TOWARDS DOMESTICATION OF KIHANSI WILD COFFEE (*Coffea kihansiensis*): POPULATION DYNAMICS, PROPAGATION AND DIVERSITY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF THE SOKOINE UNIVERSITY OF AGRICULTURE.

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EXTENDED ABSTRACT

Potential of this wild coffee can be utilized as genetic resource for coffee breeding program and/or domestication but unfortunately the population structure, propagation potential and genetic variability of the discovered Kihansi wild coffee is poorly known. Therefore, characterization and population study is highly needed hence, this study aims to (i) Assessing the population dynamics of *Coffea kihansiensis* (ii) Optimizing vegetative propagation of *Coffea kihansiensis* (iii) Characterizing morphological traits of Kihansi wild coffee (*Coffea kihansiensis*) and (iv) Assessing the genetic diversity of Kihansi wild coffee. Population dynamics assessment using quadrat method was done. Variability of population density, lowest in lower upper and upper block while highest population density was observed in lower spray. High proportional of matured plant were observed in both two seasons with lowest number of dead plants. In optimizing vegetative propagation, sand as propagation media provided promising results with good rooting ability, high survival rate and highest root number compared to decomposed rice husks (DRH) and un-decomposed rice husks (UDRH). About 62.52 % and 72.42 % population variation detected based on vegetative and reproductive traits respectively. Plant habit, branching habit, angle of primary branches, berry and cherry length, width and diameter are the most significant traits expressed the morphological variations. These variations were confirmed in genetic diversity study using SSR markers. About 97 % of the total variation was detected within populations. Four clusters varied at 60 % were obtained. These findings are useful in planning conservation and management strategies while providing insight on utilization of this coffee as genetic resource for coffee improvement.

Key words: *Coffea kihansiensis*, genetic variability, population density and improvement.

DECLARATION

I, FARAJA MAKWINJA, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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The above declaration is confirmed by;

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DEDICATION

This work is dedicated to my beloved parents, my brothers and juniors of mine namely Francis Makwinja, Gorge Makwinja and Gracious Makwinja wherever they are. I encourage them to reach this level.

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
CBD	Coffee Berry Disease
CRD	Complete Randomized Design
CTAB	Cetyltrimethylammonium bromide
DI	Diversity Index
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic acid
DRH	Decomposed Rice Husk
EDTA	Ethylene Diamine Tetraacetic Acid
IBA	Indole -3- Butyric Acid
ICO	International Coffee Organization
IPGRI	International Plant Genetic Resource Institute
IUCN	International Union for Conservation of Nature
KWC	Kihansi wild coffee
LS	Lower Spray
LU	Lower Upper
Msl	Mean sea level
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PVP	Polyvinylpyrrolidone
r.p.m	Revolution per minutes

TaCRI	Tanzania Coffee Research ...
TAE	Tris Acetate EDTA
TE	Tris EDTA
UDRH	Undecomposed Rice Husk
UPGMA	Unweighted pair group method with arithmetic mean
US	United State
US	Upper spray
VR	Variance ratio

CHAPTER ONE

1.0 INTRODUCTION

1.1 Coffee Classification

Coffee belongs to genus Coffea in a family Rubiaceae, is the one of the largest tropical plant family. There is a range of published taxonomies for this genus but according to Fazouli *et al.* (2005) there are more than 100 species belonging to this genus and only two species are of economically importance Coffee Arabica (*Coffea arabica* L) and Coffee Robusta (*Coffea canephora* Pierre) (Tounekti *et al.*, 2017). According to Anthony et al. (2001) 70% of worldwide coffee production depends on Coffee Arabica. *C. arabica* is tetraploid specie (2n=4x=44) and is self-fertile while other coffee species are diploid (2n=2x=22) and are generally incompatible (Masumbuko *et al.*, 2006). *C.arabica* has two distinct botanical varieties of *C. arabica* var. *arabica* (usually called Typical) and *C. arabica* var. *bourbon* (Hue, 2005).

1.2 Origin, Domestication and Distributions of Coffee

Coffee is originated at Ethiopia and recognized as its center of diversity of coffee. It is thought that coffee was introduced to Yemen from Ethiopia around the 16th century (Anthony *et al.*, 2002), where practitioners of Sufism first recorded consumption around 1450 (Vega, 2008). Domestication and use of coffee in Ethiopia dates back some 2000 years ago. Some legends of its early consumptions even date it back, around 1000 BC. During the old back of domestication coffee was used only by Oromo native people. It was brought and traded to Yemen around 600 years and then spread all over the world especially Latin America (Ferwerda, 1976). Also, introduction to other parts of world, Java, Amsterdam, and La Réunion was done at the beginning of the 18th century. Coffee then spread rapidly to the Americas and Indonesia and other places around the world

(Anthony *et al.*, 2002). Then it was distributed in Africa, Mauritius, Madagascar and Comoro islands, Tropical Asia and Australia (Davis *et al.*, 2006, 2011; Davis, 2010, 2011). In Tanzania introduction of coffee were made at different periods and were from different origins i.e. Ethiopia, India, Java, Jamaica, Bourbon and Aden. In Tanzania *C. arabica* is grown in Kilimanjaro, Arusha, Tanga, Mbeya, Morogoro and Ruvuma regions, while *C. canephora* is cultivated mostly in the northwestern region of Kagera and some extents in Morogoro region (Coffee annual report, 2017).

1.3 Importance of Coffee Industry to Agriculture in Tanzania

Coffee plays a significant economic role globally as well as serving as a major source of foreign earnings in many coffee producing countries. Produced in about 80 countries (Musoli *et al.*, 2009), an estimated 125 million people in Latin America, Africa and Asia depend on it for their livelihoods (Osorio, 2002). Coffee is the third largest export crop after cashewnuts and it contributes approximately $ 151 million to export earnings. The industry provides direct income to about 400 000 smallholders who produce 90 percent of the Tanzania's coffee. Tanzania is endowed with abundant land with appropriate altitude, temperature, rainfall and soil suitable for high quality Arabica and Robusta production. Throughout the country coffee is produced under two systems of pure stand and coffee inter-cropped with banana. Small holder who produce 95 percent of the coffee on average plot sizes of 2 ha and Estates produce the remaining 5 percent (Coffee annual report, 2017).

1.4 Status of Wild Coffee and its Potential for Conservation

The wild crop species provide the wider selection potential for breeder in crop improvement; their utility was recognized in breeding programs of major crops in the 1940s and 1950s (Puckett *et al.*, 2014). Prescott-Allen (1986, 1988) reviewed on the importance of wild genes in crop improvement. Both the molecular technologies and

conventional technologies are available for breeding and cultivar development that allow for the incorporation of more distantly-related taxa in these improvement procedures. Wild species provide genetic resources for crop improvement including low caffeine content, increased yields, or increased resistance to pests and pathogens such as coffee berry disease (CBD) caused by *Colletotrichum kahawae*, coffee rust caused by (*Hemileia vastatrix*), *Meloidogyne* root nematodes and the coffee berry borer (*Hypothenemus hampei*) (Hein *et al.*, 2006; Silvestrini *et al.*, 2007; Dessalegn *et al.*, 2008; Boisseau *et al.*, 2009).

Natural habitats of wild coffee species are reported to be found in tropical forestry of Africa, encompassing a wide geographical range that stretches from Guinea in West Africa to Eastern Africa (Charrier and Berthaud, 1987). Center of origin and primary diversity of most coffee Arabica species are allocated in the highlands of Southern Ethiopia (Anthony *et al.*, 1987). The tributaries of Congo River also represent the rich center of diversity (Dulloo *et al.*, 2001). Wild coffee population can play crucial in breeding; due to low genetic diversity of coffee, selection from wild species have become high priorities for researchers (Lashermes *et al.*, 2000). Diverse coffee varieties have been developed as a results of artificial pollination between cultivated coffee varieties and wild species or naturally within forest habitats, developing plants verifying in drought tolerance, disease resistance, coffee been size and taste (Kufa, 2006; Tesfaye, 2006).

Coghlan (2004) reported the discoverer of three natural decaffeinated coffee varieties from the natural wild coffee occurring population in Ethiopia. Also, towards utilization of wild coffee species to coffee improvement Barre *et al.* (1998) reported the inter-crossing between *C. pseudozanguebariae* (caffeine free) and *C. liberica var. dewevrei* and investigated the inheritance of caffeine and heteroside. He found the caffeine content of F_1 hybrid was lower than parental average. So far high number of improved varieties of

other crops other than coffee has been worked and released by incorporating with genes from wild related species. Sunflower with gene of salt resistance from wild species has been reported (Lexer *et al.*, 2004). Escalant (2004) reported that, a wild non-edible diploid banana species has been used as a source of resistance in banana hybrid against black sigatoka. Hence, this all statement justified the potential of the wild species to sustainability of agriculture.

Tanzania is one of the main areas for wild coffee species diversity, occupying the highest number of natural occurring coffee species (16 species) second after Madagascar (57 species) (Davis *et al.*, 2004). About eight coffee wild species out of sixteen found in Tanzania are endemic to Eastern arc forestry. They including *C. bridsoniae* Bridson, *C. fadenii*, *C. costatifructa* *C. kimbozensis*, *C. lulandoensis* and Kihansi wild coffee (*Coffea kihansiensis*). Only three species (*C. kihansiensis*, *C. bridsoniae* and *C. fadenii*) are endemic in Kihansi gorge forest, southern of Udzungwa Mountains (Davis *et al.*, 2004). *C. kihansiensis* is listed as critically endangered species under IUCN redlist (IUCN, 2001). However, the conservation status of this wild coffee specie, its population dynamics as well as diversity/varieties is poorly known. This specie can be useful as genetic resource for improvement of cultivated coffee. The characterization of different accessions from the population of wild coffee in the Kihansi Gorge may help in the identification of disease resistant genotypes, breeding for high yielding, improvement of agronomic characteristic, improving coffee quality and or development of new coffee variety.

1.5 Objectives

1.5.1 Overall objective

This study intended to explore the potential of this discovered wild coffee specie for future development of the coffee industry.

1.5.2 Specific objectives

i. Assessment of population dynamics of Kihansi wild coffee (C. *kihansiensis*)

ii. Optimization of propagation techniques of Kihansi wild coffee (C. *kihansiensis*)

iii. Assessment of morphological diversity of Kihansi wild coffee (C. *kihansiensis*)

iv. To assess the genetic diversity of Kihansi wild coffee (C. *kihansiensis*)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diversity of Coffee

Diversity assessment can be done through morphological, genetically and or biochemical techniques in order to assess the variability of species or individual within a population. Morphological analysis complements the genomic analysis especially in wild species as a strategy of increasing the utilization of these wild species (Kasem *et al.*, 2010, 2011). However, automation of morphological analysis is paramount as allow the screening of larger population. Gessese *et al.* (2015) reported use of morphological markers to characterize diversity of coffee in southern Ethiopia. Also, studies on morphological diversity of coffee done by Catter (1992) reveal high variations in morphology. The assessment of diversity with morphological traits was also done on Ethiopian tetraploid and hexaploidy Wheats (Bekele 1984). Barley (Demisse, 1996). Coffee (Montagnon and Bouharmont, 1996) and Sorghum (Ayana and Bekele, 1998). However, morphological variability is often limited since the characters are mainly affected by environment. Moreover, morphological traits might be insufficient to differentiate among pairs of closely related species and ecotypes since not all genetic differentiation results in morphological differentiation (Siva and Krishnamurthy, 2005). Therefore, currently different molecular markers have been developed as complementary strategies approaches in genetic diversity assessment (Terzi *et al.*, 1999). These techniques are reported to be very useful in breeding program as replacement in conventional approaches of individual selection.

2.2 Molecular Techniques for Genetic Characterization

Characterization of genetic diversity of plant species provides insight into evolutionary history, taxonomy and ecological aspect of the extent and distribution of genetic diversity (Hodgkin *et al.*, 2001; Syamsuard *et al.*, 2002; Weising *et al.*, 2005). Analysis of genetic

diversity using molecular marker is also essential to detect the population bottlenecks in threatened species (Luikart, 1998). Also the information of genetic variation provide insight on choice of management for capturing and maintaining available variation (Neel and Ellstrand, 2003). Several works have been done in exploring the molecular techniques in different aspect and these techniques differ in aspect of cost, efficiency, sequence specificity and repeatability. These techniques include conventional RFLP (Herrera *et al.*, 2001; Crouzillart *et al.*, 2004), PCR- based techniques such as RAPD (Anthony *et al.*, 2001a; Anthony *et al.*, 2001b; Cristancho *et al.* 2004) as well as microsatellite marker (Lashermes *et al.*, 1995).

Microsatellite marker (simple sequence repeats and sequence characterized amplified region (SCARs)) are based on sequence specific primers with limited transferability across the species but more reproducible (Kathurima *et al.*, 2011). Utilization of these techniques provide the easiness in breeding work perspective, the development of simple sequence repeat (SSR) markers for *Coffea spp.* (Moncada and McCouch, 2004; Poncet *et al.*, 2007) has provided a key resource for investigating genetic diversity in the genus. Studies include hybridization (Anthony *et al.*, 2000*a*; Ruas *et al.*, 2003; Tesfaye *et al.*, 2007; Gomez *et al.*, 2010), domestication (Anthony *et al.*, 2002*a*, *b*), cultivated genepools (Prakash *et al.*, 2005), breeding (Cubry *et al.*, 2008), and *in situ* and *ex situ* conservation (Krishnan *et al.*, 2013*a*, *b*).

2.3 Population Structure and Dynamics of Wild Coffee

Mostly, coffee species in Africa inhabit in forest types generally occurring in humid evergreen forests (Davis *et al.*, 2006). High range of natural occurring coffee species are reported to be found in high-density forest of 1300 m a.s.l and 1600 m a.s.l suggesting the

optimal altitude of wild coffee. Senbeta (2006). as reported in Gole *et al.* (2001) found the existence of natural occurring coffee in undergrowth of Montane forest in Ethiopia at altitude around 1400 m a.s.l and 1900 m a.s.l. Friis (1979) reported the existence of natural wild coffee population in Banja forests in altitude of 1620 m a.s.l.

Generally, the abundance and extent of wild coffee population differ depending on the geographical regions. The environmental factors and level of interference by human could be the main factors that affect the population and pattern of distribution within the forest. Ethiopia reported to be the center of diversity and center of origin of coffee species, the natural forest areas with occurrence of natural resource including the wild coffee species encounter serious challenges of loss of biodiversity due to anthropogenic impact (Kufa, 2011). However, some of wild coffee concurring with other plant in natural forest (Taye, 2006) by adopting various biotic and abiotic stress. Hence, it is imperative to conserve the precious natural resources and environment by implementing the sustainable conservation, management and utilization of maximum biodiversity. Cognizant of importance of coffee. Bellachew and Sacko (2009) emphasize the need of population dynamics assessment and immediate conservation measure to overcome the loss of biodiversity. To meaningfully conserve the genetic diversity of a taxon, knowing the population is essential and hence this should become one of the principal strategies in the conservation efforts of species to ensure success.

2.4 Genetic Conservation and Domestication of Coffee

Cultivation of coffee started in Southwestern of Ethiopia around 1500 years ago. It was thought that coffee introduced to Yemen from Ethiopia around 6th century (Antony *et al.*, 2002), where practitioner of Sufism first recorded consumption around the 1450 (Vega,

2008). From Yemen, two genetic base spread, known as Typica and Bourbon and give rise to the most of the present commercial cultivar of Arabica coffee grown worldwide. Several authors reported that the genetic base of Typica type of coffee consist of single plant cultivated in Amsterdam at the beginning of 18th century, introduced from Java, whereas Bourbon genetic base consist of trees introduced from La Reunion. Coffee spread to the America and Indonesia in the form of self-fertilized seeds with intense of reduction in genetic diversity leading to genetic bottleneck outside of center of origin (Anthony *et al.*, 2002).

Interest in coffee genetic resources and its conservation increased during the second half of the 20th century as awareness of the threats to these genetic resources due to deforestation, and the lack of variability in the crop increased (Anthony *et al.*, 2007a)
Two important commercial coffee, Arabica and Robusta are reported to have low genetic variability (Anthony *et al.*, 2002). However, *Canephora*, is estimated to have 10 times the genetic variability than *C. arabica* (Lashermes *et al.*, 2000). Hence this species is mainly utilized in crossing for coffee improvement to increasing the genetic variability together with utilization of other taxa such as *C. eugenioides*, *C. congensis*, (Lashermes *et al.*,1999), also in situ population in its center of diversity and origin still have significant genetic variability for many agronomical characters (Teressa *et al.*, 2010). These and other wild species represent the ultimate source of genetic diversity of coffee. However, threats to these genetic resources are immense and their loss would ultimately lead to significant genetic erosion of the coffee gene pool. Countering the loss of genetic resources has been the main motivation behind implementation of collecting expeditions and establishment of *ex situ* field gene banks in many coffees producing countries and emphasis on the conservation of wild coffee species has been given more priorities.

Coffee seeds are recalcitrant or exhibit intermediate storage behavior, making preservation of germplasm through seed banking problematic (Dulloo *et al.*, 1998). Hence, complementary conservation strategies are essential for effective and sustainable conservation of the maximum range of genetic diversity to include *in situ* and *ex situ* conservation strategies (Dulloo *et al.*, 1998). With the development of molecular marker technologies, genomic tools are now available for the advancement of conservation of crop genetic resources.

2.5 Optimization Techniques for Vegetative Propagation of Coffee Cuttings

Coffee plant can be propagated asexually or sexually however sexual propagation using coffee seeds are predominantly used especially in Arabica coffee due to sufficient true breeding while Selected clones of *Coffea canephora* are not propagated by seed as it is an allogenous cross-pollinated plant (Smith *et al.*, 2002). Due to short live of coffee seed it is mandatory to plant seeds within two months after harvesting (Clarke *et al.*, 1988). Vegetative propagation methods applicable to coffee, including cuttings, grafting and tissue culture normally somatic embryogenesis using leaf section as explant commonly practiced and recommended for coffee (Krishna, 2011). To express high hybrid vigour in arabica coffee many countries experience use of vegetative propagation by rooting of cuttings for larger scale farming (Tadesse, 2014). Wamatu *et al.* (1992) reported that there was inconsistency in rooting when different plant parts were used for vegetative propagation. Optimization of vegetative propagation of coffee was successful when rooting hormone was incorporated with different rooting media and cuttings. This was confirmed by Tadesse (2014) who optimized on vegetative propagation of Arabica coffee using media containing topsoil and sand at ratio of 3:1. Results were 90 % success of rooted cuttings.

In vitro methods have also been used for propagation and two ways are commonly used that is micro-cutting and somatic embryogenesis and various approaches have been considered for in vitro multiplication of coffee as apical meristem and axillary bud culture (Cameiro and Ribeiro. 1998). This type of multiplication is able to produce larger number of plantlets from single plants (Shibli *et al.*, 1997) that are genetically identical to their mother plants. But Clarke *et al.* (1988) reported that, this technique requires switching to different media with different concentration to enable success rate. Teresa *et al.* (2006) reported that there is a majority of media containing mixture of different concentration of auxin and cytokinin for somatic embryogenesis induction. Dublin (1981) showed that it is possible to produce embryogenesis from leaf explant using cytokinin (6-Benzylaminopurine) alone, this confirmed by Yasuda *et al.* (1995).

