GENETIC DIVERSITY OF CULTIVATED COFFEE (*Coffea arabica* L) IN TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

ABSTRACT

Ten new genotypes and eighteen old cultivars of *Coffea arabica* from different areas in Tanzania as well as two reference cultivars (N 39 and KP 423) were characterized morphologically and using coffee DNA microsatellite (SSR) markers. The objective of this study was to determine the level of morphological and molecular variation within and between new and existing coffee varieties in Tanzania. The Morphological characterization was done using 25 character coffee descriptors (IPGRI, 1996), while the molecular characterization 30 SSR markers were used. Both quantitative and qualitative morphological traits were recorded and the binary data were subjected to cluster and principal component analysis. Morphological variation among the genotypes was less than 25%. Four main groups were formed and improved hybrids cultivars except N39-1 were clustered in one group with the traditional cultivars N39 and KP423. Genotypes from *Coffee arabica* growing regions were clustered in separate groups. There was very little morphological variation within the varieties. Principal component analysis revealed clear separation of genotypes from Kilimanjaro and Arusha from the other group. The hybrid N 39 was grouped with the old cultivars from different regions. The first two principal components accounted for a cumulative variance of 60%. The study showed narrow genetic base of cultivated *C. arabica* in Tanzania. Furthermore, total alleles detected 82% were polymorphic and specific. In the cluster analysis, the hybrid varieties clearly separated from the other cultivated cultivars from different regions as well as the control N 39 and KP 423 based on SSR. The study demonstrated low morphological variation and hence low genetic variation among the varieties and emphasized the need to broaden the genetic base of Arabica coffee in Tanzania. In addition, the study demonstrated low molecular variation within the varieties indicative of high genetic consistency.

DECLARATION

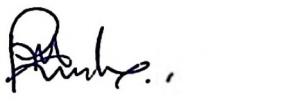
I, Robert Samwel Ngomuo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

21st March 2014

Robert Samwel Ngomuo — Date

(MSc. Candidate)

The above declaration is confirmed



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Prof P.M. Kusolwa — Date

(Supervisor)

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DEDICATION

This work is dedicated to my parents, Samwel K. Ngomuo and Ngiwanza Aron, my wife Evangeline, my daughter Pendo Robert, my son Ron, my brothers, sister and relatives and special dedication to my mother who laid down the foundation of my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP	Amplified Fragment Length Polymorphism
COSTECH	Commission for Sciences and Technology
EDTA	Ethylenediamine tetraacetic acid
IPGRI	International Plant Genetic Resource Institute
JC	Jaccard's coefficient
NTSYs	Numerical taxonomy and multivariate analysis system
PCA	Principle component analysis
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SDS	Sodium dodecyl sulfate
SSR	Simple sequence repeats
SUA	Sokoine University of Agriculture
TaCRI	Tanzania Coffee Research Institute
Taq	*Themus aquaticus*
TBE	Tris-borate EDTA
TE	Tris- EDTA
Tris	Tris (hydroxymethyl) methylamine
UPGMA	Unweighted pair group method with arithmetic

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

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. and *Coffea canephora* Pierre are the two most important beverage species in the *Rubiaceae* family. While the former is self-fertile, the latter is self-incompatible. *C. arabica* considered as a high quality coffee and contributes more than 70 % of the world coffee production (Lashermes *et al.*, 1997; Carneiro, 1999; Anthony *et al.*, 2001a; Anthony *et al.*, 2002; Stieger *et al.*, 2002).The primary centre of genetic diversity for *C. arabica* is the highlands of south-western Ethiopia and the Boma Plateau of Sudan (Lashermes *et al.*, 1999). There are also reports of *C. arabica* populations on the Imatong and Marsabit mountains in Sudan and Kenya, respectively (Berthaud and Charrier, 1988).

1.2 Origin and Geographical Distribution of Coffee in Tanzania

Tanzania has had coffee introductions from the centre of coffee genetic diversity, Ethiopia, and from both Typica and Bourbon base populations. These were all *C. arabica*, the only tetraploids (2n = 4x = 44) in the genus *Coffea* that also happens to be self-fertile. The country also has several native diploid coffee species, e.g. *C. zanguebariae* Lour, *C. eugenioides* Moore, *C. mufindiensis* Bridson and *C. canephora* Pierre. The distribution of diploid coffee species in Tanzania has not been fully documented. *C. zanguebariae* however, grows at low altitude; it has been documented in Zanzibar Island (Purseglove 1981). Samples have been gathered in the Coast region at Utete and Mkongo, the east side of Morogoro Region (Kitulangalo) and in Uzigua which lies along the Dar es Salaam-Tanga road. *C. mufindiensis* has been found on the highlands i.e. over 1600 m., in

Mamiwa and Mufindi, Iringa Region (Berthaud *et al.*, 1982). *C. eugenoides* grows on the western part of Tanzania and *C. canephora* in the northwest.

In Tanzania *C. arabica* is grown in Kilimanjaro, Arusha, Tanga, Mbeya, Morogoro and Ruvuma Regions, while *C. canephora* is cultivated mostly in the north-western region of Kagera and some extents in Morogoro Regions. Introductions of coffee into Tanzania were made at different periods and were from different origins i.e. Ethiopia, India, Java, Jamaica, Bourbon and Aden. The extent of genetic diversity present in Tanzania cultivated Arabica coffee is unknown (Masumbuko *et al.*, 2006). This is a limitation to the improvement of this crop. Tanzanian coffee improvement programs to develop elite cultivars of both arabica and robusta, initiated in early 1932 at the now call Tanzania Coffee Research Institute (TaCRI), has released 15 new lines of Arabica with outstanding selections from a multiple cross programs involving Coffee Berry Disease (CBD) resistant donor parents such as Rume Sudan (R gene), Hibrido de Timor (T gene), K7 (k gene) and the high yielding, good quality but are highly susceptible cultivars (N39 and KP 423) to both CBD and CLR (Kilambo *et al.*, 2006). Their unique features include tall stature, true breeding and resistance to the two major fungal diseases of coffee namely CBD and Coffee Leaf Rust (CLR). They are also high yielding with good bean and liquor quality that compares to N 39 and KP 423 (Kilambo *et al.*, 2006).

1.3 Economic Importance of Coffee

Economically, coffee is the most important agricultural commodity which stands second only to oil in terms of international trading on the world market (Alemayehu *et al.*, 2010). Coffee is very important product in Tanzanian agriculture, currently ranking second amongst the major sources of traditional agricultural exports of foreign exchange earnings. Coffee contributes approximately 5 % of Tanzania's total export earnings and 24% of the country's total crop export earnings (TaCRI report of March, 2011).

Tanzanian coffee is produced by small-holders that account 90 % and estate 10 %. The total area under *Coffea arabica* is 178 500 hectare and *Coffea canephora* is 51 000. Average coffee production for the past 30 years has been 50 000 tonnes clean coffee with a peak of approximately 68 000 tonnes in 2011/12. Arabica coffee produced in Tanzania is renown over the world for its fine flavour quality, and Robusta for its "natural Robusta" flavor. Several agronomic and technological differences between these two species are responsible for their individual market value. It is grown in over 80 tropical and subtropical countries and involves at least 20 million coffee-farming families around the world (Vega *et al.*, 2011). In all coffee growing regions there are more than 450 000 farmers and countrywide the lives of more than 4.5 million people depend on coffee.

1.4 Strategies to improve Coffee in Tanzania

Over the past years, coffee breeders have tried to widen the genetic base of Arabica coffee by having more introductions and undertaking hybridization programs to create variability (Lashermes *et al.*, 1999). As new coffee varieties are continuously being developed through hybridization, there is need to determine the level and sources of morphological variation within and between new and existing coffee varieties. Genetic consistency within varieties is essential to quality assurance for any agricultural product as is believed that morphological variability in coffee plantations is adverse to the product quality (Hue, 2005). While the Tanzanian coffee industry targets high quality coffee, the observed morphological variation within coffee varieties might have impact to the quality. Therefore there is a need to determine the morphological variability within Tanzanian coffee varieties.

TaCRI has developed ten new lines of Arabica coffee which are outstanding selections from a multiple cross program involving CBD resistant donor parents such as Rume

Sudan (R gene), Hibrido de Timor (T gene), K7 (k gene) and the high yielding, good quality but susceptible cultivars such as N39, KP 423 (Kilambo *et al.*, 2006). Their unique features include tall stature, true breeding and resistance to the two major fungal diseases of coffee namely Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR). They are also high yielding with good bean and liquor quality that compares to N39 and KP423 (Kilambo *et al.*, 2006). The study aims at investigating the morphological genetic diversity of the new realized as well as the old traditional *Coffea arabica* varieties cultivated in Tanzania which would contribute to the improvement of these new varieties.

1.5 Characterization of Coffee Genotypes

Genetic variation in Arabica coffee has previously been characterized using morphological and yield-related traits and phylogenetic relationships established (Lashermes *et al.*, 1996). Morphological markers are classical method to distinguish variation based on the observation of the external morphological differences such as the size and shape of the leaf and of the plant form, the colour of the shoot tip, the characteristics of the fruit, the angle of branching and the length of the internodes (De Vienne *et al.*, 2003). These descriptors do not show distinctive characters because they are influenced by environmental and genetic interaction (Tshilenge *et al.*, 2009). In other countries descriptors used to characterize coffee included those developed by the International Plant Genetic Resources Institute (IPGRI) (IPGRI, 1996) and (Walyaro, 2006). These descriptors discriminate coffee varieties based on distinctive characteristics among accessions. Coffee is one of the major plantation crops having high economic importance that calls for continuous efforts to develop better genotypes for sustainability and to meet the ever changing demands of agro-climatic conditions and the commercial markets.

