed: 7/01/2011].

Wagner, M. R., Cobbinah, J. R. and Bosu, P. P. (2008). Forest Entomology in West Africa Tropical Forest: Forest Insects of Ghana. Second edition. Springer, Dordrecht, Netherlands. Pp 7-22.

Weir, B. S. and Cockerham, C. C. (1984). Estimating Fstatistics for the analysis of populations structure. *Evolution* 38: 1358‑1370.

Wilkes, G. (1992). Strategies for Sustaining Crop Germplasm Preservation, Enhancement, and Use. Consultative Group on International Agricultural Research Research, CGlAR Secretariat, Washington, D.C.,United States. Pp 5-61.

Williams, J. W., Jackson, S. T., Kutzbach, J. E. (2007). Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Science, USA* 104: 5738-5742.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalsky, J. A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers useful in genetic markers. *Nucleic Acids Research* 18: 6531-6535.

Wright J. W. (1976). Introduction to Forest Genetics. Academic Press New York. Pg. 253.

Wu, H. X. and Ying, C. C. (1997). Genetic parameters and selection efficiencies in resistance to western gall rust, stalactiform blister rust, needle cast and sequoia pitch moth in lodgepole pine. *Forest Science* 43(4): 571-581.

Xia, T., Meng, L., Mao, K., Tian, B., Miehe, G. and Liu, J. (2008). Genetic variation in the Qinghai-Tibetan plateau endemic and endangered conifer *Cupressus gigantea*, detected using RAPD and ISSR markers. *Silvae Genetica* 57(2): 85-92.

Yang, R., Dhir, N. K., Yeh, F. C. and Hiratsuka, Y. (1997). Geographic variation in susceptibility of Alberta lodgepole pine to western gall rust. *Canadian Journal of Forest Resources* 27: 3198-1405.

Yeh, F. C. (2000). Population genetics. In: Forest Conservation Genetics: Principles and Practice. Young, A., Boshier, D and Boyle, T. (editors). CSIRO Publishing. Collingwood, Australia. Pp 21-37.

Yeh, F. C., Yang R. C., Boyle T. B. (1999). POPGENE version 1.32. Microsoft Windows-based freeware for population genetic analysis. http://www.ualberta.ca/~fyeh/index.htm. [Accessed: 8/01/2011].

Yin, T. M., DiFazio, S. P., Gunter, L. E., Jawdy, S. S., Boerjan, W. and Tuskan, G. A. (2004) . Genetic and physical mapping of *Melampsora* rust resistance genes in *Populus* and characterization of linkage disequilibrium and flanking genomic sequence. *New Phytologist* 164: 95–105. http://www.esd.ornl.gov/PGG/Yin_newphyt_04.pdf. (Accessed: 8/01/2011).

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20 (2): 176-183.

Zobel, B. and Talbert, J. (1991). Applied Forest Tree Improvement. Waveland Press Inc., Illinois. Pg 505.

Zobel, B. and Talbert, J. (1984). Applied Forest Tree improvement. John Wiley & Sons, Inc., New York. Pp 2-297.

APPENDICES

Appendix 3.3.1 Details on *C. pentandra* seed collection in Ghana

Ecological zone	Accessions	Locality	GPS Readings	
			Latitude	Longitude
Dry Semi-deciduous Forest Inner Zone (DSDFZ-Inner)	AFR 1	Afrisepakrom	N 07°20'24.8''	W 002°11'53.1''
	NKW 3	Abofour Nkwankwa	N 07°12'02.2''	W001°46'12.2''
	ADU 1	Adugyan	N07°15'14.3''	W001°24'07.2''
	ASE 1	Asieso-via Ofinso	N07°16'36.5''	W001°49'59.0''
	AKO 1	Akomadan	N07°22'26.8''	W001°55'07.2''
	NKW 1	Abofour Nkwankwa	N07°11'57.5''	W001°46'14.0''
	NKW 2	Abofour Nkwankwa	N07°12'01.9''	W001°46'17.8''
	TNK 1	Timber Nkwanta	N07°08'22.6''	W001°24'01.5''
	BUF 1	Buabeng-Fiema	N07°42'23.6''	W001°42'33.2''
	AYE 2	Ayerede	N07°32'24.2''	W001°37'08.1''
	AYE 1	Ayerede	N07°29'22.4''	W001°37'04.7''
	AKD 2	Akomsadumase	N07°32'42.8''	W001°44'21.3''
	DYN 1	Dua Yaw Nkwanta	N07°12'01.3''	W002°07'10.9''
	TAN 1	Tanaso	N07°16'01.9''	W002°13'46.0''
	ABF 1	Abofour	N07°08'38.0''	W001°44'10.8''
	NKIN 1	Nkinkasu	N07°20'22.1''	W001°54'50.9''
	AKD 1	Akomsadumase	N07°32'43.8''	W001°44'21.9''
	MSH 1	Mampong Scrab Hills	N06°58'58.9''	W001°27'26.3''
	MSH 2	Mampong Scrab Hills	N06°59'01.3''	W001°27'27.2''
	SPY 1	Sunyani Prison Yard	N 07°20'31.6''	W002°19'49.8''
	SPY 2	Sunyani Prison Yard	N07°20'31.9''	W002°19'49.5''
	SPY 3	Sunyani Prison Yard	N07°20'24.8''	W002°20'04.8''
	NKIN 2	Nkinkasu	N07°20'21.0''	W001°54'59.0''
	SKD 1	Sunyani Koforidua	N07°12'11.3''	W002°07'10.9''
Dry Semi-deciduous Forest Zone-Outlier (DSDFZ-Outlier)	KEC 9	Kecheibi		
	KUE 1	Kue		
	KUE 2	Kue		
	KEC 10	Kecheibi		
	ODO 6	Odomi		
	ODO 4	Odomi		
	NKW 7	Nkwanta		
	KUE 3	Kue		
	PAW 5	Pawa		