2.6 Adaptation of Coffee Seedlings

Overall, drought and unfavorable temperatures are the major climatic limitations for coffee production. Under field conditions, plant performance in terms of growth, development, biomass accumulation and yield depend on acclimatization ability to the environmental changes and stresses, exercising specific tolerance mechanisms that involve a complex network of biochemical and molecular processes (Wang *et al.*, 2003). Abiotic stresses such as extreme temperatures, drought and salinity or chemical toxicity represent serious limitations to coffee production (Bray *et al.*, 2000). Both low (20 °C) and high temperature (35 °C) affects the growth and development of coffee seedlings.

The strongest climatic limitation to growth and development of coffee crop are temperature and humidity (Damatta *et al.*, 2006). Several authors emphasized this focusing on the physiology of Arabica coffee (Rena *et al.*, 1994; Barros *et al.*, 1999; Carr, 2001; Maestri *et al.*, 2001) and Robusta coffee (DaMatta and Rena, 2001, 2002a, b;

DaMatta, 2004a). The optimum mean annual temperature range for arabica coffee is 18-21 °C, while in regions with a mean annual temperature below 17 - 18 °C, growth is largely depressed. For Robusta coffee, the optimum annual mean temperature ranges from 24 to 30 °C (Willson, 1999).

Robusta is much less adaptable to lower temperatures than arabica coffee (DaMatta, 2006). The optimum temperature for germination of arabica coffee is around 30 - 32 °C, where it takes about 3 weeks to germinate while if planted at 17 °C it requires 3 months (Moraes, 1963; IBC,1985). Germination is inhibited above 35 °C (Barros *et al.*, 1999). During the first week of plant development optimal temperature for seedlings growth is in range of 23 - 30 °C. Therefore, frequent water irrigation with recommended shade trees are well applied to mitigate the effect of temperatures in coffee plantations.

Robusta coffee can be grown between sea level and 800 m, whereas Arabica coffee grows best at higher altitudes and is often grown in hilly areas from 1200 m and above. The optimum annual rainfall range is 1200 - 1800 mm for Arabica coffee (Damatta *et al.*, 2006). A similar range seems to be required for Robusta, although it adapts better than Arabica to intensive rainfall exceeding 2000 mm.

Since coffee is an evergreen plant, it requires sub-soil water at all times. Thus, deep soils with good water-holding capacity are the most suitable environment for coffee growth. The soil structure must also allow good drainage because the surface feeding roots need a drier period for part of the year to slow down growth, ripen the wood and initiate flower buds, water stress is apparently essential to break the dormancy of flower buds in coffee (Paulo, 1960; Demel, 1999). Excessive moisture results in the imbalance of growth regulators and promoters and a particular hormone responsible for vegetative phase.

Excessive moisture drastically inhibits flower initiation and reduces the number of flowers (Pereila *et al.*, 2004).

2.7 Potential of Wild Coffee Species for Genetic Improvement of Cultivated spp.

All crops plants have been domesticated from wild relative's species, these species represent primary source of diversity for use in plant breeding (Kole *et al.*, 2015). The importance of these wild crop species in crop improvement was reviewed by Prescott-Allen (1987; 1988). A comprehensive survey of the use of crop wild relative genes in domesticated plants was conducted (Hajjar and Hodgkin, 2007). Up to date plant breeders seek for the genetic materials from wild population plants for crop improvement. Wild relatives have provided traits such as disease resistance, tolerance of extreme temperatures, tolerance of salinity and drought (Henry, 2014). Many studies have been conducted to evaluate the potential of wild related crop species to cultivated crop improvement and or for domestication purpose. It has been estimated that about 30% of increased crops yield in 20 centuries attributed by the use of crop wild species (Honnay *et al.*, 2012). Study of QTL in wild soya Prince *et al.* (2015) and Li *et al.* (2008) has indicated ways of improving yield in soya bean (*G. soja*) by efficiently improving water uptake. Yield QTL have been identified in inbreed lines (ILs) originating from cross between the green fruited species *Lycopersicon pennellii* and the cultivated tomato (Rao *et al.*, 2003), wild bean (wild *Phaseolus vulgaris*) (Blair *et al.*, 2006) and wheat (Singh, 2012a).

Coffee breeding programs have been striving to identify disease resistance, drought tolerance, and low caffeine content varieties from various sources of germplasm. Wild coffee gene pool responsible for number of desirable traits has been identified (Silvarolla *et al.*, 2004; Berecha *et al.*, 2014). Transferring the genes of resistance from one species

have been successfully reported, including transferring genes of resistance to coffee leaf (CLR) and coffee berry disease (CBD) from *C. canephora*, via the interspecific hybrid referred to as Hibrido de Timor (HDT, Timor Hybrid), natural cross of *C. arabica* x *C. canephora* has been reported by Kathurima *et al.* (2012). Five coffee lines coded Cr8, Cr22, Cr23, Cr27 and Cr30 have been under evaluation and their unique features include tall stature, true breeding and resistance to both CBD and CLR (Gichimu and Omondi, 2010a).

Analysis of wild *Coffea* species may support the expansion of diversity in Arabica coffee, a recently domesticated species with relatively little genetic variation. The genomes of wild relatives are being studied to better define the relationships of wild taxa with domesticated coffee (Hamon, 2015).

Therefore, a diverse coffee gene pool is of paramount importance for breeding. Particularly, cross breeding of cultivated coffee varieties and wild genetic material. This potential assumes even more significance especially by considering that different coffee species interbreed free and relative easy with high level of gene flow. Therefore, knowing the diversity of crop wild species and exploring its potential could effectively provide the addition genetic resources to coffee gene pool that can be utilized for coffee improvement hence, plant breeders could optimize the use of these species in mitigating and complete solving the limitations to coffee growers as well as the possibility to use this wild species as unique cultivars for cultivation following successful domestication.

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CHAPTER THREE

3.0 ASSESSMENT OF KIHANSI WILD COFFEE PLANT POPULATION DYNAMICS FOR CONSERVATION MEASURES

Abstract

Coffea kihansiensis is the species endemic to Udzungwa Mountain, restricted within blocks of less than 1.0 km^2, reported to be endangered species. Its population is threatened by environmental disturbance. Due to lack of information and awareness of this species, population structure and conservation status of this wild coffee is poorly known. Therefore, population dynamics study could serve as initial strategic plan for establishment of conservation measures. Quadrat method was used to study the population density of this specie; ten quadrats from each two macro-plots each of 3 m by 10 m were established. Total numbers of individuals were counted and proportions of plant classified by age class were estimated from two consecutive seasons. From each 1 m^2, 3 plants were counted. Results showed that population is high during the dry season. Highest number of matured plants was observed in both seasons, young individuals 2995 and 3405 plants in wet and dry seasons respectively were also observed. Lowest number of old plants was also observed in both seasons. Lower upper and upper spray had smallest population densities compared to lower spray. Habitat quality had effects on this population density variation between plots. Absence of fully forest tree vegetation cover in upper blocks could be possibly a reason for low plants density. Environmental factor particularly on changes of moisture and temperature also had effects on plant population and coffee seed germination physiology also. Conservation strategies through management of tree forest cover are highly encouraged. Long-term population study is suggested to be conducted for tracking consistency of population dynamic as a strategy for conservation and management of this wild coffee species.

3.1 Introduction

Coffee belongs to genus *Coffea*, is the one of the most important agricultural commodities, ranking second international trade after crude oil. The total global production of green coffee is above 134.16 million bags with a retail sales value in excess of $ 22.7 billion during 2010 and 2011 in the world market (ICO, 2010). More than 100 million people in the coffee growing areas worldwide derive their income directly or indirectly from the produce of this crop.

Current commercial Arabica varieties and their selection originated from narrow genetic diversity (Silvestrini *et al.*, 2007). Historically evidence indicates that this base population descended from few trees that survived from various efforts to spread this coffee from its center of origin (Lashermes *et al.*, 1995). As such, coffee improvement is restricted due to narrow selection from diverse population (Lashermes *et al.* 2011). In order to diversify the genetic resources of coffee to improve quality, higher yields or develop pest and disease resistance varieties, plant breeders often utilize the natural occurring crop wild species (Hoistington, 1999; Heywood *et al.*, 2007; Lashermes *et al.*, 2011). These wild species possess desirable characteristics that can be used in improving existing crops (Maxted *et al.*, 2008). However, due to lack of awareness in paying attention to these wild crop species conservation measures and population status/structure is little known and its habitats is threatened by human activities and environment disturbance hence, there is a need of population study.

Utilization of coffee germplasm normally depends on availability and its diversity (Foley *et al.*, 2011) Availability of Kihansi wild coffee is reported to be threatened by environmental disturbance (Lovejoy, 1995). This call for *in situ* and *ex situ* conservation

of the little available genetic diversity of the wild coffee population. *In situ* conservation is the preferable solution, because it sustains the mechanisms of natural selection and adaptation to changing site and environmental conditions (Charrier and Berthaud, 1990; Tewolde, 1990; Meilleur and Hodgkin, 2004). The purpose of conserving wild species for sustainable utilization population size (density) and trend of population fluctuation (dynamics) should be assessed by capturing the extent and distribution of species in its natural habitat as well as plant structure aggregated by age (plant age composition) (Honnay *et al.*, 2012).

Tanzania is endowed with abundant land with appropriate altitude, temperature, rainfall and soil suitable for high quality coffee production. In the other hand, being the diversified in altitude for coffee production. Tanzania is the one among the main area of coffee species diversity, housing the number of natural occurring coffee species around 16 species such, *C. bridsoniae* A.P. Davis and Mvungi, *C. fadenii* Bridson (Shengena hills of the pale mountain), *Coffea costatifructa* Bridson *Coffea eugeniodes* S.Moore, *Coffea kimbozensis* Bridson, *Coffea lulandoensis* Bridson and *Coffea mongensis* Bridson and is second after Madagascar (Davis *et al.*, 2011). Kihansi wild coffee (*C. kihansiensis* A.P. Davis and Mvungi) is among the endemic species found in Southern arc of Udzungwa Mountain in Morogoro, Tanzania (Davis *et al.*, 2004). This species is found in humid evergreen escapement forests in slightly sloping at Kihansi usually near the gorge, near the river on humus rich soil at altitude range of 800 to 900 m above sea level. This species is reported to be endangered under IUCN red-list (2010) criterion in restricted blocks (less than 1.0 km^2) with declining population size (Davis *et al.*, 2004). Therefore, population dynamics and structure assessment of this species is highly needed for biodiversity conservation measure as well as for sustainably utilization of this wild coffee species for coffee improvement.

The aim of this study was to provide the assessment of population dynamics and population structure within Kihansi wild coffee species in Kihansi gorge specifically the following research questions were addressed (i) how many individuals exist in this habitat? (ii) Is there any variation in population density between natural coffee garden blocks? (ii) Does the population increase, decrease or remain constant between seasons in a year? (iii) What is composition in terms of the population structure (aggregated by age?).

3.2 Material and Methods

3.2.1 Study area

The study was conducted at Kihansi gorge forests (8°35'S; 35°51'E) in Udzungwa Mountain, Morogoro Tanzania. Four experimental blocks (Upper wetland, Lower upper wetland, Lower wetland and Mhalala block) were within 800 m to 900 m above sea level.

3.2.2 Assessment of population dynamics (density)

From each block, ten plots were demarcated based on distributions of wild coffee population. Each block had the dimension of 100 m × 10 m. From each block four randomly macro-plot each of 10 m × 3 m was established based on proportion and distribution of wild coffee plants present in each sampling block. Total of five quadrats (5 m × 2 m) in each macro-plot was established randomly and subjectively and used as representative sampling areas based on methodology described by (Carly *et al.*, 1998). Estimation of number of plants per unit area was done by counting the total number of plants in each quadrat where eventually plant density was estimated by using formula described by Carly *et al.* (1998).

$N = (A/a) \times n$

Where: N = the estimated total population size

A = the total study area

a = the area of the quadrat

n = the number of plants per quadrat

3.2.3 Estimation of plant age structure/stages

From each quadrat total number of plants estimated were observed and categorized into three plant stages disaggregated by age (old plant, matured plants and younger plants/seedling) based on visual observations and descriptors for coffee developed by (IPGRI) , plant height, leaf appearance, stem diameter and color, presence and absence of fruits, plant canopy and bending of plants as criterion.

3.3 Data Analysis

Data analysis was done by estimating the number of plants in a unit space and plant composition disaggregated by age per unit area in different blocks in various seasons, wet and dry season of year 2016. Histogram was drawn for plant population parameter comparison in time and space as described by Thompson (2002).

3.4 Results

3.4.1 Population density

Plants population in terms of space was observed (Fig. 3.1). Results showed that total of 8705 coffee plants were estimated in three coffee garden plots of 3 000 m^2 total areas, estimated in two seasons this means each 1 m^2 occupied by approximately 3 plant. Out of total population, 4180 plants were estimated during wet season in year 2016, in the same area. Lower spray was occupied by highest proportional of plants population of 1880 (44.9 %) while the lowest proportional of plants population of 1 040 (25.1 %) were observed at upper spray. During the dry season of the same year, a total of 4525 plants were estimated (Fig. 3.1). The highest number of plants of 1990 (43.9 %) was observed in lower spray while the lowest number of plants of 1220 was observed in lower upper

spray. Meanwhile in both seasons results shows that lower upper spray maintains almost constant proportional of plants population per units' area compared to the other coffee garden blocks (Appendix. 1) where variation of plant density was detected.

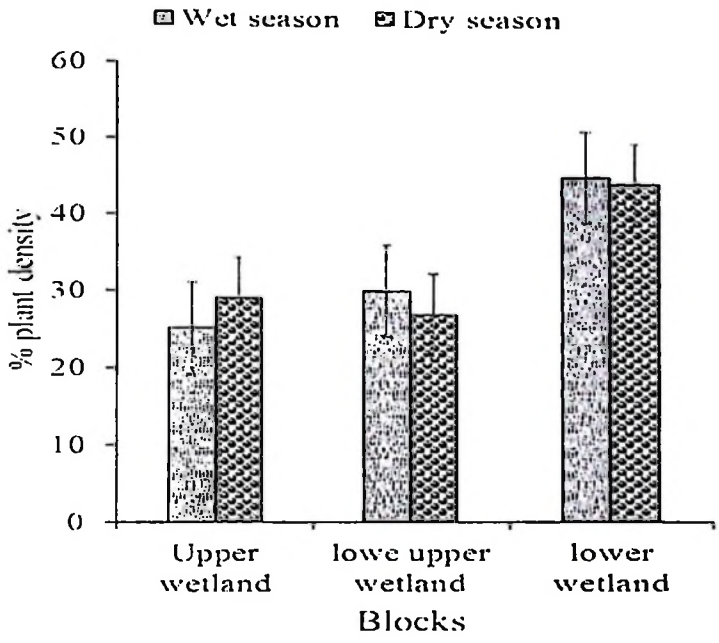

Figure 3.1: Kihansi wild coffee plant population from two seasons (wet and dry) in a different three blocks

3.4.2 Plant population composition disaggregated by age

Seasonal variations of plant population were observed in terms of plant composition disaggregated by age. In lower spray gardens highest proportional of plants was occupied by matured plants in wet and dry season where by a total of 930 and 1 050 matured plants were counted in this block during the wet season and dry season respectively. The same scenario of domination of matured plants was observed in lower upper and upper wetland gardens in both wet and dry season whereby fewer old individuals were observed in all three blocks (Fig. 3.2). However, the highest number of 185 dead or old plants of wild coffee was observed in upper spray during the wet season and 480 at lower spray during the dry season of year 2016 (Appendix 2).

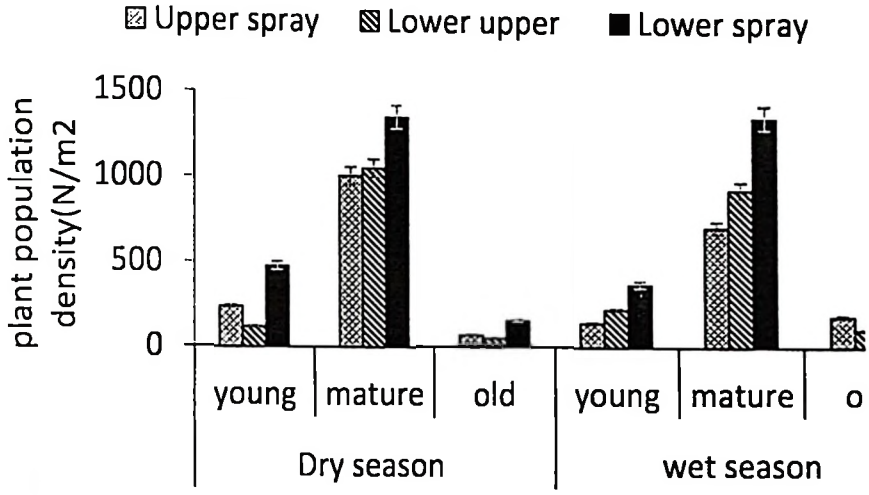

Figure 3.2: Variation of plant composition of wild coffee population disaggregated by age in a unit block in two seasons

3.4.3 Plant population dynamics

Population increase and decrease was also checked. Highest number of plants was observed during the dry season of year 2016 whereby a total of 4 525 plants were counted in three plots of 3 000 m^2. In the same season both lower spray and upper spray occupy the highest number of plants of 1 990 and 1 315 plants respectively compared to the wild coffee gardens in lower upper spray were lowest number of plants were counted. During the wet season 4180 plants were counted in the same constant plot area. In this season lowest number of plants were observed in upper spray (1 040) and lower upper spray (1 260) while highest number of plants were counted in lower spray (1 880). The highest number of plants during dry season accompanied by the highest number of the young seedling (840) and matured plants (3 405) in a population. However, there were slightly small differences of population size (55 plants) in lower upper spray during wet and dry season compared to other experimental blocks in term of population size during the year. Highest differences of population size between seasons were observed at upper wetland block followed by lower upper wetland and surprisingly less number of plants during the wet season of the same year 2016 (Appendix 2).

3.5 Discussion

Plant population density of these natural occurring wild coffee species between blocks with time was studied. Highest number of plant population was counted in lower spray in both seasons with small variation from season to season. This phenomenon indicated the population stability at this coffee garden. Lower spray block is characterized by high-density forest tree cover with high soil moisture content where; shade trees regulate sunlight and maintain relative humidity. In contrast to that, lowest population was detected in upper spray; this block is characterized by absence of forest tree vegetation cover. Hence, these habitat characteristics could be the reason of highest and lowest populations of lower spray and upper spray respectively.

Habitat quality and the effect to external environmental factors such as variation of temperature and soil moisture could be the major reasons for this plant population change over time in different blocks. Knowledge of habitat quality, properties and their effects on plant survival and growth, ranged from seed output to seedling survival, plant establishment and hence population density (Laubach *et al.*, 2010). Poor habitat resulted poor and stunted growth therefore poor plant establishment. Sawichik *et al.* (2003) suggested that population density of any plant species at a given natural site depends on the characteristics of site area (balance of vegetation composition), quality (absence of pest and diseases) and its ecological condition (favorable temperature and moisture and good soil structure).