In addition, the study will provide the knowledge on the genetic structure and diversity that will help to reveal the available genetic variability and identify population that might be of beneficiaries to the coffee breeding programs. Furthermore genetic consistency within varieties is essential to quality assurance for any agricultural product and it is believed that morphological variability in coffee plantations is adverse to the product quality (Hue, 2005). Therefore, there is a need to determine the causes of morphological variability within Tanzanian coffee varieties.

1.6 Molecular Characterization of Coffee

Molecular markers have been used to study diversity in coffee. Orozco-Castillo *et al.* (1994) used random amplified polymorphic DNA (RAPD) markers to detect genetic diversity between coffee species and *C. arabica* genotypes. The narrow genetic base of commercial cultivars of coffee was confirmed when cultivated and sub-spontaneous accessions of *C. arabica* were analysed by RAPD markers (Lashermes *et al.*, 1996). RAPD markers have also been used to study genetic diversity of Ethiopian *C. arabica* (Anthony *et al.*, 2001; Aga *et al.*, 2003). Masumbuko *et al.* (2003) used RAPD markers for analysis of Tanzanian cultivated *C. arabica.* The results showed a uniform genetic distance, implying limited genetic variability. Using amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) markers. Anthony *et al.*, (2002) were able to show a successive reduction of genetic diversity during the dissemination of coffee from its primary centre of diversity in Ethiopia.

This study reports the use of SSR markers, a PCR-based marker system that combines most of the advantages of AFLP and microsatellite analysis with the universality of RAPD (Zietkiewics *et al.*, 1994; Kantety *et al.*, 1995). Different types of molecular

markers, such as RAPD, AFLP, RFLP and SSR, have been used in coffee genetic studies (Lasherme *et al.*, 2001, Combes *et al.*, 2000, Maluf *et al.*, 2005). Genome sequencing projects have generated different classes of markers such EST-SSRs. EST-SSRs are microsatellite markers (SSR) developed from ESTs (Expressed Sequence Tags). With the increasing of ESTs datasets, the SSR identification and primer design are done in time and cost-effective manner.

Selecting and assessing genotypes in a given germplasm using SSR markers may optimize and facilitate breeding processes by separating closely related genotypes, thus increasing the efficiency and orientation of future crossings and genetic studies by clearly identification of the genotypes of interest as the germplasm has been used in breeding programs as a source of resistant genes against rust, anthracnose, bacteria and nematodes.

Previous studies on coffee germplasm characterization, including analyses using molecular markers, reported low genetic diversity in *C. arabica*, both wild and cultivated genotypes (Lashermes *et al.*, 1993, Orozco-Castillo *et al.*, 1994, Cros *et al.*, 1998, Combes *et al.*, 2000; Anthony *et al.*, 2002). Justifications for this low variability probably include narrow genetic base, reproduction system and the fact that a small number of coffee plants were introduced from its centre of origin for cultivation in producing countries.

Even though the inbred lines selected by the Tanzania Coffee Research Institute breeding program are well known and thoroughly evaluated, individual and precise identification cannot be achieved using only traditional descriptors. New legislation on commercial exploitation of genetic resources requires correct cultivar identification for both legal protection and intellectual property requirements. Therefore, in this work, molecular

However, conserving the whole populations is practically impossible due to resources limitation. Thus, there is a need to identify and conserve potential populations with the maximum possible genetic diversity, which depends on the availability of genetic diversity information. Hence, any effort towards generating information on the genetic pattern of Arabica coffee populations, especially using DNA molecular techniques, is very important. There is limited use of molecular genetic diversity information of Arabica coffee in the improvement programs. Thus, generating genetic diversity information is an important minute's parameter in the future efforts of Arabica coffee genetic resources conservation and sustainable utilization (Alemayehu *et al* 2010). The extent of genetic diversity present in Tanzania cultivated Arabica coffee is unknown and hence the limitation to the improvement of the crop.

1.8 Objectives of the Study

1.8.1 General objective

The study aimed to determine the morphological and genetic diversity of released new varieties and old cultivars of *Coffea arabica* in Tanzania.

1.8.2 Specific objectives

i. To determine morphological diversity of new released *C. arabica* varieties and old cultivars from different coffee growing regions.

ii. Evaluate genetic variability between new varieties and old cultivars of cultivated *C. arabica* L in Tanzania using SSR markers

CHAPTER TWO

2.0 LITERATURE REVIEW

Coffea arabica L. and *Coffea canephora* Pierre are the two most important beverage species in the *Rubiaceae* family. While the former is self-fertile, the latter is self-incompatible. However, *C. arabica* is the species of economic importance, which is cultivated at altitudes of 1000 – 2000 m above sea level, in the northern highlands (Arusha and Kilimanjaro Regions) and southern highlands (Morogoro, Mbeya, Iringa, and Ruvuma Regions). Geographically, most of the coffee species originated from tropical African countries: Ethiopia for the tetraploid *C. arabica*, and Central and West African countries for other coffee species (Berthaud and Charrier, 1988). During the early centuries, these coffee species were disseminated to other parts of the world where they are produced in mass nowadays. However, the Arabica coffee plants in major producing areas such as Latin and Central America, and Asian countries are believed to have a narrow genetic bases attributed by the few seeds/plants used for dissemination, the successive genetic reduction due to human impacts and reproduction nature, especially for Arabica coffee which is autogamous (Anthony *et al.*, 2002; Stieger *et al.*, 2002: Raus *et al.*, 2003).

Emphasis in the coffee breeding programme is now being directed towards breeding for disease resistance. Hence knowledge of genetic diversity in coffee is critical for future coffee improvement. This has been necessitated by the severe losses incurred by coffee farmers due to coffee berry disease (CBD) and coffee leaf rust (CLR). Genetic diversity of coffee can be assessed using different techniques that range from the traditional morphological techniques to the modern DNA-based molecular markers. The use of morphological techniques in diversity study of plants is limited by the influence of

environmental factors and growth stage of the plant (Weising *et al.*, 2005). In addition, they are also a few in numbers and require lengthy follow-up during the whole growth stage especially in perennial plants like coffee. In response to the limitation of morphological techniques, the more effective technique based on protein, isoenzymes, was developed. However, its application was limited due to inefficiency to detect within species differences in Arabica coffee (Berthaud and Charrier, 1998). Previous studies on *Coffea* germplasm characterization, including analyses using DNA markers, reported low genetic diversity in *C. arabica*, both wild and cultivated genotypes. Justifications for this low variability include narrow genetic base, reproduction system and the fact that a small number of coffee plants were introduced for cultivation in producing countries.

2.1 Morphological Characterization of Coffee Genotypes

In Tanzania, the variations of Arabica coffee were characterised based on plant height, plant girth, canopy diameter, inter-node length, number of primary branches, bearing primary branches, number of flowers, fruit set and yield (Marandu *et al.*, 2004). These descriptors do not show distinctive characters because they are influenced by environmental and genetic interaction (Tshilenge *et al.*, 2009). In other countries descriptors used to characterize coffee included those developed by the International Plant Genetic Resources Institute (IPGRI) (IPGRI, 1996) and (Walyaro, 2006). These descriptors discriminate coffee varieties based on distinctive characteristics among accessions.

2.2 Molecular Characterization of Arabica Coffee Using DNA Markers

Several molecular markers are now used to measure the genetic variations of a different plant species (Hendre *et al.*, 2008 and Yigzaw *et al.*, 2008). These include: RAPDS, inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs), random amplified

polymorphism DNA (RAPD), and amplified fragments length polymorphism (AFLP). Of these markers the simple sequence repeats (SSRs) is the most preferable for the study of the diversity of *C. arabica* (Aggarwal *et al.*, 2007, and Hendre *et al.*, 2008). The findings from work done in Ethiopia on the genetic diversity studies of arabica coffee indicated genetic variations among the studied populations (Alemayehu *et al.*, 2012). On the other hand the analyzing expressed sequence tags (ESTs) for the *Coffea* species (Aggarwal *et al.*, 2007) found 46 % of dinucleotides and 26 % of trinucleotides. However, the trinucleotides repeats were the most frequent SSR class of markers observed in ESTs of *C. canephora* (Poncet *et al.*, 2006). The higher frequency of trinucleotides class could be attributed to the lower mutation events in coding regions of the genome represented by the EST. Using AFLP and SSRs markers, Anthony *et al.* (2002) were able to show a successive reduction of genetic diversity during the dissemination of coffee from its primary centre of diversity in Ethiopia. AFLP and SSR markers were used to assess polymorphism between and within cultivars from Typical and Bourbon, and relating these to four Yemen cultivars and eleven sub-spontaneous accessions from the primary centre of diversity. Molecular markers have been used to study diversity in coffee. Orozco-Castillo *et al.* (1994) used random amplified polymorphic DNA (RAPD) markers to detect genetic diversity between coffee species and between *C. arabica* genotypes. The narrow genetic base of commercial cultivars of coffee was confirmed when cultivated and sub-spontaneous accessions of *C. arabica* were analysed by RAPD markers (Lashermes *et al.*, 1996). RAPD markers have also been used to study genetic diversity of Ethiopian *C. arabica* (Anthony *et al.*, 2001; Aga *et al.* 2003). Masumbuko *et al.* (2003) used RAPD markers for analysis of Tanzanian cultivated *C. arabica*. The results showed a uniform genetic distance, implying limited variability. Using amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) markers, Anthony *et al.* (2002) were able to show a successive reduction of genetic

diversity during the dissemination of coffee from its primary centre of origin. Overall genetic variability of *C. arabica* is very restricted, not only in cultivated materials but also in wild accessions. This is a consequence of the mode of reproduction, of the narrow centre of origin and of low number of seeds used for world dispersion (Maluf *et al.*, 2005).