Moist Semi-deciduous Forest Zone (MSDFZ)	KWA 1	Kwaso	N06°34’55.4’’	W001°27’0.5.0’’
	EDW 1	Edwenase	N06°34’01.6’’	W001°27’05.0’’
	EDW 2	Edwenase	N06°34’01.6’’	W001°27’27.7’’
	EDW 3	Edwenase		
	GYE 1	Gyedam	N06°21’36.5’’	W001°15’19.6’’
	AOF 1	Ashanti Ofoanse Abono Lake	N06°23’49.0’’	W001°18’10.8’’
	ABB 1	Bosumtwi	N06°32’22.22’’	W001°25’48.4’’
	KWA 2	Kwaso	N06°38’09.8’’	W001°26’54.1’’
	BIB 1	Bibiani	N06°27’18.5’’	W002°18’47.0’’
	BIB 2	Bibiani		
	DAN 1	Danaso	N06°42’08.6’’	W001°27’26.2’’
	PRE 1	Preteanse	N06°32’22.2’’	W001°27’26.2’’
	PEA 1	Peanse		
	KOK 1	Kokofo	N06°30’23.7’’	W001°31’34.0’’
	PAK 1	Pakyi Number 1		
	PAK 2	Pakyi Number 2		
	UST 1	KNUST		
	UST 2	KNUST		
	UST 3	KNUST		
	ACH 1	Achianse	N06°27’51.9’’	W001°18’04.4’’
	AKK 1	Akokoaso	N06°09’09.5’’	W001°02’17.5’’
	KYE 1	Kyerenfaso	N06°06’36.5’’	W001°23’40.0’’
Moist/Wet Evergreen Forest Zone (M/WEFZ)	BUS 1	Busia Beach	N04°48’14.1’’	W001°56’26.2’’
	BUS 2	Busia Beach		
	BUS 3	Busia Beach		
	BUS 4	Busia Beach		
	MAM 1	Manfia Amanfi	N05°39’25.4’’	W002°17’35.9’’
	NKA 1	Nkafoa	N05°07’27.3’’	W001°15’26.6’’
	SOF 1	Sofokrom Ichanban	N05°09’27.3’’	W001°42’16.2’’
	POK 1	Pokuase	N05°40’58.4’’	W001°16’25.5’’
	NKA 3	Nkafoa	N05°07’35.2’’	W001°14’38.6’’
	NKA 2	Nkafoa	N05°07’27.7’’	W001°15’26.3’’
	ADI 1	Adisadel College	N05°07’20.0’’	W001°15’41.6’’
	DMF1	Dunkwa Mfoum	N05°57’29.1’’	W001°47’19.7’’
	DMF 2	Dunkwa Mfoum	N05°57’10.2’’	W001°48’08.4’’
	YAM 1	Yamoransa	N05°07’45.2’’	W001°13’48.9’’
	ASA 1	Asankaragua		
	MAA 1	Mankesim		
	TWE 1	Twepease		

Guinea Savanna Zone (GSZ)	BAW 1	Bawku
	BAW 2	Bawku
	BAW 3	Bawku
	BAW 4	Bawku
	BAW 5	Bawku
	BAW 6	Bawku
	BAW 7	Bawku
	BAW 8	Bawku
	BAW 9	Bawku
	BAW 10	Bawku
	BAW 11	Bawku
	BAW 12	Bawku
	BAW 13	Bawku
	KON 1	Kongo
	KON 2	Kongo
	KON 3	Kongo
	KON 4	Kongo
	KON 5	Kongo
	KON 6	Kongo
	KON 7	Kongo
	KON 8	Kongo
	KON 9	Kongo
	KON 10	Kongo
	KON 11	Kongo
	KON 12	Kongo
	KON 13	Kongo
Total	98	

Note: 1. Ecological zone = Zone with distinct vegetation type. 2. Accessions = Trees from which seeds were collected. 3. Locality = Town of location of accession

Appendix 3.4.1 Analysis of variance of mortality rates among 80 accessions of *C. pentandra* at the nursery from September-November Screening (SNS).

Source of variation	SS	df	MS	F	P-value	F-crit
Between accessions	5.3834	79	0.068	1.725	0.002*	1.366
Within accessions	6.319	160	0.039			
Total	11.703	239				

Note: * = Significant differences in mortality rates among accessions

Appendix 3.4.2 Analysis of variance of mortality rates among 59 accessions of *C. pentandra* at the nursery from March-May Screening (MMS).