Since, coffee is a shade loving plant (DaMatta *et al.*, 2006), in direct sunlight coffee plants show high incidence of premature death (Steiman, 2003). Shading buffers extreme temperature variations and provide a microclimate which attenuates extreme temperature of air and preserve soil surface humidity (Bote and struik, 2011) which favors growth and

development of coffee plants. From the study, high dense forest trees maintained continuous soil moisture in lower spray block favoring high growth of coffee. Therefore, this calls for holistic conservation strategies of tree forest vegetation cover in upper spray block to provide shade and soil moisture conservation for growth and survival of individual coffee plants.

Proportion of matured plants was greatest surpassed young seedlings and old plants in all blocks in both seasons. Highest number of young seedlings with fewer old plants was observed during the dry season, this could be the effect of coffee seed germination physiology. Germination of coffee is slow and uneven as a result that occurs simultaneously in the embryo and endosperm (Farias *et al.*, 2015). After germination, coffee cotyledons grow by absorbing the endosperm and turn green (Marracini, 2005). The first seed parts to emerge from the soil are the cotyledons. It requires 3 to 4 weeks for the cotyledons to completely deplete the endosperm (da Silva, 2004). Emergence of coffee seedling from the soil starts 50 to 60 days after sowing in the warmer areas (Eira *et al.*, 2006). When temperatures are lower the emergence may increase to 90 days (Vario, 1976). Therefore, temperature variations it may require 3 to 4 months of coffee seed to emerge. Wet season in Morogoro lasts for 3 to 4 months, also Kihansi gorge is favored moisture during the wet season, probability of seed to germinate is high during that time however, emerged seedlings during the wet season counted at dry season. Hence, its effective number could be seen during dry season. These all mentioned reasons possibly acted as environmental factor affects wild coffee population size as observed also by Morais *et al.* (2006), Siebert (2002) and McNaughton and Jarvis (1983).

Obviously, germination is faster under optimal conditions when environmental effects such as variations in daylight temperatures and soil water potential are absent. For germination to occur in coffee seeds, it is necessary that the embryo break the barrier

imposed by the endosperm tissues surrounding the embryo (da Silva *et al.*, 2008). This tissue layer is called "cap region", it consists of high cellulose and hemicellulose and polysaccharides content (da Silva *et al.*, 2005; Eira *et al.*, 2006). In order for the germination process to occur, some enzymes may play an essential role in weakening the cell walls, softening of the cap region and allowing radicle protrusion. α-Galactosidase, β-mannosidase and endo-β-mannanase enzymes are usually considered as the main ones responsible for hydrolysis of mannans present in the cap region during germination of coffee seeds (Marracini *et al.*, 2001; Nunes *et al.*, 2009; Joet *et al.*, 2013). These enzymes act in the hydrolytic degradation of cell walls, allowing radicle protrusion (da Silva *et al.*, 2004). All these events and occurred in coffee seed germination as a results of water imbibition. Hence in absence of enough soil moisture, seed cost rapture as a result of water imbibition and finally seed germination is impaired (Eira *et al.*, 2006). In a combination of seed biology and time taken for coffee seed from germination to full emergence could be strong reason for the dry season to have high population especially young seedling plants. Also, endogenous dormancy related to process such as embryo immaturity and balance between promoters and inhibitors could also contribute to prolonged seed germination (Batista *et al.*, 2014). Population structure disaggregated by age characterized by presence of sufficient population of seedlings and adults recorded in the present study indicates successful regeneration of coffee wild species of Kihansi in its habitat as supported by Pokhriyal *et al.* (2010).

According to the nature of dispersion of plants and characteristics of habitat of this wild coffee, the random sampling technique used where highly recommended in order to obtain the high representative information to infer about population. Lowest variations detected in number of plants between sampling units suggest the precision of this

methodology used (Silva *et al*., 2017). Therefore, for any establishment of management strategies in Kihansi wild coffee specie, random sampling using quadrat methods is recommended.

3.6 Conclusion and Recommendations

3.6.1 Conclusion

Lowest plant densities in all three coffee gardens were observed with highest number of matured plants and lowest number of old plants. These results may be as results of poor habitat quality as explained by low moisture content and absence of full canopy and vegetation for growth and survival of this species. Highest number of matured plants within a population means the species is regenerated and it's the advantage for its survival.

3.6.2 Recommendations

Recruitment of viable seeds, germination, seedling establishment and seedling growth are indicators of the regeneration potential of any plant community. Optimizing the vegetative propagation techniques for mass production of young seedling *ex situ* and reintroduction in coffee garden in Kihansi gorge could serve as increase of population density in different blocks. Forest trees and vegetation cover management in upper spray and lower upper spray highly needed to provide optimal conditions for coffee growth.

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CHAPTER FOUR

[illegible] WILD COFFEE *Coffea* [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

canephora (known as Robusta coffee) dominated the coffee world (Woldemariam *et a*
2002). Coffee is one of the most economically important crops produced in about 8
tropical countries with an annual production of nearly seven million tons of green bea
(Musoli *et al.*, 2009). Coffee can be grown in wide range of climatic conditions howeve
like other larger plantations crops like tea, sisal and cashew, its production success
depends on highly productive hybrids that combine different characteristics of intere
such as high yield, disease resistant and good cup quality (Van der Vossen, 2001).

Coffee propagation can be done through sexually or asexually (Vegetative). Howeve
propagation of coffee by seeds is associated with slow, asynchronous germination a
making it hard to obtain uniform seedlings (Eira *et al.*, 2006). However, for rapid seedli
multiplication and distribution, propagation of seed is preferable. Tissue culture thou
somatic embryogenesis is much applicable now days and has been tested in differe
explants such as leaves, stems and embryos (Berthouly and Etienne, 2000). Howev
limitations such as long regeneration period and high running cost have led to hindran
in utilization of these techniques. In *C. arabica*, use of somatic embryogenesis has be
useful in inducing soma-clonal variation (Etienne *et al.*, 2002), also serving as ma
propagation methods and resolving the narrow genetic diversity of coffee and creati
variability (Berthouly and Etienne, 2000).

Many countries experience in utilization of vegetative propagation (cuttin
layers/suckers, micro-propagation and grafting), rooting of cuttings is the one mos
preferred (Oloyede *et al.*, 2004). Leaf-bud cuttings and tip cuttings are also commo
used (Hartmann, 2002). Vegetative propagation in larger scale planting is constrain
mainly, by type of rooting media and environmental conditions in propagating structu

(Tadesse, 2014). Moore *et al.* (1975) reported that to initiate rooting, temperatures within the propagation boxes should be within a range of 25 °C to 28 °C. Grafting is appropriate in behaving plant growth and ensures that the genetic characteristics of the cultvar is maintained (Oliveira *et al.*, 2004), and contributes to increase in productivity (Fahl *et al.*, 1998).

Therefore, to maintain specific characteristics of each genotype of coffee tree especially in wild coffee where different accessions are available and genetic content are unknown, cutting is used as a means of propagation (Weigel, 2002; Partelli *et al.*, 2006b). Vegetative technique is mainly practices in other plantation crops such as tea (Olaniyi *et al.*, 2014). Performance of vegetative propagation mainly depends on the types of species propagated due to difference in their genetic content (Nanda *et al.*, 1968). Therefore, this work intends to optimize the vegetative propagation of Kihansi wild coffee species.

4.2 Material and Methods

4.2.1 Study area

Study was conducted at Sokoine University of Agriculture (SUA) under shade house conditions, environment maintained: (Temperature 25 °C, humidity, 70 % and 50 % sunlight penetration).

4.2.2 Plant materials and experimental layout

Cuttings used for propagation were semi-hard wood stem of mature Kihansi coffee plant. They were prepared during dry season when coffee trees have accumulated enough food in order to optimize the chance of success of root formation and regeneration of new sprouts. The media used for propagation were pure sand, decomposed rice husk and undecomposed rice husks. These media were obtained after screening from a pilot trial of different media such as black soils, forest soils and red soils and as recommended by

(Ofori-Gyamfi, 1998) for propagation of cuttings. The experiment was laid down in complete randomized design with three replications. Hundred pieces of stem cuttings were used in each set of rooting media.

4.2.3 Cutting preparation and propagation

Pieces of cuttings were prepared from active shoots of matured wild coffee. Some parts of the harvested shoots composed of hard wood, semi hard woods and soft wood leafy cutting. Collected plant shoots for cutting preparation were directly dipped into tap water to balance the level of transpiration shock from foliar parts. Using a clear and clean sharp knife, cuttings were prepared as recommended by Opoku-Ameyaw *et al.* (2000). Leaves were halved to reduce the transpiration loss then dipped in water prior for preparation of cuttings.

4.2.4 Preparation of propagator and planting of cuttings

The propagation structures used in the experiments were bin propagator surrounded by aluminum sheet measured 1.6 m length, 0.9 m width and 0.5 m height (Plate 4.1). Three of them each of 1.44 m^2 were constructed and placed in shade house. Stones of 0.2 m thick formed the base material followed by gravels, then on top of main rooting media. To maintain moisture, irrigation was done per day to moist the media prior for propagation. For each of cuttings, about half of it was dipped into the IBA (Indole-3-butryic acid) powder in order to stimulate root formation (Yeboah *et al.*, 2011). The cuttings were inserted into the soil about 2 cm deep, watered and maintained to about 70 % humidity (Tanhan *et al.*, 2007).

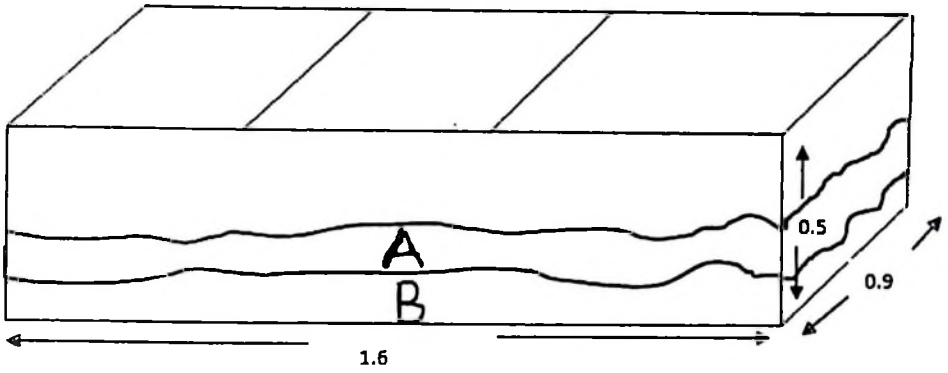

Figure 4.1: Propagation structure (bin propagator) A: Layer of propagating media and B: Layer of base materials (gravel)

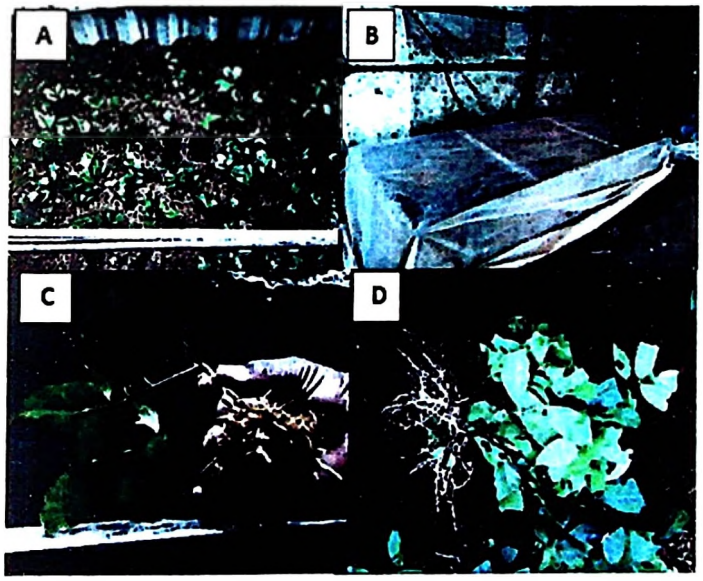

Plate 4.1: *C. kihansiensis* vegetative propagation of cuttings, A: Preparation of cage and planting B: Covering propagator by transparent sheet to maintain humidity, C: Uprooting one plant for confirmation of root formation, C: Uprooting and harvesting cuttings for pot transplanting.

4.3 Data Collection and Analysis

Data were collected on number of leaf drops in each treatment, number of sprouting shoots, and number of dead or wilting cuttings out of total planted shoots. Leaf drops and numbers of sprouted cuttings were recorded weekly from date of planting, while the

number of root lateral number per plants and root length per plant were measured and recorded 4 months from date of planting. GENSTAT software 16th Edition, one-way analysis of variance (ANOVA) was used to analyses the data. Comparison between means of treatment was carried out according to Duncan Multiple range test at $P \leq 0.05$. F-test declared significant differences.

4.4 Results

4.4.1 Effect of rooting media on rate of leaf drops of wild coffee stem cuttings

Larger number of leaf drops was observed from cutting planted on undecomposed rice husk as rooting media. In the first week, rate of 70 % was observed in sand media, 33% in decomposed rice husks and 74 % in undecomposed rice husks. Eventually a reduced leaf drops were observed in subsequent to week 6 where noticed no significant difference on leaf drops (Fig. 4.1).

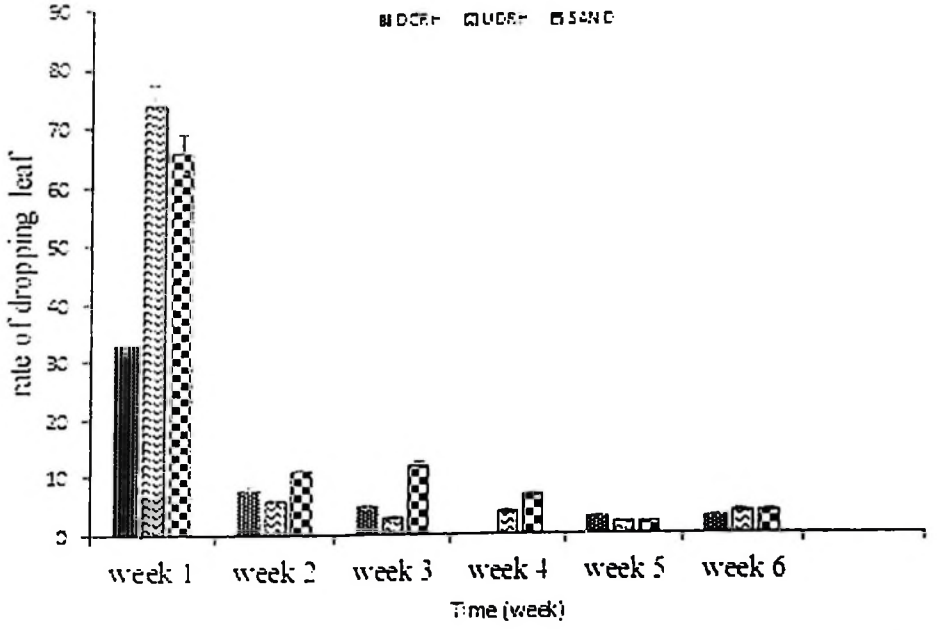

Figure 4.2: Effect of rooting media on rate of leaf defoliation of wild coffee stem cuttings

Plate 4.2: Wild coffee stem cuttings planted in undecomposed rice husks

4.4.2 Effect of rooting media on survival of wild coffee cuttings

Total number of planted cuttings remained green and active throughout the experiment without wilting sign or drying were counted and reported. Proportional of 90 % cuttings survived in sand media, 80 % survived in undecomposed rice husk media (UDRH) while only 75 % were able to survive in decomposed rice husk (Table. 4.1). Sand demonstrated better performance on number of surviving regenerating cuttings compared to other two rooting media. These variations observed in survived cuttings between propagated rooting media were significantly difference at $P \leq 0.05$ (Appendix 6), where high numbers of cuttings survived in sand media. Lowest numbers of survival cuttings were observed in undecomposed rice husk media as shown in (Table. 4 1).

Table 4.1: Mean number of cuttings survived in different rooting media

Treatment	Survival
Decomposed rice husks (DRH)	75.3a
Sand	92c
Undecomposed rice husks (UDRH)	82d
Mean	83.10
Standard error of mean	0.742
Standard error of variance	4.35
%CV	1.5
P value	0.01
F test	Significant

Means followed by the same letter in the same column show insignificant different at $P \leq 0.05$.

4.4.3 Sprouted cuttings

Cuttings were observed to be sprouted when they had one or two buds exceeding 0.5 cm in length, results indicated that there was high number of sprouted cuttings in the sand rooting media. Over 50 % of propagated cutting in sand media sprouted with production of new flushes of young leaves, but in decomposed rice husk 28 % had sprouted cuttings. Undecomposed rice husk had 24.16 % of sprouted cuttings (Fig. 4.3).

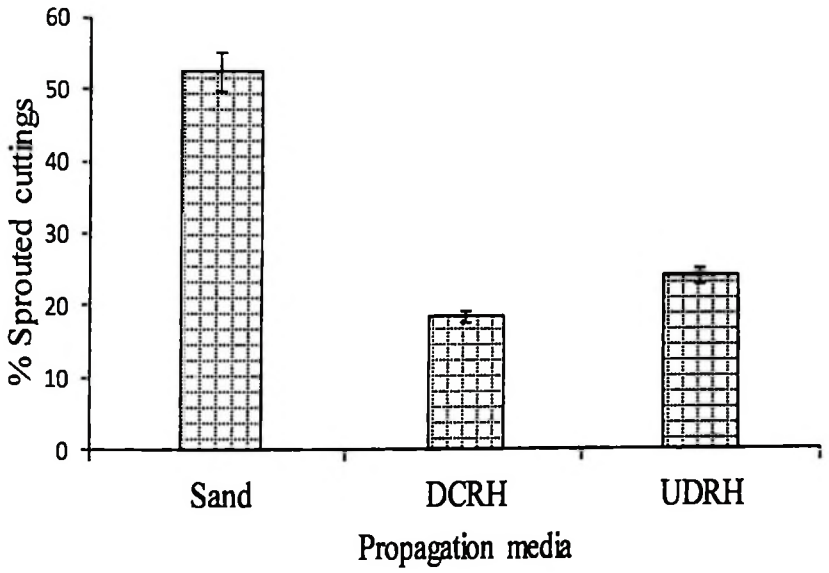

Figure 4.3: Proportional of sprouted cuttings of Kihansi wild coffee from different propagation media

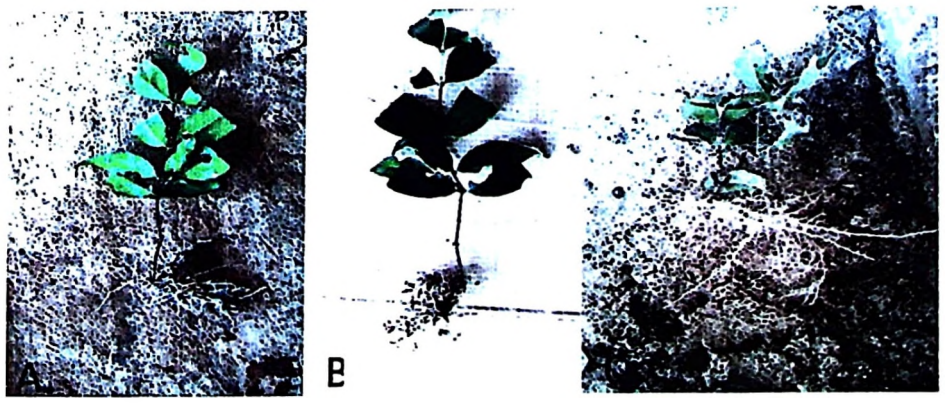

Plate 4.3: Wild coffee stem cuttings [A]undecomposed rice husks [B]decomposed rice husks and [C]sand as propagation media

4.4.4 Effect of propagation media on number of roots of cuttings

Non-significant difference was detected in number of roots per cutting in different rooting media ($P \leq 0.05$) (Appendix 3). However, for cuttings raised in undecomposed rice husks had a slight higher mean number of roots per cutting (2.6) followed by sand media (2.4) while the lowest numbers of roots (2.2) were observed in decomposed rice husks rooting media (Table 4.2).