A combination of restriction fragment length polymorphism (RFLP) markers and genomic *in situ* hybridisation (GISH) were used to investigate the origin of the *C. arabica* species. Hybridisation between *C. eugenoides* and *C. canephora* was identified as the origin of *C. arabica* (Lashermes *et al.*, 1999). Identification of DNA introgression fragments from *C. canephora* in four *C. arabica* lines and assessment of polymorphism among *C. arabica* and *C. canephora* accessions was done using microsatellites. Analysis managed to group *C. arabica* accessions from the *Typica* and *Bourbon* genetic bases separately according to their genetic origin. *C. canephora* from Central Africa was grouped with a canephora derived hybrid, while *C. canephora* from West Africa was separated from the rest of the accessions (Anthony *et al.*, 2002).

2.3 Use of Microsatellite Markers in Diversity Study of Coffee

Microsatellite (SSRs) markers which are short tandem repeats of DNA sequence of one to six base pairs were used to study the genetic diversity of Arabica coffee collections with different geographical origin and historical backgrounds (Alemayehu *et al.*, 2010). Microsatellites (SSR) markers 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 different DNA based techniques, microsatellite (SSRs) markers are the recently used techniques in

the genetic study of plants. Their use as a molecular marker has advantages over other techniques as it fulfils most of the good characteristics of genetic markers such as highly polymorphic and reproducible, locus specific and "co-dominant". Because of this, today SSRs are the markers of choice for many genetic studies. By virtue of their extreme polymorphism, SSR loci are considered to be ideal markers for population genetics. SSRs are highly abundant and exhibit extensive degrees of polymorphism in eukaryotic and prokaryotic genomes. They are found in protein-coding and non-coding regions with SSRs being more abundant in non-coding regions than in exons. DNA sequence knowledge is imperative in the design of appropriate primers for the assay.

The homology of flanking regions of SSRs allows the transferability of microsatellite loci between closely related species, besides the possibility of comparative map construction among them. Transferability may reduce costs, opening new perspectives for the development of population genetic studies. The high rate of transferability has already been reported for plant species and among animals for the human and chimpanzee species, besides the dog and the fox. The cross-species transferability of microsatellites is very variable among groups of animals, plants and fungi. The molecular markers are useful for the construction of genetic maps and identification of markers linked to the genes that control agronomic characteristics, which may open the possibilities for marker-assisted selections. Different classes of genetic markers have been developed for *Coffea* species. Molecular breeding, the newly emerging discipline, based on polymorphic DNA markers/techniques offers possibilities to overcome the difficulty of morphological characterization. SSR markers have proven to be most desirable in various genetic studies on germplasm characterization and linkage analysis due to their codominant nature, stability, abundance, sensitivity, ease and speed of analysis, minimal sample requirements and suitability for automation.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The research was carried in five *Coffee arabica* growing regions namely Kilimanjaro, Arusha, Tanga, Mbeya and Ruvuma. Laboratory works were conducted at Sokoine University of Agriculture (SUA) from November 2011 to May 2012.

3.2 Plant Materials

The test materials included 10 new varieties coded N39-1, N39-2, N39-3, N39-4, N39-5, N39-6, N39-7, KP423-1, KP423-2 and KP423-3. These varieties were evaluated alongside two commercial Arabica cultivars, N39 and KP423 as control cultivars. The new varieties have been developed by Tanzania Coffee Research Institute (TaCRI). They are true breeding lines with tall stature and have been tested both in the laboratory and in the field and proven to be resistant to two major fungal diseases of coffee CBD and CLR.

3.2.1 Plant materials for DNA extraction

Three trees per each genotype were used for collection sites where leaf samples of indigenous cultivars were collected from five regions, namely Kilimanjaro, Arusha, Tanga, Ruvuma and Mbeya whereas new hybrid varieties were collected from the mother stock garden in TaCRI Lyamungu. The sampling procedures were conducted specifically to make sure each genotype was included.

Two young leaves were picked from each coffee tree for DNA extraction. The leaves were wrapped in plastic bags and kept in a cool box at 0°C and then stored at – 20°C prior

to DNA extraction. Before DNA extraction the leaves were surface sterilized with 70% ethanol to remove contamination from other organisms followed by rinsing in sterile distilled water.

3.2.2 DNA Extraction and PCR amplification

DNA was extracted from frozen young leaves of each genotype following a modified CTAB protocol Wang *et al.* (1996) and modified by Aga *et al.*, 2003) for plants. The modification of protocol was made by additional of mecaptomethanol to reduce the phenolics compounds found in coffee. DNA quantity and quality were assessed using 1 % Agarose Gel Electrophoresis

PCR was performed using 30 pairs of primers in 20µl reaction volume containing 2µl (10x PCR buffer), 0.045u (0.18µl) Polymerase enzyme, 0.2u (0.4µl) dNTP's, 0.5nM (1µl) of forward primers and reverse primers, 1.875nM (1.2µl) of MgCl2, 1µl DNA and 13.22µl of de-ionised water. Amplification was carried out in a GeneAmpR PCR System 9700 using the following program: 10 min initial denaturation step at 94°C, followed by 35 cycles of denaturing at 94°C for 30s, at specific annealing temperature of each primer for 30s and extension at 72°C for 1 min with a final extension step at 72°C for 5 min.

Amplified DNA products were separated on 2 % agarose gel in 0.5x TBE buffer. After casting the gel, 7µl of PCR product was loaded in the well per each genotype and allowed to run on a horizontal electrophoresis unit containing the same running buffer (0.5x TBE) at 110 V and a current of 50 mA for 2.5 hr. The gel was stained with 0.5mg/ml of ethidium bromide for 20min. Then bands were visualized on a trasilluminator (UV Tech) and finally images were captured using digital camera ready for scoring

3.3 Experimental Design and Data Collection

A randomized complete block design (RCBD) with twenty trees per plot with three replications was used. Data for morphological characterization of cultivated commercial and hybrid varieties were collected from field established in TaCRI experimental plots and in farmer fields in respective regions using descriptors and protocol developed by the International Plant Genetic Resource Institute (IPGRI, 1996) and Walyaro (2006). Quantitative descriptors were taken as the mean value of three measurements made on six trees per replicate and then calculating the mean to get an overall figure per replicate

The collected data include plant habit, plant height, overall appearance, vegetative development, branching habit, angle of insertion of primary branches, stipule shape, stipule arista length (mm), young leaf colour, leaf shape, leaf apex shape, leaf length (mm), leaf width (mm), leaf petiole length (mm), leaf petiole colour, young shoot colour, mature leaf colour, venation pattern, domatia pilosity, shape of domatia, shape of the aperture, position of the domatia, inflorescence position, inflorescence on old wood, number of flowers per axil, number of flowers per fascicle, number of fascicle per node, inflorescence stalk length (mm), corolla tube length (mm), number of petals per flower, anther insertion, number of stamens per flower, fructification duration (days), fruit colour, fruit shape, absence/persistence of fruit ribs, endocarp texture, fruit disc shape, calyx limb persistence, fruit length (mm), fruit width (mm), fruit thickness (mm), pulp thickness, harvest duration, seed length (mm), seed width (mm), seed thickness (mm), seed colour, and seed shape (Appendices 2 and 3).

Data for morphological characters was collected from ten released hybrid varieties in TaCRI Lyamungu and from the old commercial cultivars from farmer's field from Kilimanjaro, Arusha, Tanga, Mbeya and Ruvuma Regions. Data was collected from three

coffee trees per accessions for all listed characteristics as per descriptors each coffee tree treated as replicate.

3.4 Data Analysis

3.4.1 Morphological data

Morphological data were organized into a matrix and subjected to cluster analysis using GenStat discovery edition 4. Variables were segregated into discrete factors (e.g., Fruit colour -light red, pink, and yellow); rank-ordered factors (e.g., fruit size - very small, small, medium, large and very large); integers (e.g., number of flowers per inflorescence) and numerical variable (e.g., average internodes' length). The clustering was done using DAISY (dissimilarity matrix calculation) function and unweighted pair-group method with arithmetic average [UPGMA] (Venables *et al.*, 2006). Quantitative data were subjected to ANOVA using GenStat Discovery Edition 4 as well as to perform a principal component (PC) analysis using the quantitative variables. In this procedure, first a similarity matrix was calculated and was used to calculate Eigen values and scores for the accessions. The accessions were then plotted on two dimensions using the first two principal components (PC1 and PC2).

3.4.2 Molecular data

The captured band images were scored and the scores were arranged in a binary matrix as 1 for presence and 0 for absent of bands as well as their corresponding base pairs sizes of the bands. For DNA markers, SSRs (Prasad *et al.*, 2008) primers was be used to compare variations of nucleotide bases of Arabica hybrid accessions and indigenous *C. arabica* from different regions.

For the molecular data the genotypic diversity among arabica hybrid accessions and old commercial coffee cultivar was determined using Shannon's information index. Genotypic frequency, allele frequency and a number of alleles were analyzed using computer software, GenStat discovery Edition 3 version 7. Cluster analyses was performed by unweighted pair- group method using arithmetic averages (UPGMA) and weighted pair- group method using arithmetic averages (WPGMA). A dendrogram was drawn based on Nei's genetic distances using UPGMA by using GenStat.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Morphological diversity

Low variations in qualitative characters were observed among all genotypes in most of the qualitative characters (Table 1). Characters such as leaf shape, anthocyanin colouration and undulation of the margin, depth of the secondary veins, leaf domatia, fruit shape, fruit colour and adherence to the branch contributed less to variation of genotypes. Significant variations were found in plant shape and fruit size. Genotypes from IB, and LLN were Ellipsoid, MB4, LK, RM, KLM and T was cylindrical in shape while the rest were conical. The fruit sizes were medium for MT, MBK and LU and large size for the rest. The hybrids lines were morphologically very similar to N39 and KP423 especially for plant height, canopy diameter and intensity of ramification. They were tall with large canopy diameter and medium intensity of ramification. The variation were observed for N39-7 with red purple colour while the rest were red and in fruit shape were N39-1 was cylindrical and N 39-5 obviate and the rest were oblong and roundish.

