Characterization of the Diversity of Coffee Genotypes in Kenya by Genetic, Biochemical and Beverage Quality Profiles

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DECLARATION

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

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DEDICATION

To my loving husband Elijah

To our daughter Kendi

To our son Kelvin

To the entire coffee community.

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LIST OF ACRONYMS AND ABBREVIATIONS

A.D	Anno Domini
AFLP	Amplified Fragment Length Polymorphism
BBC	Bacterial Blight of Coffee
CBD	Coffee Berry Disease
CBK	Coffee Board of Kenya
CGA	Chlorogenic acid
CGL	Chlorogenic acid lactones
CLR	Coffee Leaf Rust
CRF	Coffee Research Foundation
CRS	Coffee Research Station
Cv (s)	Cultivar(s)
DCM	Dichloromethane
DFA	Discriminant Factor Analysis
DNA	Deoxy-ribonucleic acid
FAO	Food and Agricultural Organization
FAQ	Fair Average Quality
GC	Gas Chromatograph
GDP	Gross Domestic Product
HDT	Hibrido de Timor
HMF	Hydroxylmethyl furfural
ICO	International Coffee Organization

IS	International Standard
ISO	Internal Organization for Standardization
IR	Infrared
MAS	Marker Assisted Selection
MATAB	Mixed Alkyltrimethylammonium Bromide
MASL	Meters Above Sea Level
MS	Mass Spectroscopy
NAD	Nicotinamide Adenine Dinucleotide
NIST	National Institute of Standards and Technology
NIR	Near infrared
NIST	National Institute of Science and Technology
PCA	Principal Component Analysis
PC	Principal Component
PCR	Polymerase Chain Reaction
QA	Quinic acid
RAPD	Randomly Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
rDNA	Ribosomal DNA
RFLP	Restriction Fragment Length Polymorphism
R11	Ruiru 11
SAH	*S*-adenosyl-L-homocysteine
SAM	*S*-adenosyl-l-methionine

SCAA	Specialty Coffee Association of America
SNK	Student-Newman-Keuls
SL28NS	SL28 not sprayed
SL28S	SL28 sprayed
SPE	Solid Phase Extraction
SSRs	Short Sequence Repeats

ABSTRACT

Coffee has been one of the most important products for the Kenyan economy for decades, and a high proportion of the coffee produced in Kenya is considered as the best quality coffee in the world. The natural variation of coffee places it in a widening spectrum of differentiated food products which include wines, beers, and cheeses among others. The possibility of coffee producers capturing price differentials will depend on consumers recognizing valuable characteristics produced at the green coffee production level. This study was conducted to: assess the genetic diversity among coffee genotypes in Kenya using molecular markers, evaluate the sensory characteristics of different *Coffea arabica* genotypes grown in Kenya, characterize *Coffea arabica* genotypes grown in Kenya by determination of their biochemical components and assess the level of association between sensory and biochemical variables. Genetic diversity of coffee genotypes was assessed using RAPD primers and microsatellites. Coffee samples for sensory and biochemical characterizations were processed by the wet method. Seven sensory variables namely; fragrance/aroma, flavour, aftertaste, acidity, body, balance and overall were assessed and scored together with three process control variables (uniformity, clean cup and sweetness) by a panel of seven trained cuppers on a 10-point scale. All the sensory parameters (including the process control parameters) were added together to constitute the total score which was a reflection of the broad coffee quality performance. Caffeine, oil, trigonelline, total chlorogenic acids (CGA), and sucrose were analyzed in green coffee samples using specific methodologies and quantified on dry weight basis. The sensory and biochemical data obtained were subjected to

analysis of variance and multivariate analysis using Costat, R-statistics, SPSS and XL-STAT statistical programs.

This study confirmed the low genetic diversity in Arabica coffee genotypes evaluated with dissimilarity of less than 5%. The study also widened the information on genetic diversity of coffee germplasm available for breeding programmes in Kenya since previous work was biased to commercial cultivars and donors of resistance to diseases. The molecular diversity shown among the Hibrido de Timor (HDT) derivatives could be exploited in breeding programmes especially by subjecting more intensive molecular characterization and consequently selecting elite lines among them. Analysis of variance indicated significant differences among the forty (40) *ex-situ* conserved coffee genotypes evaluated alongside two commercial varieties for sensory characteristics. Cluster analysis grouped the forty two (42) coffee genotypes into two major groups. The first group comprised of twenty seven (27) coffee genotypes, most of which were characterized by low beverage quality. The second cluster comprised of the remaining fifteen (15) coffee genotypes, most of which were relatively better in beverage quality. Ninety two percent (92%) of the fourty ex-*situ* conserved genotypes scored 80 points and above in mean total score qualifying them as specialty coffee. In molecular analysis, Ennareta and Geisha 11 clustered with non-introgressed Arabica genotypes and also revealed high beverage quality. The kind of diversity presented could be exploited with the aim of recommending some accessions for commercial cultivation. Sensory characteristics of the five advanced breeding lines Cr8, Cr22, Cr23, Cr27 and Cr30 together with the check cultivars SL28 and Ruiru 11 showed considerable

variations. Diversity was observed in some of the genotypes due to seasons and sites where they were grown. However, their sensory characteristics were similar to those of the check cultivars SL28 and Ruiru 11. In all the genotypes evaluated in this study, the levels of caffeine, trigonelline, oil, total chlorogenic acids (CGA) and sucrose agreed with documented values in Arabica coffee. *Ex situ* conserved coffee genotypes were most diverse in green bean caffeine, chlorogenic acids and sucrose contents compared to the commercial varieties. HDT had the highest amount of caffeine (1.68%) while Dilla Alghae had the lowest amount (0.77%). Moka Cramer had the lowest level of total chlorogenic acids (6.13%) while Barbuk Sudan had the lowest amount of sucrose (5.11%). Correlation of the cup quality and biochemical attributes showed that trigonelline significantly correlated to body, flavour, aftertaste and overall. Total chlorogenic acids negatively correlated with all sensory parameters while sucrose positively correlated significantly with all the sensory parameters. Higher green bean trigonelline and sucrose content can be improved together with desirable cup quality. Similarly, desirable cup quality traits and low green bean caffeine content can be selected simultaneously. Incorporating biochemical components analysis as a complementary method of evaluating coffee genotypes would generate additional information on the coffee genotypes and may reveal further diversity and potential for eventual exploitation.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 **Background information**

Coffee is an important export crop and a major foreign exchange earner for many countries in the developing world. It is the second most commonly traded commodity in the world after crude oil providing a livelihood to about 25 million coffee farming families around the world (Pare, 2002). Coffee beans are the seeds of a perennial evergreen tropical plant, which belongs to the family *Rubiceae* and genus *Coff*ea. Two species namely arabica (*Coffea arabica* Linnaeus) and robusta (*Coffea canephora* Pierre) are cultivated commercially (Lashermes *et al*., 1999; Anthony *et al*., 2002a; Pearl *et al*., 2004) and to a limited extent liberica *(Coffea* liberica) and excelsa (*Coffea excelsa*) (Charrier and Eskes 2004). Arabica coffee accounts for about 70% of the world coffee production (Anthony *et al*., 2002a). The natural variation of coffee places it in a widening spectrum of differentiated food products which include wines, beers, and cheeses among others (Roseberry, 1996). The possibility of producers capturing price differentials will depend on consumers recognizing valuable characteristics produced at the green coffee production level (Samper, 2003).

1.2 Global coffee production and its economic importance

Coffee is grown in over 70 countries in the tropical and subtropical regions of the world. It accounts up to 75% of the export revenue of many of these nations and provides a livelihood for about 25 million coffee farming families around the world. Coffee is the second most commonly traded commodity in the world after crude oil

(International Coffee Organization (ICO), 2010). For administrative and other reasons, the ICO has divided coffee production into four groups on the basis of the predominant type of coffee produced by each member country (Table 1). These groups are; Colombian mild Arabicas**,** other mild Arabicas, Brazilian and other natural Arabicas and Robusta.

Table 1: Coffee producing countries in the world.

Coffee categories	Producers
Colombian mild Arabicas	Colombia*, Kenya, United Republic of Tanzania
Other mild Arabicas	Bolivia, Burundi, Cameroon, Congo Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, United States, Guatemala, Haiti, Honduras, India, Indonesia, Jamaica, Madagascar Malawi, Mexico*, Nicaragua, Nigeria, Panama, Papua New Guinea, Peru, (Puerto Rico), Rwanda, Venezuela, Zambia, Zimbabwe
Brazilian and other dry processed Arabicas	Brazil*, Ethiopia, Paraguay
Robustas	Angola, Benin, Brazil, Cameroon, Central African Republic, Côte d'Ivoire, Democratic Republic of the Congo, Ecuador, El Salvador, Equatorial Guinea, Gabon, Ghana, Guinea, India, Indonesia, Laos, Liberia, Malaysia, Madagascar, Nigeria, Philippines, Sierra Leone, Sri Lanka, Thailand, Togo, Trinidad and Tobago, Uganda, Vietnam*

Note: * main producing country under each coffee category.
Source: International Trade Centre, (2002)

The trend in global coffee production for a period of five years is shown in Table 2. In the year 2004, coffee was the top agricultural export for 12 countries and in 2005; it was the world's seventh-largest legal agricultural export by value (FAO, 2007). Coffee accounted for exports worth approximately US$13.5 billion in coffee year

2008/09 (October–September) and an estimated US$ 15.4 billion in 2009/10 when some 5.6 million tonnes (93.4 million bags) were shipped (International Trade Centre, 2011). Coffee therefore is an important commodity in the world economy. Most of the world's green coffee beans are produced in Latin America and in particular in Brazil, which has led world production since 1840.

Table 2: Trend in global coffee production

(millions of bags of green coffee, 60 kg each)

Coffee types	Coffee producing regions	Coffee years				
		2005/06	2006/07	2007/08	2008/09	2009/10
Arabicas	Brazil	28.4	29.1	30.3	32.2	32.5
	Colombia	12.6	12.6	12.5	8.7	9
	Other America	23.1	23.1	24.2	22.8	21.8
	Africa	6.7	7.4	7.7	7.1	7.2
	Asia and Pacific	3.9	4.2	4.4	4.4	4.9
Robustas	Brazil	9.3	10.2	10.7	10.6	10.9
	Other Latin American	0.5	0.5	0.4	0.4	0.4
	Vietnam	13.8	19.3	16.5	18.5	18
	Indonesia	7.2	6.4	6.9	8.1	8.6
	Other Asia and Pacific	5.5	5.4	5.3	5.5	6.2
	Cőte d'Ivoire	2	2.8	2.6	2.4	1.9
	Uganda	1.7	2.2	2.6	2.6	2.4
	Other African producers	2.8	2.9	2.7	2.8	2.4
	Global totals	117.4	126.1	126.8	126.1	126.2

(Source: International Trade Centre, 2011)

1.3 Coffee production in Kenya

The first coffee seed was introduced to Kenya by French Missionaries around 1900 A.D. (Mwangi, 1983). Kenya produces Arabica coffee, and has the reputation of producing some of the best mild coffees in the trade. This is due to the varieties used, edapho-climatic conditions, good agronomic practices, careful harvesting and processing. The desirable quality attributes are derived from inherent genetic characteristics of selected coffee varieties, climatic conditions and proper field and post harvest management. Coffee is grown in three altitude zones in Kenya, the high altitude (over 1700 m above sea level), the medium altitude (between 1580 m and 1760 m) and the low altitude (1520m–1580m) above sea level (Jaetzold and Schmidt, 1993). The recommended cultivars in Kenya are K7 for low altitude areas (with serious cofee leaf rust), SL28 and SL34 for low to medium areas with good rainfall (Mwangi, 1983), and Ruiru 11 suitable for all coffee growing areas because it is resistant to Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR) (Opile and Agwanda, 1993). Within the country, coffee produced by two distinct sectors, namely plantations (estates) and the small-scale producers. There are about 500,000 smallholder farmers organized in 500 co-operatives whereas there are about 1,200 plantations.

In Kenya, coffee is mainly grown in three regions, namely: East of Rift Valley (comprising areas around Mt Kenya, the Aberdare ranges and Machakos), West of Rift Valley (comprising of Kisii highlands, Mt Elgon area and the North Rift valley) and Taita Hills in the coast. Of the estimated 160, 000 hectares of land under coffee, the East of Rift Valley region accounts for about 82%, Western of Rift for 17% and the Taita Hills for only 1%. Over the last 10 years, North Rift Valley has

increasingly become important as far as the future of coffee production in Kenya is concerned. The production area and yield trend for a period of five years is shown in Table 3.

Table 3: Hectarage and average yield of clean coffee by sector in Kenya

	Coffee Sector	05/06	06/07	07/08	08/09	09/10
Coffee by area in Ha	Co-operative	128000	121000	118000	120000	120000
	Estate	42000	42000	37000	40000	40000
Production in tonnes	Co-operative	27000	28400	22300	29400	22300
	Estate	21300	25000	19700	24600	19700
Average yield kg/ha	Co-operative	211	235	189	270	186
	Estate	506	595	532	616	493

Source: Economic survey, 2011.

Coffee in Kenya is mainly wet processed with a small proportion being dry processed (commonly known as "buni"). The primary unit operations involved in the wet processing of coffee include; harvesting, sorting, pulping, fermenting, washing, and grading drying, storage and conditioning. Each of these steps has an influence on the final quality of coffee (Mburu, 2004). In the dry mills, parchment coffee is hulled and graded into seven grades according to size, shape and density by use of mechanically agitated sieve graders. Currently there are two coffee marketing systems. The central auction system conducted every Tuesday of the week and the direct sales system commonly referred to as the "second window". In the auction system, licensed coffee dealers buy coffee through competitive bidding

whereas in the direct sales, Marketing Agents negotiate with the buyer and a sales contract is signed and registered with the Coffee Board of Kenya (CBK).

1.4 Economic importance of coffee in Kenya

The agricultural sector is the main driver of Kenyas' economy directly contributing 26% of the annual Gross Domestic Product (GDP) (Economic survey, 2010). The sector accounts for 65% of Kenya's total exports and provides more than 70% of informal employment in the rural areas. Kenya Vision 2030 has identified agriculture as one of the key sectors to deliver the 10% annual economic growth rate envisaged under the economic pillar. Coffee has remained an important export crop in Kenya since its introduction by missionaries in the early 1900s' (Mwangi, 1983). Coffee sector plays a fundamental role in foreign exchange earning, tax income, households income and employment opportunities. It also stimulates the industrial and the service sector such as agro-chemical industries, fertilizer industries, education and medicare. Currently, the coffee sector contributes approximately 10% of foreign exchange earning and is ranked fourth after horticulture, tourism and tea. The industry contributes to food and social security through trade and interdependence among various sectors in the economy. The quantities of coffee produced have been fluctuating. Coffee production increased by 10% from 48.3 thousand tonnes in 2005/06 crop year (Economic survey, 2007). However, the agricultural sector performed poorly in fiscal year 2007/08 due to adverse weather and the post election crisis contracting by 5.4% compared with a positive growth of 2.1% attained in 2007 (Economic survey, 2009). Prices of fertilizers and other farm inputs also increased, further aggravating the situation in the sector. Despite the

observed decline in coffee yields, the average prices paid for 100kg of clean coffee doubled in 2010 (Economic survey, 2011). The traditional export destinations for the Kenya coffee have been Germany (30%), Benelux (Belgium, Netherlands, and Luxembourg) (12%) USA and Canada (11%), Sweden (7%), Finland (6%) and UK (6%), (Statistical abstracts, 2008). However, in 2009, four (4) new emerging markets were identified which included China, Japan and Russia.

1.5 Problem Statement and Justification

The recommended cultivated traditional coffee varieties in Kenya are K7, SL34, and SL28. Cultivar SL28 and SL34 were selected at the former Scott Laboratories (now the National Agricultural Laboratories, (NARL) situated at Kabete) on a single tree basis (Jones, 1956). The prefix "SL" is an acronym for Scottish Laboratories where the variety was selected. The name is completed by a serial number "28 and 34" for the selections. These cultivars give excellent cup quality but are susceptible to Coffee Berry Disease (CBD), Coffee Leaf Rust (CLR) and Bacterial Blight of Coffee (BBC) which are expensive to control (Walyalo, 1983). The cultivar K7 cultivar was selected at Lengetet Estate in Muhoroni from the French Mission Coffee (Jones, 1956). The cultivar has resistance to some races of CLR and tolerant to CBD (Walyalo, 1983).

In order to alleviate the problem of coffee diseases, an extensive breeding program, at the Coffee Research Foundation (CRF), Kenya, saw the release of an Arabica coffee cultivar, Ruiru 11 in 1985 (Nyoro and Sprey, 1986). The variety name has the prefix "Ruiru" referring to the location of the Kenyan Coffee Research Station

where the variety was developed. The name is completed by an additional two code numbers, "11". The first code number denotes the type of variety as a one way cross between two designated parent populations and the second number defines the sequence of release, in this case the first release. The male parents are of outstanding selections from a multiple cross programme involving CBD resistance donors, Rume Sudan (*R* gene), HDT (T or CK-1 gene) and K7 and the good quality but susceptible cultivars such as SL28, SL34, Bourbon (B) and a drought resistant selection (DRI).The female parents are advanced generations (F3, F4 and F5) of the cultivar Catimor, ex Colombia, which is a hybrid of HDT and Caturra (Omondi *et al.*, 2000). This variety is mainly reproduced by controlled hand pollination (Agwanda, 1993). A pollination team induces flowering by irrigation of the male parents, collects pollen from these flowers and uses this pollen to fertilize female parents. The cultivar Ruiru 11 is a composite of about 60 F1 hybrid sibs each derived from a cross between a specific female and male population (Omondi *et al.*, 2001).

Due to the challenges encountered with reproducing Ruiru 11, five coffee breeding lines coded Cr8, Cr22, Cr23, Cr27 and Cr30 have been under evaluation at CRF with the prospect of releasing them as commercial varieties. Their unique features include tall stature, true breeding and resistance CBD and CLR. The five lines were selected as individual tree from backcross progenies involving SL4, N39, Hibrido de Timor (HDT) and Rume Sudan as the donor varieties and the traditional commercial cultivars SL28, SL34 and K7 as the recurrent parents. Van der Vossen, (2005)

recommended exhaustive testing of the cup quality of new cultivars before releasing them for commercial use.

A coffee gene bank is maintained at Coffee Research Station (CRS) whereby *C. arabica* accessions from Ethiopia, Sudan, Kenya, Tanzania, Angola, India, Reunion, Portugal, South and Central America (Jones, 1956) are conserved *ex-situ*. Some of these conserved genotypes have been used as progenitors in the main breeding program at CRS (Walyaro, 1983). With the shifting consumer trends it implies that quality specific selection will depend on the exploitation of unused genetic diversity. However, in spite of its significance, this *Coffea* collection has not been subjected to thorough characterization.

Market studies show that consumers are more discriminating about differences between groups of coffee, including distinctions based on product origin, taste characteristics, such as smoothness, aroma and acidity, organic characteristics, and other factors (Commission for Environmental Co-operation, 1999). The diversity of some coffee genotypes in Kenya using morphological characteristics have been determined Walyaro (1983) and Gichimu and Omondi (2010b). However, morphological markers are reportedly inefficient because they are generally dominant traits, they often exhibit epistatic interactions with other genetic traits and can also be influenced by the environment (Weising *et al*., 2005). Evaluation of genetic diversity of coffee genotypes in Kenya using molecular markers is desirable. Much work has been done in characterizing coffee germplasm in Kenya based on sensory variables (Owuor, 1988; Njoroge *et al.* 1990; Ojijo, 1993; Omondi, 2008).

A lot of work has already been done in attempting to understand the biochemical composition of green and roasted coffee beans and to associate such chemicals with the cup quality (Bertrand *et al*., 2003). The link between such studies and the genetic improvement of quality is lacking in Kenya.

Coffee has been one of the most important products for the Kenyan economy for decades, and a high proportion of the coffee produced in Kenya is considered as the best quality coffee in the world. Cup quality is linked to premium prices and stimulates farmers in Kenya to improve the quality of their coffee. Each day, this interest in quality is translated into bottom-line purchasing decisions. Organoleptic methods are widely utilized in the selection of the new cultivars and also act as a tool to support new cultivars and as a tool to communicate to consumers the peculiar traits of the products. This study envisages evaluating the genetic diversity of coffee genotypes in Kenya by biochemical components as a complementary tool in coffee analysis. Knowledge of the nature, composition and levels of the chemical substances in relation to specific sensory attributes would be of immense value in appraisal of coffee quality. Such an approach integrated at early stages may detect finer differences between the breeding lines and the traditional cultivars.

1.6 Objectives

The general objective of this study was to characterize the diversity of coffee genotypes in Kenya.

1.6.1 Specific objectives

I. To assess the genetic diversity of coffee genotypes in Kenya using molecular markers.

II. To evaluate the sensory characteristics of different *Coffea arabica* genotypes grown in Kenya.

III. To characterize *Coffea arabica* genotypes grown in Kenya by determination of their biochemical components.

IV. To assess the level of association between sensory and biochemical variables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, distribution and genetic variability of *C. arabica*

Two types of coffee are consumed worldwide, Robusta (*Coffea canephora* P.) and Arabica (*Coffea arabica* L.). The genus *Coffea* is diverse and reported to comprise about 103 species (Davis *et al*., 2006). *Coffea arabica* is the only tetraploid (2n = 4x = 44) species in this genus and self-fertile, while other species are diploid (2n = 2x = 22) and generally self-incompatible (Charrier and Berthaud, 1985). *C. arabica* contain two genomes that originated from two different diploid wild ancestors, *C. canephora* and *C. eugenioides* Moore (Lashermes *et al*., 1999). Genomic analysis using Restriction Fragment Length Polymorphism (RFLP) of chloroplast DNA (cpDNA), which is maternally inherited, supports the notion that *Coffea eugenioides* donated the maternal genome while analysis of ribosomal DNA (rDNA) demonstrated that *Coffea canephora* donated the paternal genome (Lashermes *et al.*, 1995). Although highly homozygous, *C. arabica* contains a considerable amount of fixed heterozygosity in relation to its allotetraploid origin.