Table 4.2: Mean comparison of number of roots

Treatment	Number of roots
decomposed rice husks (DRH)	2.2a
Sand	2.4a
Undecomposed rice husks (DRH)	2.6a
Mean	2.4
Standard error of mean	0.098
%CV	38.8
P value	2.33
F test	Not significant

(Means followed by the same letter in the same column are not significant different at $P \leq 0.05$)

4.4.5 Root length

Significant difference was observed in root length of wild coffee cuttings in different rooting media (Appendix 4). Decomposed rice husk shows high mean root length (11.37 cm), and undecomposed rice husk had lowest value of root length (8.17cm) below the sand media (9.04 cm) (Table 4.3).

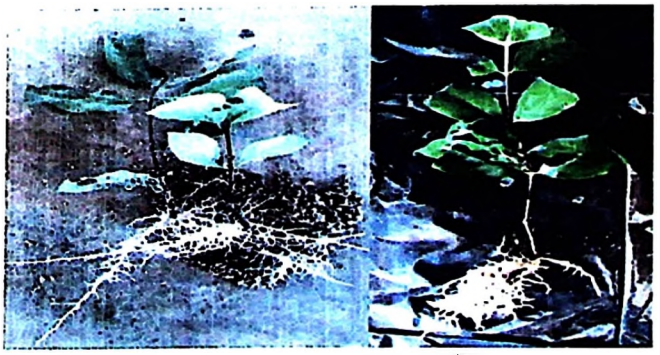

Plate 4.4: Stem cutting of Kihansi wild coffee with different root length propagated from sand (left) and undecomposed rice husks (right)

Table 4.3: Mean comparison of root length (cm) in different propagation media under Duncan multiple range test (DMRT)

Treatment	Root Length (cm)
DRH	11.37b
Sand	9.04a
UDRH	8.17a
Mean	9.7
Standard error of mean	0.258
Standard error of variance	1.073
%CV	21.3
P value	0.01
F test	significant

Means followed by the same letter are not significant different at $P \leq 0.5$.

4.5 Discussion

The significant performance of cutting on sand rooting media could be attributed to better aeration and water drainage. High aeration is responsible for promoting root development and cutting survival (Olabunden and Fawusi, 2003; Amri *et al.*, 2009). Also, rooting performance depends on the type of medium and its physical properties used in the propagating structure. The beneficial effect of root formation in stem-cuttings may be affected by physical and chemical characteristics of rooting substrates including media texture, structure, bulk density, porosity, water-holding capacity, pH, temperature and availability of oxygen (Kapadiya *et al.*, 2017). Therefore, the type of media used can have a major effect on the rooting and growth of cuttings. An appropriate media generally has to have an optimal volume of pore space and adequate oxygen diffusion rate for root respiration (Fonteno and Nelson, 1990). According to Caron *et al.* (2000), media physical properties should not be constrained to just measurements of air filled porosity, water holding capacity and bulk density, but also good aeration. The highest number of survival cutting, rooted cuttings and sprouted cuttings recorded in sand may be attributed to such characteristics (Ali, 2014).

Although, these observations indicated that it is possible to propagate wild coffee species using sand as propagation media, the study observed that cuttings with short root usually obtained. This could be attributed to lack of enough nutrients for roots elongation. Shiembo *et al.* (1999) and Tsobeng (2011) suggested that a good rooting media should contain substantial levels of nutrients for better initiation of rooting. Therefore, this justify the high success rate of sand as rooting media achieved with applied rooting hormone which help in root initiation process of cuttings essential for vegetative propagation. Rooting hormone enabling faster production of rooted cutting material which is essential for vegetative propagation (Fogaca and Fett-Neto, 2005). Also, rooting hormone is known to increase rooting regeneration and rooting time together with uniformity of rooting (Hartmann *et al.*, 2002).

Undecomposed rice husk and sand had high rate of leaf drops in first week. This was attributed by the effect of temperature and moisture conditions. Initially, cuttings lack roots for water uptake under high temperature condition. Therefore high transpiration occurred hence, plant dropped leaves in order to minimize water loss. Moore *et al.* (1975) reported that for better stem cutting development temperature should not exceed 28 °C. In this study sometimes maximum temperature was recorded as higher as 30 °C which is beyond the favorable temperature for cuttings development (25 °C to 28 °C). However, polythene cover maintained mist sprayed therefore, played significant reduction of leaf drops.

4.6 Conclusion and Recommendations

4.6.1 Conclusion

In propagation of Kihansi wild coffee for easy root formation and high survival rate of planted cuttings, sand could be recommended as propagation media of this coffee. For high root number and with optimal survival rate and easy availability of undecomposed

rice husks could be suggested to replace sand medium for propagation of cultivated commercial cultivar. this was due to due to their similarity and their performance in root growth and development during cuttings propagation.

4.6.2 Recommendations

From the results obtained from this study the following could be recommended. Sand is the best media and should be recommended for initiation of roots and development of cuttings of Kihansi wild coffee however, to obtain high number of roots and have optimal survival rate undecomposed rice husk could be used in replacement of sand. However, more research is needed in combination with different media to increase the efficiency of rooting media for propagation of wild coffee.

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CHAPTER FIVE

5.0 MORPHOLOGICAL DIVERSITY OF KIHANSI WILD COFFEE (*Coffea Kihansiensis*)

Abstract

Coffee belongs to genus *Coffea* in family Rubiaceae and is mostly grown in tropical and subtropical regions. Coffee genus comprises 103 species of which *Coffea arabica* L. and *Coffea canephora* L. are the most important commercial species. *Coffea arabica* is a commercially important high-quality coffee with low genetic diversity. Of recent, a discovery of wild coffee variety (*Coffea kihansiensis*) in Tanzania widens a genetic source for improvement of cultivated coffee. However, genetic characteristics and utilizations of wild coffee require characterization of genotype in Kihansi gorge. Morphological variability evaluated at different sites of Kihansi wild coffee garden were carried out using coffee descriptors based on IPGR. Hence, this work presents the morphological diversity of Kihansi wild coffee and elucidating traits for domestication and genetic improvement. Results showed morphological variability. 62.52 % of the total variation from vegetative morphological traits and 72.42 % of total variations from reproductive morphological traits. Plant height, branching habit, plant habit, angle of insertion of primary branches, berry and cherry size showed significant morphological variations. These results suggest potential of these traits in grouping and selection for coffee improvement and or used as cultivars. Conservation should be based on the morphological variation observed. Morphological variations reported in these coffee species should be confirmed if it is genetic. Hence, molecular analysis is highly needed.

5.1 Introduction

Coffee belongs to genus *Coffea* with two species of economically importance, *Coffea arabica* L. which is self-sterile species and *Coffea canephora* which is self-fertile

however, 70 % of total coffee production is covered by *Coffea arabica* (Alemayehu *et al.*, 2010). Coffee stand second after oil in terms of international market trading (Vega. 2008) and plays economical significant role globally as well as serving as major source of foreign earning in many producing countries (Louise *et al.*, 2008), an estimated 125 million people in Latin America Asia and Africa depend on it on their livelihoods (Osorio, 2002). Despite many challenges accoutered in coffee its production increases over past 50 years (ICO, 2014) though it is difficult to maintain this production trend due to many raised problems like increase in production cost as well as higher incidence of pest and diseases. Plant breeders try to mitigate these challenges through developing improved varieties with desirable characteristics like (larger seed size, high yielding, pest and disease resistance) preferred by coffee growers.

Breeders evaluate materials from genetic resources in available germplasm and incorporate into cultivated varieties. Surprisingly there is low genetic variability of coffee due to narrow geographic origin, self-fertilizing nature, and the historical or selective bottleneck in its agriculture adoptions (Terresa *et al.*, 2010) posing limitation to coffee improvement (Chaparro *et al.*, 2004).

Therefore, there is a need to find a way of adding up the value of the genetic resource in available germplasm that can be used as materials for coffee genetic improvement. Crop wild species are less or more closely related to adapted crops and reported to be as rich in genetic resource for coffee improvement (Sotowo *et al.*, 2013). They may be critical source of genes for pest and disease resistance. However, the morphological and genetic diversity of these wild and natural occurring species in poorly known, hence may limit its utilizations by plant breeder for crop improvement.

Kihansi wild coffee was discovered by Davis *et al.* (2004) in restricted blocks along Kihansi gorge. Like, other coffee wild species, Kihansi wild coffee specie can harbor the potential genes for coffee genetic improvement. There is need for systematic variability analysis to quantify the level of variations in this wild coffee specie.

Study on morphological characters provides an efficient tool to select the most important characters to be considered in breeding program. For coffee a number of descriptors have been developed to study the variation among coffee germplasm accessions (IPGRI, 1996; Sukshkumar *et al.*, 2013). Descriptors are used to discriminate phenotypic characters of germplasm materials. They show distinctive characters of accessions and the highly heritable characters that are easily seen by eye and are equally expressed in all environments. The study of the morphological characters provides an efficient useful tool to select the most important characters for inclusion in breeding programmes.

Morphological analysis complements to genomics analysis (Kasem *et al.*, 2010, 2011) as strategies for increasing utilization of coffee wild species in plant improvement also allow the screening of larger population for genetic analysis. Kathurima (2012) reported that morphological variability in coffee plantations is adverse to the product quality hence should be considered. Therefore, this study aims to characterize morphological variations that exist within the Kihansi wild coffee (*Coffea kihansiensis*), this will certainly boost the identification of variation existing within this specie and probably the use of these variations in commercial coffee improvement and or domestication of this specie.

5.2 Materials and Methods

This study was conducted at Kihansi gorge located at Udzungwa mountain Southern part of Morogoro region in Tanzania. A survey was carried out in-situ along sites at Kihansi where delineated patterns of natural gardens of wild coffee (*C. kihansiensis*) are

distributed over different altitudes along Kihansi gorge distributed in four separate blocks based on altitude and location of the hills namely: Lower Upper Spray, Upper Spray Forest, Upper Spray Mhalala and Lower Spray wetland. Based on survey carried out, morphological descriptors applied on randomly selected 231 samples (Appendix 1).

5.2.1 Plant materials

Kihansi wild coffee population accessions located at four sites in gorge were used as individuals for plant morphological variation characterization. A total of 231 wild coffee accessions out of 5400 plant population from four sites were used. Morphological characterization was done by assessing each plant sample using 24 descriptors developed by International Plant Genetic Resource Institute (IPGRI, 1996) and by Waryaroo (2006) (Appendix 7). About 35 coffee morphological descriptors of agronomic importance (Appendix 7) were used as described by International Plant Genetic Resources Institute (IPGRI, 1996). Coffee plant age groups and plant growing stages were considered in morphological characterization. Both vegetative and reproductive phases of wild coffee plants were used in individual sampling for qualitative and quantitative character analysis. Descriptive analysis was performed for distributions of 231 accessions out of 5400 plant population from the four major coffee garden sites at Kihansi.

5.2.2 Data analysis

Data collected from describing vegetative and reproductive parameter were presented using box plots with respective standard deviations from means for each scored descriptor. In addition, Multivariate analysis was performed using Genstat software 16th Edition. Important traits in each principal component that significantly contributed to the variation observed were identified as suggested by Jonson and Wichern (1998).

5.3 Results

5.3.1 Distribution and variability of morphological traits

Analysis of variability of morphological parameters (vegetative and reproductive) was performed and presented in box plot (Fig. 5.1). Branching habit, plant habit, fruit length, fruit width, fruit thickness, leaf width, seed width and seed weight show variations among the accessions. Wide distribution was detected corresponding to high standard deviation from the general mean. Angle of insertion of primary branches, leaf apex shape, leaf shape, and young leaf color and plant height showed no variability among the accessions. Seed thickness and seed length show very small insignificant variations among the accession compared to other parameters (Fig. 5.1).

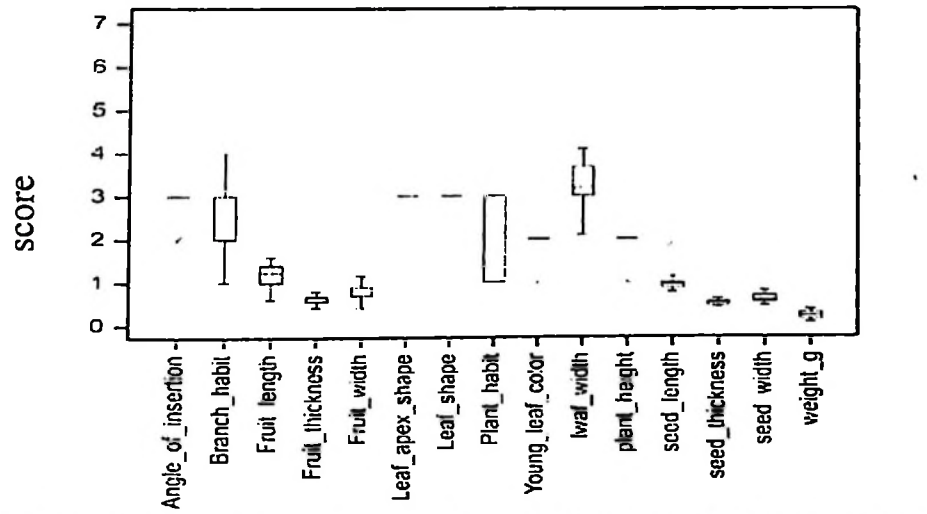

Figure 5.1: Variation and distribution of Kihansi wild coffee (*C. kihansiensis*) accessions among morphological descriptors assessed

5.3.2 Distributions of vegetative morphological descriptors

5.3.2.1 Plant habit

In this study, 231 wild coffee accessions were distributed into only two categories, shrub and bush type. More than 87 % of the observed populations was less than 5 m in terms of

height with no distinct trunk and classified as shrub. Remaining 13 % was bush type with less than 5 m but with possession of one or more trunk (Fig. 5.2).

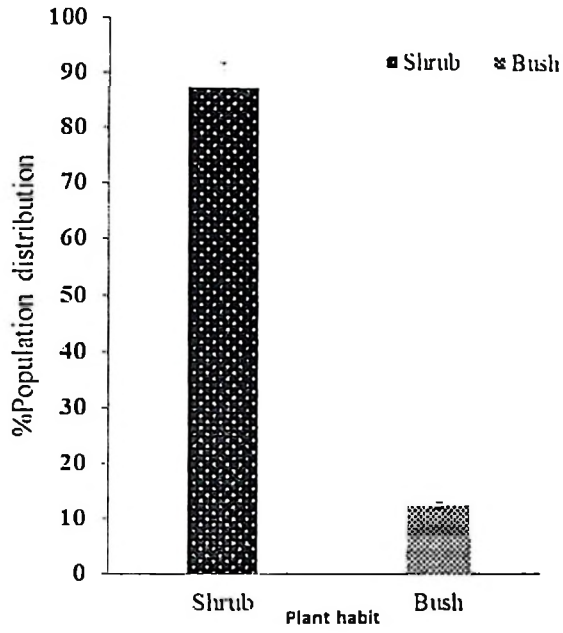

Figure 5.2: Distribution of Kihansi wild coffee 231 plants population in Plant habit morphological traits.

5.3.2.2 Plant height

Observation show that four categories of accessions was present. very short with length less than 0.5 m were 49 %, tall plant with characteristics of having length between 1m and 2 m were 34 % , very tall plant with higher than 2 m were 12 %. Only 4 % are short plants (Fig. 3.3 and Plate 5.1).

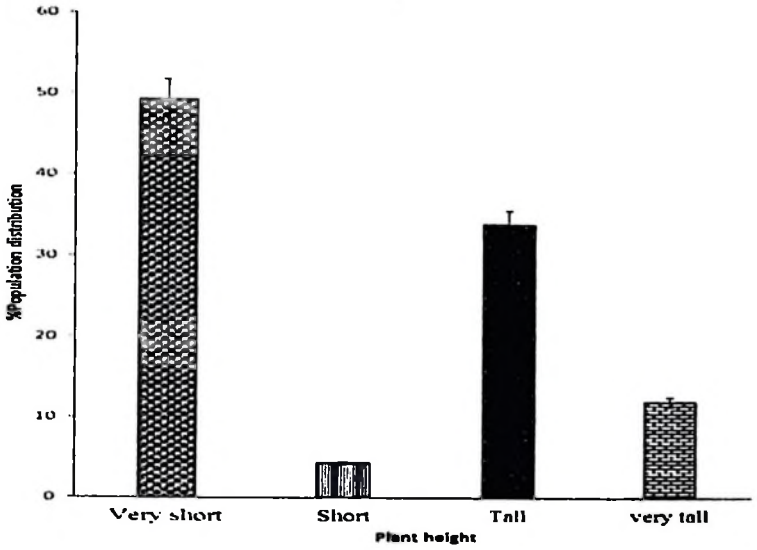

Figure 5.3: Distribution of 231 accession of Kihansi wild coffee in different categories of plant height

Plate 5.1: Plant height (very short and tall) of Kihansi wild coffee accessions observed

5.3.2.3 Branching habit

Basing on branching habit distribution of the 231 accessions observed, 36.4 % population has many branches with few secondary branches, 32.5 % has many primaries branches, and 17.8 % had very few primary branches while remaining 13 % had many primary branches with many secondary branches (Plate 5.2 and Fig. 5.4).