Source of variation	SS	df	MS	F	P-value	F crit
Between progenies	0.235	59	0.004	2.599	0.00*	1.436
Within progenies	0.184	118	0.002			
Total	0.419	176				

Note: * = Significant differences in mortality rates among accessions

Appendix 4.3.1 Accessions used for progeny trial at Abofour.

No	Ecological zone	Accession	Locality
1	DSDFZ-Inner	ABF 1	Abofour
2		ASE 1	Asieso-Via Offinso
3		AYE 1	Ayerede
4		AYE 2	Ayerede
5		BUF 1	Buabeng-Fiema
6		DNY 1	Dua Yaw Nkwanta
7		MSH 1	Mampong Scrap Hills
8		NKIN 1	Nkinkaso
9		NKIN 2	Nkinkaso
10		SKD 1	Sunyani Koforidua
11		TAN 1	Tanaso
12	DSDFZ-Outlier	KEC 1	Kecheibi
13		KEC 9	Kecheibi
14		KUE 1	Kue
15		KUE 2	Kue
16		KUE 3	Kue
17		ODO 6	Odomi
18		PAW 5	Pawa
19	GSZ	BAW 1	Bawku
20		BAW 10	Bawku
21		BAW 4	Bawku
22		BAW 5	Bawku
23		KON 13	Kongo
24		KON 8	Kongo
25		KON 9	Kongo
26	M/WEFZ	MAA 1	Mankesim
27		MAM 1	Manso-Amenfi
28		NKA 1	Nkafoa
29		NKA 2	Nkafoa
30		POK 1	Pokuase
31		SOF 1	Sofokrom Ichanban
32	MSDFZ	DAN 1	Danaso
33		ACH 1	Achianse
34		EDW 1	Edwinase
35		GYE 1	Gyedam
36		KWA 1	Kwaso
37		WOA 1	Woarakese

Note: 1. Ecological zone = Zone with distinct vegetation type. 2. Accessions = Trees from which seeds were collected. 3. Locality = Town of location of accession

Appendix 4.3.2 Illustration of the estimation of selection differential, narrow sense heritability and genetic gain in individual accessions

From equation 3 (See section 4.3.3), V_G was estimated as:

$V_G = MS_1 - MS_3/r$

From the analysis of variance table (Table 4.3.1 and Appendix 4.4.1),

$MS_1 = 4547.05$, $MS_3 = 2008.95$ and $r = 3$

$V_G = (4547.05 - 2008.95)/3 = 846.03$

From the analysis of variance (Table 4.3.1 and Appendix 4.4.1),

$V_E = MS_3/r = 2008.95/3 = 669.65$

From equation 2, V_P was estimated as:

$V_P = V_G + V_E = 846.03 + 669.65 = 1515.68$

From equation 1, h^2 was estimated as:

$h^2 = V_G/V_P = 846.03/1515.68 = 0.56$

Taking accession DNY 1 for instance. From equation 5, selection differential/deviation (S) was estimated as:

$S = Xs - X\mu$

Xs = sample mean (mean height increment of accession DNY 1)

Xμ = population mean (mean height increment of all the 37 accessions)

$S = 177.00 - 106.24$

$= 70.76$ cm/yr (See table 4.4.1)

$$\text{Percentage deviation} = \frac{\text{Deviation}}{\text{Mean}} \times 100\ \%$$

$$= \frac{70.76}{106.24} \times 100\ \%$$

$= 66.60\ \%$

Percentage gain = percentage deviation x heritability

= 66.60 x 0.56

= 37.3 % (Table 4.4.1)

Genetic gain = percentage gain x heritability

= 37.3 x 0.56

= 20.89

This procedure was repeated for all the accessions. The results produced are as shown in Table 4.4.1.

Appendix 4.3.3 Illustration of the estimation of selection differentials and genetic gain in the 22 selected accessions (accessions with mean height performance above the overall mean. These are ranked 1-22 in Table 4.4.1).

S = Xs - Xμ.

where;

Xs = sample mean (mean height increment of the 22 selected accessions)

Xμ = population mean (mean height increment of all the 37 accessions)

S = 132.60 - 106.24

= 26.36 cm/yr

Percentage deviation = $\frac{26.36}{132.60}$ x 100 %

= 19.88 %

Percentage gain = percentage deviation x heritability

= 19.88 x 0.56

= 11.13 %

Appendix 4.4.1 Analysis of variance in height growth.

Source of Variation	df	SS	MS	F	P-value	F crit
Between accessions	36	163693.80	4547.05	2.26	0.00	1.58
Blocks	2	27205.45	13602.73	6.77	0.20	8.12
Within accessions	72	144644.50	2008.95			
Total	110	335543.70				

Appendix 4.4.2 Analysis of variance in diameter growth.

Source of Variation	df	SS	MS	F	P-value	F crit
Between accessions	36	7815.45	217.10	1.65	0.03	1.58
Blocks	2	44.52	22.26	0.17	0.84	3.12
Within accessions	72	9452.41	131.28			
Total	110	17312.38				

Appendix 4.4.3 Analysis of variance in mortality rates.