The centre of origin of the genus *Coffea* is mainly confined to the plateau of southwestern Ethiopia and on the Boma plateau of Sudan (Lashermes *et al*., 1999; Anthony *et al.*, 2002a). Populations of *C. arabica* have also been reported in Mount Imatong (Sudan) and Mount Marsabit (Kenya) (Berthaud and Charrier, 1988). On the other hand, the centre of origin of other coffee species overlaps elsewhere in the central and western parts of Africa (FAO, 1968a). Therefore, *C. arabica* follows the

typical distribution features of polyploids, that is, peripheral expansion outside the range of distribution of the other diploid species of the genus (FAO, 1968b).

Early domestication and selection of the species was carried out by Arabs who introduced it into Yemen probably in the 13 and 14th century (Wintgens, 2004). All species of *Coffea* are woody, ranging from small shrubs to large robust trees with heights of up to 10 meters. Phenotypic variation between species is wide.; some are deciduous while others are evergreen; leaves range in colour from yellow and dark green to bronze and purple-green and vary in size, (*Coffea liberica.*) has the largest leaves); fruit size ranges from that of a small pea to a good-sized plum. The coffee flowers consist of a white fine lobe corolla, a calyx of five, stamens and a pistil. The ovary is at the base of the corolla and contains two ovules that if duly fertilised produce two coffee beans (Charrier and Eskes, 2004). Wind pollination maybe of prime importance, however coffee blossom also attract insects which contribute to the pollination process. An illustration of coffee blossom is shown in Plate 1. The time taken from flowering until the maturation of the coffee berries varies according to the variety, climatic conditions, and agricultural practices. *C. arabica* takes 6-9 months while Robusta takes 9-11 months (Wintgens, 2004).

Plate 1: Coffee Blossom
(a bee can be seen)

The genus *Coffea* is not only endowed with enormous morphological variation, but also with adaptation to a wide range of environments, provided there is no frost. The plants grow from sea level to 2600 meters altitude above sea level, in habitats ranging from under shade to without shade, from sandy to humic soils and from flooded habitats (example *Coffea congensis* Frohener) to arid areas example *Coffea rhamnifolia* (Chiov.) Bridson, (FAO, 1968a). Illustrations of some morphological diversity of some coffee genotypes are shown in Plate 2, Plate 3, and Plate 4. Features of some berries of different coffee genotypes are shown in Plate 5 and Plate 6.

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Plate 2: ***Coffea arabica*** **variety Purpurascens**

Plate 3: ***C. eugenioides***

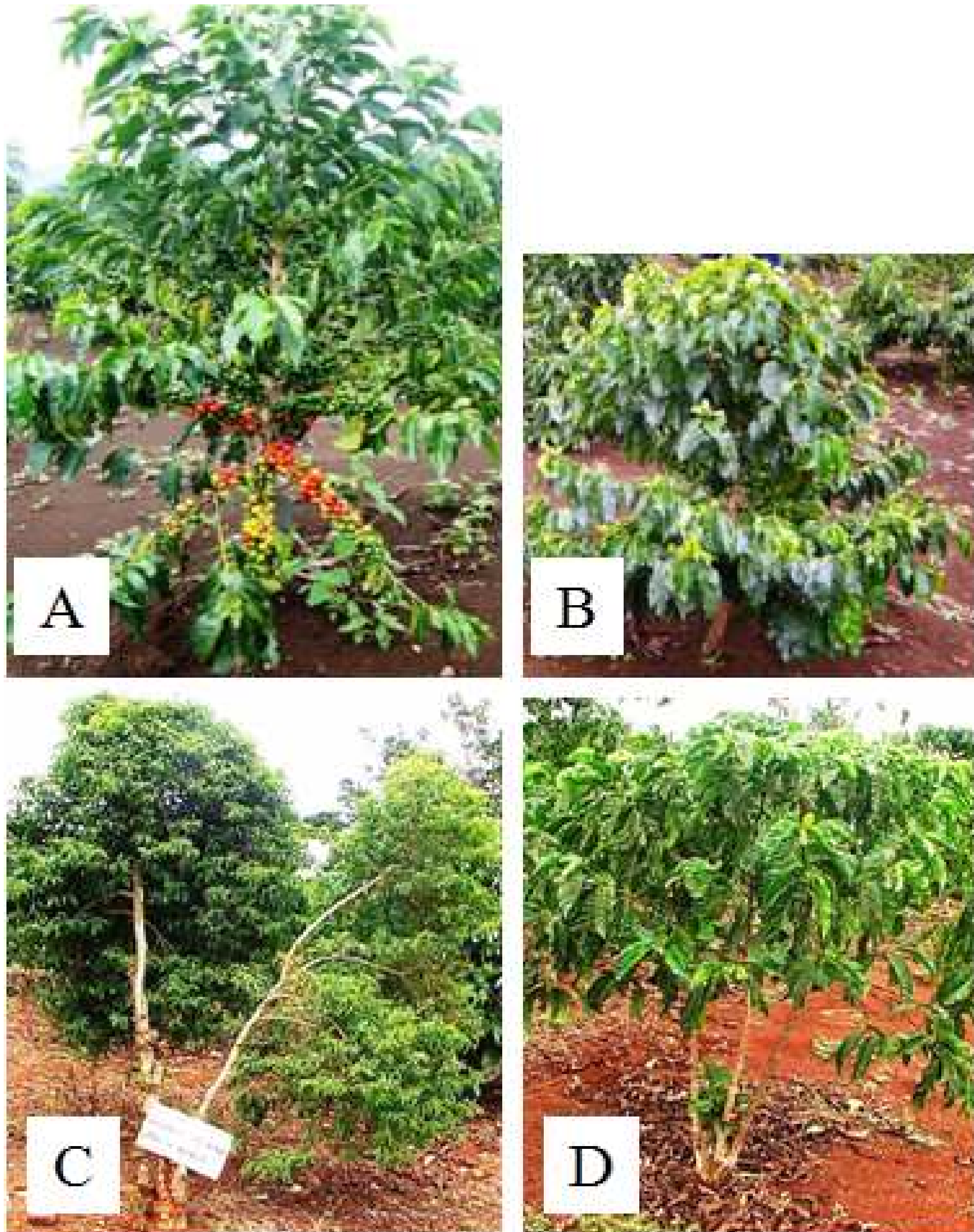


Plate 4: Canopy characteristics of different coffee genotypes;

(A) Open tall statured advanced breeders' line, (B) the compact Ruiru 11 hybrid, (C) the bushy *Coffea eugenioides* and (D) the robust *Coffea canephora* (Robusta coffee)

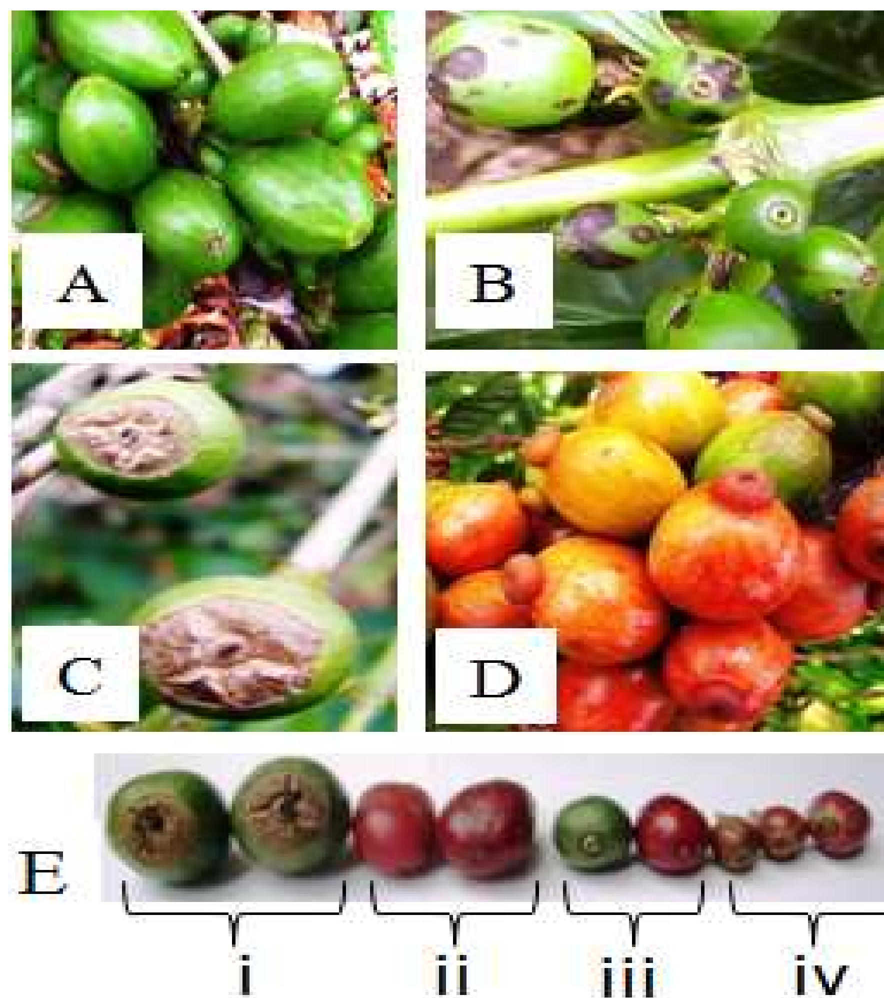


Plate 5: Features of berries of different coffee genotypes

(A) typical Arabica coffee berries, (B) berries of the Cultivar SL28 infected by Coffee Berry Disease, (C) the large round fruit with prominent disc end of Polysperma, (D) the prominent navels of a variety of Robusta and (E) different sizes of berries (i) Polysperma, (ii, typical Arabica, (iii) Robusta and (iv) *Eugenioides*

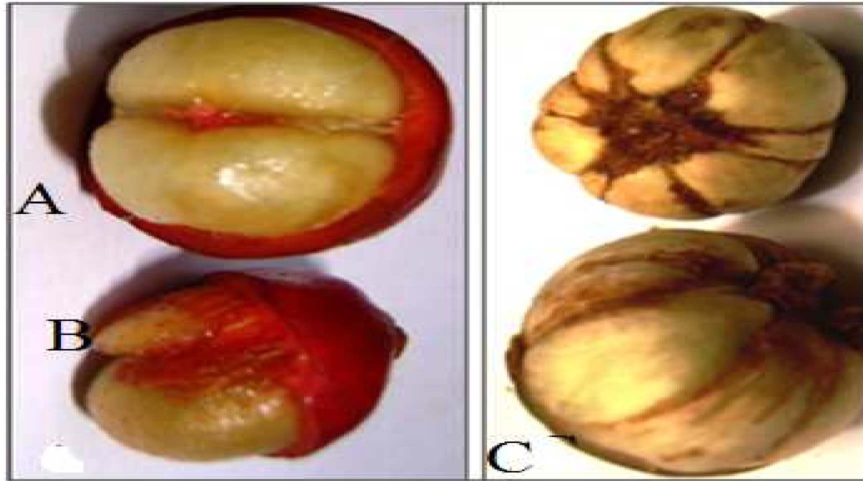


Plate 6:Coffee berries showing different distinguishing features

(A) Characteristic normal two beans (B) Occasional one bean accompanied by a malformed twin (C) Polysperma typically with multiple beans per pod.

C. arabica is characterized by low genetic diversity (Lashermes *et al.,* 1996), which is attributable to its reproductive biology and recent evolution. Among other things, the low variability is reflected in its susceptibility to most coffee diseases. Although breeders have managed to exploit this low variability to develop improved coffee varieties, transfer of traits of agronomic importance from other *Coffea* species is desirable. One avenue of such transfer is by use of HDT. HDT is a spontaneous inter-specific cross between *C. arabica* and *C. canephora* that was observed as an atypical tree in a *C. arabica* field planted in 1927, in the island of Timor (Bettencourt, 1973). Progenies of this hybrid, mainly three accessions (numbers 832/1, 832/2 and 1343), have been and continue to be used worldwide as the main source of resistance to various pests including CBD, CLR and nematodes (*Meloidogyne spp*). Molecular genetic analysis of derivatives of these progenies have demonstrated that they variously contain an estimate of 9-29% of the *C. canephora* genome, and they constitute a considerable source of diversity for

Arabica coffee improvement (Lashermes *et al.*, 2000b). Breeding programmes utilizing these progenies have given rise to introgressed cultivars like 'IAPAR59' in Brazil, 'Variedad Colombia' in Colombia 'IHCAFE 90' and 'Costa Rica 95' in Central America, Ruiru 11 in Kenya and 'Sln 12' in India (Anthony *et al.*, 2002a). The continued use of the derivatives of HDT for Arabica coffee breeding emphasizes the importance of these materials and introduction of genes from diploid relatives of *C. arabica*. Coffee genetic resources are conventionally conserved as trees in field gene banks. So far no efficient procedure is available for the long term storage of coffee seeds (Florin *et al.,* 1995).

Important collections of *C. arabica* are maintained at Coffee Research Station (CRS) Ruiru. The coffee germplasm conserved *ex-situ* at Coffee Research Station (CRS) Ruiru, has many *C. arabica* accessions from Ethiopia, Sudan, Angola, India, Reunion, Portugal, South and Central America and some from Kenya (Millot, 1969). Genetic diversity analysis among such accessions is vital for efficient utilisation of the available germplasm.

2.2 Methods of assessing genetic diversity

2.2.1 Molecular techniques

Different methods such as morphological, biochemical and molecular markers are available to estimate the genetic diversity within and among genotypes. A variety of techniques have been utilized to measure genetic variation of coffee species in Kenya. Walyalo (1983) evaluated the diversity of eleven varieties (8 were from the Kenya coffee gene bank accessions and three (3) were commercial cultivars) using

morphological characteristics. Gichimu and Omondi, (2010b) also determined the morphological diversity among some newly developed and existing commercial cultivars in Kenya. However, morphological markers are reportedly inefficient because they are generally dominant traits, they often exhibit epistatic interactions with other genetic traits and can also be influenced by the environment (Weising *et al*., 2005).

The importance of molecular markers for genetic improvement in perennial crops like coffee is immense. It allows selection of desirable genotypes at an early growth stage (at seedling stage), on a large number of breeding lines, reduce the number of backcross cycles required to restore the quality of the recurrent parent and for simultaneous improvement of different traits (Lashermes *et al*., 2000b). A variety of molecular techniques have been developed to measure genetic variation at both interspecific and intraspecific levels in a number of plant species. Recent advances in the field of plant molecular genetics have resulted in the development of a series of DNA markers. Of these, Short Sequence Repeats (SSRs) or microsatellites (Weber and May, 1989), Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al.,* 1980), Randomly Amplified Polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al*., 1990), and Amplified Fragment Length Polymorphism (AFLP) (Vos *et al*., 1995) are the most popular. Only the molecular markers applied in this study are discussed below.

2.2.1.1 RAPD

RAPD was first used by Williams *et al*., (1990) to examine human DNA samples. This method is based on the fact that using short arbitrary primer sequences; they can by chance anneal on random sequences within the genome in close proximity and in opposite orientation to be amplified in a PCR programme. RAPD marker technique is quick, easy and requires no prior sequence information (Welsh and McClelland, 1990). In coffee, RAPD technique was used to study the genetic diversity and relationships among *Coffea* species (Lashermes *et al.,* 1993, Orozco-Castillo *et al.,* 1994; Anthony *et al.,* 2001). The technique has also been used successfully to analyze the genetic diversity among cultivated and sub-spontaneous accessions of *Coffea arabica* (Lashermes *et al.,* 1996). Agwanda *et al*. (1997) conducted a study to identify RAPD markers associated with CBD resistance and to identify markers which could be used to select against the genetic background of CBD resistance donors. However, Gichuru *et al.* (2007, 2008) were not able to regenerate polymorphic bands with the RAPD primers reported by Agwanda *et al.* (1997). Destpite such shortcomings, RAPD markers have been applied to study general diversity. Masumbuko *et al.* (2003) demonstrated that RAPD markers were able to determine variability in the Tanzanian cultivated *C. arabica* accessions clustering them according to geographical locations. A study conducted by Aga *et al.* (2003) on forest *C. arabica* in Ethiopia also demonstrated that the RAPD technique could be applied for measuring the degree of variability within, and between forest *C. arabica* L. populations. Tshilenge *et al.* (2009) established high variability in the Congolese *Coffea canefora* var. robusta genepool using RAPD and Inter-simple sequence repeat (ISSR) markers. Like it is for many crops, evaluation

of the genetic diversity and available resources within the genus *Coffea* is an important step in coffee breeding (Cubry *et al*., 2008).

2.2.1.2 Microsatellites

A microsatellite is a short DNA sequence that is repeated many times within the genome of an organism. Repetitive DNA consists of simple homopolymeric tracts of a single nucleotide type [poly (A), poly (G), poly (T), or poly (C)] or of large or small numbers of several multimeric classes of repeats (Van Belkum *et al*., 1998). The number of repeats at a particular locus is hypervariable between individuals of the same species. Simple sequence length polymorphism caused by the variation in the number of repeats can easily be detected by PCR using pairs of primers designed from unique sequences bordering the SSR motifs. It is for this reason that microsatellites can be used for genetic fingerprinting. Anthony *et al*. (2002b) studied the genetic diversity within and among Typica-, Bourbon- and subspontaneous-derived accessions using six SSR loci and identified two alleles which discriminated the Typica derived accessions from the Bourbon derived accessions. Microsatellites have been applied to identify *C. arabica, C. canephora* and related species (Combes *et al*., 2000). They have also been used to investigate polymorphisms among wild and cultivated *C. arabica* accessions (Rovelli *et al*., 2000; Anthony *et al*., 2002b; Baruah *et al*., 2003; Moncada and Couch 2004) and to analyze the introgression of DNA fragments from *C. canephora* and *C. liberica* into *C. arabica* (Lashermes *et al*., 2000, Lashermes, *et al*., 2010; Prakash *et al*., 2002; Gichuru *et al*., 2008). In Kenya, Gichuru (2007) showed Sat 235 and Sat 172 to be linked to CBD resistance while, Omondi *et al.* (2009) reported SSR polymorphism

between a disease resistance donor, (Rume Sudan), and a susceptible cultivar, (SL28). This study expect to use molecular markers to assess overall genetic diversity of coffee genotypes, in Kenya.

2.2.2 Organoleptic characterization of coffee

Organoleptic relates to the attributes perceptible by the senses. The human senses have been used for centuries to evaluate the quality of foods. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Martens, 1999). Coffee quality is assessed organoleptically by trained coffee tasters (Van der Vossen, 1985; Agwanda, 1999). The distinct flavour of brewed coffee is certainly the main reason for its wide popularity and almost universal appeal as a refreshing beverage (Petracco, 2001). The desirable aroma and taste of brewed coffee is formed during roasting of green coffee beans. The international standard ISO-5492 (2008) gives a list of terms used in sensory analysis of coffee. In that standard, flavour is defined as a complex combination of olfactory (pertaining to the sense of smell), gustatory (pertaining to the sense of taste) and trigeminal (oro-nasal chemesthesis) sensations perceived during tasting. Coffee aroma is composed of the gaseous chemical components of roasted coffee beans, which escape as gases after the coffee beans are ground and as vapors' during brewing (Lingle, 2001). Coffee taste is composed of water-soluble organic and inorganic natural chemical components of roasted and ground coffee beans which are extracted as liquids during the brewing process (Lingle, 1996).

Acidity has been recognized as an important attribute of the sensory quality in coffee. The International Standard ISO-5492 (2008) defines acidity as a basic taste produced by dilute aqueous solutions of most acid substances. Some of the acids contributing to this sensation are formed during the development of the coffee bean while some are generated during roasting (Ginz *et al*., 2000). Acidity rises from the presence of hydrogen ions from the ionization of constituent acids (both inorganic and weak organic) in aqueous solution. Among the coffee tasters, sourness has a particular connotation, generally unfavorable, whereas, acidity is a favorable characteristic Washed Arabicas (or milds) usually have fine acidity whereas dry processed Robustas are neutral with varying degrees of harshness (Clarke, 1987). Mouthfeel is a tactile sensation perceived by buccal mucous membranes, along with the thermal response due to the beverage's temperature (Petracco, 2001). In coffee tasting, body is the sense of weight or heaviness that coffee brew exerts in the mouth sometimes referred to as mouth-feel.

Different countries have over time developed their own methodologies for assessment of coffee quality. In Kenya, Colombia and Ethiopia for example, liquor quality is determined on the basis of the level of acidity, body, and flavour of the brew (Devonshire, 1956; Moreno *et al*., 1995; Asfaw, 2008). The coffee industries rely on human sensory panels that are trained to discriminate degrees of smell and taste in coffee. It is difficult to relate the output of analytical instruments to human perception because the chemosensory systems of smell and taste use information gathered from the interaction of complex chemical mixtures with the biological sensors without separation of individual components (Sneath *et al.,* 2002). The

international coffee market is increasing demand for products of unique characteristics or of high beverage quality. The term ‘specialty coffee’ originated in the United States where it was initially used to describe the range of coffee products sold in dedicated coffee shops, to differentiate them from those available through general retail outlets (International Trade Centre, 2011). Due to the growth and the accompanying proliferation of specialty products, Specialty Coffee Association of America (SCAA) developed a standard protocol for the certification of specialty coffee (Lingle, 2001). The SCAA methodology has been designed in such a way that the entire panel employs the same terminologies when evaluating coffee. When panellists with the same level of training employ the same terminologies, results generally show good inter-panel agreement and are comparable (Martin *et al*., 2000). However, the interpretation of the underlying sensory dimensions responsible for the perceived differences may differ between panels due to differences in individual panellist’s understanding and use of certain attributes (Risvik *et al*., 1992; Hunter and McEwan, 1998). Regardless of the approach being used to analyze the sensory attributes of the coffee, panels require extensive training before the panel can become a reliable sensory instrument (Findlay *et al*., 2006).