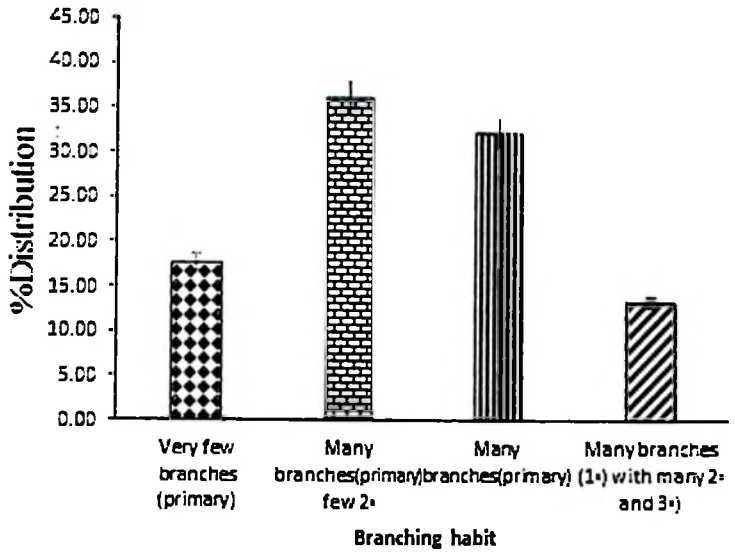

Figure 5.4: Distributions of 231 accession of Kihansi wild coffee to categories of branching habit

Plate 5.2: Branching habit (Many branches and few branches) observed in Kihansi wild coffee accession

5.3.2.4 Angle of insertion of primary branches

Observation show that more than 66 % of population accessions were characterized as semi-erect in terms angle of branches in its primary branches, and 33.33 % population characterized as having horizontal type of angle of insertion. Over three quarter of individual in this population has primary branches which are semi-erect and remaining quarter has horizontal branches (Plate 5.3 and Fig. 5.5).

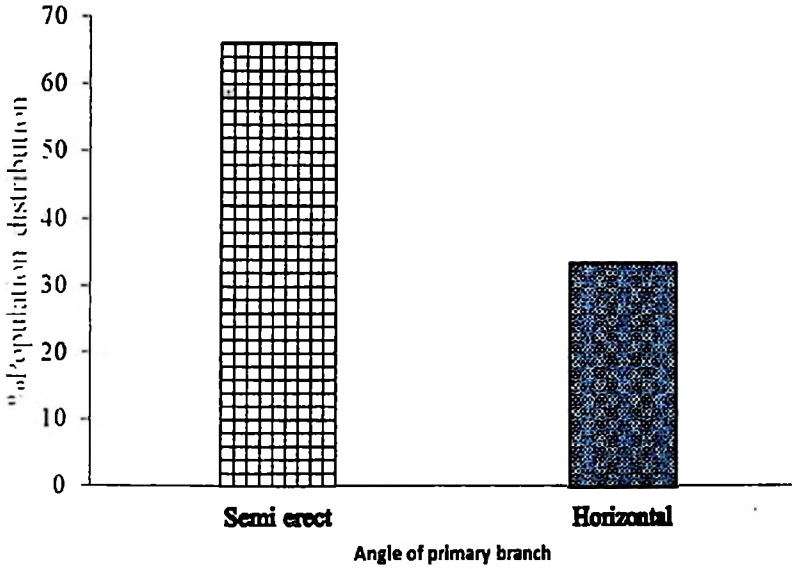

Figure 5.5: Distribution of population of 231 accessions to angle of insertion to primary branching

Plate 5.3: Branching habit (Horizontal and semi- erect) of Kihansi wild coffee

5.3.2.5 Young leaf color

Colors of young leaves were characterized as green (88.74 %), brownish type (6.5%) and remaining 4.8% were greenish. Young leaf color dominated in whole population (Fig. 5.6)

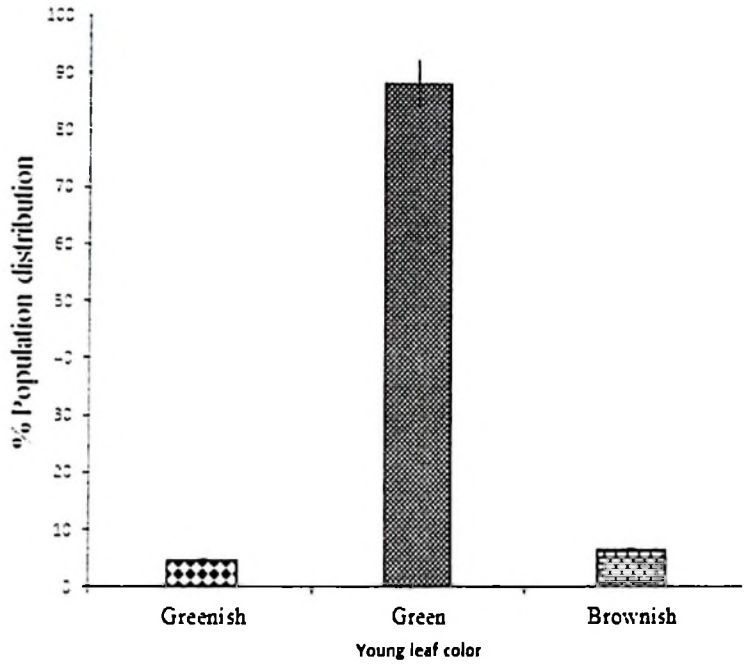

Figure 5.6: Distribution of 231 accessions of wild coffee to leaf color morphological characteristics

Plate 5.4: Young leaf colors (brownish and greenish) observed in Kihansi wild coffee accessions

5.3.2.6 Leaf shape

In this study, elliptic and ovate shape type were observed (Plate 5.5) from all population. However, elliptic type of leaves dominated for over 97 %, remaining population of 3 % observed as ovate type shape (Fig. 5.7)

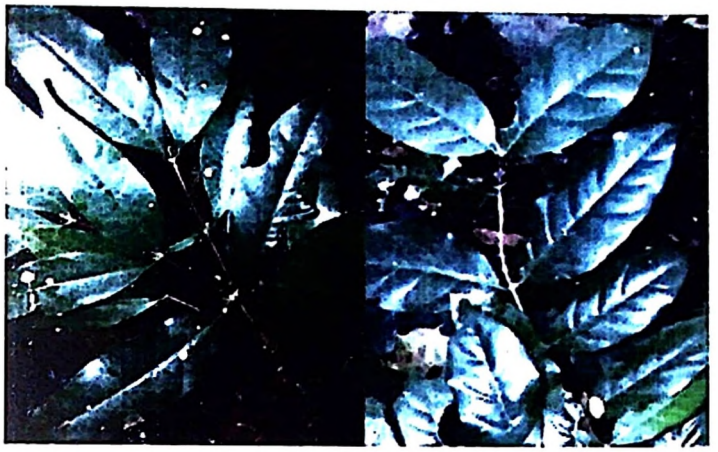

Plate 5.5: Different leaf shape (Elliptic-left and ovate-right) observed in Kihansi wild coffee population accessions

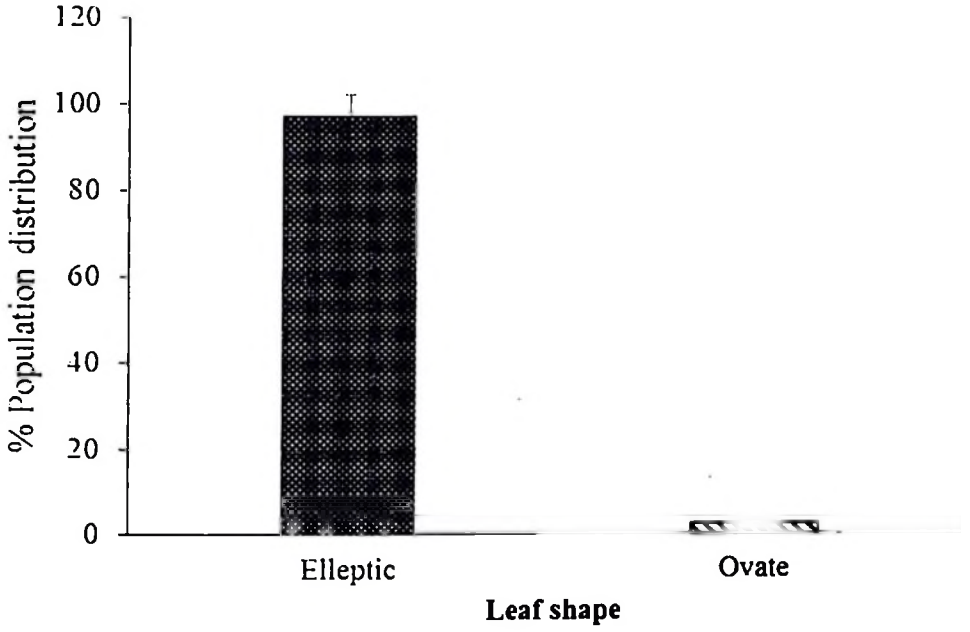

Figure 5.7: Distribution of Kihansi wild coffee of 231 accessions into leaf shape morphological characterization

5.3.2.7 Leaf apex shape

Fewer number of accessions were characterized into obtuse types, observing the leaf apex shape they were 6 (1.3 %) of total accession observed under this study. The majority of accessions observed 225(98.7 %) were categorized to acute shape of leaf apex (Fig. 5.8).

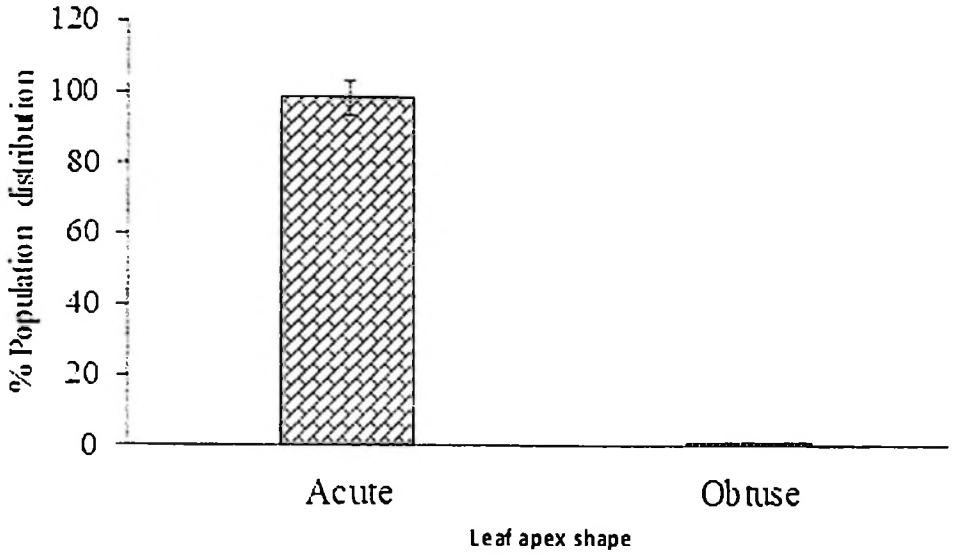

Figure 5.8: Kihansi wild coffee plant accessions distribution in leaf apex shape

5.3.2.8 Leaf width

Characterization of Kihansi wild coffee species based on leaf width were categorized into three different groups, 80.95 % of accessions had leaf width between 3 cm and 4 cm and 15.15 % had mean leaf width of greater than 4 cm while 3.9 % were accessions with less than 3cm mean leaf width (Fig. 5.9).

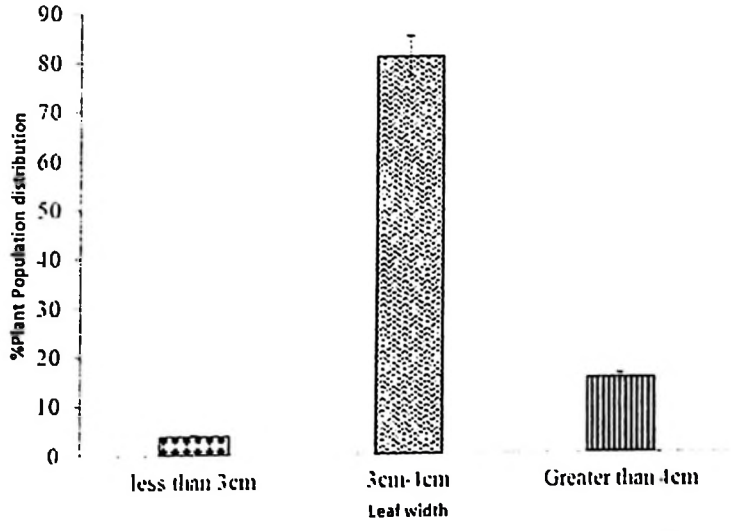

Figure 5.9: Distribution of Kihansi wild coffee accession to leaf width morphological qualitative trait

5.3.3 Qualitative reproductive morphological descriptors

5.3.3.1 Fruit shape

Morphology of the fruits were assessed in terms of fruit shape whereby 66 % of total accession produced roundish shaped fruits, and 34 % of fruits had oblong shape. High percentage of oblong type fruits was found in the upper wetland plots and the rest were found in lower wetland and lower upper (Fig. 5.10).

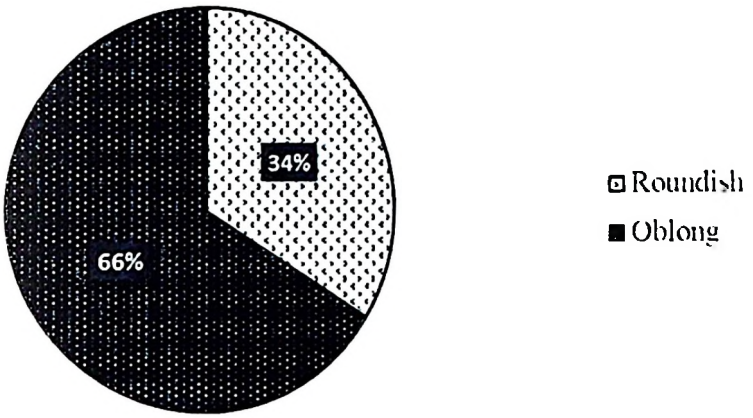

Figure 5.10: Distribution of wild coffee accession into fruit shape categories.

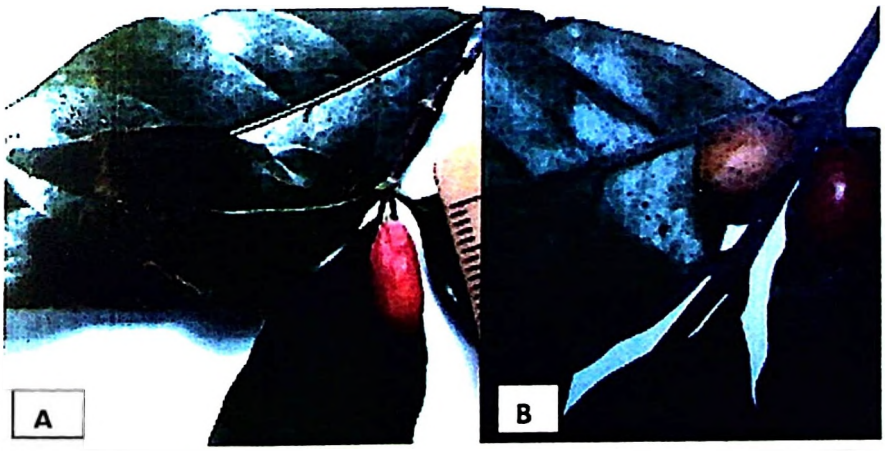

Plate 5.6: Fruit shape (A: oblong B: Oblong) of matured Kihansi wild coffee accessions

5.3.3.2 Fruit color

The study found no variations of fruits color within accession of Kihansi wild coffee. Orange fruit color dominated. However, the fruit color changed during the fruit development from yellowish to full yellow and then to orange upon maturation (Plate 5.7).

Plate 5.7: Fruit color of mature A: unripen, B: matured ripened and C: dried fruits of Kihansi wild coffee

5.3.3.3 Inflorescence position

Also, inflorescence was arranged on the axillary position of the branches while no inflorescence on the terminal position of the branches observed. Observation also shows the absence of inflorescence in the old wood, demonstrated that 100% had inflorescence on the primary and secondary branches (Plate 5.8).

Plate 5.8: Inflorescence position (axillary) as observed in vertical branches of Kihansi wild coffee plants accessions

5.3.3.4 Fruit filling coefficient

The ratio of cherry number over berry number in all accessions assessed, 100 % had complete fruit filling ratio, 1:1 of cherry and bean number.

5.3.4 Quantitative reproductive morphological descriptors

5.3.4.1 Variation in fruit dimension

Observation showed a wide distribution of fruit size in length and width. Minimum seed length observed was 6.6 mm while maximum fruit length was 16 mm and overall means length was 12.2 mm (Table 5.1). Also, fruit width ranged from 2 mm to 11.6 mm with overall mean of 8.2 mm. Fruit thickness was observed in a narrow range of 4.2 mm to 7.9 mm.

Slightly variations between the maximum and minimum seed size were observed in wild coffee accessions. Seed length, seed width and seed thickness had minimum value of 7.7 mm, 4.5 mm and 4 mm respectively while maximum value with 19 mm, 7.9 mm and 6mm. A wide variation of distribution of accessions observed on single berry weight recorded of 0.06 g as minimum and 0.5 g as maximum berry weight (Table 5.1).

Table 5.1: Fruit and seed dimension of Kihansi wild coffee accessions indicated by range of minimum and maximum value with general mean.

Parameter (mm)	Minimum – Maximum	Mean
Fruit length	6 - 1.6	12.2
Fruit width	2 - 1.16	8.2
Fruit thickness	4.2 - 7.9	6.2
Seed length	7.7 – 19	9.4
Seed width	4.5 - 7.9	6
Seed thickness	4 – 6	5.1
Single berry weight	0.6 – 5	2

5.3.4.2 Comparison a of bean size of Arabica coffee in relation to Kihansi wild coffee

The coffee seed are elliptical or egg shaped. possessing the longitudinal furrow on the plane surface and the outer covering is formed by the hard-pale brown endocarp (Eira *et al.*, 2006). Measurements done with many seeds indicate that coffee Arabica seed are 10 mm to 18 mm long and 6.5 mm to 9.5 mm wide (Eira *et al.*, 2006). The bean size of Kihansi wild coffee showed roundish shape or oblong type of fruits with soft brownish endocarp. Seed of Kihansi wild coffee had 7.7 mm to 19 mm long with 4.5 mm to 7.9 mm wide (Table 5.2).

Table 5.2: Comparison of bean size for *Coffea arabica* L., *Coffea racemose* and *Coffea kihansiensis*

Species of coffee	Seed length (mm)	Seed width(mm)	Reference
C. Kihansiensis	7.7 to 19	4.5 - 7.9	Observation
Coffee arabica L	10 to 18	6.5-9.5	Eira *et al.* (2006)
Coffee racemose	3 to 3.5	5 to 7	Eira *et al.* (2006)

5.3.4.3 Principal component analysis

The principal component analysis (PCA) and percentage contribution of each component to the total variation in qualitative morphological traits are presented (Table 5.4). The results showed that 4 principal components with Eigen value greater than a unit accounted for 62.52 % of the total variations in the populations were obtained with relative discriminating power of PCA as revealed by Eigen values of 1.64 (highest) in PCA 1 and 1.02 (lowest) in PCA 4. The first principal component accounted about 20 % of the total variations whereby second PCA accounted 15.86 % of the total variations. The highest contribution to the variations was shown in plant growth habit (0.54), plant height (0.51), and branching habit (0.44) in PCA 1 while leaf width (0.68), branching habit (0.42) and

angle of insertion of primary branching had the highest component in PCA 2 (Table 5.3). Young leaf color (0.52), angle of insertion (0.35) and branching habit (0.32) also shown to be more in contribution to the total variations in PCA 3(Table 5.3).