Source of Variation	df	SS	MS	F	P-value	F crit
Between accessions	36	4.47	0.12	2.13	0.00	1.58
Blocks	2	0.33	0.16	2.82	0.07	3.12
Within accessions	72	4.19	0.06			
Total	110	8.99				

Appendix 4.4.4 Variation in resistance levels within an accession.

Note: A and B, respectively, show resistant and susceptible progenies of the same accession (mother tree).

Appendix 5.4.1 Variation among five populations of *C. pentandra* base on RAPD polymorhism using Chi-Square (χ^2) Analysis.

Locus	Degree of freedom	Chi-Square (χ^2)	Probability (0.05)	Inference
RL1	4	1.170	0.883	DAP
RL2	4	1.104	0.894	DAP
RL3	4	2.034	0.730	DAP
RL4	4	2.776	0.596	DAP
RL5	4	3.623	0.459	DAP
RL6	4	2.776	0.596	DAP
RL7	4	1.766	0.779	DAP
RL8	4	0.797	0.939	NDAP
RL9	4	2.891	0.576	DAP
RL10	4	2.340	0.670	DAP
RL11	4	2.770	0.597	DAP
RL12	4	5.619	0.229	DAP
RL13	4	4.590	0.332	DAP
RL14	4	4.319	0.365	DAP
RL15	4	0.942	0.919	DAP
RL16	4	2.166	0.705	DAP
RL17	4	1.064	0.900	DAP

Note: 1. Chi-square shows whether there are difference among populations or not.
2. When computed Chi-square statistic exceeds the critical for 0.05 probability level, the null hypothesis of equal distribution (in this case equal number of alleles among populations) is rejected. Following this, 16 (94.12 %) of the loci show there are difference among populations
3. DAP = Differences among populations, NDAP = No differences among populations

Appendix 5.4.2 Variation among five populations of *C. pentandra* base on RAPD polymorhism using G-Square test.

Locus	Degree of freedom	G-Square	Probability (0.05)	Inference
RL1	4	1.165	0.883	DAP
RL2	4	1.135	0.889	DAP
RL3	4	1.673	0.796	DAP
RL4	4	2.944	0.567	DAP
RL5	4	3.691	0.449	DAP
RL6	4	2.944	0.567	DAP
RL7	4	1.796	0.773	DAP
RL8	4	0.807	0.937	NDAP
RL9	4	2.888	0.577	DAP
RL10	4	2.451	0.653	DAP
RL11	4	3.030	0.533	DAP
RL12	4	5.926	0.205	DAP
RL13	4	4.829	0.305	DAP
RL14	4	3.568	0.467	DAP
RL15	4	1.464	0.833	DAP
RL16	4	2.857	0.582	DAP
RL17	4	1.336	0.855	DAP

Note: 1. G-square shows whether there are differences or not. Unlike the Chi-square test, the G-values are additive.

2. When computed G-square statistic exceed the critical for 0.05 probability level, the null hypothesis of no differences among populations is rejected. Following this, 16 (94.12 %) loci show there are difference among populations.
3. DAP = Differences among populations, NDAP = No differences among populations

Appendix 5.4.3 Ewens-Watterson Test for neutrality among five populations of *C. pentandra* base on RAPD polymorhism.

Locus	n	Obs. F	Min F	Max F	Mean*	SE*	L95*	U95*	Inference
RL1	34	0.7521	0.5000	0.9429	0.7667	0.0246	0.5017	0.9429	N
RL2	34	0.7279	0.5000	0.9429	0.7694	0.0248	0.5017	0.9429	N
RL3	34	0.9699	0.5000	0.9429	0.7654	0.0243	0.5017	0.9429	Nn
RL4	34	0.5321	0.5000	0.9429	0.7668	0.0249	0.5017	0.9429	N
RL5	34	0.5103	0.5000	0.9429	0.7700	0.0248	0.5017	0.9429	N
RL6	34	0.5321	0.5000	0.9429	0.7617	0.0249	0.5017	0.9429	N
RL7	34	0.5234	0.5000	0.9429	0.7647	0.0247	0.5017	0.9429	N
RL8	34	0.6825	0.5000	0.9429	0.7676	0.0242	0.5017	0.9429	N
RL9	34	0.5754	0.5000	0.9429	0.7614	0.0257	0.5017	0.9429	N
RL10	34	0.6769	0.5000	0.9429	0.7648	0.0254	0.5017	0.9429	N
RL11	34	0.6533	0.5000	0.9429	0.7671	0.0254	0.5017	0.9429	N
RL12	34	0.6014	0.5000	0.9429	0.7669	0.0251	0.5017	0.9429	N
RL13	34	0.5867	0.5000	0.9429	0.7712	0.0239	0.5017	0.9429	N
RL14	34	0.9383	0.5000	0.9429	0.7678	0.0253	0.5017	0.9429	N
RL15	34	0.8036	0.5000	0.9429	0.7588	0.0259	0.5017	0.9429	N
RL16	34	0.7244	0.5000	0.9429	0.7577	0.0262	0.5017	0.9429	N
RL17	34	0.8567	0.5000	0.9429	0.7566	0.0261	0.5017	0.9429	N

Note: 1. The "Obs. F" is compare to the "L*95" and "U*95". If "Obs. F" is within this confidence interval, the locus is neutral; otherwise, it is not. Following this 16 (94.12 %) of the 17 loci are neutral.