2.2.2.1 Some factors affecting coffee quality

Coffee beverage quality is a complex characteristic which depends on a series of factors. Genetic factors have been associated with the quality of coffee. The two species of *Coffea* that have acquired worldwide economic importance are Arabica and Robusta. The difference in these coffees are recognized commercially with Robusta usually selling at prices 20–25% lower than Arabica (Esteban-Díez *et al.,*

2004,) Arabica beans are highly valued by the trade, as they are considered to have a finer flavour than Robusta. Bertrand *et al.* (2005) reported that introgression of genes from the *C canephora* genome could have a negative impact on the cup quality of cultivars derived from the Timor Hybrid. However some introgressed F1 hybrids have been found to have similar or superior to traditional cultivars for certain attributes, such as acidity or aroma (Bertrand *et al.,* 2006).

The environment has also a strong influence on coffee quality (Decasy *et al*., 2003; Gichimu and Omondi, 2010a). The interaction between the genotypes and the environment has also been studied. Walyaro (1983) reported relatively lower genotype by environment interaction effects on cup quality characters. Van der Vossen (1985) reported non-significant genotype by environment interaction effects on quality characters, such as bean size and cup quality. However, Agwanda *et al.* (2003) reported significant genotype by environment interaction effects on coffee bean and liquor quality.

The quality of the soil and specifically the balance between the different nutrients is important for the cup quality (Yadessa *et al.,* 2008). Amber beans (smooth yellowish coffee beans) were observed to be produced on trees suffering from iron deficiency (Robinson, 1960). The coffee beverage produced from roasted amber beans was described as full in body but lacking acidity (Devonshire, 1956). Iron deficiency in coffee trees in Kenya was shown to be caused bythe high alkanity in the soil (Robinson, 1960).

Climate, altitude, and shade play an important role through temperature, availability of light and water during the ripening period have a strong influence on flowering, bean expansion, and ripening (Harding *et al*., 1987). Dessalegn (2005) reported that if other factors are kept constant, better quality coffee can be found at higher altitudes, while low land coffee were found to be somewhat bland, with considerable body. The slowed-down ripening process of coffee berries at higher elevations (lower air temperatures), or under shading, allows more time for complete bean filling (Vaast *et al.,* 2006), yielding beans that are denser and far more intense in flavour than those grown at lower altitudes (or under full sunlight). The slower maturation process should therefore play a central role in determining high cup quality, possibly by guaranteeing the full manifestation of all biochemical steps required for the development of the beverage quality (Silva *et al.,* 2005).

Post harvest techniques also influence the quality of coffee. Green coffee is traditionally produced either wet or dry processing. In the dry process, whole coffee fruits are dried in the sun while in the wet process, ripe coffee cherries are mechanically de-pulped and the mucilaginous residues are degraded during a 'fermentation' step and then washed off and sun dried. The coffee from the two processing systems is hulled mechanically to obtain green coffee. Wet processed Arabica has been reported as being aromatic with fine acidity and some astringency, while dry processed Arabica is less aromatic and less acidic but with greater body (Clifford, 1985). The metabolic reactions that occur during wet-processing help generate pleasant cup quality attributes (Selmar *et al*., 2006). Natural dry-processed Arabicas from Brazil and Ethiopia have been reported to have low acidity, less

marked aroma, but much stronger body, which is important in espresso coffees (Illy and Viani, 2005). Cup quality may also be influence by the degree of roast, grind and, brewing methods among other factors (Lingle, 1996).

2.2.3 Characterization of coffee genotypes by biochemical composition

Coffee cup quality is based on the characterization of a large number of factors including taste and aroma. These factors are related to the biochemical content of roasted beans. These compounds rise from a smaller number of biochemical compounds present in green beans. There have been various investigations of the chemical composition of green coffee beans (Clifford, 1985; Montagnon *et al*., 1998; Farah *et al*., 2006). Green coffee biochemical composition of has been used to discriminate between Arabica and Robusta (Martin *et al*., 1998; Fischer *et al*., 2001). The biochemical composition and beverage quality has also been used to compare Arabica hybrids grown at various elevations in Central America (Bertrand *et al*., 2005). Caffeine, chlorogenic acids, sucrose and trigonelline have been used for characterization of coffee species as well as varieties within a species (Bicchi *et al*., 1995; Ky *et al*., 2001). These biochemical compounds are important in beverage quality since they are aroma precursors. Correlations between coffee cup quality and some chemical attributes may be used as an additional tool for coffee quality evaluation (Farah *et al.* (2006). Some of the major biochemical components of green coffee bean are discussed below.

2.2.3.1 Carbohydrates and their contribution to coffee quality

Carbohydrates are the most abundant constituents in raw coffee beans accounting for more than 50% of the bean dry weight (Njoroge, 1987, Wrigley, 1988). Green coffee beans contain a wide range of different carbohydrates which can be grouped as simple sugars, disaccharides, oligosaccharides and polysaccharides. The principal low molecular weight carbohydrate or sugar in green coffee is sucrose. Sucrose is one of the main sugars in the coffee beans, varying from 5% to 9.5% of dry matter basis (dmb) in *Coffea arabica* and from 4% to 7% of dmb in *Coffea canephora* (Ky *et al*., 2001). Sucrose is the main contributor of reducing sugars which are implicated in Maillard reactions occurring during the roasting process (Grosch, 2001). Sucrose as the most abundant, act as aroma precursors that affect both taste and aroma of the beverage (Maria, *et al,* 1994).

The polysaccharides consist mannans or galactomannans, arabinogalactan-proteins and cellulose (Redgwell *et al*., 2002). Small amounts of pectic polysaccharides and xyloglucan has also been found (Oosterveld *et al*., 2003). The importance of carbohydrates in coffee can be attributed not only to their high concentration in the bean but also to the complex changes they undergo during the roasting process which contribute to the organoleptic appeal of the coffee beverage. Roasting is an essential step in coffee production for the formation of various types of flavour compounds. The content and nature of sugars in the green beans is important in the development of flavour and pigmentation during roasting (Flament and Bessière-Thomas, 2002). The conversion of carbohydrates contributes significantly to the formation of these compounds (Maria *et al.,* 1994). Heating during roasting hastens the inversion of

sucrose to reducing sugars. Furan derivatives are the principal products of decomposition of monosaccharides and higher sugars (Flament and Bessière-Thomas, 2002). Compounds such as phosphates, acids and alkalis tend to catalyse the pyrolysis of sugars and amino compounds. Sulphur containing amino acids react readily forming sulphur derivatives of furan such as furfurylthiol (Dart and Nursten, 1985). These compounds are particularly important organoleptically as they possess rich roasted and coffee-like aromas. The roasting process is responsible for opening the cell-wall matrix resulting in the solubilisation of polysaccharides upon extraction (Leloup and Liardon, 1993).

Sucrose represents the major transport form of photosynthetically assimilated carbon in coffee and its metabolism plays a key role, particularly in sink tissues such as fruits (Lalonde *et al.,* 1999). Higher sucrose contents in Arabica green have been shown to partially explain its better cup quality (Ky *et al.,* 2001). Considering its importance, it would therefore be of interest to evaluate Kenya coffee genotypes for sucrose levels.

2.2.3.2 Alkaloids in coffee

Coffee plants contain two different kinds of alkaloids derived from nucleotides. Caffeine (1, 3, 7-*N*-trimethylxanthine) and theobromine (3, 7-*N*-dimethylxanthine) are purine alkaloids while, trigonelline (1-*N*-methylnicotinic acid) is a pyridine alkaloids. The physiological functions of alkaloids are not completely understood, but they are considered to participate in plant chemical defences (Ashihara and

Crozier, 1999). Caffeine has been related to the pharmacological effects of coffee and trigonelline has been associated with flavour formation coffee roasting.

2.2.3.2.1 Caffeine and its biosynthesis

The most important sources of caffeine are coffee (*Coffea* spp.), tea (*Camellia sinensis*), guarana (*Paullinia cupana*), mate (*Ilex paraguariensis*), cola nuts (*Cola vera*), and cocoa (*Theobromacacao*) (Suzuki *et al*., 1992). The amount of caffeine found in these crops vary with the highest amounts found in guarana (4–7%), followed by tea leaves (3.5%), mate tea leaves (0.89–1.73%), coffee beans (1.1–2.2%), cola nuts (1.5%), and cocoa beans (0.03%) (Clifford *et al*., 1990). In *C. arabica* seedlings, caffeine occurs mainly in leaves and cotyledons and is essentially absent in roots and the older brown parts of shoots (Zheng and Ashihara, 2004). Chemically, caffeine remains stable during coffee roasting except for minute amounts that sublime although roasting has been reported to cause a reduction in caffeine content (Franca *et al.,* 2005; Hecˇimovic´ *et al.,* 2011).

Caffeine was isolated from tea and coffee in the early 1820s, but the main biosynthetic and catabolic pathways of caffeine were not fully established until the year 2000 (Kato *et al*., 2000). The xanthine skeleton of caffeine is derived from purine nucleotides. Main caffeine biosynthetic pathway is a four step sequence consisting of three methylation and one nucleosidase reactions (Figure 1. The initial step is the methylation of xanthosine by a *S*-adenosyl-l-methionine (SAM). In the process SAM is converted to *S*-adenosyl-L-homocysteine (SAH) which in turn is hydrolysed to L-homocysteine and adenosine (Ashihara *et al*., 2008). The last two

steps of caffeine synthesis are also catalysed by SAM-dependent N-methyltransferase(s).

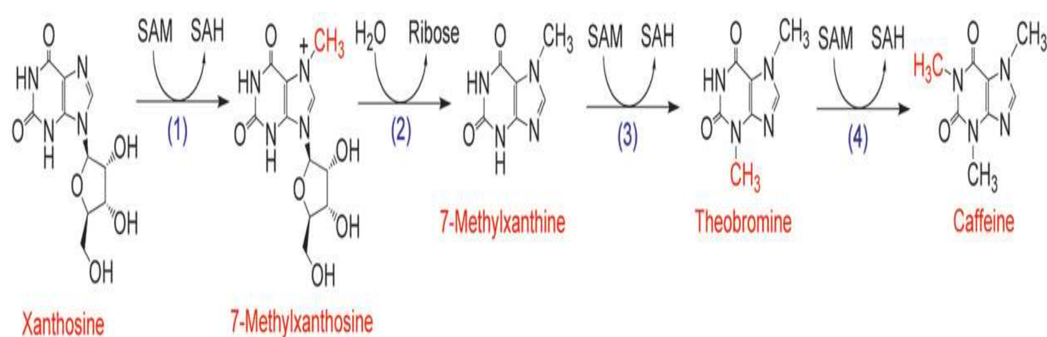


Figure 1: Caffeine biosynthesis

Source: Ashihara *et al*. (2008)

The exact biological role of caffeine and related purine alkaloids of plants is still unclear, although there are some hypotheses. In the chemical defense theory, it has been proposed that caffeine protects young leaves and fruit from predators (Hollingsworth *et al*., 2002) and that the caffeine released by the seed coat prevents germination of other seeds (allelopathic or autotoxic theory) (Friedman and Waller, 1983). In agreement with these proposals, it is known that caffeine is accumulated in both the seeds and young leaves of coffee plants (Ashihara and Suzuki, 2004). Strong supporting evidence for the chemical defense theory has recently been obtained by Uefuji *et al*. (2005), who demonstrated that leaves of transgenic tobacco (*Nicotiana tabacum*) plants engineered to produce caffeine were less susceptible to insect feeding compared to control leaves that did not contain caffeine.

Caffeine is probably the most frequently ingested pharmacologically active substance in the world and presents a characteristic bitter taste that is important to coffee flavour (Trugo, 1985). It accounts for 10-30% of the bitterness of coffee brew detected by taste (Viani, 1985). After its oral ingestion, caffeine is absorbed, and distributed to various tissues, and broken down to metabolites with variable pharmacological actions, which are then excreted (Mandel, 2002). In the human, slightly more than 80% of administered caffeine (1,3,7-trimethylxanthine) is metabolized by demethylation to paraxanthine (1,7- dimethylxanthine) via liver cytochrome P-450 1A2, and about 16% is converted to theobromine and theophylline, (3,7- and 1,3-dimethylxanthine, respectively) (Benowitz *et al*., 1995). Caffeine is a mild stimulant, which acts on the central nervous system and increases the metabolic rate. Consumption of caffeine equivalent to that found in a couple of cups of coffee has been shown to improve alertness and enhance concentration. Lieberman *et al.* (2002) examined whether moderate doses of caffeine would reduce adverse effects of sleep deprivation and exposure to severe environmental and operational stress on cognitive performance. They found that even in the most adverse circumstances, moderate doses of caffeine could improve cognitive function, including vigilance, learning, memory, and mood state. When cognitive performance is critical and must be maintained during exposure to severe stress, administration of caffeine may provide a significant advantage. A dose of 200mg appeared to be optimal under such conditions. Smith (2002) conducted a study aimed at determining whether a realistic drinking regime (multiple small doses - 4 x 65 mg over a 5-h period) produced the same effects as a single large dose (200 mg). The smaller doses were selected so that the amount of caffeine present in the body

after 5 hours would be equivalent to that found with the single dose. The results showed that in both consumption regimes caffeine led to increased alertness and anxiety and improved performance on simple and choice reactive tasks, a cognitive vigilance task, a task requiring sustained response and a dual task involving tracking and target detection. Rogers *et al* (2003) compared the mood, alerting, psychomotor and reinforcing effects of caffeine in caffeine non-consumers and acutely (overnight) withdrawn caffeine consumers. The reinforcing effect of caffeine was evident from an effect on drink intake, but drink choice was unaffected. Caffeine increased self-rated alertness of both caffeine consumers and non-consumers; however, for some of the non-consumers this was associated with a worsening of performance.

Commercially cultivated coffee plants contain substantial quantities of caffeine; *C. arabica* beans usually contain 1.2–1.4% (DWB) and *C. canephora* 1.2–3.3% (Mazzafera and Calvalho, 1992). Demand for decaffeinated coffee has increased gradually since the early 1970s. Sales of decaffeinated coffee in the world have achieved a 12% share of the total market, estimated to be worth more than US$4 billion (Heilmann, 2001). Decaffeination sometimes interferes with the organoleptic characteristics of the coffee. However, according to Vitzthum (2005) modern methods of decaffeination carried out correctly may minimally affect the organoleptic quality of the beverage. In Kenya a lot of studies have been conducted to evaluate caffeine levels in tea leaves. Studies conducted in Kenya showed that withering may influence the caffeine concentration in tea compared to normal withers, since the levels in physically withered teas was less (Owuor *et al.,* 1987).

Mohammed and Suleiman, (2009) compared teas from Kenya and China. The caffeine content in the tea leaves samples analysed varied from 1.40% in Kenya sample to 2.80% in China sample, with a mean of 1.83% and coefficient of variation 11.6 percent. The levels of caffeine in certain tea brands (chai mara moja, kericho gold, sasini, finlays premium) in the Kenyan market were found to be within the documented range (Wanyika *et al*., 2010).

Wanyika *et al.* (2010) evaluated caffeine content in some soluble coffee found in the Kenyan domestic market. It is important to note that most soluble coffees are made from Robusta coffee. Despite its importance, information on caffeine levels in coffee genotypes existing in Kenya is limited. At the same time, when new varieties are developed it is important to bench mark them with the existing varieties in caffeine content.

2.2.3.2.2 Trigonelline and its biosynthesis

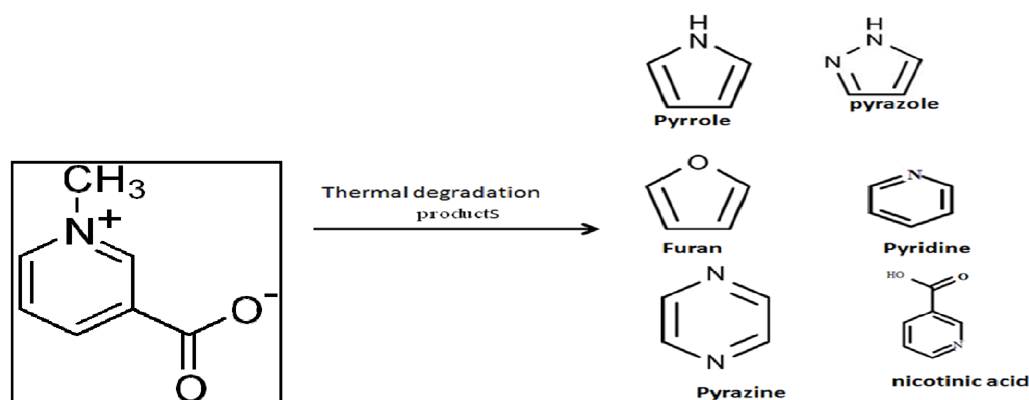
Trigonelline is an alkaloid with chemical formula $C_7H_7NO_2$ and a molecular weight of 137.138 g/mol. Structural formula of trigonelline is shown in Figure 2. Trigonelline was first isolated from seeds of fenugreek (*Trigonella foenum-graecum*) (Johns, 1885). Common foods containing trigonelline include barley, cantaloupe, corn, onions, peas, soybeans, and tomatoes (Beckstrom-Sternberg and Duke, 1996). Trigonelline exposure also occurs when crustaceans, fish, or mussels containing trigonelline (Ito *et al*., 1994) are consumed. Coffee is a significant source of trigonelline where it occurs naturally in green coffee. Trigonelline levels in *C. arabica*, have been reported to range from 0.88% to 1.77% dmb (Ky *et al*., 2001),

1% to 1.94% dmb) and 1.52% to 2.9% dmb (Mazzafera, 1991). In *C. canephora* levels reported ranges 0.75% to 1.24% dmb (Ky *et al.*, 2001) and 0.91% to 1.94% (Martin *et al.*, 1998).

The direct precursor of trigonelline is nicotinic acid (Joshi and Handler, 1960). In plants, nicotinic acid is produced as a degradation product of Nicotinamide Adenine Dinucleotide (NAD) (Wagner and Backer, 1992; Zheng and Ashihara, 2004). Trigonelline and its metabolic synthesis form [^{14}C] nicotinic acid are distributed in all parts of coffee seedlings (Zheng and Ashihara, 2004). Biosynthesis of trigonelline has been found to be high in young developing coffee leaves and declines in aged leaves. Trigonelline also accumulates in fruits of *Coffea arabica* during growth, and accumulates finally in seeds. Shimizu and Mazzafera (2000) found that trigonelline accumulated in the seeds is converted to nicotinic acid during germination, and is used for the NAD synthesis. In this case, trigonelline acts as a reservoir of nicotinic acid in plants.

Trigonelline is a pyridine derivative known to contribute indirectly to the formation of appreciated flavour products including furans, pyrazine, alkyl-pyridines and pyrroles during coffee roasting (Ky *et al.*, 2001). Some thermal degradation products are shown Figure 2. Demethylation of trigonelline during coffee roasting generates nicotinic acid, a water-soluble B vitamin also known as niacin. Nicotinic acid produced during coffee roasting is highly bioavailable in the beverage, in contrast to natural sources where it is present in bound form (Trugo, 2003). Coffee is a significant source of this vitamin in the diet (Trugo *et al.*, 1985). However, contents

in commercial coffee may be highly influenced by coffee species, variety, geographical origin and roasting conditions (Ky *et al*., 2001). Despite the significance of trigonelline to coffee flavour development, no studies are reported on the levels of this variable in Kenyan coffee genotypes.



Trigonelline

Figure 2: Structural formula of trigonelline and some of its thermal degradation product

(Source: Trugo *et al*., 1985)

2.2.3.3 Occurrence of chlorogenic acids in coffee

Chlorogenic acids (CGA) are esters of *trans*-cinnamic acids, such as caffeic, ferulic and *p*-coumaric acids, with quinic acid (QA) (Clifford, 2000). CGA are products of the phenylpropanoid pathway, one branch of the phenolic metabolism in higher plants that is induced in response to environmental stress conditions such as infection by microbial pathogens, mechanical wounding, and excessive UV or high visible light levels (Herrmann, 1995). Plant phenolic acids are synthesized from phenylalanine and tyrosine via the shikimic acid pathway, which converts simple carbohydrate precursors, derived from glycolysis and the pentose phosphate shunt

(phospho-enol-pyruvate and D-erythrose-4-phosphate), into aromatic amino acids. (Farah and Donangelo, 2006)

Chlorogenic acids (CGA) are the highest occurring acids in coffee and have been analyzed extensively (Carelli *et al.,* 1974; Rees and Theaker, 1977; Van der Stegen and Van Duijn, 1980; De Azevedo *et al.*, 2008). The structural formula of chlorogenic acid is shown in Figure 3.

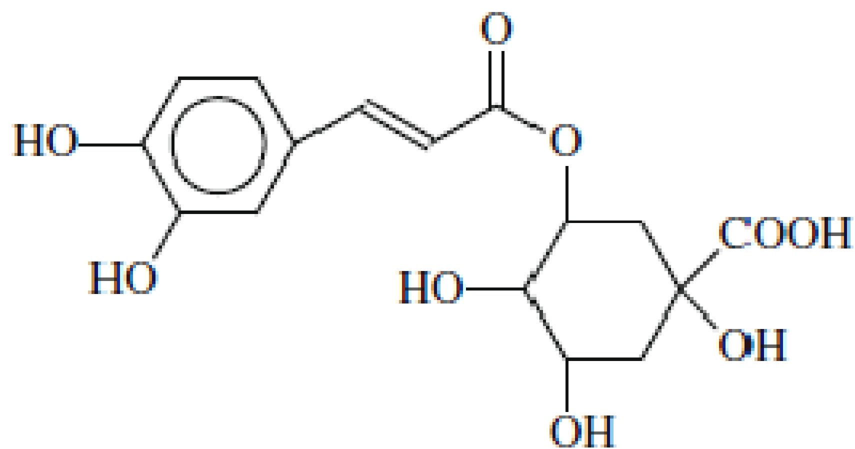


Figure 3: Structural formula of chlorogenic acid
(Source: Marinova *et al.,* 2009)

The major CGA subgroups are quinic acid esters with caffeic acid [caffeoylquinic acids (CQA) and dicaffeoylquinic acids (diCQA)] or with ferulic acid [feruloylquinic acids (FQA)] and represent 98% of all CGAs (Clifford, 1985; Morishita, *et al*., Kido, 1989). There are also some minor compounds, such as esters of Feruloyl-Caffeoylquinic acids (FCQA), Caffeoyl-Feruloylquinic acids (CFQA) or p-Coumaric acid (p-CoQA).