Table 5.3: Four first principal components (PCA) of Eigen value greater than a unit morphological (vegetative) descriptors

Traits	PC1	PC2	PC3	PC4
Plant habit	0.54	0.14	0.04	0.25
Plant height	0.51	0.16	0.24	0.27
Branching habit	0.44	0.42	-0.32	0.08
Angle of insertion	-0.42	0.37	-0.35	0.22
Young leaf color	0.19	-0.19	-0.52	-0.32
Leaf shape	0.14	-0.25	0.05	0.84
Leaf apex shape	-0.11	0.28	0.67	-0.06
Leaf width	-0.12	0.68	-0.09	0.08
Eigen value	**1.64**	**1.2**	**1.12**	**1.02**
% variation	**20.29**	**15.86**	**13.92**	**12.65**

Some individual accessions are quite dispersed in a biplot graph (Fig. 5.11) For example accessions 50, 48, 227 in branching habit; 208, 81, and 209 in leaf width; 228, 219, 140 and 147 in plant habit. A large proportional of studied accessions showed similarities by concentrating together as indicated in biplot (Fig. 5.11).

Moreover, results showed that 72.42 % of the total variation is contributed by the reproductive morphological descriptors in first four principal components. Principal component 1 accounted 20.29 % of the total variation; whereby fruit length is 0.28, fruit thickness -0.56, and seed width 0.42. These traits contribute greatly than other descriptors, fruit thickness contributed highly but negatively.

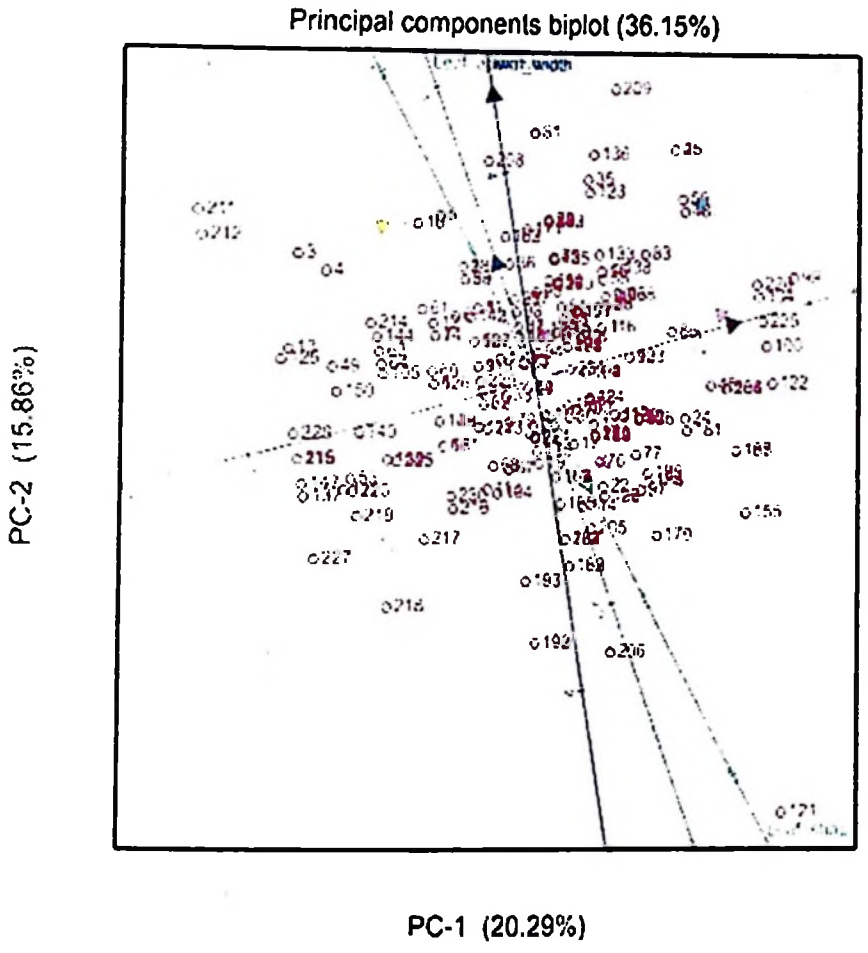

Figure 5.11: Biplot of two PCA for vegetative morphological descriptors

Principal component 2 accounted about 15.86 % of the total variations with seed length of 0.51, seed thickness 0.66, and weight of single berry 0.40 contributed more to the total variation compared to other descriptors in this component. In comparative to PC3, 15.17 % of the total variations contributed by this component where width (0.79) fruit, and seed width (0.45) had more contribution to the variations in this component. The discriminating power shown by highest Eigen value (1.95) in PC1 and lowest Eigen value (0.96) in PC4. Highly dispersion of characters in quantitative reproductive descriptors was observed in biplot of first and second PCA (Fig. 5.12); fewer individuals were confined at the center.

Table 5.4: Eigen value and percentage variations of the four first PC of quantitative morphological (reproductive) descriptors

TRAITS	PC 1	PC2	PC3	PC4
Fruit length	0.40	0.09	0.28	0.38
Fruit thickness	-0.56	0.13	0.12	0.02
Fruit width	0.20	-0.18	0.79	0.24
Seed length	0.28	0.51	0.21	-0.60
Seed thickness	0.31	0.66	-0.21	0.25
Seed width	-0.42	0.29	0.45	-0.27
Weight (g)	-0.38	0.40	0.01	0.55
Eigen Value	1.95	1.16	1.07	0.96
%Variation	27.51	16.30	15.17	13.44

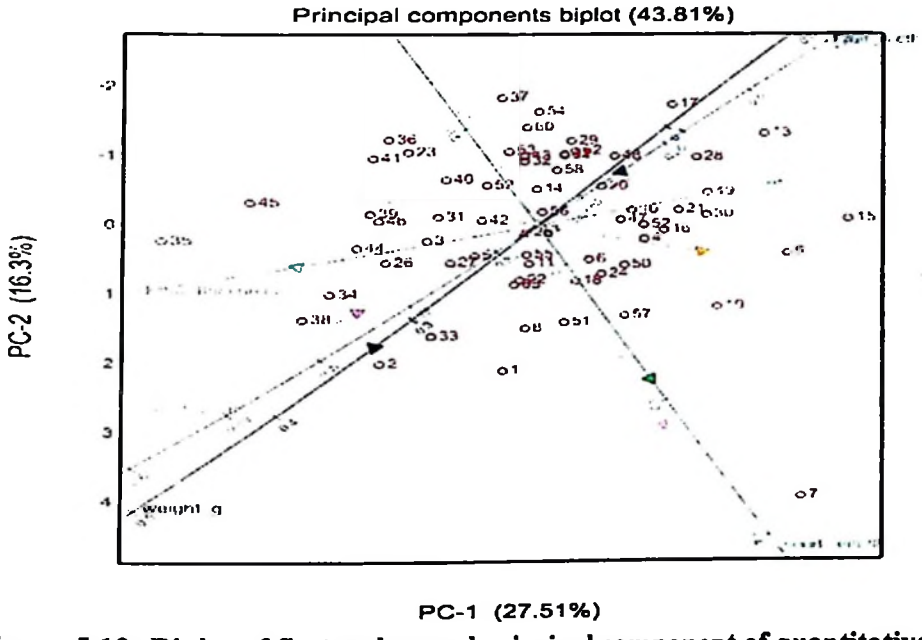

Figure 5.12: Biplot of first and second principal component of quantitative morphological (reproductive traits)

5.3.4.4 Correlation of morphological descriptors of *Coffea kihansiensis*

Among descriptors; fruit length, fruit width, seed thickness, seed width, fruit thickness and single beery weight showed less with a positive correlation. A positive and significance correlation observed between fruit thickness and fruit width (r= 0.4) was also observed between single berry weight and fruit thickness (r= 0.3). However, most of descriptors showed negative correlation with each other as fruit width had negatively

correlation with all other parameters (Table 5.5) Seed width had negative correlations with seed thickness. Fruit thickness and seed thickness also had negatively correlations to each other.

Table 5.5: Correlation of seed and fruit dimension as reproductive morphological descriptors. FL=Fruit Length (cm), FW=Fruit width (cm), FT=Fruit Thickness (cm), SL=Seed Length (cm), SW=Seed Width (cm), ST=Seed Thickness (cm), SBW=Single Berry Weight (g)

	FL	FW	FT	SL	SW	ST	SBW
FL	1						
FW	0.174	1					
FT	-0.194	-0.194	1				
SL	0.101	-0.168	0.101	1			
SW	-0.116	-0.002	0.405**	0.03	1		
ST	0.2	-0.046	-0.227	0.218	-0.11	1	
SBW	-0.171	-0.049	0.321*	-0.125	0.184	0.047	1

*, **Correlation is significant at probability value of 0.05 and 0.01 respectively.

5.4 Discussion

5.4.1 Distribution of vegetative morphological characteristics of *Coffea kihansiensis.*

About 87 % of the coffee wild in Kihansi is bush type and only 13 % of the accession represented shrubs type. The garden is dominated by spatial distribution of short plants, tall plants and very tall plants within a population of KWC. This result can describe KWC population dominated by bush type with many short plants as supported Davis *et al.* (2004) who described the presence of plants with height range from 1.5 m to 3m length. Presence of horizontal and semi erect type of branches in trees indicated that there are two quite different of plant populations existing within KWC. Similar results were obtained by Davis and Mvungi (2004) observed horizontal to semi erect branches with grey to brown, slightly rough.

Over 88 % of population characterized, their young leaves were of green color. Small proportions of accessions were distributed to other groups of leaf color such as brownish. Very significant variations of plant accession were observed in leaf shape however, only two groups of these characters were observed as elliptical by 97 % and ovate only 3 %. In comparison to the findings obtained by Davis and Mvungi (2004), who characterized the leaf as elliptic to broadly elliptic and sometimes ovate to elliptic obovate with dimension of 3.5m-10m×1.5cm -5.5cm suggest the presence of the diverse accessions in this population. Although, this observed difference is phenotypically characterized.

5.4.2 PCA of vegetative morphology traits

Principle component analysis has shown that the plant habit, plant height, branching habit in PC1 and leaf width in PC2 provide maximum and significant contributions to the total variations. This observation is supported by data presented in boxplot which shows that branching habit, plant height, leaf width and plant habit had large standard deviations indicating a wide distributions and deviations of individuals from the mean population

Biplot of PC1 and PC2 showed individuals within a population of plants concentrated at the center of the biplot. This close relatedness is the indicator of the similarities among individuals within a population of KWC. However, some individuals observed dispersed far away from the center. Accessions close to plant habit line were 34, 220, 99, and 206, 208 and 81 were close to leaf width line. Accessions; 50, 48, 227 and 217 were close to branching habit showing distinctness from other individuals in a population. This is the indicator of distinctness and variability of individuals to some of the parameters used in the study.

In light of the results obtained from the PCA and box plot, it may be possible to deduce that more than half (62.52 %) of the variation obtained from vegetative morphological

parameters was primarily due to branching habit. plant habit. leaf width and plant height. These results corresponds to the findings by Gessese *et al.* (2015). Kebede *et al.* (2007) and Wrigley (1988) who evaluate the phenotypic diversity of Ethiopian coffee. They reported that 53 % of variations were attributed to plant habit. branching habit. young leaf color and number of nodes.

5.4.3 Reproductive morphological traits

Both qualitative and quantitative reproductive traits were accessed, only two distinct groups of accessions had oblong type of fruit shape and others of roundish type. However, in terms of fruit color, only orange fruit color was observed which developed from the greenish to yellowish up to orange upon ripening. Significant variation was observed in quantitative traits of this population. Fruit width (0.2 mm -11.6 mm), fruit length (6mm - 16mm), and fruit thickness (4.2 mm - 7.9 mm) shows wide distributions among the accessions with wide range (minimum value to maximum). This variability in different traits is proof to the existence of different accessions within a population and can be used as factor for clustering this population. These findings were partially agreed with that described by Davis and Mvungi (2004) who found the ellipsoid to ellipsoid- obvoid type of fruit shape existed in a KWC population with size of 9.5-12.6 mm × 5-9.2 mm. yellowish to orange berries at maturity. Seeds with 7.7-19 mm × 4.5-7.9 mm (length × width), 4-6 mm thickness observed in a population possibly attributed to environmental variations. These findings support previous study of diversity reported by Musoli *et al.* (2009), Cubry *et al.* (2008) and Cubry *et al.* (2005).

5.4.4 PCA of reproductive morphological parameters

First, second and third PC with an Eigen value of larger than a unit in reproductive morphological traits accounted about 72.42 % of the total variations where by fruit length,

fruit thickness, seed width and weight of single berry in PC1, seed length seed thickness and weight of single berry in PC2 as well as fruit width in PC3 contribute more to the total variation observed. It is possible to deduct that selection and clustering this population could be effectively if much consideration would be based on reproductive traits (fruit characteristics). These findings were supported by Tounekti *et al.* (2017) who worked on diversity of Arabica coffee in Saud Arabica. They found that the first 2 principal components PC1 and PC2 with percentage variation of 51 % and 15 % respectively with fruit length, fruit width, fruit thickness and berry weight contribute greatly to the total variation and were used as selection criterion.

According to Chahal *et al.* (2002), characters with largest absolute values closer to the unity within the first principal component PC1 and PC2 influence the clustering more than those with lower absolute values closer to zero. This was also later supported by Olika *et al.* (2011), who reported that coffee bean length, hundred bean weight, leaf length and leaf width contributed to the variation among Limmu coffee accessions. The existence of morphological diversity among coffee accessions was further confirmed by many authors who reported the variability of coffee accessions morphologically (Yigzow, 2005; Kebede, 2008; Bayetta, 1997 and Gichimu, 2010).

5.5 Conclusion and Recommendations

5.5.1 Conclusion

Morphological variation was detected in this population of Kihansi wild coffee in both reproductive and vegetative traits however, large variation were detected on reproductive morphological traits over reproductive traits, these traits were revealed in principle components and in box plots and will be used as significant morphological traits in

selection of accessions in improving productivity of coffee cultivar. Also, can be used to identify morphological traits using branching habit, leaf color, plant height and plant habit, angle of insertion of primary branches, berry size and cherry size.

5.5.2 Recommendations

Variations detected from this population of wild coffee had potential in breeding program of cultivated coffee. Effective selection for various traits from this population and combination of these parameters for breeding in cultivated coffee varieties could have enabled the potentially utilization of this wild species to coffee crop improvement and/or domestication. Numerous and more traits of important should be evaluated for their morphological variation in order to have more traits that can be used for coffee improvements.

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CHAPTER SIX

6.0 GENETIC CHARACTERIZATION OF KIHANSI WILD COFFEE (*Coffea kihansiensis*) USING MOLECULAR MARKERS

Abstract

Kihansi wild coffee (*Coffea kihansiensis*) has it is origin at Udzungwa mountain in Kihansi gorge. Its populations exist as wild species in Udzungwa forest. There is limited use of molecular genetic diversity information of Kihansi wild coffee in the coffee improvement programs. Thus, generating genetic diversity information is an important parameter in the future efforts of coffee genetic resources conservation and sustainable utilization. Wild species serve as new source of genes for developing disease resistance varieties, heat tolerance, and improvement of different agronomic characters. In this study the genetic diversity of *C. kihansiensis* collections were studied using 11 microsatellite (SSRs) markers. The results indicated low genetic diversity of this wild species. However, 97% of total variations were detected within population. *Coffea kihansiensis* with larger population genetic distances were clearly separated into four groups where by each group differ from another by 60 %. The detected variations correspond to morphological results observed. The results suggest the potential variation detected leading to utilization of the accessions in coffee improvement and conservation program. For future survival of this species proper management should be established, between population variation should be maximized through inter crossing of individual between population.

6.1 Introduction

Coffee belongs to the genus *Coffea* in the Rubiaceae family and is mostly grown in the tropical and subtropical regions (Berthaud and Charrier, 1988). Of the 100 species in the genus *Coffea*, *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* P. (Robusta

coffee) are the two most important commercial species with. *C. arabica* is mostly used due to its excellent quality attributes. Coffee is one of the most important crops second only to oil (Pendergrast, 1999), at the same time one of the most neglecting crops in the world with regard to genetic conservation (Teworde and Egziabher, 1990).

Coffee reported to have narrow genetic diversity especially for Arabica coffee which is autogamous (Orozco-Castillo *et al.*, 1994; Lashermes *et al.*, 1996; Carneiro, 1999; Anthony *et al.*, 2002; Stieger *et al.*, 2002; Raus *et al.*, 2003). Even though, the overall genetic diversity of *Coffea arabica* is believed to be less polymorphic as compared to its diploid relative species. The populations in its place of origin and diversity have high genetic variability based on different DNA-based molecular markers techniques (Lashermes *et al.*, 1995; Lashermes *et al.*, 1996; Lashermes *et al.*, 1997; Anthony *et al.*, 2001a; Anthony *et al.*, 2001b; Moncada, 2004). These populations exist in different forms: as wild coffee that are inaccessible and non-used, forest and/or semi-forest coffee and garden (landraces) coffees (Senbeta and Denich, 2006).

Genetic diversity of coffee can be assessed using different techniques that range from the traditional morphological techniques to the modern DNA-based molecular markers. A number of DNA-based techniques are in use in different coffee genetic studies. These include the conventional RFLP method (Crouzillat *et al.*, 2004) and the different PCR-based methods such as RAPD (Anthony *et al.*, 2001a; Anthony *et al.*, 2001b; Aga *et al.*, 2003; Cristancho *et al.*, 2004), AFLP (Anthony *et al.*, 2001a; Coulibaly *et al.*, 2001; Steiger *et al.*, 2002) and microsatellite (SSRs) markers (Lashermes *et al.*, 1995).

These molecular marker techniques have many advantages such as: not subjected to environmental factors and growth stage of the plant, and the potential of existing in unlimited numbers, covering the entire genomes (Weising *et al.*, 2005). Of the various

DNA based techniques, microsatellite (SSRs) markers are techniques used in the genetic study of plants. They are short tandem repeats of DNA sequence of one to six base pairs. Their use as a molecular marker has advantages over other techniques as it fulfills most of the good characteristics of genetic markers such as highly polymorphic and reproducible. locus specific and co-dominant.

Different studies have been done to access the genetic diversity of the coffee species. Lashermes *et al.* (1960) analyzed the genetic diversity among cultivated species and sub-spontaneous accession of *C. arabica.* Sylvian (1958) witnessed the existence of great variations among the wild coffee plants in Ethiopia. Although, a lot of work has been done to explore the variation existing in cultivated coffee species, less work has been done in wild coffee species in their natural occurring habitat may be due to lack of awareness, inaccessible as well as less attention to these species. In addition, these species may possess genes for coffee improvement. This work was aimed at utilization of the molecular DNA marker to explore the genetic variability of Kihansi wild coffee accessions.

6.2 Material and Methods

6.2.1 Plant materials

A total of 150 KWC plant leaf samples were collected from different coffee gardens in respective blocks such that, 50 accessions were collected each from upper wet land, lower wetland, and lower upper wetland in Udzungwa mountain in Morogoro. Collected young leaves from each site were stored in 1.5 ml Eppendorf tubes and kept in cool ice box before transporting to molecular biology laboratories located at SUA and kept at -20 ^{0}C prior for DNA extraction.