Table 1: Variation of qualitative characters in *Coffea arabica* genotypes in Tanzania

Genotype	Plant shape	Plant height	Canopy diameter	Intesity of ramification	Leaf shape	Anthrocianin colouration	Margin undulation	Depth of 2 vein	Leaf domatia	Fruit shape	Fruit colour	Adherence to branch	Fruit size	Seed colour	Seed shape
N39-1	Conical	Tall	Large	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Cylindrical	Medium	Large	Cream	Cylindrical
N39-2	Conical	Tall	Large	Medium	Eliptic	Absent	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
N39-3	Conical .	Tall	Large	Medium	Eliptic	Absent	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
N39-4	Conical	Tall	Large	Medium	Eliptic	Absent	Medium	Medium	Absent	Red	Oblong	Medium	Large	Cream	Oblong
N39-5	Conical	Tall	Large	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Obovate	Medium	Large	Cream	Oblong
N39-6	Conical	Tall	Large	Medium	Eliptic	Present	Medium	Medium	Absent	Red	Oblong	Medium	Large	Cream	Oblong
N39-7	Conical	Tall	Large	Medium	Eliptic	Present	Medium	Medium	Absent	Red purple	Roundish	Medium	Large	Cream	Oblong
KP423-1	Conical	Tall	Large	Medium	Eliptic	Present	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
KP423-2	Conical	Tall	Large	Medium	Eliptic	Absent	Medium	Medium	Absent	Red	Oblong	Medium	Large	Cream	Eliptic
KP423-3	Conical	Tall	Large	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Oblong	Medium	Large	Cream	Eliptic
N39	Conical	Tall	Medium	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
KP423	Conical	Tall	Large	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
MAB (MB1)	Conical	Tall	Medium	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
IB(MB2)	Ellipsoid	Tall	Medium	Medium	Elliptic	Absent	Weak	Medium	Absent	Red	Elliptic	Medium	Large	cream	Oblong
MB3	Conical	Tall	Large	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
MB 4	Cylindical	Tall	Large	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
LLN	Ellipsoidal	Tall	Medium	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Roundish	Medium	Large	Cream	Oblong
MT	Conical	Tall	Large	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Medium	Cream	Oblong
MBK	Conical	Tall	Large	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Medium	Cream	Oblong
LU	Conical	Tall	Medium	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Medium	Cream	Oblong
LK	Cylindical	Tall	Large	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
RM	Cylindical	Tall	Large	Medium	Elliptic	Absent	Medium	Deep	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
KLM	Cylindical	Tall	Large	Medium	Elliptic	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
T(N39)	Cylindrical	Tall	Medium	Medium	Lanceolate	Absent	Medium	Medium	Absent	Red	Elliptic	Medium	Large	Cream	Oblong
M	Conical	Tall	Medium	Medium	Lanceolate	Absent	Strong	Deep	Absent	Red	Roundish	Medium	Large	Cream	oblong
MW	Conical	Tall	Medium	Medium	Lanceolate	Absent	Strong	Deep	Absent	Red	Roundish	Medium	Large	Cream	oblong
NR(KP)	Conical	Tall	Medium	Medium	Lanceolate	Absent	Strong	Deep	Absent	Red	Roundish	Medium	Large	Cream	oblong

Results of the cluster analysis are presented in Fig. 1. Degree of similarity varied from 20 to 25% and four main groups were formed after the similarity index was considered for clustering (Fig. 1). The hybrids cultivars except N39-1 were clustered in one group with the traditional cultivar N39 and KP423 while other genotypes from different regions were clustered in separate group. The other genotypes 27 also clustered together in shared sub-clusters.

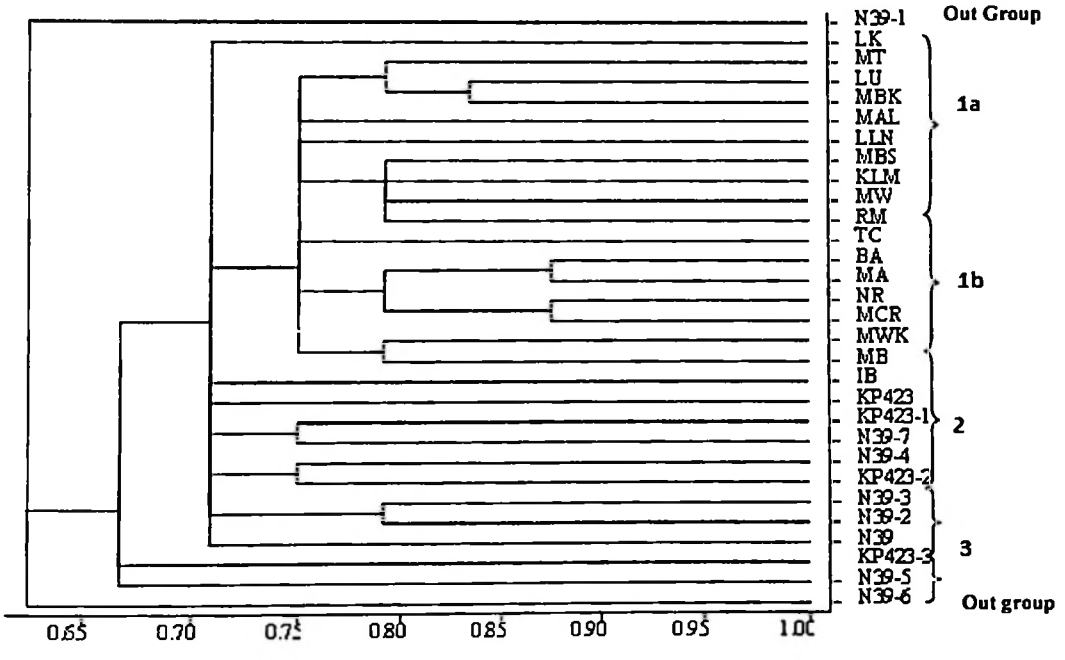

Figure 1: Cluster dendrogram illustrating morphological diversity between thirty coffee genotypes characterized using 24 morphological descriptors

Quantitative morphological data are presented in Table 2, all the test materials were not significantly different in leaf length, leaf width, flower number, seed width and seed thickness. However, significant variations were obtained in internodes length, weight of 100 dry fruits, seed length, and seed length/width ratio and weight of 100 dry seeds. The genotypes from Lushoto had significantly shorter internodes length than the rest of the test materials. On the other hand, genotype from Lilondo in Ruvuma Region had a longer interned length than the rest of the genotypes. Genotype KP 423-3 had a bigger seed size as evident from its significantly bigger seed length and seed length/width ratio as compared to the rest of the genotypes. All the test materials were significantly different from each other in weight of 100 dry seeds while N 39 recorded significantly more seed thickness than KP 423 and other genotypes.

Table 2: Variation of quantitative characters in the coffee genotypes

Genotype	Internodes length	Leaf length	Leaf width	No of flower	Seed length	Seed width	Seed L/W ratio	Seed thickness	Wt. of 100 dry seeds
MAL	6.45	13.65	6.40	21.90	11.50	7.50	1.53	4.19	24.30
IB	4.70	12.75	5.10	19.40	11.55	7.10	1.63	3.98	24.80
MBS	5.75	14.85	6.90	20.80	11.55	7.07	1.63	3.85	25.77
MB	4.30	09.70	4.70	21.70	11.64	7.08	1.64	3.75	25.87
LLN	7.85	16.60	7.20	26.40	11.25	7.55	1.49	4.09	24.00
MT	6.45	12.25	6.25	18.10	10.80	7.72	1.40	4.03	24.62
MK	5.00	13.45	5.19	19.00	11.20	8.04	1.39	4.01	25.30
LU	6.00	13.10	5.20	19.80	11.03	7.57	1.46	4.02	24.92
NR	4.67	12.57	6.16	21.20	12.90	6.43	2.01	3.87	24.78
MA	5.16	13.12	5.25	20.70	12.00	6.85	1.75	3.51	24.45
MCR	3.83	12.54	5.82	21.20	12.50	7.11	1.76	3.79	24.85
LS1	3.69	12.68	6.20	21.80	12.55	7.00	1.79	3.57	25.40
TC	4.35	13.20	6.30	21.60	12.10	6.40	1.89	3.34	25.20
LS2	3.55	12.65	5.95	21.40	12.25	6.30	1.94	3.20	25.30
LK	5.55	12.25	5.75	15.40	10.95	7.50	1.46	3.27	25.05
RM	5.65	12.40	6.20	17.20	12.50	6.43	1.94	3.63	26.05
KLM	6.00	13.10	6.25	18.10	12.60	6.75	1.87	3.28	26.22
N-39	5.80	15.25	6.75	16.60	12.40	7.10	1.75	5.50	26.50
N 39-3	5.65	12.85	6.30	15.80	15.00	7.40	2.03	3.50	24.10
N 39-2	5.95	12.10	5.75	16.20	12.00	7.80	1.54	5.00	24.57
N 39-1	6.30	12.85	6.00	16.40	10.40	8.10	1.28	2.90	18.51
N39-6	6.10	11.80	5.40	18.10	12.20	6.20	1.97	3.90	22.90
KP43-3	6.20	12.65	5.60	15.40	15.30	8.00	1.91	3.90	31.30
N 39-5	5.30	11.95	5.90	16.20	7.00	5.00	1.40	4.00	23.20
N 39-7	5.60	12.40	5.9	17.10	14.00	8.00	1.75	3.00	31.40
KP423-1	5.55	12.25	5.75	15.40	14.00	10.00	1.40	3.00	31.30
N 39-4	5.65	12.40	6.2	17.20	13.00	9.00	1.44	6.00	30.20

The variability is mostly explained by the weight of 100 dry seeds followed by the period between flowering and ripening, plant height and internodes length in that order (Table 2). Results of the principle component analyses indicated that the first two Principal components explained 35 and 25% (a total of 60%) of the total variation (Table 3).