Chlorogenic acids (CGA) are an important group of non-volatile compounds in green coffee. They play an important role in the formation of roasted coffee flavour and have a marked influence in determining coffee cup quality (Farah *et al.*, 2006). They are known to be responsible for coffee pigmentation, aroma formation, bitterness and astringency (De Maria *et al.*, 1995). During coffee roasting, CGA are partially degraded as a result of pyrolysis, generating phenolic lactones and other derivatives. Cinnamoyl-1, 5-quinolactones (CGL) are the main CGA lactones in roasted coffee, being produced through the loss of a water molecule and formation of an intramolecular ester bond between positions 1 and 5 of QA (Farah, *et al.*, 2005). Along with CGA, CGL also contribute to coffee flavour despite their low concentrations, (Ginz and Engelhardt, 2000). Chlorogenic acid is fairly unstable and decomposes into caffeic and quinic acid. This breakdown increases the total acid content of the beverage which creates an acerbic (bitter and sour) taste in the beverage (Lingle, 1996). *C. canephora* species have low quality beverage and are known to produce more chlorogenic acids (8-13%) than *C. arabica* (7-8%), (Clifford, 1985; Ky *et al.*, 1999; Guerrero *et al.*, 2001). Using chlorogenic acid composition, Moreira *et al.* (2001) were able to discriminate Brazilian Arabica green coffee samples.

Antioxidants are organic molecules which can prevent or delay the progress of lipid oxidation. Their ability to do this is based mainly on their phenol-derived structure. Recently, the interest in using antioxidants of natural origin in food has increased, because they also appear to be suitable antioxidants for the prevention of diseases associated with the process of lipid peroxidation (Gordon, 1996; Stahl, 2000;

Valenzuela, *et al*., 2003). Hydroxycinnamic acid compounds have been described as chain-breaking antioxidants, probably acting through radical-scavenging, which is related to their hydrogen-donating capacity, and their ability to stabilise the resulting phenoxyl radical (Siquet, *et al*., 2006). The major chlorogenic acid compounds present in coffee are differentially absorbed and/or metabolized in humans, with a large inter-individual variation. According to (Monteiro *et al.,* 2007), urine does not appear to be a major excretion pathway of intact CGA compounds in humans. Farah *et al*. (2008) has demonstrated that chlorogenic acids from green coffee extract are highly bio-available in humans. Several beneficial health effects have been attributed to CGA and may be largely explained by their potent antioxidant activities (Pereira *et al.,* 2003). Some *in vitro* and *in vivo* pharmacological properties of CGA are hypoglycemic, antiviral, hepatoprotective and immunoprotective activities (Basnet, 1996; Tatefuji *et al*., 1996; Hemmerle *et al*., 1997). Cinnamoyl-1, 5-quinolactones (CGL) the main chlorogenic acid lactones have also been studied for their potential hypoglycemic effects (Shearer *et al*., 2003) and for their actions at opioid and adenosine brain receptors (De Paulis *et al*., 2002, De Paulis *et al*., 2004). However, the study of polyphenols is quite complex because of heterogeneity of the different molecular structures and scarcity of data on bioavailability as well as on biotransformation.

2.2.3.4 Lipids in coffee

Coffee lipids are located in the endosperm of green coffee beans (Wilson *et al*., 1997) and only a small amount of the coffee wax is located on the outer layer of the bean.The lipid content of Arabica coffee beans averages 15% whilst Robusta coffee contains

around 10% (Speer *et al.,* 1993). Coffee oil is composed mainly of triacylglycerols with fatty acids in proportions similar to those found in common edible vegetable oils. The main fatty acids present in the coffee oil are myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1) and behenic acid (C22:0) (Folstar, 1985).

Coffee oil contains a high percentage of unsaponifiables, including about 19% total free and esterified diterpene alcohols, about 5% total free and esterified sterols, and the remainder very small quantities of other substances, such as tocopherols (Speer and Kölling- Speer 2001). The main diterpenes in coffee are pentacyclic diterpene alcohols mainly cafestol, kahweol and 16-O-methylcafestol. Cafestol is present in both Arabica and Robusta while kahweol is absent in Robusta. During the roasting process dehydrocafestol and dehydrokahweol are formed as decomposition products from cafestol and kahweol (Kölling- Speer *et al*., 1997). The amounts of both compounds increase with raising roasting temperatures but also depend on the contents of cafestol and kahweol in the green coffee (Speer *et al*., 1991; Kölling- Speer *et al*., 1997). Nevertheless, using the ratio of cafestol and dehydrocafestol, the formation of this decomposition product is suitable as an objective characteristic for the roasting degree of coffees (Kölling-Speer *et al*., 1997). The structural formulae of diterpenes are shown in Figure 4.

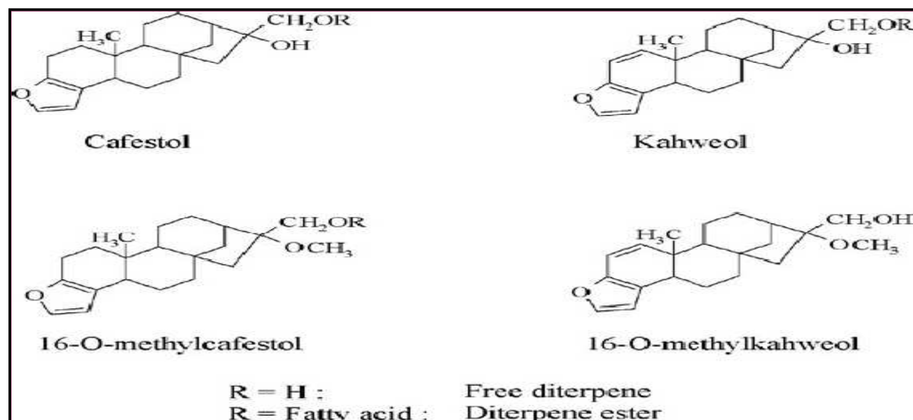


Figure 4: Structural formulae of diterpenes.

(Source: Speer, and Ko¨lling-Speer, 2006).

Coffees with higher oil contents have give better roasts (Northmore, 1968). During roasting the oil is expelled to the bean surface, forming a layer which may trap volatile aromas, preventing the immediate loss of these compounds (Clifford, 1985; Arnaud, 1988). The oil therefore, plays an important role in the overall presentation of coffee flavour although the oil is poorly extracted into the coffee brew. Nevertheless, considerable amount of oil may be found in coffee brew depending on the brewing method. Boiled coffee (decanted without filtering) lead in the amount of oil that can be extracted while paper filters are most effective at retaining oil droplets, allowing only about 10 mg/l in the brew (Petracco, 2001).

2.2.3.5 Biochemical components analysis by near infrared spectroscopy

The standard analytical techniques usually offer a high level of accuracy and precision, they also show some handicaps, such as high costs, high labor input and delay in reporting. As demand for rapid and cheaper controls is growing, wet chemistry methodologies are being replaced by dry methods (Rubayiza and

Meurens, 2005). Molecular absorption spectroscopy based on ultraviolet, visible and infrared radiations is widely used for the identification and quantitative analysis of inorganic, organic and biological molecules. Infrared absorption spectroscopy is one of the powerful tools for structural identification of molecules by measuring the absorption of different frequencies of infrared radiation by the matrix being measured. The most important use of infrared has been the identification of organic compounds, because the infrared spectra can provide unique fingerprints for organic molecules. The use of near infrared (NIR) has become very useful in the analysis of agricultural products. Near infrared spectroscopy is based on the absorption of electromagnetic radiation by matter (Osborne *et al*., 1993). When applied to food products, this technique is of analytical use as it can extract a large amount of information concerning biochemical composition. NIR has been applied as an analytical tool in discriminating between different tea varieties (Budínová *et al*., 1998), to study the composition and adulteration of virgin olive (Baeten and Aparicio, 2000; Vlachos *et al*., 2006), and to identify Thai aromatic rice (Theanjumpol *et al.,* 2005). Previous study on coffee has shown that NIR spectral methods seem effective for authenticating coffee varieties (Esteban-Díez *et al.,* 2007), to discriminate Robusta and Arabica (Davrieux *et al*., 2001) to quantify the Robusta variety content of roasted coffee samples (Pizarro *et al*., 2007), as a means for controlling coffee adulteration (Posada *et al*., 2009). NIR spectroscopy has already been used to predict the contents of trigonelline and sucrose (which are aroma precursors of appreciated flavours), chlorogenic acids, caffeine and oil in green coffee (Bertrand *et al*., 2003). Near infrared spectroscopy (NIRS has been proved efficient to discriminate Robusta and Arabica (Pizarro *et al*., 2007, Davrieux

et al., 2001), to determine the origin of green coffees and the ratio Robusta/Arabica in coffee blends. Calibration equations are established by regression techniques taking into account a limited number of predictors, which are absorbencies at certain wavelengths.

The standard analytical techniques usually offer a high level of accuracy and precision, they also show some handicaps, such as high costs, high labor input and delay in reporting. In addition, many standard techniques involve the destruction of the test sample, which could be a handicap in the case of valuable and scarce materials. As demand for rapid and cheaper controls is growing, wet chemistry methodologies are being replaced by dry methods (Rubayiza and Meurens, 2005). Since 1991 the Centre de coopération International en Recherche en Agronomique pour le Développement (CIRAD) France has been developing near infrared spectroscopy (NIRS) databases for green coffee (more than 5000 references) and roasted coffee (more than 4000 references) (Davrieux *et al*., 2001). NIRS has been proved efficient to discriminate Robusta and Arabica (Davrieux *et al*., 2001; Pizarro *et al*., 2007), to determine the origin of green coffees and the ratio Robusta/Arabica in coffee blends. NIRS has already been used to predict the contents of trigonelline and sucrose (which are aroma precursors of appreciated flavours), chlorogenic acids, caffeine and oil in green coffee (Bertrand *et al*., 2003).

CHAPTER THREE

3.0 ASSESSMENT OF GENETIC DIVERSITY AMONG COFFEE GENOTYPES IN KENYA USING MOLECULAR MARKERS.

3.1 INTRODUCTION

The coffee conserved *ex-situ* at Coffee Research Station (CRS) Ruiru in germplasm field plots has many *C. arabica* accessions from Ethiopia, Sudan, Kenya, Tanzania, Angola, India, Reunion, Portugal, South and Central America (Millot, 1969). Some of these conserved genotypes have been used as progenitors in a main breeding programme at CRS (Walyaro, 1983). The coffee breeding programme successfully transferred resistance to CLR and Coffee Berry Disease (CBD) from *C. canephora*, via the interspecific hybrid referred to as Hibrido de Timor (HDT, Timor Hybrid) (*C. arabica* x *C. canephora*) (Nyoro and Sprey, 1986). The cultivar developed from this breeding regime (Ruiru11) is suitable for all coffee growing areas because it is resistant to CBD and CLR (Opile and Agwanda, 1993). In spite of it is significance, this *Coffea* collection has not been evaluated for genetic variability mainly at DNA level. According to Jump *et al.* (2008) there is heavy reliance on plant genetic diversity for future crop security in agriculture and industry. However they observed that genetic diversity for natural populations receives less attention. Like it is for many crops, evaluation of the genetic diversity and available resources within the genus *Coffea* is an important step in coffee breeding (Cubry *et al.*, 2008). As new coffee varieties are continuously being developed through hybridization, there is a need to determine the level and sources of genetic variation within and between new and existing coffee varieties (Gichimu and Omondi, 2010a). Genetic

consistency within varieties is also essential to quality assurance for any agricultural product. Hue (2005) reported that morphological variability in coffee plantations is adverse to the product quality. Reduced genetic diversity is also reported to compromise the ability of populations to evolve to cope with environmental changes and thus reducing their chances of long-term persistence (Frankham *et al*., 2002). Determination of genetic diversity/consistency is therefore important not only in coffee but also to other crops. Walyaro (1983) determined the diversity of eleven coffee genotypes using morphological characteristics. Gichimu and Omondi (2010b) also determined the morphological diversity among advanced breeders lines and existing commercial coffee cultivars in Kenya. However, morphological markers are reportedly inefficient because they are generally dominant traits, they often exhibit epistatic interactions with other genetic traits and can also be influenced by the environment (Weising *et al.,* 2005). Lashermes *et al.* (1996a) reported that genetic factors are more accurately tested by molecular markers. This study utilized RAPD and microsatellites (SSRs) to assess the genetic diversity of *ex-situ* conserved genotypes, commercial varieties and upcoming coffee varieties in Kenya.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials

Twenty four (24) coffee genotypes comprising of one Catimor (Line 90), four commercial varieties, five advanced coffee selections and fourteen non-commercial accessions, were used in this study (Table 4). Trees of these coffee genotypes are available in the commercial fields, experimental sites and germplasm conservation plots at Coffee Research Station (CRS), Kenya. Young, fully expnaded and disease-

free leaves were picked from second and third nodes from the growing tips (Plate 7) of the coffee branches for DNA extraction.

Table 4: Status and sources of the coffee genotypes in this study

Genotypes	Status	Introduced from
Marsabit	Museum accession	Wild from Northern Kenya
Geisha 11	Museum accession	Kitale, Kenya
Columnaris	Museum accession	Puerto Rico
Grafts	Museum accession	Not known
Moca	Museum accession	Yemen
N39	Museum accession	Lyamungu Tanzania
C. eugenioides	Museum accession	Nandi Forest, Kenya
Harar	Museum accession	Ethiopia
Ennareta	Museum accession	Ethiopia
Laurina	Museum accession	LA Reunion
Hibrido De Timor	Museum accession	Portugal
Pretoria	Museum accession	Guatemala
K7	Commercial variety	Kenya
SL34	Commercial variety	Kenya
SL28	Commercial variety	Kenya
Blue Mountain	Museum accession	Guatemala
Robusta	Museum accession	Uganda
Cross 8 (Cr8)	Advanced Selection	Kenya
Cross22 (Cr22)	Advanced Selection	Kenya
Cross 23 (Cr23)	Advanced Selection	Kenya
Cross30 Cr30)	Advanced Selection	Kenya
Cross 27 (Cr27)	Advanced Selection	Kenya
Catimor - Line 90	Breeding material	Colombia
Ruiru11-Line 5	Commercial variety	Kenya

Plate 7: Sampling coffee leaves

3.2.2 Extraction of genomic DNA

Genomic DNA was extracted from the fresh young leaves by the method of Diniz *et al.* (2005) with minor modifications using Mixed AlkylTriMethylammonium bromide (MATAB). The harvested leaves were wiped with 70% ethanol and 0.5g weighed and placed in a motar. Liquid nitrogen was added and the leaves crushed to fine powder by use of a pestle. Lysis and extraction buffers (Appendix 1) were added to the powder (1ml each) and crushing continued. The mixture was transferred to a 2ml plastic bottle and incubated at 62°C in a water bath for 20-30 minutes with regular shaking. After incubation, 1 ml of chloroform/isoamyl-alcohol mixture, (24:1) was added to each bottle and vigorously shaken and then centrifuged at 13000 rpm for 5 minutes in a desktop micro-centrifuge. The supernatants were carefully pipetted out into new 2 ml plastic bottles. Twenty to thirty micro litres of RNase (10 mg/ml) was added to the supernatants and incubated at 37°C in a water-bath for 30 minutes. A volume of isopropyl alcohol equal to the volume of each

supernatant was added into each bottle, and mixed gently by inverting the tubes several times to precipitate DNA. The suspended DNA was centrifuged at 13000 rpm for 5 min and a DNA pellet was obtained and the supernatant was carefully removed. The DNA pellets were then washed with 200µl of 70% ethanol and centrifuged at 13000 rpm for 3 minutes. The ethanol was drained by decanting or micro-pipetting, and the pellets dried in a vacuum centrifuge for 20 minutes. The pellets were dissolved overnight in 20-40 µl of TE (Tris-EDTA) (depending on pellet size) at 4°C. Estimation of DNA quantity was done by agarose gel electrophoresis. The stock solution and or their 10^{-1} dilution in 1% agarose gel (QBiogene, France) were used and were visually compared to standardized Lambda DNA ladders (Promega, Madison, WI, USA). This procedure also allowed assessment of the DNA quality attributes such as degradation and contamination that distort the migration of DNA. Genomic DNA was diluted to 1ng/µl and stored at -20°C ready for use.

3.2.3 Amplification of coffee genomic DNA

RAPDs were the main primers used due to their ease of use and availability of primers at CRF. The method of Lashermes *et al*. (1996b) and modified by Agwanda *et al*. (1997) was used for RAPD analysis. Twenty one (21) arbitrary decamer oligonucleotides (Operon) were preselected and a subset (Table 5) showing clear amplifications were selected for analysis of the entire panel of study genotypes. The PCR reaction mix was in 25 µl containing, consisting of 5 µl of genomic DNA (1ng/µl), 7.5 µl of dNTPs (500 µM; 1/10 dilution of the 5 mM dNTPs in Appendix 2), 2.5 µl of buffer (10X, Promega), 2.0 µl of $MgCl_2$ (25 mM, Promega), 0.1µl of

Taq DNA polymerase (Promega), 1 µl of primers (10 µM, Appligene) and 7.0 µl of PCR water. Amplification was carried out in a Flexigene thermocycler (TECHNE, USA). The amplification program started with one cycle of initial denaturation at 94°C for 5minutes followed by 45 cycles of 1 min at 94°C (denaturation), 1 min at 35°C (annealing), and 2 min at 72°C (elongation). The final extension was done at 72°C for 7 min to ensure that the primer extension reaction was completed. The RAPD products were electrophoresed in 1.8% (w/w) agarose gel and then visualised in a UV trans-illuminator after staining in ethidium bromide solution.

Table 5: List of RAPD primers used for PCR analysis of 24 coffee genotypes

Primer	Sequence	Primer	Sequence
1. OPI-07	CAGCGACAAG	12. OPM-04	GGCGGTTGTC
2. OPJ-19	TGAGCCTCAC	13. OPX-20	CCCAGCTAGA
3. OPK-03	CCAGCTTAGG	14. OPY-10	CAAACGTGGG
4. OPE-05	TCAGGGAGGT	15. OPE-04	GTGACATGCC
5. OPE-08	TCACCACGGT	16. OPF-12	ACGGTACCAG
6. OPE-18	GGACTGCAGA	17. OPF-13	GGCTGCAGAA
7. OPF-15	CCAGTACTCC	18. OPF-16	GGAGTACTGG
8. OPF-17	AACCCGGGAA	19. OPI-20	AAAGTGCGGG
9. OPG-03	GAGCCCTCCA	20. OPL-18	ACCACCCACC
10. OPG-05	CTGAGACGGA	21. OPY-15	AGTCGCCCTT
11. OPN-18	GGTGAGGTCA		

For microsatellites analysis, two primers, Sat 235 (with forward sequence of TCGTTCTGTCATTAAATCGTCAA and reverse sequence of GCAAATCATGAAAATAGTTGGTG) and M24 (with forward sequence of GGCTCGAGATATCTGTTTAG and reverse sequence of TTAATGGGCATAGGGTCC) were used. The two microsatellites were selected

based on results by Gichuru (2007) and Omondi *et al.* (2009). The DNA was amplified by the methodology described by Combes *et al*. (2000). Amplification was in 25 µl PCR reaction mix containing 5 µl of 5 ng/µl genomic DNA, 2.5 µl of buffer (10X, Promega), 2.5 µl of $MgCl_2$ (25 mM, Promega), 7.5µl of dNTPs (250µM), 1 µl each of right and left primers (2 µM, Eurogentec), 0.2µl of *Taq* DNA polymerase (5U/µl, Promega), 5.5 µl of double distilled water. The PCR programme consisted of an initial denaturation of 5 min at 94 °C followed by 5 cycles of 45 sec of denaturation at 94 °C, 1min primer annealing at 60 °C reducing by 1 °C every cycle, elongation for 1 min at 72 °C and 30 cycles of 45 sec of denaturation at 90 °C, primer annealing at 55 °C for 1 min and elongation at 72 °C for 1 min 30s and final extension of 8 min at 72 °C. The PCR products were visualized in 2.3 % agarose gel with Ethidium bromide staining.

3.2.4 Scoring and analysis of bands

The bands were scored for presence (1) and absence (0) in the various genotypes. The data was organized into a matrix and subjected to cluster analysis using R statistical software. A dendrogram was constructed using dissimilarity matrix calculation function and unweighted pair-group method using arithmetic averages (UPGMA) (Venables *et al*., 2006). The R command 'g clus' was used to reorder the genotypes within a cluster keeping them contiguous to each other. The cluster dendrogram constructed was used to estimate the genetic diversity among the 24 genotypes indicating how closely related or different they were.

3.3 RESULTS

Among the twenty one (21) RAPD primers tested, 16 primers showed amplification out of which 14 produced clear bands that could be clearly scored (example shown in Plate 8). The total number of fragments observed among the coffee genotypes based on the 14 RAPD primers was 83 (Table 6). The number of bands produced per primer ranged from 2 to 12. Ten out of the 14 primers generated 35 polymorphic fragments. The other four primers did not show any polymorphism (Example given in Plate 9). Robusta and C. eugenioides gave rise to most of the diversity observed while the Arabica accessions variously shared bands with these two species. The two microsatellites tested also showed varying polymorphism amongst the genotypes (Plate 10).