6.2.3 DNA extraction

DNA extraction was performed as described by Krizman *et al.* (2006) with little modification. Young leaves of 250 g were taken from first and second leaves of KWC plants and placed in 1.5 mls tube containing 2 g of sterilized washed sand and grounded using sterilized pestle in presence of 600 µl of extraction buffer as described by Krizman *et al.* (2006). The composition of buffer contain (1.21 g of Tris base, 0.74 g of EDTA, 8.2 g of NaCl, 2 g of CTAB) per 100 liter of distilled water with little modification by added 2 g of PVP, and 5 g of charcoal and 80 mls of βeta- mecaptoethanol both per 100 l of distilled buffer to remove phenols and to improve the purity of the coffee DNA during the extraction (Khanuja *et al.*, 1999). The ground leaf tissue solution was incubated in water bath at 65 °C for 30 minutes and regularly shaken three times during the incubation. Following incubation, the solution was inverted several times after adding 600 µl of Chloroform: Isoamyl- alcohol (24:1) to bind protein and centrifuged for 10 minutes at 14 000 rpm. The 500 µl of the top layer was removed into new clean tube and mixed well with 600 µl of ice cold isopropanol and 60 µl of ammonium acetate (7.5 M, pH=8.0) and incubated into freezer at -20 °C for 30 minutes for DNA precipitation. The content was centrifuged for 10 minutes at 14 000 rpm, carefully supernatant was removed without disturbing the pellet. Pellet was then washed with 600 µl of 70 % ethanol to remove salts and any supernatant. This was followed by incubation at -20° C for 7 minutes. The resultant solution was centrifuged at 7 000 rpm for 7 minutes and the supernatant removed. The pellets were eventually opened for air dry and remove any remaining alcohol. The pellet was finally dissolved in 50 µl 1×TE buffer ready to use or stored in -20 °C.

6.2.4 Genomic DNA analysis

Following DNA extraction, few samples were used for quantification and quality analysis using 0.8 % agarose gel prepared in 1× TAE buffer where 4 µl of DNA sample was

loaded for electrophoretic migration followed by addition of 5µl of ethidium bromide as staining agent for DNA fragments. For electrophoretic migration and quality control 5 µl of molecular DNA ladder was loaded parallel to KWC DNA samples in same 0.8 % agarose gel. Electrophoresis was run in 45 minutes and gel viewing was performed in dark room for good image and its picture was taken by digital camera (Fig. 6.1 and 6.2).

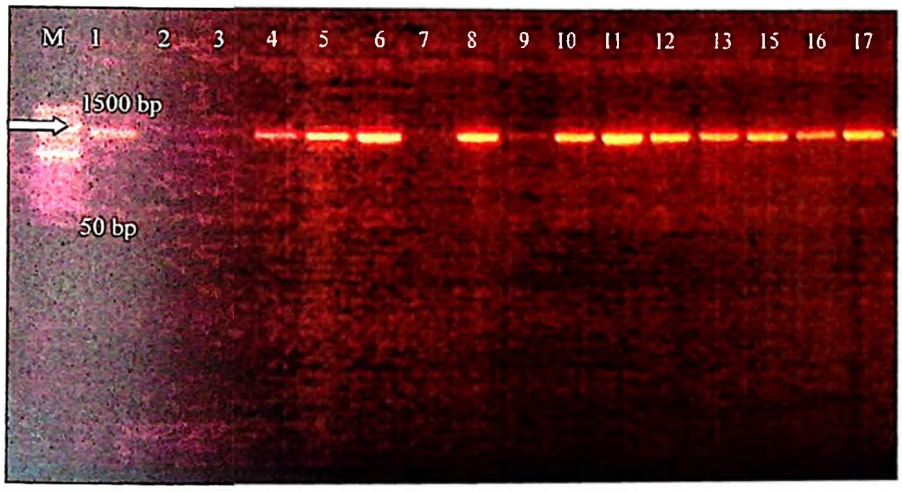

Figure 6.1: Genomic DNA from *Coffea kihansiensis* accession (Lower upper wetland garden) as observed on 0.8% agarose gel using 50 bp ladder

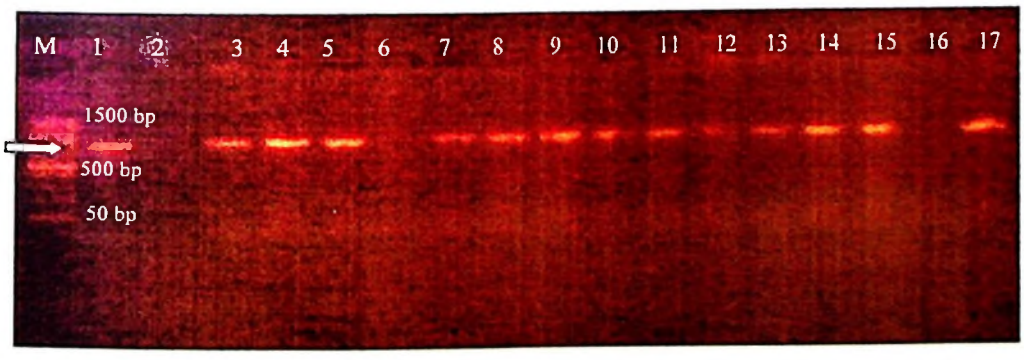

Figure 6.2: Genomic DNA from *Coffea kihansiensis* accession (upper wetland garden) as observed on 0.8% agarose gel using 50 bp ladder

6.2.5 PCR amplification

PCR was performed in 25 µl as total reaction volume containing 12.5 µl of AmpliTaq Gold® Master Mix (Applied Biosystems product), 9.5 µl od deionized water, 1 µl of forward primer (10 nM) and 1 µ of reverse primer (10 nM) and 1 µl of extracted template DNA. Total of 15 SSR primers were used. Amplification was carried out on a GenAmp® PCR system using the following program: 10 minutes of initial denaturation step at 94 °C, followed by 35 cycles of denaturing at 94 °C at 0.5 minutes., annealing at 50 °C at 0.5 minutes and extension at 72 °C for 1 minutes and final extension at 72 °C for 7 minutes.

Table 6.1: List of SSR molecular markers used for characterization of Kihansi wild coffee accessions

No	SSR locus code	5'.3' Forward primer/5'.3' Reverse primer	Source of organism	Reference
1	Ssr R105	F:CACCAATTCCACTGACAATG R:TCCCTGCCAACACACTTC	*C.canephora*	Maluf *et al.*, 2005
2	Ssr R126	F:GCACAATCACTCCCAAAG R:TGACGGCCTACTACTTACAG	C.*canephora*	Maluf *et al.*, 2005
3	Ssr CMA263	F:TGCTTGGTATCCTCACATTCA R:ATCCAATGGAGTGTGTTGCT	*C.canephora*	Maluf *et al.*, 2005
4	Ssr R278	F:TGTAGATTTGAAACCCAATC R:AAGTCTCGACAAGTTTTGAC	*C.canephora*	Maluf *et al.*, 2005
5	Ssr CMA008	F:CATTCTGGTCCTGATGCTCT R:TCATTCACTTATTAACGTCCATC	*C.arabica*	Maluf *et al.*, 2005
6	Ssr CMA055	F:TTGAGCAAAAACCCTATTCC R:TAAACCCAAAAAGACCACAA	*C.arabica*	Maluf *et al.*, 2005
7	ssr CM5	F: GTAACCACCACCTCCTCTGC R:TGGAGGTAACGGAAGCTCTG	*C. arabica*	Maluf *et al.*, 2005
8	ssr CM 8	F:GCCAATTGTGCAAAGTGCT R:ATTCATGGGGCCTTTGTCTT	*C. arabica*	Maluf *et al.*, 2005
9	ssr CM 16	F:TGGGGAAAAGAAGGATATAGACAAGAG R:GAGGGGGGCTAAGGGAATAACATA	*C. arabica*	Maluf *et al.*, 2005
10	ssr CM 17	F:CCAGCCTTTTCACAATTCTCACCC R:TGCCCCCTAGATATGGTACAAGCTTTC	*C arabica*	Maluf *et al.*, 2005
11	ssr CM 2	F:TGTGATGCCATTAGCCTAGC R:TCCAACATGTGCTGGTGATT	*C. arabica*	Maluf *et al.*, 2005

6.2.6 Gel electrophoresis

A 2% agarose gel was prepared by dissolving 2 g of agarose in 100 mls of 1× TAE buffer then heated in a microwave to dissolve the agarose powder completely until clear gelatinous solution with no bubbles was homogenized. Volume of 2 µl of Ethidium bromide was added in 100 mls of agarose solution after cooling the agarose. A volume 5 µl of PCR products were loaded in the wells with 5µl of ladder of 50 bp serve as reference of DNA molecular size. Electrophoresis was run at 140 v for 1:00 hour, and then gel picture was taken for bands scoring.

6.2.7 Data analysis

The polymorphic bands were scored as 1 (present) or 0 (absent) for the same amplified fragments. Major allele frequency, number of alleles per locus, gene diversity and polymorphism information content (PIC) were analyzed using Power-Marker V 3.25 to access the value of each primer. Genetic distance (GD) and pairwise values of F-Statistics (Fst) between populations were calculated using Power Marker V 3.25. The relationship between various germplasm collections was displayed as a dendrogram constructed using NTSYS –PC 2.1 software (Rohlf, 1995) based on Unweighted pair wise group method using arithmetic averages (UPGMA).

Figure 6.3: SSR profile of upper spray block using ssrCM 105 marker viewing in agarose gel of 1.2%, 50bp ladder used as a marker

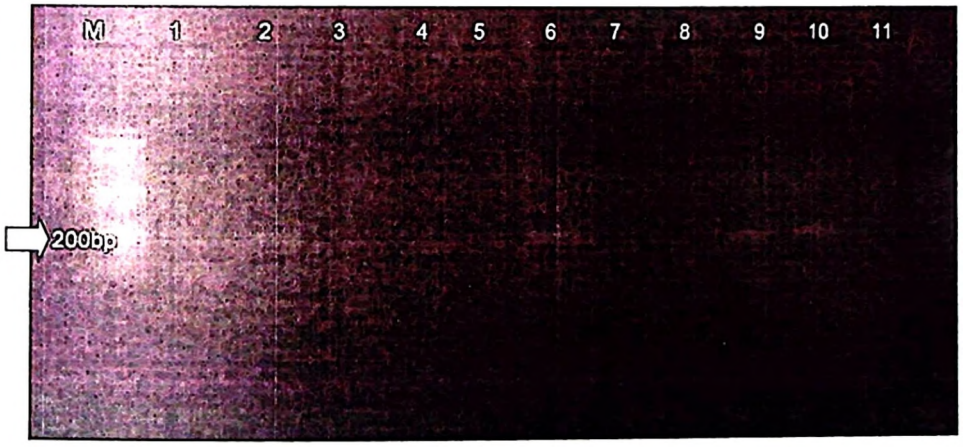

Figure 6.4: SSR profile of wild coffee accessions using ssrCM17 from Lower spray showing band patterns viewed from 1.2% agarose gel, 50bp ladder used

6.3 Results

6.3.1 Genetic diversity

A total of 150 Kihansi wild coffee accessions were amplified using 12 SSR markers (Table 6.1). Seven markers did not amplify in most of the accessions, therefore were eliminated from the analysis. The remaining five primers were amplified 87 monomorphic bands with maximum of two bands per primer pairs. The polymorphism

information content (PIC) for the SSR loci ranged from 0.09 to 0.25 and the average PIC value was 0.17 (Table 6.2). The genetic diversity ranged from 0.09 to 0.30 with an average of 0.19. Results revealed zero heterozygosity to all loci, where the major allelic frequency of 0.95 was observed in primer ssrCM17. 0.93 in primer ssrCM28 and 0.87 in primer ssrCM2 with an average of 0.89.

Table 6.2: Genetic parameters of efficient amplified SSR markers used

Marker	Major. Allele. frequency	Number of observation.	Allele Number	Genetic diversity	PIC
ssrCM2	0.87	150	2	0.22	0.20
ssrCM8	0.93	150	2	0.12	0.12
ssrCM17	0.95	150	2	0.09	0.09
ssrCMA263	0.86	150	2	0.24	0.21
ssrR105	0.82	150	2	0.30	0.25
Mean	0.89	150	2	0.19	0.17

6.3.2 Analysis of molecular variance

Blocks and plots of accession were provided the organization of the AMOVA. In the total genetic variance based on the blocks, 3 % of the total variations were observed between blocks and remaining 97 % was explained by the individual difference within blocks (Table 6.3).

Table 6.3: Analysis of molecular variance of Kihansi wild coffee accession

Source of variation	Degree of freedom	Sum of Square	Mean Square	Estimate Variance	Probability value
Among blocks population	2	0.704	0.352	0.006	0.03
Within block Population	67	13.648	0.204	0.204	0.97
Total	69	14.352		0.210	1

6.3.3 Genetic relationship

Based on genetic similarities and differences, accessions of Kihansi wild coffee clustered into four groups of different genotypes, most of accessions in lower spray and upper spray coffee garden blocks were grouped into cluster 1, cluster 2 formed by only accessions from the Upper spray blocks, cluster 3 were formed by the accession from all three blocks of upper spray, lower spray and lower upper while in cluster 4 were formed by the accession from upper and lower upper blocks.

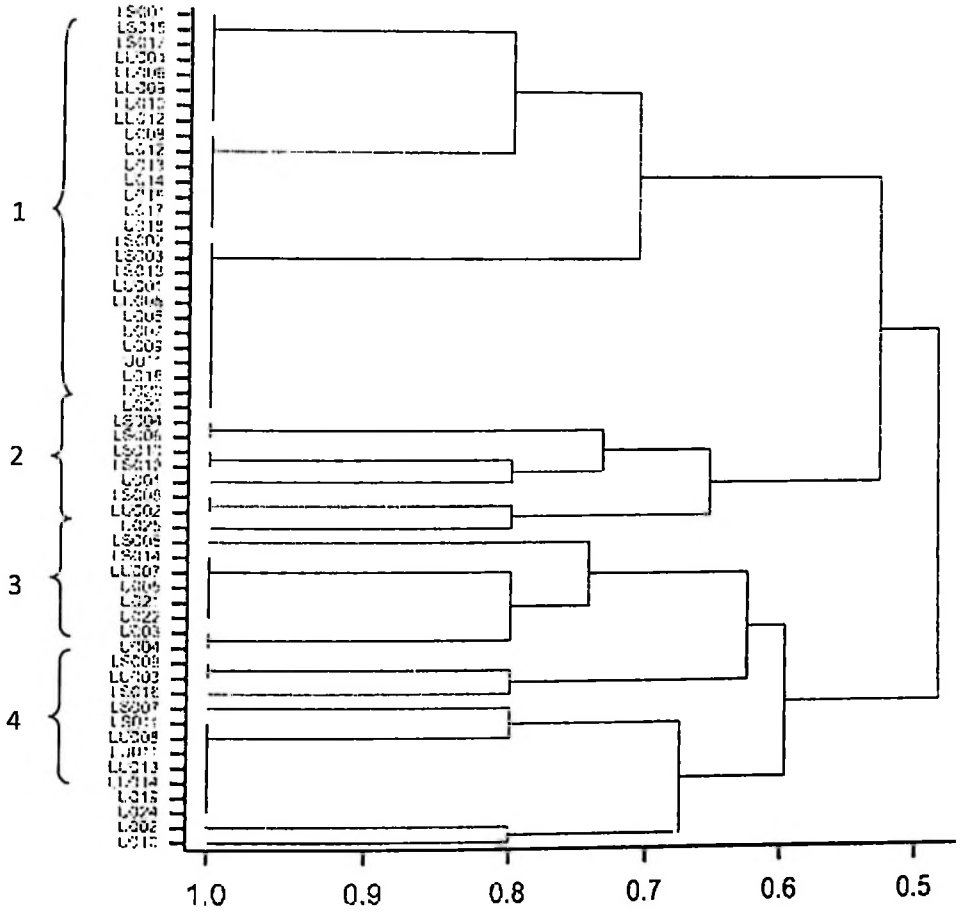

Figure 6.5: Dendrogram building by UPGMA methods based on the genetic distance estimated by 150 accessions of Kihansi wild coffee

6.3.4 Genetic distances between Kihansi wild coffee genotypes

Table 6.4 present genetic distances of 150 Kihansi wild coffee from three population from different three coffee blocks. Genetic distance ranged from lowest value (0.212) between

the genotypes found in upper spray blocks to largest value (0.246) between the genotypes within lower upper and genotypes within lower spray. Also, genetic distance of 0.212 was observed between genotypes of lower upper spray block and lower upper spray block.

Table 6.4: Genetic distance of Kihansi wild coffee based on coffee blocks garden

	Lower upper	Upper spray	Lower spray	
	******			
Lower upper	0.246	******		
Upper spray	0.212	0.184	******	
Lower spray	0.241	0.212	0.246	******

6.4 Discussion

Polymorphic information content, genetic diversity index and genetic distance found out in this study it reflect lowest genetic diversity among 150 genotypes of the Kihansi wild coffee. The lowest genetic diversity observed is evidence that the marker used was inadequate for detecting the variations within the species as supported by Combes *et al.* (2000) and Anthony *et al.* (2002). These findings are in disagreement with the work of Agwanda *et al.* (1997), Anthony *et al.* (2000), Aga *et al.* (2003), Masumbuko *et al.* (2003) and Maluf *et al.* (2005) who found the existence of high genetic variation among the wild coffee genotypes using the SSR markers. However, the extent of distributions, area sampled and plant characteristics such as mode of reproduction and generation time are some of the important parameters that determine the level of genetic variability in a species (Essayas *et al.*, 2003). Also, these findings are in agreement with those reported in global study of diversity of cultivated and wild coffee species using SSR markers that SSR markers are adequate for assessing intra-specific and interspecific variations and for detecting and information on genetic diversity (Cubry *et al.*, 2005; Cubry *et al.*, 2008 and Musoli *et al.*, 2009).

Based on cluster analysis, four main groups formed composed wild coffee genotypes from different blocks of Kihansi wild coffee gardens (Fig. 6.4). There is no such clear distinction between genotypes from different blocks of coffee garden. These findings are in agreement with those obtained by Oljiram (2006) and Zhang *et al.* (2013). Based on genetic distance and, analysis of molecular variance disclosed in the study, highest variations are existing in the coffee wild genotypes within the blocks compared to variations detected between blocks of Kihansi wild coffee genotypes. This highest variation within blocks can be attributed due to wild coffee adaptations towards the environmental conditions at the time of introduction, growth and development (Dussert *et al*, 2003; Chaparro *et al*., 2004; Khan *et al*., 2015). However, between blocks population variations observed in this study is very low compared to that observed in another species study (Ayana *et al*., 2000; Aga *et al*., 2003; Tesfaye, 2006; Balemi, 2007) and this may be contributed by low sample size, sampling method or evolution process of that Kihansi wild coffee specie. The highest variation within block populations may be caused by two reasons, either mode of reproduction or natural selection of wild coffee species (Hamrik and Godt, 2004). Meyer (2002) reported that there is partial out-crossing by pollen and seed (partial selfing) for which some levels of gene flow expected that could result in high genetic diversity in Arabica coffee (Aga *et al*., 2003; Tesfaye, 2006; Oljira, 2006 and Balemi, 2007).