Table 3: The first two Principal Components (PC) of the morphological characters

Variables	PC 1	PC 2
Athrocyanin colouration	-0.24222	-0.25424
Canopy diameter	- 0.31587	-0.04143
Depth of secondary vein	0.37341	-0.20239
Fruit colour	0.16842	0.21948
Fruit shape	-0.09760	-0.28857
Fruit size	0.08954	-0.23356
Internodes length	-0.29705	0.25175
Leaf length	0.04441	0.22227
Leaf width	0.07060	0.08684
Leaf shape	-0.29831	0.13763
Margin of undulation	0.34085	-0.23935
Number of flowers	0.30561	0.12241
Plant shape	0.03686	0.09044
Seed length	-0.11897	-0.40332
Seed length width ratio	0.21687	-0.33750
Seed thickness	-0.07878	0.13287
Seed width	-0.34835	0.04927
Seed shape	-0.12094	-0.25507
Weight of 100 dry seeds	-0.24026	-0.35392

The genotypes N 39 and KP 423 were found to be the most uniform recording intra-variation of less than 5 %. On the other hand, the genotypes from different regions were the most variable with an intra-variation of close to 13 %. The new lines portrayed an intra-variation of less than 10 %. From Table 2, number of flowers, margin of undulation and depth of secondary vein most to PC1 while internodes length and leaf length contributed most to PC2.

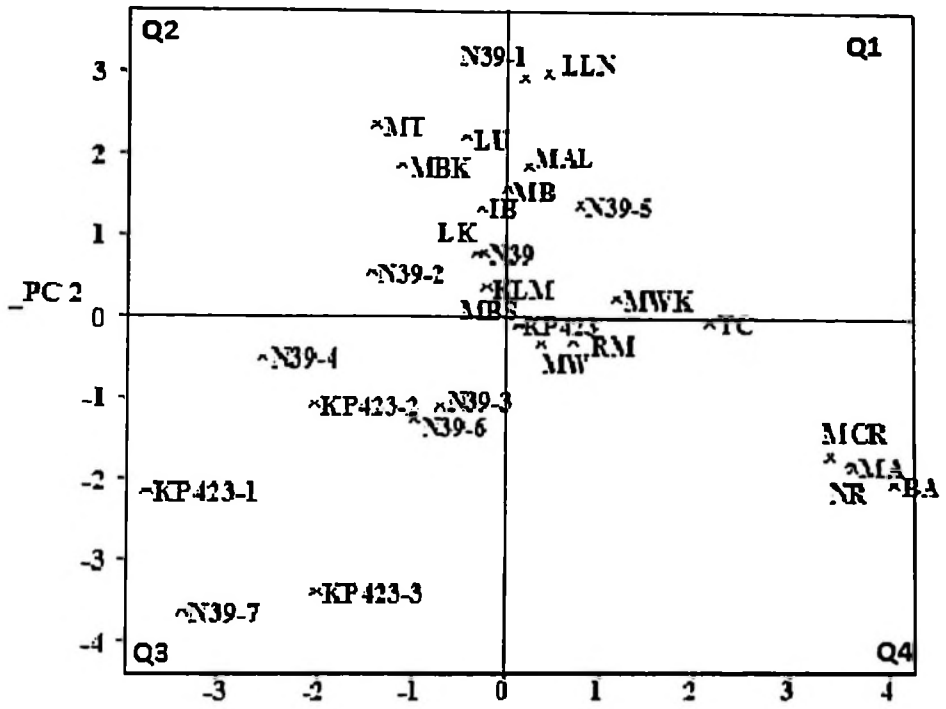

Figure 2: Bi plot analysis of *C. arabica* accessions presenting diversity among genotypes as determined by morphological variables.

The traits adherence to branch, intensity of ramification, presence or absence of leaf domatia, seed colour and plant height did not contribute at all to both PC1 and 2. The two dimensional presentation of the thirty genotypes is presented in Fig 2. Improved cultivars, KP 423-1, N 39-7, KP 423-3, N39-4, KP 423-2 N 39-3 and N 39-6 were grouped in a third quadrant. The other hybrids (N39-2, N39-1) and control N39 were placed in second quadrant together with samples from Kilema (KLM), Lekura (LK), Mtua (MT), Mbiku (MBK), Luahita (LU), Iboya (IB) and Mbozi (MBS). Furthermore the hybrid N 39-5 was in the first quadrant with sample from Lilondo (LLN), Malonji (MAL), Mweka (MWK), and Mbimba (MB). Finally the fourth quadrant contained samples from Mwika (MW), Tchibo (TC), Rombo (RM), Machare (MCR), Bazo (BA), Ngurudoto (NR), and the standard KP 423. Mbeya Region (Iboya, Mbimba, Mbozi, Malonji) and Ruvuma Region (Luahita, Mbiku, Mtua, Lilondo), and as well as the traditional cultivars N39 and KP423

found in this group. Genotypes from Machare, Arusha and Tanga form the separate group on the lower right side of the PCA graph (Fig 2).

4.1.2 Genetic diversity

4.1.2.1 Genetic differences and similarities among *Coffea arabica* in Tanzania

After DNA extraction the quantification of the products was conducted by running the individual genotype product through the 1.5% gel for 1.5 hr a shape bands were observed to satisfy the quality of the DNA is good for running PCR.

4.1.2.2 Genetic diversity evaluation of coffee Arabica genotypes

Separation of the bands for each genotype was observed in Plates 1 and 2. The amplification products were separated in 3% agarose gels in 1x TBE buffer and detected by staining with ethidium bromide 0.1µl after electrophoresis, run for one and half hours at 55volts (Sambrook *et al.*, 1989). Plates 1 and 2 show a sample DNA bands amplified from the *Coffea arabica* genotypes using different SSR markers.

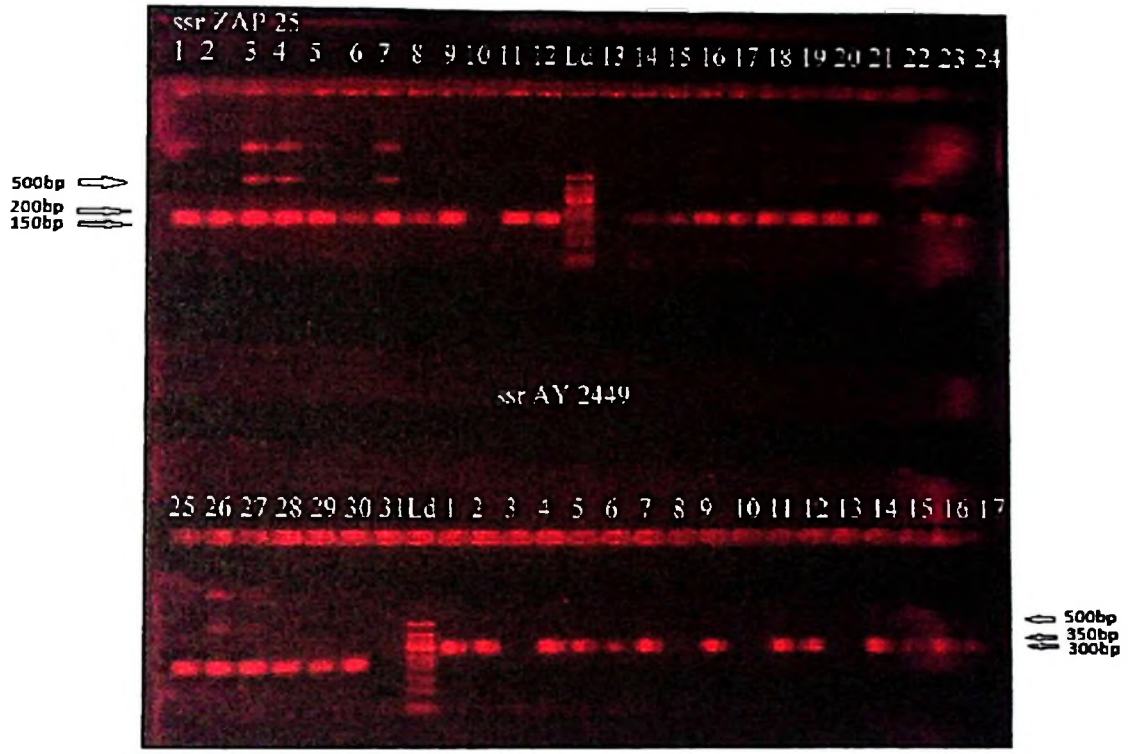

Plate 1: Amplification of DNA from coffee genotypes using SSR ZAP 25 (genotypes 1-30) and SSR AY 2449(genotypes 1-17)

From the total of 30 SSRs primer used, to detect allele's .Alleles were detected per each genotype and on electrophoresis they gave bands of different sizes (Plate 1). The detected bands ranged from 300 to 350 bp when SSR AY 2449 primer was used for amplification. On the other hand when ssr ZAP used as primer it gave the bands with sizes ranging from 150 to 200 bp. (Plate 1).