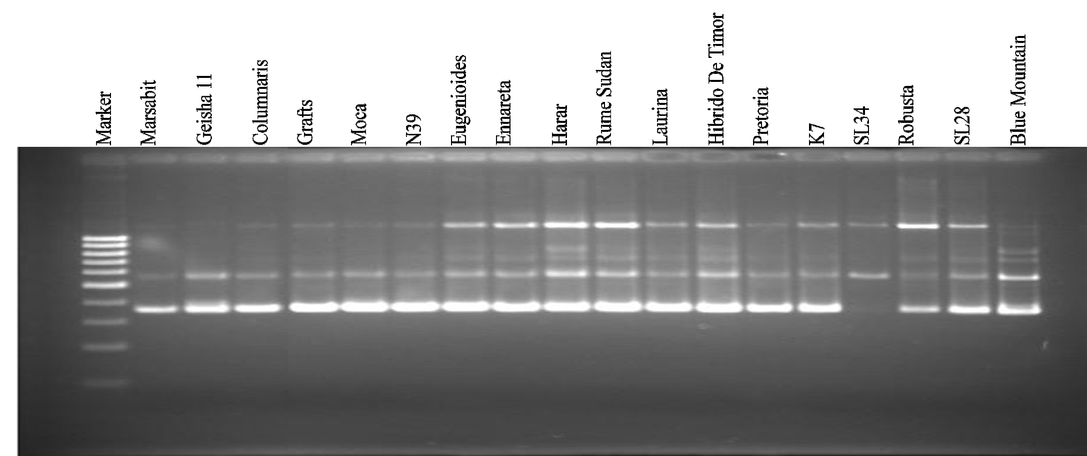


Plate 8: A panel of RAPD profiles generated by primer OPK-03 in the coffee genotypes

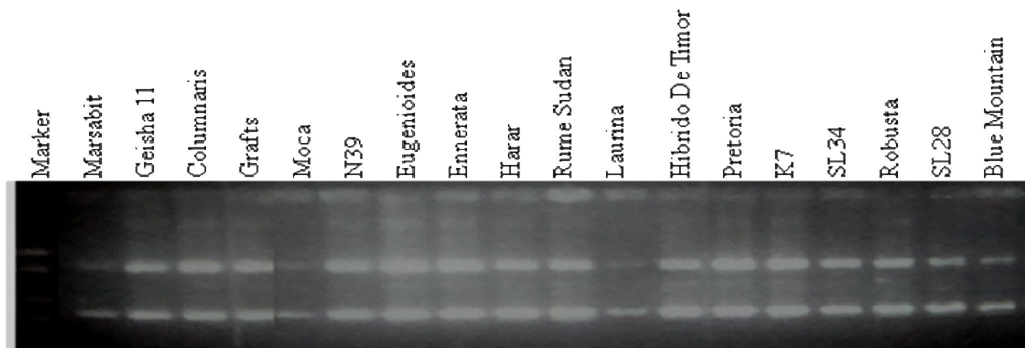


Plate 9: A panel of RAPD profiles generated by primer OPE-08 in coffee genotypes

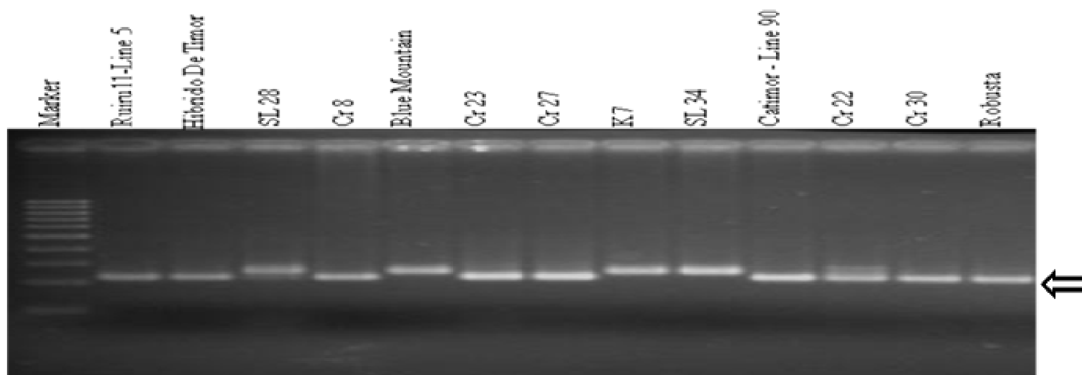


Plate 10: A polymorphic band pattern generated by Sat 235 on the coffee genotypes.

The lower band (arrowed) is a marker for a Robusta genome fragment also present in HDT and its derivatives.

Table 6: Results of analysis of 24 coffee accessions using RAPD primers

Primer	Total bands	Polymorphic bands	% polymorphic
OPI-07 - CAGCGACAAG	12	10	83
OPJ-19 - TGAGCCTCAC	9	4	44
OPK-03 - CCAGCTTAGG	5	2	40
OPE-05 - TCAGGGAGGT	4	1	25
OPE-08 - TCACCACGGT	2	0	0
OPE-18 - GGACTGCAGA	8	1	13
OPF-15 - CCAGTACTCC	4	0	0
OPF-17 - AACCCGGGAA	5	0	0
OPG-03 - GAGCCCTCCA	4	4	100
OPG-05 - CTGAGACGGA	4	1	25
OPN-18 - GGTGAGGTCA	7	2	29
OPM-04 - GGCGGTTGTC	4	0	0
OPX-20 - CCCAGCTAGA	7	6	86
OPY-10 - CAAACGTGGG	8	4	50
	83	35	42

Cluster dendrogram constructed using polymorphic bands was used to estimate the genetic diversity of the twenty four coffee accessions (Figure 5). The genotypes separated into three main clusters. *C. eugenioides* clustered alone in the first cluster, Arabica accessions dominated the second cluster while the third cluster contained Robusta, Ruiru 11, Hibrido de Timor and Catimor. The R command ‘g clus’ which was used to reorder the genotypes within and among clusters and keeping them contiguous to each other depicted Robusta and *C. eugenioides* as the most distantly related. Except for *C. eugenioides*, the maximum dissimilarity index observed was 0.10.

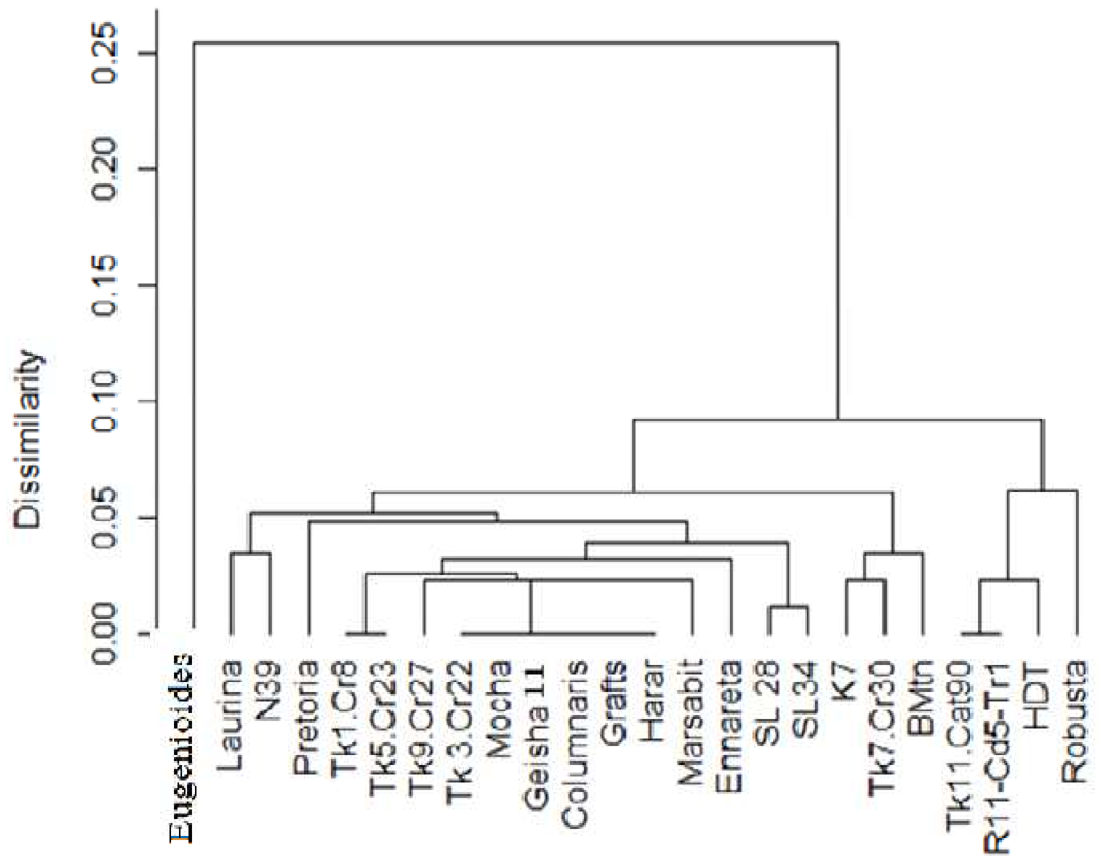


Figure 5: Cluster dendrogram illustrating genetic diversity among twenty four genotypes analysed using RAPD

3.4 DISCUSSION

Molecular markers have been widely applied in studying the diversity of coffee. The results demonstrate that RAPD markers were able to determine variability among the coffee accessions tested. Lashermes *et al.,* 1993; Agwanda *et al.,* 1997; Anthony *et al*., 2001; Aga *et al.,* 2003 and Masumbuko *et al*., 2003, among others also reported success in use of RAPDs in genetic characterization of *Coffea* species. Although HDT, Ruiru 11 and Catimor 90 clustered together with Robusta, it was apparent from the general analysis that the coffee accessions clustered according to the three different species namely *C. eugenioides*, *C. canephora* (Robusta) and *C. arabica* (Arabica). Thus, for rapid improvement in breeding work, widening of the existing genetic diversity through interspecific hybridisation is desirable. Similar observation was made by Lashermes *et al*. (1993) and Agwanda *et al.* (1997). Close genetic proximity was observed among the existing commercial varieties in Kenya, namely SL28, SL34, K7 and Blue Mountain. This agrees with the work of Agwanda *et al.* (1997) and Hue (2005) which revealed high genetic similarity between Kenyan commercial varieties. In this study, the accession Marsabit which is a wild accession from Northern Kenya clustered with K7 which confirmed the findings of Lashermes *et al*. (1996b) that cultivar K7 was closely related to an accession collected in Marsabit Mountain. Considering that the coffee genotypes evaluated in this study originated from diferent countries (Kenya, Puerto Rico, Tanzania, Ethiopia, Reunion, Portugal, Yemen, Guatemala and Colombia), the similarities observed among Arabica genotypes, attests to the narrow genetic diversity among Arabica coffee as reported in other studies (Lashermes *et al.,* 1993). Comparatively, higher genetic diversity has been reported among wild coffee populations than within

cultivated genotypes (Anthony *et al.,* 2000; Aga *et al*., 2003; Masumbuko *et al.,* 2003, Maluf *et al*., 2005; Masumbuko and Bryngelsson, 2006).

HDT, Catimor Line 90 and Ruiru 11, were found to be genetically divergent from the rest of the varieties and bearing close relationship to Robusta coffee. Similar observations were made by Lashermes *et al.* (1996b) and Agwanda *et al.* (1997). Different lines of HDT have been used worldwide to breed coffee varieties that are resistant to different pathogens. As would be expected, different accessions of HDT derivatives have different levels of introgressed *C. canephora* genome (Lashermes *et al.,* 2000; Silveira *et al.,* 2003). This could explain the close relationship observed between HDT, Ruiru 11 and Catimor Line 90 to Robusta. On the other hand, the cultivar Ruiru 11 is a composite F1 hybrid between lines of the variety Catimor, (as the female parent), and male selections most of which have HDT in their pedigree. The breeding programmes to develop the male parents involved backcrossing and selfing at various selection stages which affected the amount of Robusta genome passed on to the next generation. This explains the wide range of diversity observed between HDT and its derivatives (Catimor Line 90, Ruiru 11 line 5, Cr8, Cr22, Cr23, Cr27 and Cr30) analysed in this study.

CHAPTER FOUR

4.0 EVALUATION OF THE SENSORY CHARACTERISTICS OF DIFFERENT *COFFEA ARABICA* GENOTYPES GROWN IN KENYA.

4.1 INTRODUCTION

The value of coffee lies within the pleasure and satisfaction it gives to the consumer through its flavour and desirable physiological and psychological effects. Coffee quality, especially liquor or cup quality, determines both the relative price and usefulness of a given consignment of coffee (Walyaro, 1983; Roche, 1995; Agwanda *et al*., 2003). Therefore production and supply of coffee with excellent quality is crucial in the producing countries. Coffee quality assessment is done organoleptically by trained coffee tasters (Van der Vossen, 1985; Owuor, 1988; Agwanda, 1999). In Kenya, sensory evaluation has been applied to determine the influence of various processing activities on coffee quality. Most of the earlier work was oriented towards improving the processing conditions and led to the recommendation of a two stage fermentation procedure (Wootton, 1960, 1963, 1965). Sensory evaluation was also applied in studying the genesis of various coffee defects and methods for their elimination were established (Wootton, 1963**,** Northmore 1964). Walyaro, (1983) studied the organoleptic quality of eleven coffee genotypes and recommended organoleptic assessment as a sufficient and reliable method for use as a basis of selection in coffee quality improvement programs. Since the release of Ruiru 11 in 1985, most of the work done in the area of quality assessment in Kenya has concentrated in comparing this cultivar with the traditional coffee varieties (Owuor, 1988; Njoroge *et al*., 1990; Ojijo, 1993; Agwanda, 2003; Omondi, 2008). That continuous assessment accumulated evidence over the years,

showing that the beverage quality of Ruiru 11 does not deviate significantly from that of the traditional cultivars like SL28. Nevertheless, Van der Vossen, (2005) recommended exhaustive testing of the cup quality of new cultivars before releasing them. Knowing the sensory characteristics of the new upcoming coffee varieties alongside the existing ones is important for rolling out the genotypes to the coffee industry. Similarly, sensory evaluation of coffee genotypes under *ex-situ* conservation may demonstrate their diversity and potential for eventual exploitation. The diversity of *ex-situ* conserved coffee genotypes, commercial varieties and upcoming coffee varieties in Kenya were assessed organoleptically.

4.2 Characterization of 40 *ex-situ* conserved coffee germplasm and two cultivars by beverage sensory characteristics

4.2.1 Materials and methods

4.2.1.1 Study site and plant materials

The ex-situ conserved genotypes were maintained only at Coffee Research Station (CRS) Ruiru, the headquarter of Coffee Research Foundation (CRF). The area lies within the upper Midland two agro-ecological zone (UM 2) at latitude 1° 06'S and longitude 36° 45'E. The altitude is about 1620 meters above sea level (MASL), (Jaetzold and Schimidt, 1983). The area receives a bimodal mean annual rainfall of 1063mm and the mean annual temperature is 19°C. The soils are classified as a complex of humic nitisols and plinthic ferrasols. They are well drained, deep reddish brown, slightly friable clays with murram sections occasionally interrupting. A total of forty (40) *C. arabica* genotypes obtained from CRS germplasm conservation site (Millot, 1969) were used in this study and were analysed together with SL28 and K7

which are commercial varieties grown in the same plot (Table 7). Many of them are elite genotypes that have been used in breeding programs. The *ex situ* conserved accessions were selected based existing information and possibility of future utility of the coffee. These trees were maintained under natural conditions with minimum inputs.

Table 7: List of *ex-situ* conserved and commercial coffee genotypes for sensory analysis

No.	Genotypes	Introduced from	Site
1	M63	Kitale, Kenya	CRS
2	Dalle	Ethiopia	CRS
3	Dilla Alghe	Ethiopia	CRS
4	1225VI	Ethiopia	CRS
5	Angustifola	NARL, Kenya	CRS
6	Arousi	Ethiopia	CRS
7	Barbuk Sudan	National Agricultural Labs, Ken	CRS
8	Blue Mountain	Guatemala	CRS
9	Dilla	Ethiopia	CRS
10	Drought Resistant I (DRI)	French Mission Selection	CRS
11	Drought Resistant II (DRII)	French Mission Selection	CRS
12	Ennareta	Ethiopia	CRS
13	Erecta	NARL, Kenya	CRS
14	Eritrean Moca	Ethiopia	CRS
15	F53	Kitale, Kenya	CRS
16	G53	Kitale, Kenya	CRS
17	G 5B	Kitale, Kenya	CRS
18	Geisha 11	Kitale, Kenya	CRS
19	Geisha 12	Kitale, Kenya	CRS
20	Gimma Galla	Ethiopia	CRS
21	Gimma Galla Sidamo	Ethiopia	CRS
22	Gimma Mbuni	Ethiopia	CRS
23	H1	Lyamungu, Tanzania	CRS
24	Hibrido De Timor	Portugal	CRS
25	Moca	Aden	CRS
26	Mocha (Series D)	NARL, Kenya	CRS
27	Mokka Cramers	NARL, Kenya	CRS
28	Murta	Guatemala	CRS
29	Padang	Puerto Rico	CRS
30	Plateau Bronze	NARL, Kenya	CRS
31	Polysperma	Lyamungu, Tanzania	CRS
32	Pretoria	Guatemala	CRS
33	Purpurascens	NARL, Kenya	CRS
34	SeriesC	NARL, Kenya	CRS
35	SeriesL	NARL, Kenya	CRS
36	SL4	NARL, Kenya	CRS
37	Tanganyika Draught Resistant (TDR)	Tanzania	CRS
38	Wollamo	Ethiopia	CRS
39	Yelow Amarello	Brazil	CRS
40	Zeghie Ltana	Ethiopia	CRS
41	SL28	Commercial variety Kenya	CRS
42	SL34	Commercial variety Kenya	CRS

4.2.1.2 Processing of the samples

Ripe berries (Plate 11) were harvested from a sample size of five trees for the ex-situ conserved genotypes and bulked to give one sample per genotype during the main crop of 2008 (October, November, December). The cherry samples were pulped, fermented, washed and the wet parchment dried to final moisture content of 10.5 to 11% (Mburu, 2004). The parchment was then hulled and graded to seven grades based on size, shape and density as follows: E – Elephant beans which are the largest coffee beans and are retained on screen 21. AA – Flat beans that asses through screen No. 21, and are retained on 18, (7.2 mm), AB passes through screen No. 18 and is retained on screen No. 16, (size 6.35 mm). C grade describes flat beans that passes through screen No. 16, and retained on screen No. 10, size (3.96 mm). TT are light beans extracted from AA and AB by use of pneumatic separator; Pea Beans (PB) – are retained by a piano wire screen on 12, size 4.76 mm (4.43 mm); T – Very small beans and broken bits; Grade AB was used as a representative grade for the assessment of beverage quality.

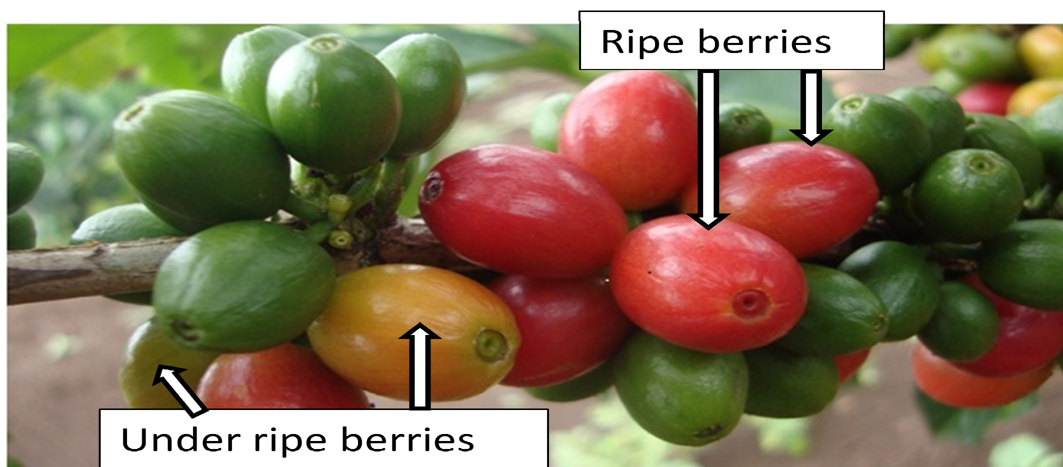


Plate 11: Coffee berries at different stages of maturity

4.2.1.3 Roasting green coffee and sensory evaluation

Roasting of the green coffee was done to attain a medium roast level using a Probat laboratory roaster within 24 hour of evaluation and allowed to rest for at least eight hours. Samples were weighed out to the predetermined ratio of 8.25g per 150 ml of water. Each coffee genotype's batch was ground individually using a Probat grinder for roasted coffee into the cup (five cups per sample). Sensory evaluation procedure described by Lingle (2001) was followed. The roasting was completed in no less than 8 minutes and no more than 12 minutes. Samples were immediately air-cooled and packaged in non-permeable bags on reaching room temperature until analysis to minimize exposure to air and prevent contamination. The roasted coffee bean samples were weighed out as whole beans (five cups of each sample) and ground immediately prior to cupping, (no more than 15 minutes before infusion with water). Clean and odor free water was used for coffee beverage preparation and was brought to approximately 200º F (93ºC) at the time it was poured onto the ground coffee. The hot water was poured directly onto the measured grounds in the cup to the rim of the cup, making sure to wet all of the grounds. The grinds were allow to steep undisturbed for 3-4 minutes before evaluation. The crust was broken by stirring gently while sniffing. A cupping form (Appendix 3.) provided a means of recording 10 sensory attributes for each coffee on a ten point scale: Fragrance/Aroma, Flavour, Aftertaste, Acidity, Body, Balance, Uniformity, Clean Cup, Sweetness and Overall. A panel seven judges used in this study was selected from a pool trained and certified by Coffee Board of Kenya and Coffe Quality Institute of America who were actively practicing in their respective companies.

Since the trees were not replicated genotypes the cuppers were considered as the reps. The set up of a cupping laboratory is shown in Plate 12.