2. L*95 = Lower limit at 95 % confidence interval, U*95 = Upper limit at 95 % confidence interval Obs. F = Observed Frequency, Min F = Minimum Frequency, Max F =Maximum Frequency, SE = Standard Error, N = Neutral, Nn= Not neutral

Appendix 5.4.4 Gene flow among five populations of *C. pentandra* base on RAPD polymorhism.

Locus	n	Ht	Hs	Gst	Nm
RL1	34	0.2392	0.2308	0.0352	13.7178
RL2	34	0.2613	0.2534	0.0302	16.0688
RL3	34	0.0292	0.0275	0.0602	7.8005
RL4	34	0.4618	0.4274	0.0746	6.1987
RL5	34	0.4828	0.4380	0.0928	4.8902
RL6	34	0.4618	0.4274	0.0746	6.1987
RL7	34	0.4711	0.4488	0.0472	10.0893
RL8	34	0.3154	0.3067	0.0276	17.6456
RL9	34	0.4237	0.3887	0.0825	5.5622
RL10	34	0.3105	0.2893	0.0681	6.8383
RL11	34	0.3354	0.3084	0.0802	5.7320
RL12	34	0.3771	0.3192	0.1535	2.7572
RL13	34	0.3955	0.3473	0.1217	3.6075
RL14	34	0.0600	0.0523	0.1278	3.4113
RL15	34	0.1848	0.1782	0.0356	13.5266
RL16	34	0.2715	0.2506	0.0768	6.0144
RL17	34	0.1382	0.1332	0.0361	13.3578
Mean	34	0.3070	0.2840	0.0751	6.1599
St. Dev		0.0202	0.0167		

Note: 1. Ht = total heterozygosity, Hs = average heterozygosity in the five subpopulations. Gst =Fst. Fst values in the range of 0.00 - 0.05, 0.05 - 0.15, 0.15 - 0.25 and 0.25-1.0 indicate low, moderate, large and very large genetic differentiation respectively (Yeh, 2000). Following this, the Gst estimated (0.0751) indicate moderate genetic differentiation among the populations.

2. Nm values above 1 indicate significant genetic similarity due to gene flow, when 1 >Nm is an indication of weak but possible gene flow, when Nm < 0.5, populations are isolated (Potter, 1990). Following this, the Nm estimated (6.1599), indicates significant genetic similarity among populations.

Appendix 5.4.5 Variation among five populations of *C. pentandra* base on ISSR polymorhism using Chi-Sequare (χ^2) Test.

Locus	Degree of freedom	Chi-Square (χ^2)	Probability (0.05)	Inference
IL1	4	2.880	0.578	DAP
IL2	4	0.000	1.000	NDAP
IL3	4	0.375	0.984	NDAP
IL4	4	0.375	0.984	NDAP
IL5	4	1.420	0.841	DAP
IL6	4	4.817	0.306	DAP
IL7	4	3.367	0.498	DAP
IL8	4	4.617	0.329	DAP
IL9	4	1.230	0.873	DAP
IL10	4	1.555	0.817	DAP
IL11	4	1.848	0.764	DAP
IL12	4	1.810	0.771	DAP
IL13	4	0.000	1.000	NDAP
IL14	4	1.810	0.771	DAP

Note: 1. Chi-square shows whether there are difference among populations or not.
2. When computed Chi-square statistic exceeds the critical for 0.05 probability level, the null hypothesis of equal distribution (in this case equal number of alleles among populations) is rejected. Following this, 10 (71.43 %) of the loci show there are difference among populations.
3. DAP = Differences among populations, NDAP = No differences among populations

Appendix 5.4.6 Variation among five populations of *C. pentandra* based on ISSR polymorhism using G-Square test.

Locus	Degree of freedom	G-Square	Probability (0.05)	Inference
IL1	4	3.579	0.466	DAP
IL2	4	0.000	0.000	NDAP
IL3	4	0.364	0.985	NDAP
IL4	4	0.364	0.985	NDAP
IL5	4	1.694	0.792	DAP
IL6	4	4.887	0.299	DAP
IL7	4	3.427	0.489	DAP
IL8	4	4.557	0.336	DAP
IL9	4	1.167	0.883	DAP
IL10	4	1.561	0.816	DAP
IL11	4	2.169	0.705	DAP
IL12	4	1.890	0.756	DAP
IL13	4	0.000	0.000	NDAP
IL14	4	1.890	0.756	DAP

Note: 1. G-square shows whether there are differences among populations or not. Unlike the Chi-square test, the G-values are additive.
2. When computed G-square statistic exceeds the critical for 0.05 probability level, the null hypothesis of no differences among populations is rejected. Following this, 10(71.43 %) of the loci show there are difference among populations.
3. DAP = Differences among populations, NDAP = No differences among populations.