Furthermore when SSR 124577 used as primer for amplification it give bands ranging from 150 to 200 bp (Plate 2). These different sizes of bands give the genotypes opportunity to be differentiated from others.

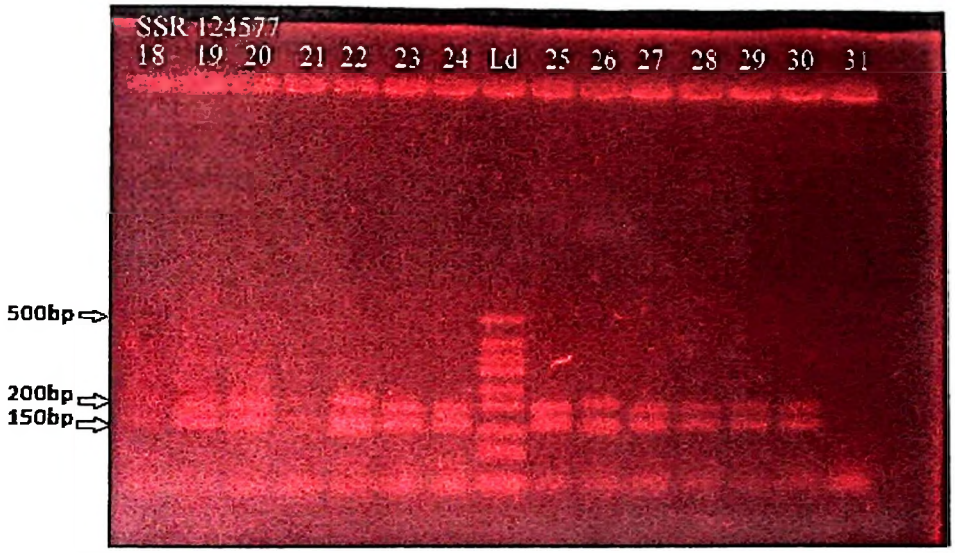

Plate 2: Amplification of DNA from coffee genotypes 18-30 using SSR 124577.

Further results from the PCR products amplified by the rest of SSR markers detected 60 alleles and out of these, 49 were polymorphism among the *Coffea arabica* genotypes. The number of loci per primer ranged from 1- 4, with the highest number exhibited by primer SSR 126 and SSR 278 while the minimum number of polymorphic loci were shown by primer number SSR A268, SSR AY 2449, SSRZAP 25, SSR CMA059 and SSR CMA151 (Table 4).

Table 4: Total number of alleles, polymorphoric alleles and rate of polymorphism (*rP*, %) for 29 SSR markers in Arabica coffee collections

SSR Code	Fragment size range(bp)	Number of alleles Total	Polymorphic
SSR 105	150 – 275	3	2
SSR 126	200 – 300	4	4
SSR 175	215 – 250	2	1
SSR 209	175 – 225	3	2
SSR 268	125 – 175	1	1
SSR 278	125 – 175	4	4
SSR 325	225 -275	2	2
SSR 338	235 -240	2	1
SSR 339	225 – 230	2	1
SSR A8783	90 – 130	1	1
SSR A8847	180 – 190	3	2
SSR AY2434	175 – 230	2	2
SSR AY2449	275 – 325	1	1
SSR ZAP25	185 – 210	1	1
SSR CMA8	110 – 155	2	2
SSR CMA055	95 – 100	2	1
SSR CMA059	125 – 175	1	1
SSR CMA151	168 – 200	1	1
SSR CMA 198	200 – 290	2	2
SSR CMA 199	150 – 200	2	2
SSR 233	225 – 250	3	2
SSR CMA263	200 – 225	2	2
SSR 124577	160 – 200	2	2
SSR 122850	150 – 190	2	2
SSR 124195	90 – 120	2	1
SSR 129793	200 – 250	2	2
SSR 123909	275 – 300	2	1
SSR 124161	325 – 380	2	1
SSR 123557	200 – 275	2	2
Total		60	49
Rate of polymorphism (rP %)		0.82	
No SSRs		29	
Average		2.07	1.69

4.1.2.3 Cluster analysis

Diversity representation of the overall studied coffee collections was also generated using the first and second principal component analysis. The first and the second principal components covered 28.7 and 8.2 % of the total variation, respectively, with a total of 36.9 %. The genetic relationship analysis revealed two clearly separated main clusters and several outside groups. The first containing genotypes N39-2, N39-3 and genotype from Marangu, second cluster contains the genotype N39-1, KP 423-1 and N39-7 and the most of indigenous genotypes. The genotypes N39-4, N39-5, KP423-3 and N39-6 formed the outside groups (Fig. 3).

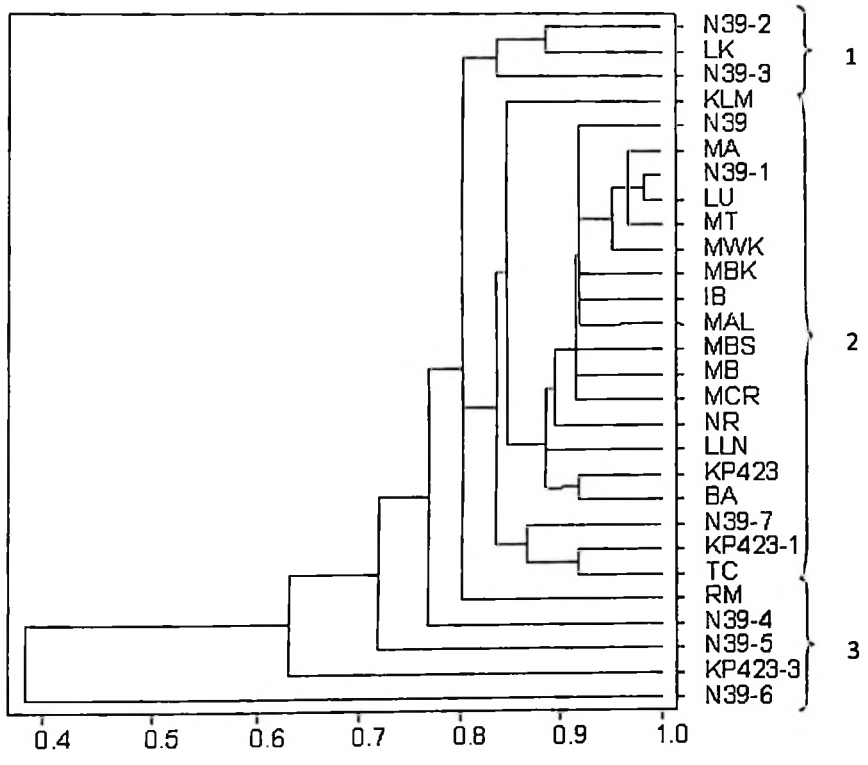

Figure 3: A Dendrogram showing the genetic similarity of *C. arabica* based on UPGMA using GenStat version 4.

4.2 Discussion

Genetic variation in Arabica coffee has previously been characterized using morphological and yield-related traits and phylogenetic relationships were established in Ethiopia (Lashermes *et al.*, 1996). However, the morphological markers are a classical method to distinguish variation based on the observation of the external morphological differences such as the size and shape of the leaf and of the plant form, the colour of the shoot tip, the characteristics of the fruit, the angle of branching and the length of the internodes (De Vienne *et al.*, 2003). In this study, morphological characteristics used to explain the variations existing in the new hybrids and the traditional cultivars from Coffee Arabica growing regions .The current results showed that the genotypes has a narrow genetic base of less than 25 %. This is different from the report of Kumar *et al.* (2008) that coffee trees differ greatly in morphology, size and ecological adaptation. The most distant related genotypes are the hybrid N39-7 and the genotype from Ruvuma. The observed difference could be attributed to hybridization program used in the development of the hybrid unlike in the genotype from Lilondo where single tree selection procedures were used as is the traditional cultivated genotype.

There was a high degree of similarity in qualitative traits of the old cultivars N39 and KP423 and the hybrid lines. The principal component analysis also produced N39 and KP 423 as being more closely related to the genotypes from different regions than to hybrids. The results could be attributed to inefficiency of morphological markers in assessing genetic variation. This concurs to the report of De Vienne *et al.* (2003) that assessing genetic variation with morphological markers can be inefficient as they are generally dominant traits, often exhibit epistatic interactions with other genetic traits and can also be influenced by the environment. Lashermes *et al.* (1996) reported that genetic factors are more accurately tested using molecular DNA markers. Agwanda *et al.* (1997) also

reported that single tree selection procedures used to develop most Arabica coffee cultivars have contributed to high level of uniformity among them.

The divergence of most of hybrids from other genotypes studied could have resulted from the fact that the hybrids are the results of the hybridization programs where selections from a multiple cross programs involving CBD resistant donor parents such as Rume Sudan (R gene), Hibrido de Timor (T gene), K7 (k gene). On the other hand, genotypes from Tanga and Arusha separated differently in the cluster diagram which shared almost all the morphological characters except the plant shape where genotypes from Arusha are cylindrical while genotypes from Tanga are conical.

The seed characters explain variation within genotypes where it loads high in the first principal component. Genotypes KP 423 (15.3mm) and N39-3 (15mm) have longer seed length than others while genotype N39-5 got the smallest (7.0 mm) (Table 2). Furthermore, genotypes KP 423-1 and N 39-7 showed more weight on 100 seed weight of 31.3g and 31.4g respectively. Lastly, genotypes from Arusha gave higher ratio (2.1) of seed length to its seed weight than the other genotypes.