Plate 12: The set up of the cupping laboratory at CRF

Fragrance (defined as the smell of the ground coffee when still dry) and aroma (the smell of the coffee when infused with hot water). The fragrance/aroma was scored on the basis of dry and wet evaluation. Three distinct steps were followed: (1) sniffing the grounds placed into the cup before pouring water onto the coffee; (2) sniffing the aromas released while breaking the crust; and (3) sniffing the aromas released as the coffee steeped.When the liquor had cooled to 160º F (about 70º C), tasting began. The liquor was aspirated into the mouth to cover as much area of the tongue and upper palate. At that point flavour and aftertaste were rated.

As the coffee continued to cool (160º F - 140º F; 70º C - 60º C), the acidity, body and balance were rated next. Balance is the assessment of how well the flavour, aftertaste, acidity, and body fit together in a synergistic combination. As the brew approached room temperature (below 100º F; 37 º C), sweetness, uniformity, and clean cup were evaluated. For these attributes, the assessors made judgments on each individual cup, awarding 2 points per cup per attribute (10 points maximum score). The attribute overall, reflected the panelists personal appraisal based on the holistically integrated rating of the sample as perceived by the individual panelist. All the sensory scores for each coffee sample were added together to constitute the total score which was a reflection of the broad coffee quality performance. During the analysis, reference was made to ISO 13300 (2006) and ISO 6668 (2008) on general guidelines for the staff of a sensory evaluation laboratory and preparation of samples for use in sensory analysis respectively.

4.2.1.4 Data analysis

Since the trees were not replicated, the seven sensory assessors were considered as the reps. Data was subjected to analysis of variance and multivariate analysis. Mean seperation was done using Student-Newman-Keuls ($SNK_{5\%}$) test by Costat. Principal component analysis, dendrograms and discriminant function analysis were done using R-statistics and XLSTAT. Where R-statistics was applied all variables were entered as numerical factors and clustered using DAISY (dissimilarity matrix calculation) function and unweighted pair-group method with arithmetic average [UPGMA] (Venables *et al*., 2006). The statistical uncertainty of resulting hierarchical cluster groups was determined by calculating approximately unbiased p-

values through multi-scale bootstrap re-sampling using the R package pvclust (Venables *et al*., 2006).

4.2.2 Results

Analysis of variance revealed the genotypes varied significantly (P<0.05) in all sensory characteristics (flavour, acidity, aftertaste, body, balance) except in fragrance/aroma (Appendix 4). The mean sensory variables of the forty (40) coffee genotypes under *ex-situ* conservation together with two (2) commercial varieties (K7 and SL34) are shown in (Table 8). The genotypes Arousi, SL34, G5B , G53, F53, Mokka Cramers, Ennareta and Murta differed significantly with Dalle, Polysperma and DRII in flavour. SL34 which is a commercial cultivar, G5B, G53, F53 and Arousi scored the highest in flavour (7.86), while Polysperma, Dalle and DRI scored below 7.0. SL34 was significantly different in acidity from DRII, Dalle, Polysperma, SL4 and Dilla Alghe. G53 which scored the highest in aftertaste (7.86) was significantly different from SL4, Angustifola, Dilla, Dalle, polysperma and DRII. SL34 had the the best balance (7.93) while Ennareta had the highest score in overall (8.07). The flavour of the genotype Erecta was decribed as lemonish that of Geisha 11 as citrus, fruity and floral while that of Mokka Cramer was described as citric. G58 and G53 were described as fruity, lemon and floral. SL34 was described citrus, floral and spicy while F53 was described as fruity, lemon, floral and spicy.

Table 8: Mean sensory attributes of 40 ex-situ conserved coffee germplam together with 2 commercial varieties

Genotypes	Sensory variables							Flavour descriptors
	F/Aroma	Flavour	Aftertaste	Acidity	Body	Balance	Overall	
DRII	7.29	6.86c	6.86e	7.07cd	7.21ab	7.07ef	6.93f	none
Dalle	7.36	6.93bc	7.00cde	7.07cd	7.00b	7.00f	7.07def	none
Polysperma	7.21	6.93bc	6.93cd	7.00d	7.14ab	7.50a-f	7.00ef	none
SL4	7.5	7.21abc	7.07b-d	7.07cd	7.21ab	7.00f	7.14c-f	none
Padang	7.29	7.14abc	7.21a-e	7.21bcd	7.21ab	7.21c-f	7.29a-f	none
Dilla	7.43	7.21abc	7.07b-d	7.29bcd	7.21ab	7.21c-f	7.29a-f	none
Dilla Alghe	7.43	7.21abc	7.14a-e	7.07cd	7.43ab	7.29b-f	7.36a-f	none
Angustifola	7.29	7.29abc	7.07b-d	7.43a-d	7.43ab	7.21c-f	7.21a-f	none
Eritrean Moca	7.43	7.21abc	7.21a-e	7.36a-d	7.36ab	7.21c-f	7.29a-f	none
Series C	7.29	7.36abc	7.29a-e	7.36a-d	7.36ab	7.14def	7.36a-f	none
Plateau Bronze	7.57	7.21abc	7.14a-e	7.36a-d	7.36ab	7.57a-f	7.29a-f	none
Gimma Galla	7.29	7.36abc	7.29a-e	7.43a-d	7.43ab	7.36a-f	7.43a-f	none
SeriesL	7.5	7.36abc	7.14a-e	7.50a-d	7.43ab	7.36a-f	7.29a-f	none
Gimma Mbuni	7.36	7.43abc	7.29a-e	7.43a-d	7.36ab	7.36a-f	7.43a-f	none
ZeghieLtana	7.29	7.29abc	7.43a-e	7.36a-d	7.43ab	7.50a-f	7.36a-f	none
DR1	7.36	7.29abc	7.36a-e	7.36a-d	7.57ab	7.36a-f	7.43a-f	none
TDR	7.21	7.36abc	7.29 a-e	7.50a-d	7.43ab	7.50a-f	7.43a-f	none
BarbokSudan	7.36	7.57abc	7.43 a-e	7.50a-d	7.43ab	7.29b-f	7.43a-f	none
M63	7.36	7.43abc	7.43a-e	7.57a-d	7.50ab	7.29b-f	7.50a-f	none
HDT	7.5	7.29abc	7.29a-e	7.50a-d	7.64ab	7.57a-f	7.43a-f	none
Yellow. Amarello	7.07	7.43abc	7.29a-e	7.71abc	7.50ab	7.57 a-f	7.64a-f	none
Geisha12	7.21	7.36abc	7.50a-e	7.57a-d	7.50ab	7.57 a-f	7.57a-f	none
GimmaG.Sidamo	7.36	7.50abc	7.43a-e	7.57a-d	7.50ab	7.57 a-f	7.36a-f	none
K7(cv)	7.5	7.50abc	7.43a-e	7.57a-d	7.36ab	7.36 a-f	7.57a-f	none
Purpurascens	7.43	7.50abc	7.43a-e	7.57a-d	7.57ab	7.43 a-f	7.50a-f	none
1225VI	7.5	7.50abc	7.43a-e	7.50a-d	7.50ab	7.57 a-f	7.64a-f	none
Pretoria	7.57	7.50abc	7.43a-e	7.71abc	7.57 ab	7.43 a-f	7.50a-f	none
Wollamo	7.86	7.57abc	7.64a-d	7.71abc	7.36ab	7.36 a-f	7.57a-f	none
Mocha Series	7.29	7.50abc	7.43a-e	7.79abc	7.71 ab	7.64a-e	7.79a-e	none
Murta	7.29	7.71a	7.57a-d	7.71abc	7.43 ab	7.64a-e	7.79a-e	none
Erecta	7.5	7.57abc	7.57a-d	7.71abc	7.57ab	7.64a-e	7.64a-f	lemonish
Geisha 11	7.5	7.57abc	7.50a-e	7.64 a-d	7.50ab	7.57a-f	8.00ab	Citrus, fruity, floral
H1	7.36	7.64ab	7.64a-d	7.86ab	7.64 ab	7.64a-e	7.71a-f	none
Blue Mountain	7.64	7.64ab	7.57a-d	7.71abc	7.64ab	7.86ab	7.79a-e	Fruity, citrus
Mocha	7.43	7.64ab	7.43a-e	7.86ab	7.79 ab	7.86ab	7.86a-d	none
Arousi	7.36	7.86a	7.64a-d	7.86ab	7.71ab	7.64a-e	7.86a-d	none
Mokka Cramers	7.57	7.79a	7.64a-d	7.64 a-d	7.71ab	7.71a-d	7.93abc	Lemon, citric
G58	7.86	7.86a	7.71abc	7.79abc	7.71ab	7.64a-e	7.79a-e	Fruity, lemon,floral
G53	7.5	7.86a	7.86b	7.93ab	7.64ab	7.86ab	7.86a-d	Fruity, lemon, floral, spicy
Ennareta	7.86	7.79a	7.71abc	7.79ab	7.64ab	7.79abc	8.07a	none
SL34 (cv)	7.5	7.86a	7.79ab	8.07a	7.79ab	7.93a	7.93abc	Citrus, floral, spicy
F53	7.79	7.86a	7.79ab	7.93ab	7.93a	7.79abc	7.93abc	Fruity, lemon, floral, spicy

Means within a column not sharing a letter are significantly different at P<0.05. cv-cultivar. Student-Newman-Keuls (SNK5%) test.

Cluster dendrogram constructed using sensory data from the forty (40) *ex-situ* conserved genotypes and the two commercial varieties was used to estimate beverage quality diversity amongst them thus indicating how closely related or diverse they were. Results of the cluster analysis are illustrated in . The genotypes first separated into two broad clusters which recorded beverage quality diversity of 47%. The first cluster contained 27 genotypes while the second cluster had 15 genotypes as determined by the degree of diversity/similarity in beverage quality. The faint line in Figure 6 shows the point at which the dendrogram was truncated by Kelley-Gardener-Sutcliff (KGS) penalty function to define supported sub-clusters. Both clusters were further subdivided into two sub clusters each giving four supported sub-clusters which separated at 22-24% level of dissimilarity. Interpretation of the cluster dendrogram was based on execution of the Kelley-Gardener-Sutcliffe penalty function which reduced the dendrograms to 4 clusters at dissimilarity level of about 18%. Sub-clustering, however continued with closely related genotypes grouping together down the dendrogram to eight sub-clusters separating at 12-15% dissimilarity which further sub-clustered to smaller groups of closer similarity (Figure 7). The most similar genotypes which recorded 0% dissimilarity were Yellow Amarello and HDT in one cluster, Geisha 11, Gimma Galla Sindamo and K7 in a separate cluster, Blue Mountain and Moca in another cluster, and Mocha Series D and Murta in their own cluster.

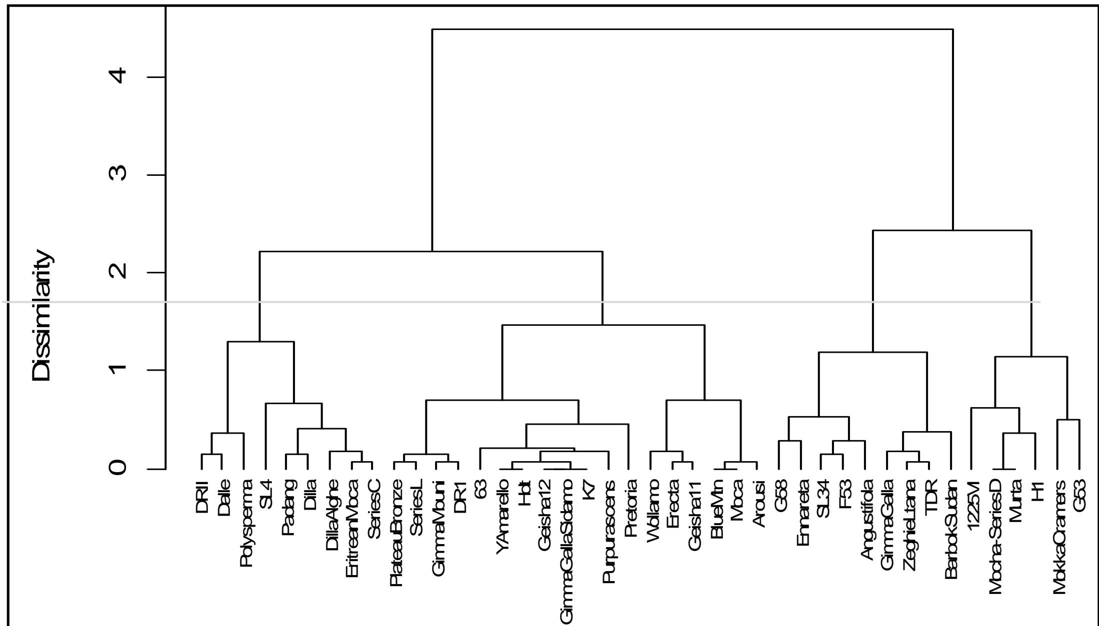


Figure 6: Dendrogram of 42 ex-situ conserved *C. arabica* genotypes construcdted by cluster analysis of 7 sensory variables

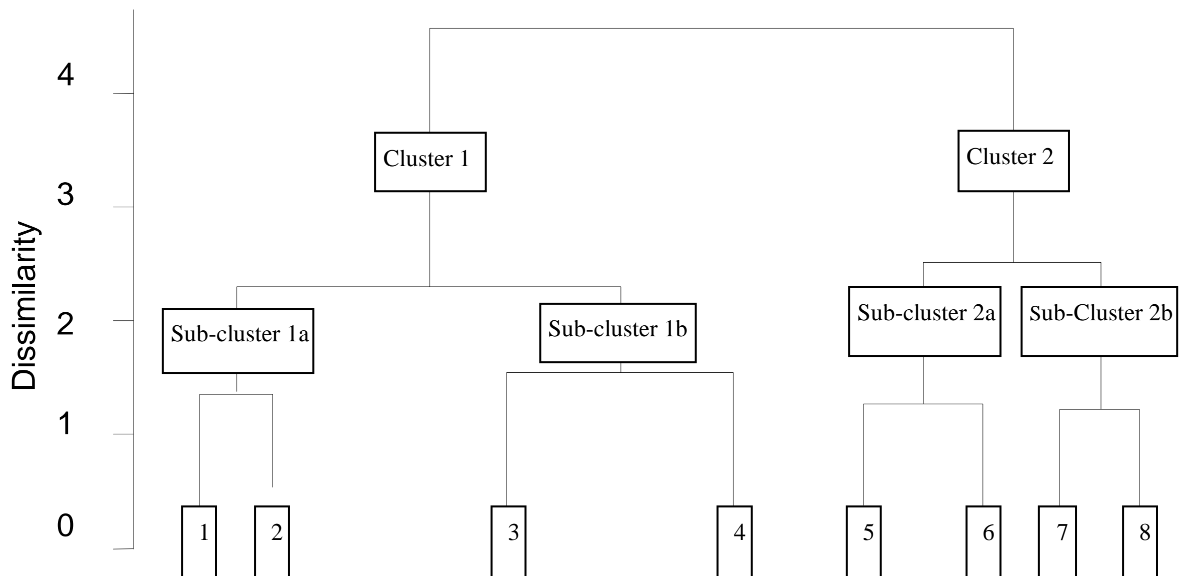


Figure 7: Pruned cluster dendrogram illustrating four supported sub-clusters

The variables clean cup, sweetness and uniformity which are process control characteristics scored maximum (10 points each) in all the genotypes. However, the scores are not shown in the table but their scores were added to the other seven sensory scores to get the total score. According to Lingle, (2001) the total score is used to classify the coffee as specialty or commercial whereby a coffee which scores 80 to 100 points is specialty grade, while 79 points and below is commercial grade coffee. Figure 8 shows the diversity of the genotypes as determined by the total scores. Ninety two percent (92%) of the fourty ex-*situ* conserved genotypes scored 80 points and above in mean total score qualifying them as specialty coffee. The genotype F53 had the highest total score (85.0) followed by SL34 which is a commercial variety with a score of 84.86. The genotypes DRII, Dalle and Polysperma scored the lowest (79.29, 79.34 and 79.71 respectively).

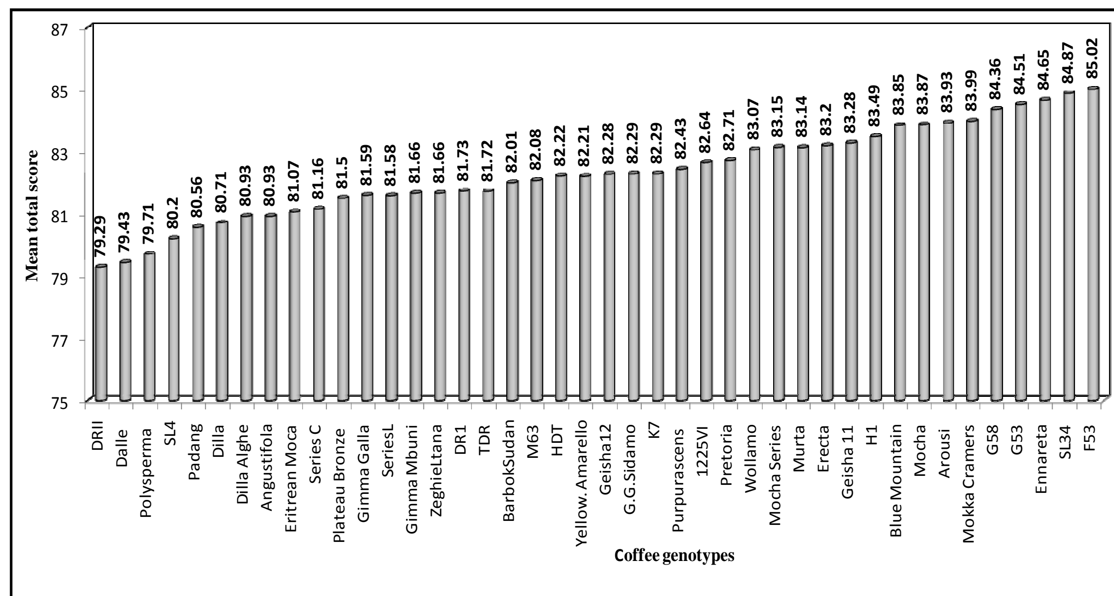


Figure 8: Diversity of 40 *ex-situ* conserved and 2 commercial coffee varieties by mean total scores.

4.2.3 Discussion

The *ex-situ* conserved coffee germplasm collections showed diversity in terms of their sensory quality. Previous studies showed SL34 (commercial cultivar) as one of the best in terms of sensory characteristics (Walyaro, 1983). Alongside SL34, G53, F53, G5B and Ennareta showed the best performance in sensory characteristics although cluster analysis gouped SL34, F53 and G5B showing they were quite similar. One common characteristic of G53, F53 and G5B is that they were selected from the same area (Millot, 1969). For a coffee to qualify as specialty, it must score 80 points and above (total score) on beverage characteristics (sensory variables and process control characteristics) (Lingle, 2001). Ninety two present (92%) of the genotypes excluding the two commercial varieties scored 80 points and above. DR11, Dalle and Polysperma scored below 80 points.

Yield has been stressed as one important parameter in breeding (Walyaro, 1983). However specialty buyers are looking for unique and differentiated coffees (Hide, 2009). The flavour descriptors used to describe some of the genotypes showed that they were unique.The flavour descriptors such as sour, winey, sweet, mellow, salt, astringent, bitter and harsh are categorized under tastes (Lingle 2001). Loss of organic matter in the coffee results in flavours described as woody, aged and grassy among others. Enzymatic activities as the coffee beans develop result in the formation of aroma compounds such as flowery, floral, coffee blossom, tea rose, fruity, citrus, lemon, berry like, herby and leguminous. Astrigency is defined as complex sensation accompanied by shrinking drawing or pluckering of the skin or mucosal surface in the mouth produced by substances such as kaki tannins or soloe

tannins International Standard, ISO 5492 (2008). Flavour descriptors encountered in this study are given in Appendix 17. Within the United States, the specialty coffee segment is the major growth area with a 20% annual growth rate and total sales in 2006 of $12.27 billion (International Trade Centre, 2011). In 2007 some specialty coffee from Kenya (5 bags) was sold at $954 per 50kg bag and this was part of some Ethiopian collection genotypes grown by an estate in Kenya.

Therefore the diversity observed in the conserved genotypes can be exploited for improvement of beverage quality in arabica coffee. These genetic resources should therefore be properly conserved in order to utilize them for genetic improvement of sensory coffee quality for the emmerging markets in the future. However there are certain drawbacks that limit efficiency and threaten security of plants in the gene bank. The plants are exposed to pests, diseases and other natural hazards such as drought, weather damage, human error and vandalism. Field gene banks are costly to maintain and, as a consequence, are prone to economic decisions that may limit the level of replication of accessions, the quality of maintenance and even their very survival in times of economic stringency. However due to their importance, considerable inputs should be applied to ensure maintenance and existence of these materials.

4.3 Diversity of Ruiru 11 progenies evaluated at Kitale, Koru and Ruiru-CRS by sensory characteristics.

4.3.1 Materials and methods

4.3.1.1 Description of study sites

The study was conducted in three different coffee growing regions in Kenya namely Ruiru, Koru and Kitale. Descriptions of Ruiru are as are given in section 4.2.1.1. Koru, CRF substation is located at geographical coordinates 00 07'S, 35^0 16'E and has an elevation of 1700MASL. The mean annual rainfall is 1720mm which is well distributed. The area receives a unimodal rainfall pattern.The soils are eutric nitosols, friable clays, and weakly acidic to neutral, rich in bases, available phosphorous and moderate inorganic matter (Jaetzold *et al*, 2006a). Kitale CRF-substation in Trans Nzoia is found at 0^0 59'S and 35^0 01'E at an altitude of 1982 MASL. The annual average rainfall is 1100 mm (unimodal rainfall pattern), most of which falling between April and September (Jaetzold *et al*, 2006a).The soils are fairly deep sandy clays/loamy clays and full of weatherable minerals. The soils are eutric nitosols, friable clays, and weakly acidic to neutral, rich in bases, available phosphorous and moderate inorganic matter.