Appendix 5.4.7 Ewens-Watterson Test for neuttrality among five populations of *C. pentandra* base on ISSR polymorhism.

Locus	n	Obs. F	Min F	Max F	Mean*	SE*	L95*	U95*	Inference
IL1	36	0.8350	0.5000	0.9460	0.7556	0.0264	0.5015	0.9460	N
IL2	36	1.0000	1.0000	1.0000	****	****	****	****	Nn
IL3	36	0.8152	0.5000	0.9460	0.7565	0.0250	0.5015	0.9460	N
IL4	36	0.8152	0.5000	0.9460	0.7731	0.0254	0.5015	0.9460	N
IL5	36	0.9442	0.5000	0.9460	0.7543	0.0254	0.5015	0.9460	N
IL6	36	0.5695	0.5000	0.9460	0.7696	0.0253	0.5015	0.9460	N
IL7	36	0.5744	0.5000	0.9460	0.7628	0.0247	0.5015	0.9460	N
IL8	36	0.6849	0.5000	0.9460	0.7672	0.0259	0.5015	0.9460	N
IL9	36	0.7646	0.5000	0.9460	0.7681	0.0252	0.5015	0.9460	N
IL10	36	0.7174	0.5000	0.9460	0.7688	0.0246	0.5015	0.9460	N
IL11	36	0.8371	0.5000	0.9460	0.7702	0.0246	0.5015	0.9460	N
IL12	36	0.7165	0.5000	0.9460	0.7583	0.0244	0.5015	0.9460	N
IL13	36	1.0000	1.0000	1.0000	****	****	****	****	Nn
IL14	36	0.7165	0.5000	0.9460	0.7716	0.0238	0.5015	0.9460	N

Note: 1.The "Obs. F" is compare to the "L*95" and "U*95". If "Obs. F" is within this confidence interval, the locus is neutral; otherwise, it is not. Following this 12 (85.71 %) of the 14 loci are neutral.

2. L*95 = Lower limit at 95% confidence interval, U*95 = Upper limit at 95% confidence interval Obs. F = Observed Frequency, Min F = Minimum Frequency, Max F =Maximum Frequency and SE = Standard Error, N = Neutral, Nn = Not Neutral

Appendix 5.4.8 Gene flow among five populations of *C. pentandra* using ISSR polymorhism.

Locus	Sample	Ht	Hs	Gst	Nm
IL1	36	0.1349	0.1238	0.0818	5.6142
IL2	36	0.0000	0.0000	****	****
IL3	36	0.1762	0.1747	0.0085	58.5578
IL4	36	0.1762	0.1747	0.0085	58.5578
IL5	36	0.0520	0.0496	0.0475	10.0188
IL6	36	0.4238	0.3633	0.1426	3.0056
IL7	36	0.4282	0.3822	0.1074	4.1560
IL8	36	0.2843	0.2524	0.1123	3.9525
IL9	36	0.2416	0.2335	0.0335	14.4340
IL10	36	0.2859	0.2721	0.0481	9.8864
IL11	36	0.1743	0.1637	0.0609	7.7156
IL12	36	0.2612	0.2481	0.0503	9.4417
IL13	36	0.0000	0.0000	****	****
IL14	36	0.2612	0.2481	0.0503	9.4417
Mean	36	0.2071	0.1919	0.0736	6.2890
St. Dev		0.0179	0.0141		

Note: 1. Ht = total heterozygosity, Hs = average heterozygosity in the five subpopulations. Gst =Fst. Fst values in the range of 0.00 - 0.05, 0.05 - 0.15, 0.15 - 0.25 and 0.25-1.0 indicate low, moderate, large and very large genetic differentiation respectively (Yeh, 2000). Following this, the Gst estimated (0.0736) indicate moderate genetic differentiation among the populations.

2. Nm values above 1 indicate significant genetic similarity due to gene flow, when 1> Nm is an indication of weak but possible gene flow, when Nm < 0.5, populations are isolated (Potter, 1990). Following this, the Nm estimated (6.2890), indicates significant genetic similarity.

Appendix 5.4.9 Variation among five populations of *C. pentandra* base on combined RAPD and ISSR polymorhism using Chi-Square (χ^2) analysis.

Locus	Degree of freedom	Chi-Square (χ^2)	Probability (0.05)	Inference
RIL1	4	1.820	0.769	DAP
RIL2	4	1.276	0.865	DAP
RIL3	4	2.183	0.702	DAP
RIL4	4	3.914	0.418	DAP
RIL5	4	4.980	0.289	DAP
RIL6	4	3.914	0.418	DAP
RIL7	4	2.797	0.592	DAP
RIL8	4	0.204	0.995	NDAP
RIL9	4	3.630	0.458	DAP
RIL10	4	1.851	0.763	DAP
RIL11	4	3.301	0.509	DAP
RIL12	4	5.746	0.220	DAP
RIL13	4	4.388	0.356	DAP
RIL14	4	4.630	0.327	DAP
RIL15	4	1.067	0.899	DAP
RIL16	4	3.494	0.479	DAP
RIL17	4	1.351	0.853	DAP
RIL18	4	2.988	0.560	DAP
RIL19	4	0.000	0.000	NDAP
RIL20	4	1.337	0.855	DAP
RIL21	4	0.375	0.984	NDAP
RIL22	4	1.420	0.841	DAP
RIL23	4	3.279	0.512	DAP
RIL24	4	1.401	0.844	DAP
RIL25	4	4.771	0.312	DAP
RIL26	4	1.686	0.793	DAP
RIL27	4	1.261	0.868	DAP
RIL28	4	1.087	0.896	DAP
RIL29	4	2.230	0.694	DAP
RIL30	4	0.000	1.000	NDAP
RIL31	4	2.230	0.694	DAP