There was very little morphological variation observed within genotypes. The mean similarity values between genotypes were higher than within varieties which underscore the low variability and the inbreeding nature of cultivated Arabica coffee. The results concurs with those of Anthony *et al.* (2001), who also demonstrated low genetic variation within Arabica coffee genotypes. Masumbuko and Bryngelsson (2006) also found similar results when comparing diploid coffee species and cultivated *Coffea arabica* L. from Tanzania. The low genetic variability within genotypes further strengthened the evidence of the narrow genetic base of Arabica coffee.

The assessment of genetic diversity is an essential component in germplasm characterization and conservation. The results derived from analyses of genetic diversity of *Coffee arabica* at the molecular level using SSR makers could be used for designing effective breeding programs aiming to broadening the genetic bases of coffee in Tanzania.

Furthermore in this study thirty, *Coffee arabica* genotypes were evaluated using thirty SSR markers. The genotypes studied represent *Coffee arabica* cultivated by farmers as well as the hybrid varieties developed by TaCRI. All the SSRs were polymorphic across all genotypes. From the total 30 SSR used, the detected alleles were assigned accordingly. One SSR primer had no amplification for the all genotypes. The total numbers of detected alleles were sixty, the number of allele per marker ranged from one to four with an average of 2.07 for the whole genome per marker. Fourtnine markers out of 60 markers detected were polymorphic with rate of 82 % polymorphism.

Results obtained from the genetic diversity study of *Coffea arabica* showed narrow genetic base as reported by Alemayehu *et al.* (2010) mainly based on pedigree and/or biochemical analyses. To our knowledge, this work represents the second study of genetic diversity and molecular characterization of Tanzanian *Coffea arabica* genotypes using molecular techniques whereby the first study used Inter simple sequence repeat (ISSR) by Masumbuko *et al.* (2006). Moreover, the study is supported by few published reports of DNA fingerprinting. SSR's were chosen for the analysis of genetic diversity of *Coffea arabica* genotypes because several works have shown that these markers are very powerful for differentiating individual germplasm accessions, particularly when they are closely related (Alemayehu *et al.*, 2010). Additionally, SSRs showed a series of advantages when compared with other DNA-based markers, such as abundance in the genome, high level of polymorphism, repeatability, co-dominance, and cost-effectiveness

(Ni *et al.*, 2002). The markers with the highest number of alleles are likely useful in distinguishing closely related *Coffea arabica* genotypes. These included Primer SSR 105, SSR 126, SSR 278 and SSR 233.

The genetic characterization using the UPGMA procedure using GenStat version 4 revealed eight clusters where all samples had a range of 0.4 – 1.0 Jaccard's coefficient of similarity. Results from the dendrogram generated based on UPGMA from similarities or a genetic distance matrix has shown an overall pattern of variation as well as the degree of relatedness among accession of cultivated *Coffea arabica.*

According to Moncada and McCouch (2004), the cultivated tetraploids were embodied approximately three fourth the amount of SSR diversity as the wild tetraploids based on the number of alleles, PIC values and similarity coefficients. Relatively high level of SSR polymorphism and genetic richness were also reported in Arabica coffee from Ethiopia (Anthony *et al.*, 2002; Moncada and McCouch, 2004). Similar results were also reported using other marker techniques such as RAPD (Lashermes *et al.*, 1996), AFLP (Lashermes *et al.*, 1996) and ISSR (Lashermes *et al.*, 1996).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Both traditional and new hybrid genotypes were evaluated in this study to demonstrate the usefulness of both morphological and molecular markers in the study of diversity of *C. arabica* genotypes in Tanzania. By using of morphological characters the genotypes from different regions were characterized based on the characters in the descriptor and the diversification recorded. Seed characters give the distinguishable evidence as they load high in the PCA. The power of microsatellite markers (SSR) was revelled, and it ability in diversity study as it give a clear discrimination of genotypes from the region of origin and the natural of genotype whether is hybrid or cold cultivar.

However, the results in both cases the results showed narrow genetic bases which concur with the previous findings by Masumbuko *et al.* (2005). The uniformity within genotypes expressed by dissimilarity values show there is limited variability within the cultivated *C. arabica* in Tanzania. The uniformity within genotypes expressed by dissimilarity values show there is limited variability within the cultivated *C. arabica* in Tanzania. The mean dissimilarity values between regions 0.70 were higher than 0.45 within regions was explained by the low variability and the inbreeding nature of cultivated Arabica coffee. Lashermes *et al.* (1996), found similar results when comparing cultivated and wild accessions of coffee. A similar study by Masumbuko *et al.* (2002) using RAPD markers showed a similar trend. By using morphological descriptor most of hybrids varieties had formed single distinguished clusters while N39-7, KP423-1, N39-1, and N39-2 were found together with other genotypes. On other hand most of genotypes from different

regions clustered together due to nature and the introduction of these materials to different regions from the centre of origin.

Furthermore, the genetic diversity of Arabica coffee cultivated in Tanzania studied using SSR markers showed the potential of SSRs markers to clearly differentiate coffee genotypes from different geographical origin suggests the possibility to use in quality control (DNA-based traceability) of Tanzanian coffee known by their areas of production.

5.2 Recommendation

The results from this study showed minimal morphological variation among the genotypes that were tested indicating low genetic variation and narrow genetic base. The information from this study can enhance utilization of coffee genetic resources in sustainable ways. This will include but not limited to conservation of its wild natural habitat or field gene banks, development of core collection and improved varieties, and other applications at molecular level such as Arabica coffee genetic map development and QTL detections to be used in marker assisted selection. In addition to SSR marker, other molecular marker techniques such SNPs should also be used to get the maximum benefits from the application of molecular techniques. There is therefore need to widen the genetic base of Arabica coffee in Tanzania by having more introductions of *C. arabica* genotypes.

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APPENDICES

Appendix 1: List of primers used in PCR

Sn	SSR Locus code	Primer Sequence		Repeat unit	References
		F: Forward	R: Reverse		
1	ssrR105	CACCAATTCCACTGACAATG	TCCCTGCCAACACACTTC	GA(18)	R&D Nestle Tours
2	ssrR126	GCACAATCACTCCCAAAG	TGACGGCCTACTACTTACAG	GA(23)	R&D Nestle Tours
3	ssrR175	GCAGTGACGCAGCAATG	AAAAGGAGAGCCAAAGCAGT	GA(20)	R&D Nestle Tours
4	ssrR209	CGGGGGTAAAAAGATTGTAA	TTGGTGGGAGGGGAGTA	GA(16)	R&D Nestle Tours
5	ssrR268	GTATCCCACAATGAAATCAC	AGTAGAATTTTCAACATATAAG	GA(19)	R&D Nestle Tours
6	ssrR278	TGTAGATTTGAAACCCAATC	AAGTCTCGACAAGTTTTGAC	GA(16)	R&D Nestle Tours
7	ssrR325	CCTTGTTGTTGGGGAATGTC	GGCTGTTCTGGGCTTTGTG	GA(23)	R&D Nestle Tours
8	ssrR338	CGAAGGCTGTCAACAACTGG	GGGATAAACAAGTTAAAGGA	GA(17)	R&D Nestle Tours
9	ssrR339	ATTATGCTCGCTGGGCTGTT	TGGGATCACTCCTGTGTCGC	CT(12)	R&D Nestle Tours
10	ssrA8783	CTTCGTATGGTTGTCTGTGT	AATGATAGGAGGCACTTGAC	GT(16)	Rovellii *et al* 2000
11	ssrA8847	GCACACATGAAAAAGATGCT	GATGGACAGGAGTTGATGG	GT(18)	Rovellii *et al* 2000
12	ssrAY2434	CGCAAATGTTTATGTCAATC	GCAACTTATGAGCCTAATCC	GA(20)	Cristancho *et al* 2002
13	ssrAY2449	CGAAAATATGCTGCCCATTG	CCGAACCCATAAGGTGTGAC	CT(20)	Cristancho *et al* 2002
14	ssrZAP25	GCGAAATCTTTCTCCCTCCC	CCGTCCTTTTCCTCGAACTC	GT(12)	Combes *et al* 2000
15	ssrCMA008	CATTCTGGTCCTGATGCTCT	TCATTCACTTATTAACGTCCATC	(CT)14	Universite Trieste
16	ssrCMA055	TTGAGCAAAAACCCTATTCC	TAAACCCAAAAAGACCACAA	(TG)18	Cristancho *et al* 2002
17	ssrCMA059	GATGGACAGGAGTTGATGGT	TTTTAACACTCATTTTGCCAAT	(CT9) (CA)8	Universite Trieste
18	ssrCMA151	GCCAGAAGAAGCTGGATGAC	ACCGTCCTTTTCCTCGAACT	(GT)8	Universite Trieste
19	ssrCMA198	AGCAACTCCAGTCCTCAGGT	TGGAAGCCCGCATATAGTTT	(TG)9 (AG)18	Universite Trieste
20	ssrCMA199	CATGCCATCATCAATTCCAT	CTAGCTAGCTGGATCAGTACCC	(CT)11	Universite Trieste
21	ssrCMA233	CAACGAGATAACTGGCAGGTC	CAAACCAATATTAGGAATAAAGAACG	CA)13	Universite Trieste
22	ssrCMA263	TGCTTGGTATCCTCACATTCA	ATCCAATGGAGTGTGTTGCT	(CT)18	Universite Trieste
23	SSR124577	GATGGCTTTTCTCCGTTATCC	GGATTCGACTGCTGGATGAT	AAG(6)	CGN
24	SSR122850	TCCAGTTTGATCAGCAACCA	CCATCTTGGGGATAGAGCAA	(AGAG)3	CGN
25	SSR124195	ATCCCCATCAGAAGACCTCA	CCTCCACCGCCTGTTTATTA	(AGC)6	CGN
26	SSR119699	GCCGTGGTGGAAGATGTACT	CGAGTTCACCAAGAACGTCA	(AGC)6	CGN
27	SSR129793	CTTGTAGCGGGGAAAATTGA	GCGATGGAAAAACCGATTAC	CACA (5) E	CGN
28	SSR123909	AGGCTTGCTGGAACTCTTGA	GAAAGACTTGTCCTTTGCCG	CTCT(7)	CGN
29	SSR124161	TGCGAAACCATTGAGAACAG	CCGGAGGATGAGATTGAAAA	CT(5)	CGN
30	SSR123557	ATCTCCTCGTTCTTCCCCAT	GCTTGTAGCAGGCAGGAAAC	CTCT(4)	CGN