Natural populations are likely to be the source of new resistance genes needed to cope with future evolution in the pathogens of a crop and other environmental constraints (Agwanda *et al*., 1997). This will especially be the case if host populations are maintained *in situ* to allow co-evolution to occur. However, preserving all populations is not possible hence, decisions about which populations to preserve must be made.

6.5 Conclusion and Recommendation

6.5.1 Conclusion

Low variations were detected in this population of Kihansi wild coffee as a results of low polymorphic information content observed however, highest variations was observed within population variations compared between population variations. Four clusters were formed despite of the low diversity in this species. Variations detected from this clusters are importance in genetic conservation of this species as well as in breeding program.Therefore, information on the amount and pattern of genetic variation of KWC populations is crucial in the planning process and could serve as one of preliminary strategy of establishment of conservation program.

6.5.2 Recommendation

For the future survival of this species variability should be maximized through crossing between individuals within and between populations. Also terms of management should be initiated for the development and survival of this coffee species. Larger populations should be tested with more markers to explore the large variability in a population of this species to increase its importance in breeding programme and commercial coffee improvement.

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CHAPTER SEVEN

7.0 CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

Study show the variability of population density in different blocks between seasons where by wet season had less population density compared to dry season with less individual's in upper spray and lower upper spray blocks during the wet and dry season respectively where by population stability was observed in the lower spray by having the constant population size thought. Larger proportional of matured plants were observed in all blocks with less old plants and young individual plants. It is revealed that the rate of population growth of these accessions is very low which contributed by the nature of the crop as well as the small coverage area (Restricted blocks).

Furthermore, this study revealed the optimized vegetative propagation techniques of this Kihansi wild coffee, semi-hard wood with two to three nodes when treated with rooting growth hormone (Indole-3-butric acid) under good control of moisture and temperature in sand rooting media for long and many roots, high survival rate of cuttings as well as high number of rooted cuttings. In view of this Kihansi wild coffee accessions it must be acknowledged that morphological variations existed especially in reproductive traits (bean size and berry size and shape), Molecular analysis of these accessions confirmed the observed morphological variability. However, not much genetic variability as expected in comparison to the extent of morphological variability and this is explained by low PIC values detected across the markers used. Most variations are found in individuals within blocks as explained by molecular variance. These accessions seem to resemble as coffee Arabica due to the fact that the same primer amplified in coffee Arabica amplified in these coffee accessions.

7.2 Recommendations

From this study the following recommendations were made

i. This population dynamics of KWC study were done only two seasons, therefore provide the useful information that can be used as starting point in assessment of change of population size of KWC over time (season) and space (blocks). Hence. study on trend of population size change over time should be done for more than two seasons.

ii. Adaptation study of this wild coffee in different growing areas should undertake for survival, development and growth assessment.

iii. Further study on other propagation techniques including tissue culture (somatic embryogenesis) so as to provide the best methods of propagation of this species that is less destructive other than using cuttings should be done and recommended

iv. Further study on DNA sequence of this wild coffee should be done in order to confirm it relatedness with commercial growing coffee Arabica and Robusta. It has to be verified with data from alignment of DNA sequence.

Finally, holistic conservation strategies should be effectively established specific to each block before the utilization of KWC to ensure sustainability. Planting of the species into different coffee producing region should be initiated in selected coffee research institutions. Eventually adaptation of wild coffee to local coffee grower areas is expected to be realized in their locality. In long run this species is expected to be useful in coffee improvement program.

APPENDICES

Appendix 1: Plant population density of Kihansi wild coffee from two seasons of year 2016

Block	Total population size($1000m^2$) Wet Season (2016)	Dry Season (2016)
Lower upper spray	1260	1220
lower spray	1880	1990
upper spray	1040	1315
Grand Total	4180	4525
Mean	1393	1508

Appendix 2: Plant population composition of Kihansi wild coffee disaggregated by age from two seasons of year 2016

	Wet season (2016)			Dry season (2016)		
Plots	Young	Mature	Old	Young	Mature	Old
Lower upper	225	930	105	120	1050	50
Lower spray	375	1355	150	480	1350	160
Upper spray	145	710	185	240	1005	70
Grand total	**745**	**2995**	**440**	**840**	**3405**	**280**
Mean	**248**	**998**	**146**	**280**	**1135**	**93**

Appendix 3: Analysis of variance for Root number of Kihansi wild coffee

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Variance ratio	Propability
Treatment	3	3.3763	1.1254	1.33	0.268
Residual	117	98.8882	0.8452		
Total	**120**	**102.2645**			

Appendix 4: Analysis of variance for Root length of Kihansi wild coffee

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance Ratio	F Probability
Treatment	3	163.26	54.42	12.71	<.001
Residual	117	501.13	4.283		
Total	**120**	**664.389**			

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Appendix 5: Analysis of variance for rooted cutting of Kihansi wild coffee

Source of variation	Degree of freedom	Sum of squares	Mean of Squares	Variance ratio	F Probability
Treatment	3	2.07	6.91	1.73	<.001
Residual	117	4.67	3.99		
Total	**120**	**2.07**			

Appendix 6: Analysis of variance of Survival cuttings

Source of variation	Degree of freedom	Sum of Squares	Mean of Squares	Variance ratio	F probability
Treatment	3	166854.7	55618.22	22699.99	<.001
Residual	117	286.667	2.45		
Total	**120**	167141.3			

Appendix 7: Descriptors for morphological traits characterization

Descriptor	Reference	Methods of assessment and scales used
37.Plant habit	IPGRI 1996	Measuring the height of coffee trees using 10 metre ruler and scored as follows: 1= Bush (< 5 m- without distinct trunk), 2= Shrub or small tree (<5 m- one or more trunks) and 3= Tree (> 5 m- single trunk).
38.Plant height	IPGRI 1996	Visual estimation: 1= Very short, 3= short, 7 = Tall and 9= Very tall
39.Overall appearance at the specific age of plant	IPGRI 1996	1= Elongated conical, 2= Pyramidial and 3= Bushy
40.Branching habit	IPGRI 1996	1= very few branches (primary), 2= Many branches (primary) with few secondary , 3 = Many branches (primary) and 4 = Many branches (primary) with many secondary and tertiary branches
41.Angle of insertion of primary branches	IPGRI 1996	Observation is done on the main stem(1= Drooping, 2 = Horizontal and 3= Semi erect
42.Young leaf colour	IPGRI 1996	1= Greenish, 2 =Green, 3 = Brownish, 4= Reddish, 5= Bronze and 6= others if any
43.Leaf shape	IPGRI 1996	1= Obovate, 2= Ovate, 3= Elleptic , 4= Lanceolate and 5= others if any
44. Leaf apex shape	IPGRI1996	1= Round, 2= Obtuse, 3= Acute, 4 = Acuminate, 5= Apiculate, Spatulate and 6= others
45.Leaf width (mm)	IPGRI 1996	Measuring and take the average of five mature (> node 3 from the terminal bud) leaves, measured at the widest part

Descriptor	Reference	Methods of assessment and scales used
46.Fruit colour	IPGRI, 1996, Walyaro, 2006	Observed on mature fruits: 1- Yellow, 2= Yellow- orange, 3=Orange, 4= Orange- red, 5=Red, 6 Red –purple, 7= Purple, 8=Purple- violet, 9= Violet, 10= Black and 11 others
47.Fruit shape	IPGRI, 1996, Walyaro, 2006	Average of five normal (not caracole) mature fruits: 1= Roundish, 2= Obovate, 3= Ovate, 4= Elliptic,5= Oblong and 6 = others
48Calyx limb persistence	IPGRI 1996	0= No and 1 = Yes
49.Fruit length (mm)	IPGRI 1996	Average of five normal mature green fruit, measured at the longest part
50.Fruit width (mm)	IPGRI 1996	Average of five normal mature green fruit measured at the thickest part
51.Pulp thickness	IPGRI 1996	Visual observation in relation to berry/bean. Scoring done using 3= Thin, 5= Intermediate and 7= Thick.
53.Harvest duration	IPGRI,1996	Days taken by the crop to ripe
54.Empty fruit rate (%)	IPGRI,1996, Walyaro.2006	Scored by floating fruits
55.100 bean weight (g)	IPGRI,1996	Calculated at (11% moisture) content as follows: ("Bean weight at 0% moisture content"x 100)/ ("Bean number "x 0.89).
56.Fruit filling coeffient	IPGRI,1996, Walyaro,2006	Ratio of bean number over cherry number. The fruit filling coefficient varies between 0 (sterility) a 2 (complete fertility).
57.Seed length (mm)	IPGRI,1996	Maximum length average of five normal mature seeds
58.Seed width (mm)	IPGRI,1996	Average of five normal mature seeds, measured at the widest part
59.Seed thickness (mm)	IPGRI,1996	Average of five normal mature seeds, measured at the thickest part.
60.Seed colour	IPGRI,1996& Walyaro, 2006	Visualized at 11% humidity and scored by using 1= Yellow, 2= Brown purple and 3= Others
61.Seed shape	IPGRI,1996& Walyaro,2006	1= Round, 2= Obovate, 3= Ovate, 4=Elliptic, 5 = Oblong and 6 =Others if any
62.Biotic stress susceptibility	IPGRI1,996&Walyaro,2006	Scored based on a susceptibility scale from 1 to 9; 1= Very low or no visible sign of susceptibility, 3= Low, 5= Intermediate, 7= High and 9= Very high.

Appendix 8: Kihansi wild coffee accessions

No	Code	Collection Area	Sampling block area	No	Code	Collection Area	Sampling block area
1	LU001	Kihansi accession	Kihansi lower upper	76	US26	Kihansi accession	Kihansi upper wetland
2	LU002	Kihansi accession	Kihansi lower upper	77	US27	Kihansi accession	Kihansi upper wetland
3	LU003	Kihansi accession	Kihansi lower upper	78	US28	Kihansi accession	Kihansi upper wetland
4	LU004	Kihansi accession	Kihansi lower upper	79	US29	Kihansi accession	Kihansi upper wetland
5	LU005	Kihansi accession	Kihansi lower upper	80	US30	Kihansi accession	Kihansi upper wetland
6	LU006	Kihansi accession	Kihansi lower upper	81	US31	Kihansi accession	Kihansi upper wetland
7	LU007	Kihansi accession	Kihansi lower upper	82	US32	Kihansi accession	Kihansi upper wetland
8	LU008	Kihansi accession	Kihansi lower upper	83	US33	Kihansi accession	Kihansi upper wetland
9	LU009	Kihansi accession	Kihansi lower upper	84	US34	Kihansi accession	Kihansi upper wetland
10	LU010	Kihansi accession	Kihansi lower upper	85	US35	Kihansi accession	Kihansi upper wetland
11	LU011	Kihansi accession	Kihansi lower upper	86	US36	Kihansi accession	Kihansi upper wetland
12	LU012	Kihansi accession	Kihansi lower upper	87	US37	Kihansi accession	Kihansi upper wetland
13	LU013	Kihansi accession	Kihansi lower upper	88	US38	Kihansi accession	Kihansi upper wetland
14	LU014	Kihansi accession	Kihansi lower upper	89	US39	Kihansi accession	Kihansi upper wetland
15	LU015	Kihansi accession	Kihansi lower upper	90	US40	Kihansi accession	Kihansi upper wetland
16	LU016	Kihansi accession	Kihansi lower upper	91	US41	Kihansi accession	Kihansi upper wetland
17	LU017	Kihansi accession	Kihansi lower upper	92	US42	Kihansi accession	Kihansi upper wetland
18	LU018	Kihansi accession	Kihansi lower upper	93	US43	Kihansi accession	Kihansi upper wetland
19	LU019	Kihansi accession	Kihansi lower upper	94	US44	Kihansi accession	Kihansi upper wetland
20	LU020	Kihansi accession	Kihansi lower upper	95	US45	Kihansi accession	Kihansi upper wetland
21	LU021	Kihansi accession	Kihansi lower upper	96	US46	Kihansi accession	Kihansi upper wetland
22	LU022	Kihansi accession	Kihansi lower upper	97	US47	Kihansi accession	Kihansi upper wetland
23	LU023	Kihansi accession	Kihansi lower upper	98	US48	Kihansi accession	Kihansi upper wetland
24	LU024	Kihansi accession	Kihansi lower upper	99	US49	Kihansi accession	Kihansi upper wetland
25	LU025	Kihansi accession	Kihansi lower upper	100	US50	Kihansi accession	Kihansi upper wetland
26	LU026	Kihansi accession	Kihansi lower upper	101	LS001	Kihansi accession	Kihansi lower spray
27	LU027	Kihansi accession	Kihansi lower upper	102	LS002	Kihansi accession	Kihansi lower spray
28	LU028	Kihansi accession	Kihansi lower upper	103	LS003	Kihansi accession	Kihansi lower spray
29	LU029	Kihansi accession	Kihansi lower upper	104	LS004	Kihansi accession	Kihansi lower spray
30	LU030	Kihansi accession	Kihansi lower upper	105	LS005	Kihansi accession	Kihansi lower spray
31	LU031	Kihansi accession	Kihansi lower upper	106	LS006	Kihansi accession	Kihansi lower spray
32	LU032	Kihansi accession	Kihansi lower upper	107	LS007	Kihansi accession	Kihansi lower spray
33	LU033	Kihansi accession	Kihansi lower upper	108	LS008	Kihansi accession	Kihansi lower spray
34	LU034	Kihansi accession	Kihansi lower upper	109	LS009	Kihansi accession	Kihansi lower spray
35	LU035	Kihansi accession	Kihansi lower upper	110	LS010	Kihansi accession	Kihansi lower spray
36	LU036	Kihansi accession	Kihansi lower upper	111	LS011	Kihansi accession	Kihansi lower spray
37	LU037	Kihansi accession	Kihansi lower upper	112	LS012	Kihansi accession	Kihansi lower spray
38	LU038	Kihansi accession	Kihansi lower upper	113	LS013	Kihansi accession	Kihansi lower spray

39	LU039	Kihansi accession	Kihansi lower upper	114	LS014	Kihansi accession	Kihansi lower spray
40	LU040	Kihansi accession	Kihansi lower upper	115	LS015	Kihansi accession	Kihansi lower spray
41	LU041	Kihansi accession	Kihansi lower upper	116	LS016	Kihansi accession	Kihansi lower spray
42	LU042	Kihansi accession	Kihansi lower upper	117	LS017	Kihansi accession	Kihansi lower spray
43	LU043	Kihansi accession	Kihansi lower upper	118	LS018	Kihansi accession	Kihansi lower spray
44	LU044	Kihansi accession	Kihansi lower upper	119	LS019	Kihansi accession	Kihansi lower spray
45	LU045	Kihansi accession	Kihansi lower upper	120	LS020	Kihansi accession	Kihansi lower spray
46	LU046	Kihansi accession	Kihansi lower upper	121	LS021	Kihansi accession	Kihansi lower spray
47	LU047	Kihansi accession	Kihansi lower upper	122	LS022	Kihansi accession	Kihansi lower spray
48	LU048	Kihansi accession	Kihansi lower upper	123	LS023	Kihansi accession	Kihansi lower spray
49	LU049	Kihansi accession	Kihansi lower upper	124	LS024	Kihansi accession	Kihansi lower spray
50	LU050	Kihansi accession	Kihansi lower upper	125	LS025	Kihansi accession	Kihansi lower spray
51	US001	Kihansi accession	Kihansi upper wetland	126	LS026	Kihansi accession	Kihansi lower spray
52	US002	Kihansi accession	Kihansi upper wetland	127	LS027	Kihansi accession	Kihansi lower spray
53	US003	Kihansi accession	Kihansi upper wetland	128	LS028	Kihansi accession	Kihansi lower spray
54	US004	Kihansi accession	Kihansi upper wetland	129	LS029	Kihansi accession	Kihansi lower spray
55	US005	Kihansi accession	Kihansi upper wetland	130	LS030	Kihansi accession	Kihansi lower spray
56	US006	Kihansi accession	Kihansi upper wetland	131	LS031	Kihansi accession	Kihansi lower spray
57	US007	Kihansi accession	Kihansi upper wetland	132	LS032	Kihansi accession	Kihansi lower spray
58	US008	Kihansi accession	Kihansi upper wetland	133	LS033	Kihansi accession	Kihansi lower spray
59	US009	Kihansi accession	Kihansi upper wetland	134	LS034	Kihansi accession	Kihansi lower spray
60	US010	Kihansi accession	Kihansi upper wetland	135	LS035	Kihansi accession	Kihansi lower spray
61	US011	Kihansi accession	Kihansi upper wetland	136	LS036	Kihansi accession	Kihansi lower spray
62	US012	Kihansi accession	Kihansi upper wetland	137	LS037	Kihansi accession	Kihansi lower spray
63	US013	Kihansi accession	Kihansi upper wetland	138	LS038	Kihansi accession	Kihansi lower spray
64	US014	Kihansi accession	Kihansi upper wetland	139	LS039	Kihansi accession	Kihansi lower spray
65	US015	Kihansi accession	Kihansi upper wetland	140	LS040	Kihansi accession	Kihansi lower spray
66	US016	Kihansi accession	Kihansi upper wetland	141	LS041	Kihansi accession	Kihansi lower spray
67	US017	Kihansi accession	Kihansi upper wetland	142	LS042	Kihansi accession	Kihansi lower spray
68	US018	Kihansi accession	Kihansi upper wetland	143	LS043	Kihansi accession	Kihansi lower spray
69	US019	Kihansi accession	Kihansi upper wetland	144	LS044	Kihansi accession	Kihansi lower spray
70	US020	Kihansi accession	Kihansi upper wetland	145	LS045	Kihansi accession	Kihansi lower spray
71	US021	Kihansi accession	Kihansi upper wetland	146	LS046	Kihansi accession	Kihansi lower spray
72	US022	Kihansi accession	Kihansi upper wetland	147	LS047	Kihansi accession	Kihansi lower spray
73	US023	Kihansi accession	Kihansi upper wetland	148	LS048	Kihansi accession	Kihansi lower spray
74	US024	Kihansi accession	Kihansi upper wetland	149	LS049	Kihansi accession	Kihansi lower spray
75	US025	Kihansi accession	Kihansi upper wetland	150	LS050	Kihansi accession	Kihansi lower spray

Appendix 9: Population dynamics of Kihansi wild coffee species in different four blocks in wet and dry season (year 2016) estimate.

Block	Total population size($1000m^2$) Wet Season (2016)	Dry Season (2016)
Lower upper spray	1260	1220
lower spray	1880	1990
upper spray	1040	1315
Grand Total	4180	4525
Mean	1393	1508

Appendix 10: Plant stage/ age class population dynamic of Kihansi wild coffee species in different blocks in wet season and dry season (year 2016).

	Wet season (2016)			Dry season (2016)		
Plots	**Young**	**Mature**	**Old**	**Young**	**Mature**	**Old**
Lower upper	225	930	105	120	1050	50
Lower spray	375	1355	150	480	1350	160
Upper spray	145	710	185	240	1005	70
Grand total	**745**	**2995**	**440**	**840**	**3405**	**280**
Mean	**248**	**998**	**146**	**280**	**1135**	**93**