Note: 1. Chi-square shows whether there are difference among populations or not.
2. When computed Chi-square statistic exceeds the critical for 0.05 probability level, the null hypothesis of equal distribution (in this case equal number of alleles among populations) is rejected. Following this, 27 (87.10 %) of the loci show there are difference among populations.
3. DAP = Differences among populations, NDAP = No differences among populations.

Appendix 5.4.10 Variation among five populations of *C. pentandra* base on combined RAPD and ISSR polymorhism using G-Square test.

Locus	degree of freedom	G-Square	Probability (0.05)	Inference
RIL1	4	2.429	0.657	DAP
RIL2	4	1.320	0.858	DAP
RIL3	4	1.733	0.784	DAP
RIL4	4	4.142	0.385	DAP
RIL5	4	5.162	0.271	DAP
RIL6	4	4.162	0.385	DAP
RIL7	4	2.890	0.576	DAP
RIL8	4	0.206	0.995	NDAP
RIL9	4	3.630	0.459	DAP
RIL10	4	1.940	0.747	DAP
RIL11	4	4.410	0.353	DAP
RIL12	4	7.236	0.124	DAP
RIL13	4	4.842	0.304	DAP
RIL14	4	3.694	0.449	DAP
RIL15	4	1.766	0.779	DAP
RIL16	4	4.070	0.397	DAP
RIL17	4	1.645	0.800	DAP
RIL18	4	3.781	0.436	DAP
RIL19	4	0.000	0.000	NDAP
RIL20	4	1.783	0.776	DAP
RIL21	4	0.364	0.985	NDAP
RIL22	4	1.694	0.792	DAP
RIL23	4	3.471	0.482	DAP
RIL24	4	1.472	0.832	DAP
RIL25	4	5.435	0.246	DAP
RIL26	4	1.493	0.828	DAP
RIL27	4	1.262	0.868	DAP
RIL28	4	1.640	0.802	DAP
RIL29	4	3.043	0.551	DAP
RIL30	4	0.000	1.000	NDAP
RIL31	4	3.043	0.551	DAP

Note: 1. G-square only shows whether there are differences or not. Unlike the Chi-square test, the G-values are additive and can, therefore, be used for more elaborate statistical designs.

2. When computed G-square statistics exceeds the critical for 0.05 probability level, the null hypothesis of no differences among populations is rejected. Following this, 27 (87.10 %) of the loci show there are difference among populations.

3. DAP = Differences among populations, NDAP = No differences among populations.

Appendix 5.4.11 Ewens-Watterson Test for neuttrality among five populations of *C. pentandra* base on combined RAPD and ISSR polymorhism.

Locus	n	Obs. F	Min F	Max F	Mean*	SE*	L95*	U95*	Remarks
RIL1	36	0.7629	0.5000	0.9460	0.7702	0.0244	0.5015	0.9460	N
RIL2	36	0.7182	0.5000	0.9460	0.7680	0.0256	0.5015	0.9460	N
RIL3	36	0.9716	0.5000	0.9460	0.7663	0.0262	0.5015	0.9460	Nn
RIL4	36	0.5210	0.5000	0.9460	0.7717	0.0253	0.5015	0.9460	N
RIL5	36	0.5044	0.5000	0.9460	0.7675	0.0247	0.5015	0.9460	N
RIL6	36	0.5210	0.5000	0.9460	0.7574	0.0265	0.5015	0.9460	N
RIL7	36	0.5145	0.5000	0.9460	0.7599	0.0256	0.5015	0.9460	N
RIL8	36	0.6785	0.5000	0.9460	0.7668	0.0255	0.5015	0.9460	N
RIL9	36	0.5900	0.5000	0.9460	0.7587	0.0265	0.5015	0.9460	N
RIL10	36	0.6729	0.5000	0.9460	0.7660	0.0255	0.5015	0.9460	N
RIL11	36	0.6679	0.5000	0.9460	0.7722	0.0246	0.5015	0.9460	N
RIL12	36	0.6000	0.5000	0.9460	0.7646	0.0257	0.5015	0.9460	N
RIL13	36	0.5873	0.5000	0.9460	0.7648	0.0253	0.5015	0.9460	N
RIL14	36	0.9416	0.5000	0.9460	0.7706	0.0249	0.5015	0.9460	N
RIL15	36	0.7890	0.5000	0.9460	0.7630	0.0251	0.5015	0.9460	N
RIL16	36	0.7342	0.5000	0.9460	0.7578	0.0258	0.5015	0.9460	N
RIL17	36	0.8639	0.5000	0.9460	0.7639	0.0265	0.5015	0.9460	N
RIL18	36	0.8347	0.5000	0.9460	0.7692	0.0253	0.5015	0.9460	N
RIL19	36	1.0000	1.0000	1.0000	****	****	****	***	Nn
RIL20	36	0.8130	0.5000	0.9460	0.7545	0.0260	0.5015	0.9460	N
RIL21	36	0.8152	0.5000	0.9460	0.7617	0.0251	0.5015	0.9460	N
RIL22	36	0.9442	0.5000	0.9460	0.7638	0.0254	0.5015	0.9460	N
RIL23	36	0.5747	0.5000	0.9460	0.7618	0.0262	0.5015	0.9460	N
RIL24	36	0.5811	0.5000	0.9460	0.7642	0.0254	0.5015	0.9460	N
RIL25	36	0.6843	0.5000	0.9460	0.7648	0.0255	0.5015	0.9460	N
RIL26	36	0.7633	0.5000	0.9460	0.7724	0.0256	0.5015	0.9460	N
RIL27	36	0.7183	0.5000	0.9460	0.7600	0.0246	0.5015	0.9460	N
RIL28	36	0.8387	0.5000	0.9460	0.7685	0.0252	0.5015	0.9460	N
RIL29	36	0.7152	0.5000	0.9460	0.7618	0.0259	0.5015	0.9460	N
RIL30	36	1.0000	1.0000	1.0000	****	****	****	****	Nn
RIL31	36	0.7152	0.5000	0.9460	0.7662	0.0243	0.5015	0.9460	N