Appendix 2: Descriptors for characterization of coffee as described by (IPGRI)

These will include: plant habit, plant height, overall appearance, vegetative development, branching habit, angle of insertion of primary branches, stipule shape, stipule arista length (mm), young leaf colour, leaf shape, leaf apex shape, leaf length (mm), leaf width (mm), leaf petiole length (mm), leaf petiole colour, young shoot colour, mature leaf colour, venation pattern, domatia pilosity, shape of domatia, shape of the aperture, position of the domatia, inflorescence position, inflorescence on old wood, number of flowers per axil, number of flowers per fascicle, number of fascicle per node, inflorescence stalk length (mm), corolla tube length (mm), number of petals per flower, anther insertion, number of stamens per flower, fructification duration (days), fruit colour, fruit shape, absence/persistence of fruit ribs, endocarp texture, fruit disc shape, calyx limb persistence, fruit length (mm), fruit width (mm), fruit thickness (mm), pulp thickness, harvest duration, seed length (mm), seed width (mm), seed thickness (mm), seed colour, and seed shape.

‹ppendix 3: Descriptors for characterization of coffee as described by (Walyaro 2006)

)ther descriptors which will be used in this study will include: internodes length on stem nd primaries, stem diameter, stem height, primaries and canopy radius, colour of mature :af, number of bearing primary branches, percent of bearing nodes, berries per nodes and ield cherries/ clean coffee, single berry weight (based on 200 mature fruits), bean single ean weight based on 200 green beans), out turn ratio, empty fruit rate and fruit filling oefficient, carcoli- bean rate (Pea berry), Bean grades above 18, 16, 14 and 12, coffee ody, acidity, bitterness astringency and beverage value, reaction to coffee wilt disease nd to little extend on coffee leaf rust and red blister diseases (Walyaro, 2006).

Appendix 4: List of genotypes used in the study.

Region	Genotype	Old cultivars
Kilimanjaro	Hybrid varieties	
	N 39-1 ,N 39-2 ,N 39-3	
	N 39-4, N 39-5, N 39-6	
	N 39-7, KP 423-1 ,KP 423-2	
	KP 423-3	
	Rombo	RM
	Kilema	KLM
	Marangu	M
	Kibosho	TC
	Machare	MCH
	Mweka	MW
	Reference cultivars	
	N 39	
	KP 423	
Arusha	Ngurudoto	NR
Ruvuma	Lilondo	LLN
	Ugano	MT
	Mbiku	MBK
	Luahita	LU
Mbeya	Malonji	MAB
	Iboya	IB
	Mbimba	MB
	Mbozi	MB2
Tanga	Lushoto 1	BA
	Lushoto 2	MA

Appendix 5: Data score sheet for the size of 30 SSR Microsatellite allele fragments indicating size range for each marker

VARIETY	ssrR105		ssrR126		ssrR175		ssrR209		ssrR268		ssrR278		ssrR325		ssrR338		ssrR339		ssrA8783		ssrA8847		ssrAY2434		ssrAY2449		ssrZAP25		ssrCMA8	
ALLELE	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
N39-2	150	150	200	240	215	250	175	210	125	175	130	160	225	275	235	235	230	230	90	130	160	200	175	230	275	325	185	210	110	150
LK	150	150	200	240	215	250	175	210	125	175	130	160	230	275	235	235	230	230	0	0	160	200	175	230	275	325	185	210	110	150
N39-3	150	150	200	240	215	250	175	210	125	175	130	160	0	0	235	235	230	230	90	130	160	200	175	230	0	0	185	210	115	155
N39-4	0	0	0	0	0	0	175	210	125	175	130	160	230	275	235	235	230	230	90	130	160	200	175	230	275	325	185	210	115	155
RM	150	150	210	275	215	250	175	210	125	175	130	160	230	275	235	235	230	230	90	130	155	190	175	230	275	325	185	210	115	155
N39-5	150	150	0	0	215	0	175	210	125	175	130	160	230	275	235	235	230	230	0	0	0	175	175	230	275	325	185	210	115	155
KLM	0	0	210	275	215	250	175	210	125	175	130	160	230	275	235	235	230	230	90	130	160	200	175	230	275	325	185	210	115	155
N39-6	0	0	0	0	215	250	175	210	125	175	0	0	230	275	235	235	0	0	0	0	0	0	0	0	0	0	185	210	115	155
N39-7	200	250	210	275	215	250	180	225	125	175	150	170	230	275	235	235	230	230	90	130	160	200	180	230	275	325	185	210	115	155
N39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KP423-1	200	250	210	275	215	250	180	225	120	160	150	170	235	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
TC	200	250	210	275	215	250	180	225	120	160	150	170	235	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
KP423-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KP423-3	0	0	0	0	0	0	180	225	125	175	150	170	230	275	240	240	230	230	0	0	0	0	180	230	275	325	185	210	115	155
LS1	250	275	210	275	250	0	175	210	125	175	0	0	230	275	240	240	230	230	90	130	130	130	180	230	275	325	185	210	115	155
KP423	250	275	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	130	130	180	230	275	325	185	210	115	155
MCR	250	275	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	130	130	180	230	275	325	185	210	115	155
MAL	250	275	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
IB	250	275	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
MB	0	0	0	0	0	0	175	210	125	175	125	150	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
N39	0	0	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
MBS	0	0	210	275	215	250	175	210	125	175	125	150	230	275	240	240	230	230	90	130	160	200	180	230	0	0	0	0	115	155
LLN	250	275	250	300	0	0	175	210	125	175	125	150	230	275	240	240	225	225	90	130	130	130	180	230	275	325	185	210	115	155
MWK	250	275	250	275	215	250	175	210	125	175	130	175	230	275	240	240	225	225	90	130	160	200	180	230	275	325	185	210	115	155
MT	250	275	0	0	215	250	175	210	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
NR	0	0	250	275	0	0	175	210	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
MBK	250	275	260	275	215	250	175	210	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
LS2	250	275	260	275	215	250	175	210	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
LU	250	275	260	275	215	250	175	175	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
N39-1	250	275	260	275	215	250	175	210	125	175	130	175	230	275	240	240	230	230	90	130	160	200	180	230	275	325	185	210	115	155
CONTR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 5: Continue

ssrCMA055		ssrCMA059			ssrCMA1511		ssrCMA198			ssrCMA199		ssrCMA233		ssrCMA263		ssr124577		ssr122850		ssr124195		ssr119699		ssr129793		ssr123909		ssr124161		ssr123557	
I	II	I	II	III	I	II	I	II	III	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
95	95	125	150	175	168	200	200	250	290	165	200	230	250	200	225	160	190	150	170	90	120	0	0	200	250	300	300	325	380	200	250
95	95	125	150	175	168	200	200	250	290	0	0	230	250	200	225	160	190	150	170	90	120	0	0	200	250	300	300	0	0	200	250
95	95	125	150	175	168	200	200	250	290	165	200	230	250	200	225	170	200	150	170	90	120	0	0	200	250	275	275	325	380	200	250
0	0	125	150	175	168	200	200	250	290	165	200	225	250	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	200	250
95	95	125	150	175	168	200	200	250	290	150	175	230	250	200	225	170	200	150	170	90	120	0	0	0	0	275	275	325	380	200	250
95	95	125	150	175	168	200	200	250	290	150	175	0	0	200	225	160	190	0	0	90	120	0	0	200	250	0	0	325	380	0	0
95	95	125	150	175	168	200	200	250	290	150	175	230	230	200	225	160	190	150	170	90	120	0	0	200	250	300	300	325	380	210	275
95	95	0	0	0	0	0	200	250	290	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	95	125	150	175	168	200	200	250	290	150	175	230	230	200	225	160	190	150	190	90	120	0	0	200	250	275	275	325	380	210	275
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	95	125	150	175	168	200	200	250	290	150	175	0	0	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	290	150	175	230	250	200	225	170	200	150	170	90	120	0	0	200	250	275	275	325	325	210	275
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	95	125	150	175	168	200	200	250	290	150	175	230	230	0	0	0	0	150	170	90	120	0	0	200	250	0	0	0	0	0	0
95	95	125	150	175	168	200	200	250	290	150	175	230	230	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	290	150	175	0	0	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	190	150	170	90	120	0	0	0	0	275	275	325	380	0	0
95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	200	150	170	90	120	0	0	200	250	275	275	325	380	0	0
95	95	125	150	175	168	200	0	0	0	150	175	230	250	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	200	150	170	90	120	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	190	150	170	90	120	0	0	200	250	275	275	325	380	210	275
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95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	190	150	170	0	0	0	0	200	250	275	275	325	380	210	275
95	95	125	150	175	168	200	200	250	275	150	175	230	250	200	225	160	190	150	170	0	0	0	0	200	250	275	275	325	380	210	275