4.3.1.2 Test materials and field layout

In selecting Ruiru 11 siblings availability of common sibings in the three selected sites, Kitale, Ruiru and Koru was considered. Ten Ruiru 11 siblings (Table 9) were found to be common in the three sites. All the sites were laid out in a Randomized Complete Block Design (RCBD) with three replications. The trees were planted at a spacing of 2 m by 2 m, with each rep having 12 trees. The coffee genotypes were

mature bearing coffee trees having been planted in adaptation trials at Ruiru and Koru in 1994 and Kitale in 1990. All agronomic practices including, change of cycle weeding, pest and disease control, fertilizer application and pruning were carried out as recommended.

Table 9: List of Ruiru 11 siblings evaluated by sensory variables

Accession code	Parentage	Reps
CRF- 03	SL28 x (RS x SL28) (B X HT) X CAT.90	3
CRF- 05	SL28 x (RS x SL28) (B X HT) X CAT.124	3
CRF- 11	SL28 X (RS x SL28) (B X HT) X CAT.86	3
CRF- 23	SL28 X (RS x SL28) (B X HT) X CAT.90	3
CRF- 41	SL28 X (RS x SL28) (B X HT) X CAT.86	3
CRF- 50	SL28 X (RS x SL28) (B X HT) X CAT.134	3
CRF- 91	SL28 X (SL34 x RS) HT X CAT.86	3
CRF- 111	SL28 X (SL34 x RS) HT X CAT.86	3
CRF- 123	SL28 X (SL34 x RS) HT X CAT.86	3
CRF- 131	SL28 X (SL34 x RS) HT X CAT.86	3

HT = Hibrido de Timor, RS = Rume Sudan, B=Bourbon

4.3.1.3 Wet processing of ripe cherries, roasting green coffee and sensory evaluation

Ripe berries were harvested from a sample size of twelve trees for each genotype and bulked to give one rep per sample during the peak harvesting period of the main crop of 2008 (October, November, December). The cherries were wet processed using standard recommended procedures as explained in section 4.2.1.2.

Roasting green coffee and sensory evaluation was done as described in section 4.2.1.3.

4.3.1.4 Data analysis

Data was subjected to analysis of variance and multivariate analysis. Mean seperation was done using Student-Newman-Keuls ($SNK_{5\%}$) test by Costat. Discriminant Function analysis was done using XL-STAT.

4.3.2 Results

Analysis of variance did not reveal significant differences ($P<0.05$) among the genotypes except in the variable overall (Appendix 5). However variations due to site were significant for all the sensory variables except for fragrance and acidity. Similarly, site by sibling ($G \times E$) interactions were also significant ($P< 0.05$) for fragrance, aftertaste, balance and overall.

Means of the sensory characteristics of of Ruiru11 progenies evaluated at Kitale, Koru and Ruiru are shown in Table 10. The fragrance of CRF-03 was not significantly ($P>0.05$) different in the three sites but at Koru the sibling gave significantly ($P< 0.05$) lower scores in all the other sensory variables than at Ruiru and Kitale. At Ruiru CRF-05 had significantly better balance and overall than at Koru and Kitale. Except for balance the sibling CRF-50 had significantly ($P< 0.05$) higher scores in all the other sensory characteristics a Ruiru than at Koru and Kitale. The other siblings differed in one variable or the other among the sites. However, the siblings CRF-11 and CRF-23 were the most stable in the three sites showing no significant differences in all the sensory variables evaluated in the three sites. The

different terms used by the sensory panel to describe the flavour of the siblings in the three sites are shown in Table 10. At Koru no flavour descriptors were given for CRF-03, CRF-23, CRF-91, CRF-111 and CRF-123. Similarly, no flavour descriptors were given for CRF-23 and CRF-41 at Kitale. The descriptors used for the siblings at Ruiru were more diverse than those used to describe coffee from the other two sites.

Table 10: Comparison of sensory performance of Ruiru11 progenies

		Sensory variables							
Progeny	Site	Fragrance	Flavour	Aftertaste	Acidity	Body	Balance	Overall	Flavour descriptors
CRF-03	Kitale	7.45a	7.48a	7.50a	7.52a	7.43a	7.48a	7.50a	Floral
	Koru	7.10a	6.81b	6.76b	6.81b	7.05b	6.90b	6.81b	none
	Ruiru	7.38a	7.36a	7.36a	7.48a	7.40a	7.43a	7.48a	Floral,fruity,lemon, tea rose
CRF-05	Kitale	7.43a	7.24ab	7.38a	7.40ab	7.40a	7.33b	7.33b	Harsh, grassy, rubbery
	Koru	7.10a	6.88b	7.00a	7.02b	7.14a	7.10b	6.98b	Grassy
	Ruiru	7.60a	7.52a	7.55a	7.74a	7.55a	7.69a	7.67a	Tea rose, honey, floral
CRF-11	Kitale	7.48a	7.33a	7.38a	7.48a	7.52a	7.52a	7.48a	Foral
	Koru	7.43a	7.33a	7.38a	7.40a	7.45a	7.36a	7.38a	Fruity
	Ruiru	7.76a	7.57a	7.57a	7.74a	7.57a	7.62a	7.69a	Lemon, floral
CRF-23	Kitale	7.50a	7.31a	7.31a	7.45a	7.43a	7.40a	7.40a	none
	Koru	7.29a	7.19a	7.17a	7.31a	7.29a	7.29a	7.26a	none
	Ruiru	7.33a	7.40a	7.48a	7.64a	7.50a	7.50a	7.50a	Fruity ,floral, lemon
CRF-41	Kitale	7.55a	7.52ab	7.48a	7.62a	7.57a	7.57a	7.60a	none
	Koru	7.36a	7.21b	7.31a	7.45a	7.48a	7.43a	7.43a	Grassy
	Ruiru	7.71a	7.67a	7.64a	7.90a	7.69a	7.81a	7.79a	Floral,
CRF-50	Kitale	7.29b	7.12b	7.12b	7.31b	7.43b	7.31a	7.29b	Floral
	Koru	7.21b	7.00b	7.11b	7.11b	7.18b	7.14a	7.04b	Lemon
	Ruiru	7.55a	7.69a	7.62a	7.90a	7.76a	7.64a	7.71a	Floral, fruity
CRF-91	Kitale	7.48a	7.45ab	7.48a	7.55a	7.43a	7.50ab	7.48b	Floral
	Koru	7.40a	7.24b	7.36a	7.38a	7.45a	7.33b	7.38ab	none
	Ruiru	7.57a	7.76a	7.69a	7.88a	7.76a	7.79a	7.81a	Lemon, floral, fruity
CRF-111	Kitale	7.43a	7.31a	7.33a	7.38ab	7.36a	7.29a	7.33a	Lemon, floral
	Koru	7.29a	7.12a	7.05a	7.12b	7.24a	7.17a	7.17a	none
	Ruiru	7.36a	7.38a	7.33a	7.62a	7.48a	7.48a	7.43a	Lemon,tea rose, floral.fruity
CRF-123	Kitale	7.29a	7.21a	7.31ab	7.33ab	7.38ab	7.26b	7.26b	Flat
	Koru	7.31a	7.14a	7.17b	7.17b	7.19b	7.24b	7.21b	none
	Ruiru	7.50a	7.64b	7.57a	7.79a	7.64a	7.64a	7.71a	Fruity, floral, lemon
CRF-131	Kitale	7.36a	7.24a	7.38a	7.38b	7.29b	7.26a	7.33b	Floral
	Koru	7.29a	7.24a	7.24a	7.45b	7.36b	7.40a	7.31b	Harsh
	Ruiru	7.57a	7.68b	7.61a	7.82a	7.64a	7.79a	7.86a	Lemon, floral, fruity

Means within a genotype for specific sensory attributes not sharing a letter are significantly different at $P<0.05$ Mean seperation done using Student-Newman-Keuls ($SNK_{5\%}$) test

The sensory data generated from the ten Ruiru 11 genotypes evaluated in the three different geographical sites was subjected to discriminant function analysis (DFA). Results of the discriminant function analysis showed that the first discriminant factors explained 82.93% total variation (Figure 9). The factors which contributed significantly to the discrimination were flavour, body, balance and overall score (

Table 11). Acidity, fragrance and aftertaste showed the least contribution to the discrimination. Using sensory variables the progenies from the three sites were not distinctly separated since overlaping of points was mostly observed.

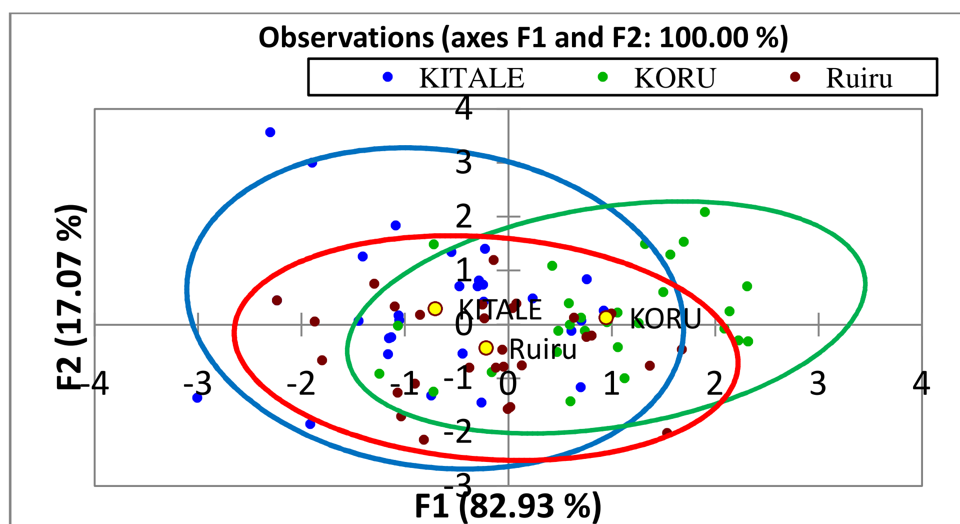


Figure 9: Discriminant factor analysis (DFA) based on combined sensory data of Ruiru 11 progenies evaluated at Koru, Kitale and Ruiru

Table 11:Contribution of the specific sensory variables to the discrimination

Variable	Lambda	F	DF1	DF2	p-value
Fragance	0.956	1.942	2	85	0.150
Flavour	0.898	4.802	2	85	0.011
Aftertaste	0.934	3.019	2	85	0.054
Acidity	0.986	0.617	2	85	0.542
Body	0.790	11.325	2	85	< 0.0001
Balance	0.895	4.982	2	85	0.009
Overall	0.763	13.226	2	85	< 0.0001

For all the siblings, the variables clean cup, sweetness and uniformity scored maximum (10 points each). These scores were added to the scores of the other sensory variables to classify the coffee as specialty grade (80 to 100 points) or commercial grade (79 and below). The diversity of the Ruiru 11 siblings evaluated at Kitale, Koru and Ruiru (CRS) by total score is shown in Figure 10. Except for CRF-03 and CRF-05 at Koru, all the other siblings attained specialty grade in the three sites. CRF-41 scored the highest in total score in each of the three sites.

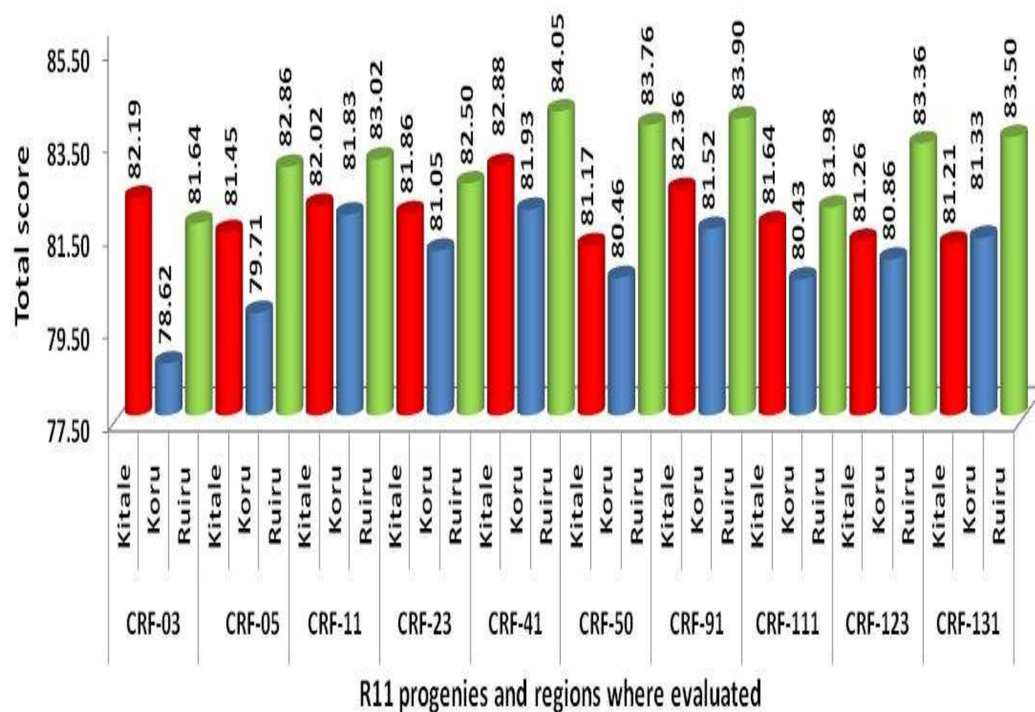


Figure 10: Mean total score of Ruiru 11 progenies evaluated at Kitale, Koru and Ruiru-CRS

4.3.3 Discussion

Most of the work done in sensory characterization of coffee varieties in Kenya has compared Ruiru11 cultivar with the traditional varieties. (Owuor, 1988; Njoroge, 1990; Ojijo, 1993 and Omondi, 2008). The main conclusion from these studies was that the quality of Ruiru 11 was comparable to that of the traditional variety SL28 with Ojijo (1993) reporting Ruiru 11 progenies as showing great beverage quality. Similarly, the ten Ruiru 11 progenies evaluated at Kitale, Koru and Ruiru indicated significant variations among them in beverage characteristics. The sensory performance of the Ruiru 11 progenies evaluated tended to differ with the regions where they were grown. Agwanda *et al*. (2003) observed that discrimination on the basis of liquor traits were best observed in the sites where moderate moisture stress occurred during bean filling stage. Areas with excess precipitation, especially during crop maturation, have a tendency to produce lower quality coffee due to irregular cherry ripening and poor conditions for drying the crop after harvesting (Van der Vossen, 2009). This could probably explain why the same genotypes performed differently in the three regions. All the siblings evaluated in the three sites scored over 80 points in total score except CRF-03 and CRF-05 evaluated at Koru. At Koru, the progenies total score ranged from 78.62 to 81.93, at Kitale 81.17 to 82.88 and at Ruiru 81.64 to 84.05. The progeny coded CRF-41 showed the best performance in the three sites by scoring the highest in total score in each of the three sites. This is a clear indication that Ruiru 11 progenies have the potential to reach the specialty quality as defined by the market (Lingle, 2001). The flavour descriptors associated with good flavour that were used to describe some of the genotypes in this study such as citrus, fruity, floral and lemon are highly appreciated

by specialty buyers. Most of those flavour descriptors were used to describe mainly the Ruiru 11 progenies evaluated at Ruiru and Kitale. That observation stresses the importance of the environment when it comes to coffee production.

4.4 Sensory profiling of beverage quality of five advanced breeding lines of Arabica coffee compared to two commercial coffee cultivars

4.4.1 Materials and methods

4.4.1.1 Description of the study sites

Tatu Estate in Ruiru lies at latitude 1° 05'S and longitude 36° 54'E and is approximately 1623MASL. The area receives a bimodal mean annual rainfall of 1063mm with the first rainy season falling between March and July; and second season falling between September and December. The mean annual temperature is 19°C with the hottest season occurring between January and April. The soils are classified as a complex of humic nitisols and plinthic ferrasols with the former being dominant in the older coffee divisions. They are welldrained, deep reddish brown, slightly friable clays with murram sections occassionally interrupting (Jaetzold and Schimidt, 1983).

Mariene CRF substation in Meru, lies at latitude 0° N, 37' and longitude 36° 'E. The altitude is 1524 MASL. The area receives a bimodal rainfall pattern with the main rains in October- November and short rains April –May. The mean annual rainfall 1063mm.The minimum temperature is 11.7^{o}C and maximum 22.8^{o}C although the pattern keep flactuating. The soils are ando-humic acrisols, friable clays, strongly acid, moderate in organic matter, very low in bases.

Machakos Agricultural Training Centre is a government institution mainly for training farmers. This site lies at latitude 1°31′S and longitude 37°16′E and has an altitude of 1600 MASL. The area is semi-arid with mean annual rainfall of 750 mm and mean annual temperature of 20.9^{o}C. The soils are luvisols, well drained, moderately deep to deep, dark red to yellowish red, friable to firm, sandy clay often with a topsoil of loamy sand are (strongly leached soils. (Jaetzold *et al.*, 2006b).

4.4.1.2 Test Materials and field layout

Five advanced coffee breeding lines coded Cr8, Cr22, Cr23, Cr27 and Cr30 were evaluated alongside two commercial Arabica cultivars, SL28 and Ruiru 11 as check cultivars. Details of the materials are shown in Table 12: List of advanced coffee breeding lines evaluated alongside two commercial Arabica cultivars, SL28 and Ruiru 11 as check cultivars.

Table 12: List of advanced coffee breeding lines evaluated alongside two commercial Arabica cultivars, SL28 and Ruiru 11 as check cultivars

Code	Parentage
Cr.2322	Clone B 15.1525 with SL28 as recurrent parent; HDT and Rume Sudan as donor parents. Also contains N39 and SL4
Cr.2222	Clone B 15.1559 with SL28 as the recurrent parent; HDT and Rume Sudan as donor parents. Also contains N39 and SL4
Cr.2722	Clone B 15.1534 with SL28 as recurrent parent; HDT and Rume Sudan as donor parents. Also contains N39 and SL4
Cr.3022	Clone B15.96 with SL28 as the recurrent parent; HDT,Rume Sudan and K7 as donor parents. Also contains SL34
Cr.0822	Clone B15.239 with SL28 as the recurrent parent; HDT and Rume Sudan as donor parents. Also contains SL34
SL28	Commercial Check cultivar
Ruiru 11	Commercial Check cultivar

At Tatu Estate in Ruiru, the five advanced breeding lines were established together with the check cultivars between 6-18th March, 2006. Twenty trees of each genotype were planted at a spacing of 1.5m x2.0m replicated three times. SL28 was established at two levels namely prayed with and not sprayed not sprayed. SL28 was sprayed with copper fungicides to control Coffee Berry Disease (CBD) and Coffee leaf rust (CLR) while the other SL28 entry was not sprayed with any fungicides. At Mariene Meru, establishment was done on 25th May 2007 under two spacing regimes (2m x 1.5m and 2.75m x 2.75m) replicated three times. SL28 was established only as not sprayed. Establishment at Machakos and Koru and were done on 25-26 May 2008 and 18-20 December 2007 respectively. The genotypes were established like at Meru except that SL28 sprayed with fungicides was included in the layout.

4.4.1.3 Wet processing of ripe cherries, roasting green coffee and sensory evaluation

At Tatu Estate, ripe berries were harvested from a sample size of twenty trees and bulked to give one sample per genotype per rep in 2008 and 2009 during the early crop (April May June) and main crops (October, November December.) At Meru, Koru and Machakos samples were harvested from a sample size of 10 trees to give one sample per rep during the main main crops (April, May, June at Meru and October, November, December at Koru and Machakos). At Meru sampling was done for three years (2009 to 2011) while at Koru and Machakos samples were taken in 2010 and 2011. All the samples were wet processed as explained in section

4.2.1.2. while roasting of green coffee and sensory evaluation were done as described in section 4.2.1.3.

4.4.2 Sensory profiling of beverage quality of five advanced breeding lines of Arabica coffee compared to two commercial coffee cultivars

4.4.2.1 Diversity of the coffee genotypes evaluated at Tatu- Ruiru

Analysis of variance performed on the sensory data revealed significant differences among the genotypes with seasons showing significant contribution. (Appendix 6). The genotypes Cr22, Cr8, Cr27 and Cr23 did not show any significant differences in the sensory characteristics in the different seasons in 2008 while in 2009 only Cr27 and SL28S were stable (Table 13). In 2008, Cr30 had significantly higher (P<0.05) acidity, body and balance during the early crop while in 2009 all the senosry variables were significantly higher (P<0.05) during the main crop except fragrance. Contrary, Ruiru 11 scored significantly lower (P<0.05) in body, balance and overall in 2008 and acidity in 2009 during the early crop. SL28NS had a significantly better (P<0.05) aftertaste and higher body during the early crop than in the main crop in 2008 while acidity was significantly higher in the main crop in 2009.