Note: 1. L*95 = Lower limit at 95% confidence interval, U*95 = Upper limit at 95% confidence Interval. Obs. F = Observed Frequency, Min F = Minimum Frequency, Max F =Maximum Frequency, SE = Standard Error, N = Neutral, Nn = Not neutral.

2. The “Obs. F" is compare to the "L*95" and "U*95". If "Obs. F" is within this confidence interval, the locus is neutral; otherwise, it is not. Following this 27 (87.10 %) of the 31 loci are neutral.

Appendix 5.4.12 Gene flow among five populations of *C. pentandra* base on combined RAPD and ISSR polymorhism.

Locus	Sample Size	Ht	Hs	Gst	Nm
RIL1	36	0.2259	0.2114	0.0641	7.3030
RIL2	36	0.2632	0.2548	0.0321	15.0816
RIL3	36	0.0292	0.0275	0.0602	7.8005
RIL4	36	0.4654	0.4171	0.1036	4.3263
RIL5	36	0.4842	0.4250	0.1223	3.5880
RIL6	36	0.4654	0.4171	0.1036	4.3263
RIL7	36	0.4741	0.4386	0.0749	6.1749
RIL8	36	0.3224	0.3205	0.0061	82.0283
RIL9	36	0.4179	0.3790	0.0930	4.8735
RIL10	36	0.3112	0.2975	0.0442	10.8244
RIL11	36	0.3137	0.2780	0.1138	3.8944
RIL12	36	0.3628	0.3005	0.1715	2.4159
RIL13	36	0.3840	0.3383	0.1189	3.7044
RIL14	36	0.0600	0.0523	0.1278	3.4113
RIL15	36	0.1997	0.1927	0.0350	13.7671
RIL16	36	0.2853	0.2537	0.1110	4.0053
RIL17	36	0.1408	0.1353	0.0390	12.3139
RIL18	36	0.1411	0.1298	0.0804	5.7175
RIL19	36	0.0000	0.0000	****	****
RIL20	36	0.1733	0.1651	0.0472	10.0928
RIL21	36	0.1762	0.1747	0.0085	58.5578
RIL22	36	0.0520	0.0496	0.0475	10.0188
RIL23	36	0.3990	0.3632	0.0897	5.0711
RIL24	36	0.4059	0.3912	0.0362	13.3188
RIL25	36	0.2714	0.2355	0.1322	3.2824
RIL26	36	0.2474	0.2350	0.0501	9.4771
RIL27	36	0.2938	0.2827	0.0377	12.7545
RIL28	36	0.1615	0.1565	0.0311	15.5531
RIL29	36	0.2543	0.2352	0.0751	6.1576
RIL30	36	0.0000	0.0000	****	****
RIL31	36	0.2543	0.2352	0.0751	6.1576
Mean	36	0.2592	0.2385	0.0799	5.7544
St. Dev		0.0204	0.0165		

Note: 1. Ht = total heterozygosity, Hs = average heterozygosity in the five subpopulations. Gst =Fst. Fst values in the range of 0.00 - 0.05, 0.05 - 0.15, 0.15 - 0.25 and 0.25-1.0 indicate low, moderate, large and very large genetic differentiation respectively (Yeh,2000). Following this, the Gst estimated (0.0799) indicate moderate genetic differentiation among the populations.

2. Nm values above 1 indicate significant genetic similarity due to gene flow, when 1>Nm is an indication of weak but possible gene flow, when Nm < 0.5, populations are also isolated (Potter, 1990). Follwing this, the Nm estimated (5.7544), indicates significant genetic similarity.