Table 13: Comparison of the sensory performance of the genotypes in different seasons for two years at Tatu, Ruiru

Year	Genotype	Sensory variables													
		Fragrance		Flavour		Aftertaste		Acidity		Body		balance		Overall	
		Ealy Crop	Main Crop	Ealy Crop	Main Crop	Ealy Crop	Main Crop	Ealy Crop	Main Crop	Ealy Crop	Main Crop	Ealy Crop	Main Crop	Ealy Crop	Main Crop
2008	Cr22	7.67a	7.43a	7.50a	7.50a	7.62a	7.45a	7.69a	7.62a	7.69a	7.43a	7.52a	7.57a	7.64a	7.67a
	Cr23	7.64a	7.40a	7.60a	7.48a	7.52a	7.43a	7.74a	7.71a	7.76a	7.55a	7.69a	7.67a	7.62a	7.64a
	Cr27	7.67a	7.48a	7.64a	7.47a	7.60a	7.48a	7.91a	7.64a	7.83a	7.64a	7.83a	7.69a	7.83a	7.64a
	Cr30	7.69a	7.24a	7.81a	7.50b	7.88a	7.43a	8.05a	7.57b	7.86a	7.55b	8.00a	7.62b	7.95a	7.69a
	Cr8	7.71a	7.50a	7.67a	7.50a	7.57a	7.38a	7.71a	7.62a	7.71a	7.49a	7.67a	7.52a	7.64a	7.52a
	R11	7.21a	7.21a	6.86a	7.17a	7.00a	7.14a	7.00a	7.26a	6.93b	7.26a	6.86b	7.31a	6.86b	7.24b
	SL28NS	7.57a	7.38a	7.57a	7.48a	7.71a	7.40b	8.14a	7.69b	7.71a	7.56a	7.86a	7.54a	8.00a	7.64a
	SL28S	7.62a	7.48a	7.62a	7.52a	7.69a	7.50a	7.74a	7.69a	7.81a	7.45b	7.67a	7.62a	7.79a	7.62a
2009	Cr22	7.39a	7.43a	7.25b	7.61a	7.30b	7.57a	7.54b	7.75a	7.55a	7.66a	7.48a	7.58a	7.52a	7.59a
	Cr23	6.89a	7.21a	6.79a	7.29a	6.82a	7.25a	6.75a	7.33a	6.86b	7.39a	7.00a	7.29a	6.96a	7.67a
	Cr27	7.39a	7.36a	7.46a	7.48a	7.50a	7.44a	7.54a	7.54a	7.68a	7.48a	7.54	7.40a	7.61a	7.35a
	Cr30	7.23a	7.39a	7.27b	7.66a	7.16b	7.61a	7.34b	7.62a	7.30b	7.52a	7.29b	7.50a	7.29b	7.50a
	Cr8	7.21a	7.21a	7.05a	7.14a	7.05a	7.11a	7.19a	7.18a	7.25a	7.29a	7.16a	7.18a	7.16a	7.62a
	Ruiru 11	7.21a	7.30a	7.00a	7.19a	6.98b	7.20a	7.14a	7.31a	7.23a	7.35a	7.12a	7.26a	7.11a	7.21a
	SL28NS	7.29a	7.39a	7.36b	7.58a	7.37a	7.49a	7.45a	7.64a	7.49a	7.58a	7.46a	7.52a	7.42a	7.25a
	SL28S	7.36a	7.44a	7.48a	7.58a	7.50a	7.55a	7.61a	7.64a	7.68a	7.52a	7.61a	7.54a	7.59a	7.58a

Means along a column not sharing a letter are significantly different ($P<0.05$). Means separated by Student-Newman-Keuls (SNK5%) test. **Key**: SL28S- SL28 Sprayed SL28, SL28NS Not Sprayed,

The flavour of Cr22 was described as floral, citrus and fruity in 2008 and flowery, caramel and citrus in 2009. Cr23 was described as herbal, lemon and fruity in 2008 while in 2009 there were no flavour descriptors were given. Cr27 was citrus and fruity in 2008 while citrus, chocolate, and floral in 2009. Cr30 was citrus, lemon, spicy, floral and fruity in 2008 and fruity in 2009. Cr8 was grassy in both years while Ruiru 11 was grassy, astringent and grassy in 2009. SL28NS was harsh in 2008 and grassy in 2009. The flavour of SL28S was described as fruity and floral in 2008 while in 2009 it was described as tea rose, lemon and juicy in 2009.

Combined sensory data was subjected to principal component analysis (PCA). Results of the principal component analysis showed that the first two principal components explained 98.91% (PC1 94.93% and PC2 3.98%) of the total variation (Figure 11). The genotypes Cr22, Cr27, Cr30 and SL28NS and SL28S were placed in the positive side of PC1 while Ruiru 11, Cr23 and Cr8 were placed in the negative side of PC1. All the sensory attributes contributed almost equally to PC1 while balance contributed the most to variations in PC2 as shown in Table 14.

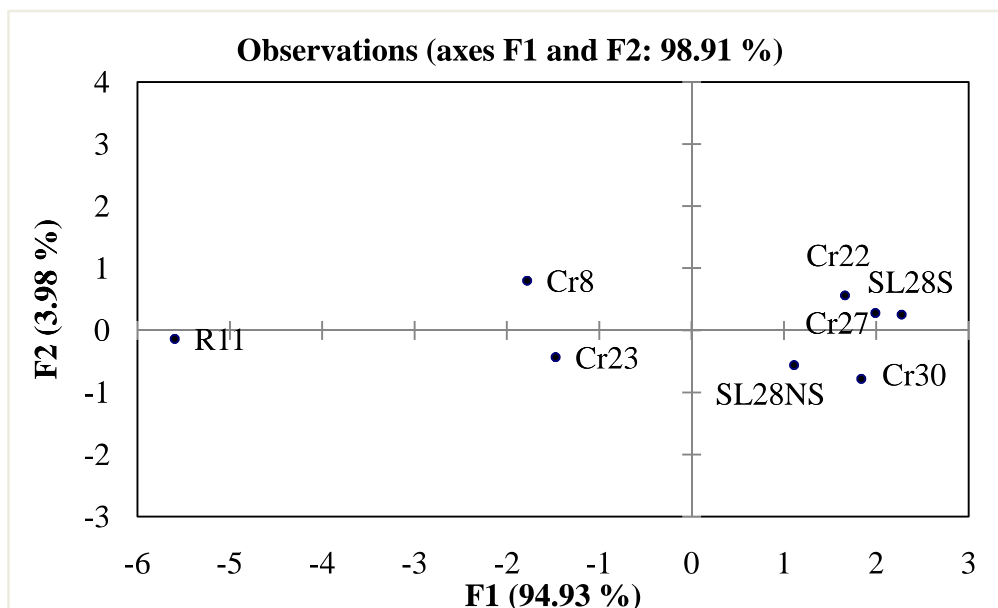


Figure 11: Principle component (PC) analysis plot of first two principle components, illustrating relationship among the coffee genotypes evaluated for two years at Tatu -Ruiru.
Key: SL28S, SL28 Sprayed; SL28NS, Not Sprayed; R11, Ruiru 11

Table 14: The first two principle components (PC) of the seven sensory variables.

Variables	PC1	PC2
Fragrance/aroma	0.384	-0.129
Flavour	0.383	-0.117
Aftertaste	0.384	-0.235
Acidity	0.385	0.071
Body	0.383	-0.217
Balance	0.340	0.911
Overall	0.385	-0.181
Eigen value	6.645	0.279
Variability (%)	94.928	3.984
Cumulative (%)	94.928	98.912

All the genotypes scored a maximum of 10 points for each of the variables clean cup, sweetness and uniformity, which were added to the scores of the other sensory variables to classify the coffee as specialty grade (80 to 100 points) or commercial grade (79 and below). The mean total scores of the coffee genotypes evaluated for two years presented in seasons is shown in Figure 12. All the genotypes attained specialty grade during the main crop for the two years. However, Ruiru 11 did not achieve specialty grade during the early crop for the two years and Cr23 in 2009. The performance of individual genotypes showed variations. For instance Cr23 which scored 83.57 during the early crop of 2008 gave the lowest score (78.07) in the same season in 2009. Cr30 scored 85.24 points during the early crop of 2008 and 80.88 points during the same season in 2009.

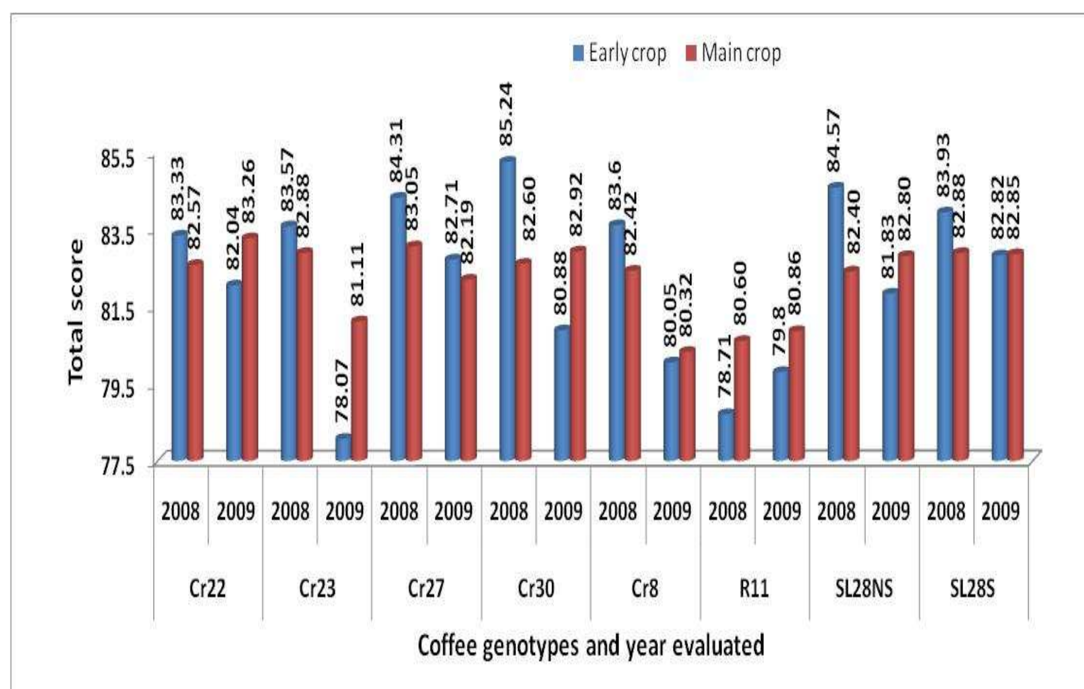


Figure 12: Mean of sensory attributes of seven coffee genotypes evaluated for two seasons at Tatu Ruiru

Key: R11- Ruiru 11, SL28S-SL28 Sprayed; SL28NS-SL28 Not Sprayed

4.4.2.2 Diversity of coffee genotypes evaluated at Mariene-Meru by sensory characteristics

Analysis of variance showed that the advanced breeding lines evaluated alongside check cultivars were variable in all the cup quality characteristics evaluated (Appendix 8, Appendix 9 and Appendix 10). Significant variations were observed in various sensory characteristics in 2009 and 2010 whereas all the genotypes showed no significant variations were observed in 2011. Variations due to spacing were also highly significant.

Results of the mean sensory characteristics of the coffee genotypes evaluated at Meru for three years are shown in (Table 16). In 2009 ,Cr22, Cr8 and Ruiru 11 were significantly different in flavour from SL28NS and Cr27. During the same year, the genotype SL28NS, had significantly higher aftertaste and acidity than Cr8, Cr22, Ruiru 11 and Cr23. In overall score, Cr22, Cr8 and Ruiru 11 had significantly lower scores than SL28NS, Cr30 and Cr27.

In 2010, the fragrance of Ruiru 11 was significantly lower than that of Cr23, Cr27 and Cr30. The genotypes SL28NS, Cr8 and Ruiru 11 differed significantly in flavour from Cr27. The aftertaste of Ruiru 11 was significantly lower than that of all the other genotypes except Cr22. Cr27 was significantly higher in acidity than that of Ruiru 11 while the body of Ruiru 11 was significantly different from that of all the other genotypes except Cr8 and Cr22. In terms of balance, Ruiru 11 scored significantly lower than all the other genotypes. The overall of Cr23 and Cr 27 was significantly higher than that of Ruiru 11.

Table 15: Mean sensory variables of 5 advanced breeding lines and 2 commercial cultivars evaluated at Meru for three years

		Genotypes						
Genotypes	Year	Cr22	Cr23	Cr27	Cr30	Cr8	Ruiru 11	SL28NS
Fragrance	2009	7.38a	7.44a	7.46a	7.41a	7.35a	7.34a	7.45a
	2010	7.53ab	7.60a	7.62a	7.62a	7.51ab	7.40b	7.51ab
	2011	7.60a	7.67a	7.63a	7.59a	7.57a	7.61a	7.54a
Flavour	2009	7.40b	7.47ab	7.55a	7.50ab	7.40b	7.40b	7.55a
	2010	7.66ab	7.69ab	7.75a	7.72ab	7.64b	7.52c	7.63b
	2011	7.61a	7.80a	7.74a	7.62a	7.61a	7.64a	7.49a
Aftertaste	2009	7.34b	7.44ab	7.50a	7.45ab	7.34b	7.42ab	7.51a
	2010	7.66ab	7.63a	7.68a	7.68a	7.61a	7.50b	7.61a
	2011	7.61a	7.69a	7.67a	7.61a	7.54a	7.59a	7.51a
Acidity	2009	7.45c	7.57b	7.66ab	7.63ab	7.45c	7.54bc	7.71a
	2010	7.58bc	7.76ab	7.78a	7.75abc	7.73abc	7.64c	7.69abc
	2011	7.64a	7.79a	7.76a	7.66a	7.60a	7.67a	7.54a
Body	2009	7.47b	7.55b	7.58ab	7.53b	7.49b	7.49b	7.66a
	2010	7.58ab	7.67a	7.66a	7.65a	7.62ab	7.55b	7.64a
	2011	7.66a	7.66a	7.67a	7.67a	7.64a	7.61a	7.59a
Balance	2009	7.40c	7.51ab	7.58a	7.52ab	7.39c	7.46bc	7.58a
	2010	7.55a	7.61a	7.59a	7.61a	7.57a	7.45b	7.59a
	2011	7.67a	7.77a	7.70a	7.65a	7.61a	7.64a	7.55a
Overall	2009	7.43b	7.54ab	7.64a	7.58a	7.43b	7.45b	7.65a
	2010	7.61ab	7.71a	7.73a	7.69ab	7.65ab	7.55b	7.66ab
	2011	7.62a	7.76a	7.71a	7.65a	7.57a	7.62a	7.55a

Means along a row not sharing a letter are significantly different ($P<0.05$). Means separated by Student-Newman-Keuls (SNK5%) test., Key:SL28NS, Not Sprayed;

In 2009, no significant differences were observed on the sensory performance of Cr22, Cr23, Cr27 and Cr30 under the two spacing regimes. Ruiru 11 recorded significantly higher scores in all sensory variables under the spacing of 2.75m x 2.75m (space 2) in 2009 than under the spacing of 1.5m x2.0m (space 1) except in fragrance and aftertaste whereas in 2010 all the sensory scores were higher under space 2. Cr8 had significantly higher scores in all sensory attributes under space 2 in 2009 and higher aftertaste and acidity in 2010 under space 2. SL28NS recorded significantly higher scores in flavour and acidity in 2009 under space 2 while in 2010 no significant variations were observed under both spacings.

The mean sensory variables for each of the coffeee genotypes compared for three years is presented in Table 16. All the advanced breeders lines (Cr22, Cr23, Cr27, Cr30 and Cr8) scored significantly higher in all sensory atributes in 2011 than in 2009. Ruiru 11 had significantly higher scores in fragrance, flavour, aftertaste, acidity and overall in 2011 than in 2010. However, body and balance were significantly higher in 2009 than in 2011. SL28NS did not show any differences in the scores for the various attributes in the three years except in acidity whereby in 2009, it was significantly higher than in 2010 and 2011.

Table 16: Comparison of sensory performance of each genotype for specific sensory attributes for the three years

		Genotypes						
Genotypes	Year	Cr22	Cr23	Cr27	Cr30	Cr8	Ruiru11	SL28NS
Fragrance	2009	7.38b	7.44b	7.46b	7.41b	7.35b	7.34b	7.45a
	2010	7.53a	7.60a	7.62a	7.62a	7.51a	7.40b	7.51a
	2011	7.60a	7.67a	7.63a	7.59a	7.57a	7.61a	7.54a
Flavour	2009	7.40b	7.47c	7.55b	7.50b	7.40b	7.40b	7.55a
	2010	7.66a	7.69b	7.75a	7.72a	7.64a	7.52ab	7.63a
	2011	7.61a	7.80a	7.74a	7.62a	7.61a	7.64a	7.49a
Aftertaste	2009	7.34b	7.44b	7.50b	7.45b	7.34b	7.42b	7.51a
	2010	7.66a	7.63a	7.68a	7.68a	7.61a	7.50ab	7.61a
	2011	7.61a	7.69a	7.67a	7.61a	7.54a	7.59a	7.51a
Acidity	2009	7.45c	7.57b	7.66b	7.63b	7.45c	7.54b	7.71a
	2010	7.58b	7.76a	7.78a	7.75a	7.73a	7.64a	7.69a
	2011	7.64a	7.79a	7.76a	7.66ab	7.60b	7.67a	7.54b
Body	2009	7.47b	7.55b	7.58b	7.53b	7.49b	7.49b	7.66a
	2010	7.58ab	7.67a	7.66a	7.65a	7.62a	7.55ab	7.64a
	2011	7.66a	7.66a	7.67a	7.67a	7.64a	7.61a	7.59a
Balance	2009	7.40c	7.51c	7.58b	7.52ab	7.39b	7.46b	7.58a
	2010	7.55b	7.61b	7.59b	7.61a	7.57a	7.45b	7.59a
	2011	7.67a	7.77a	7.70a	7.65a	7.61a	7.64a	7.55a
Overall	2009	7.43b	7.54b	7.64a	7.58a	7.43b	7.45b	7.65a
	2010	7.61a	7.71a	7.73a	7.69a	7.65a	7.55ab	7.66a
	2011	7.62a	7.76a	7.71a	7.65a	7.57a	7.62a	7.55a

Means within a genotype for specific sensory attributes not sharing a letter among the three years are significantly different at P<0.05 (Means separated by Student-Newman-Keuls (SNK5%) test). Key:SL28NS, Not Sprayed;

Various terms were used to describe the coffees during the three years of analysis. Cr22 was described as harsh in 2009, floral, herbal, chocolate in 2010, fruity and grassy in 2011. Cr23 was fruity, lemon, citrus and syrupy in 2009 while in 2010 it was floral, winey, fruity citrus, lemon and syrupy and mild lemony, floral and fruity in 2011. The terms citrus, lemon, fruity, juicy were used to describe Cr27 in 2009 and fruity, floral, lemon, juicy, honey, tea rose and citric in 2010. In 2011 it was dscribed as floral, lemon, fruity and citrus. Cr30 was described as fruity,chocolate,caramel and herbal in 2009, floral, honey, caramelly, tea rose, citrus and lemon in 2010, fruity, grassy and lemon in 2011. Cr8 was harsh in 2008, fruity, juicy, floral and lemon in 2010 and harsh, bitter and grassy in 2011. Ruiru 11 was astringent and grassy in 2009, fruity, grape fruit, herbal, harsh and grassy in 2010 and citrus like, fruity, grassy, harsh and astringent in 2011. SL28NS was Juicy, lemon, winey, floral in 2009, floral, fruity, lemon, chocolate, caramel, tea rose, honey and syrupy in 2010, floral, fruity, sweet, harsh and grassy in 2011.

For the three years of evaluation, all the genotypes attained specialty grade by scoring a total score of more than 80 points Table 17. SL28NS was ranked the first in 2009 while Cr27 was ranked the first in 2010 and Cr23 in 2011. SL28NS showed a downward trend in its performance while Cr23, Cr27 and Cr30 were ranked higly in the three years of analysis.

Table 17: Average performance of each genotype for three years.

Rank	2009		Rank	2010		Rank	2011	
	Genotype	Total score		Genotype	Total score		Genotype	Total Score
1	SL28NS	83.77a	**1**	Cr27	83.81a	**1**	Cr23	84.16a
2	Cr23	83.32a	**2**	Cr30	83.71ab	**2**	Cr27	83.90a
3	Cr30	82.57b	**3**	Cr23	83.65ab	**3**	Cr30	83.45a
4	Cr27	82.41b	**4**	SL28NS	83.34ab	**4**	Cr22	83.43a
5	Ruiru 11	81.72c	**5**	Cr8	83.35ab	**5**	Ruiru 11	83.39a
6	Cr8	81.09cd	**6**	Cr22	83.15b	**6**	Cr8	83.14a
7	Cr22	80.93d	**7**	Ruiru 11	82.61c	**7**	SL28NS	82.76a

Total score means along a column not sharing a letter are significantly different ($P<0.05$). Student-Newman-Keuls (SNK5%) test Key: SL28NS, SL28 Not Sprayed SL28S, SL28 Sprayed

4.4.2.3 Diversity of coffee genotypes evaluated at Koru and Machakos by sensory characteristics

Analysis of variance of the sensory data of the genotypes evaluated at Koru and Machakos is presented in

Appendix 11. The genotypes were significantly different in all the sensory characteristics with the site and spacing having a significant contribution to the variations observed. The mean sensory characteristics of coffee genotypes evaluated at Koru CRF substation for two years are shown in (Table 18).

In 2010, Cr27 scored significantly higher in fragrance than SL28NS, SL28S and Ruiru 11 and had significantly better flavour and aftertaste than all the other genotypes. Similarly, acidity of Cr27 was significantly higher than that of Cr22, Cr30, Cr8, SL28NS, SL28S and Ruiru 11 while balance was significantly higher than in Cr22, Cr8, SL28NS, SL28S and Ruiru 11. During that year, Ruiru 11, Cr22 and SL28NS did not show any significant sensory fluctuations due to spacing at Koru. However, Cr23 and Cr8 had significantly higher ($P<0.05$) flavour, aftertaste and overal under space 2 than under space 1. Cr30 scored significantly higher in flavour, aftertaste, acidity and body under space 2 than under space 1. Cr27 had higher balance under space 2 than under space 1.

In 2011 all the genotypes did not show any significant differences in body and fragrance. Cr30 and SL28S showed significant differences in flavour from Ruiru 11 and Cr23. The aftertaste and balance of SL28S was significantly higher than that of Ruiru 11. The acidity of Cr23 and Ruiru 11 was significantly lower than in the rest

of the genotypes. The overall score of SL28S was significantly higher than that of Cr23 and Ruiru 11. In 2011, Cr8, Cr22, Cr27, Cr30, Ruiru 11 did not how any significant ($P<0.05$) differences in all the sensory variables under the two spacings. Nevertheless all sensory variables were significantly ($P<0.05$) higher under space 2 than under space 1 for Cr23 while for SL28NS, flavour, aftertaste, balance and overall were significantly higher under space 2.

The flavour of Cr22 was described as fruity, citrus, chocolate, course astringent and rich floral in 2010, while in 2011 as floral. Cr23 was described as fruity, lemon, grassy and juicy in 2010 and as nutty in 2011. The flavour of Cr27 was described as lemon winey, tea rose, floral, citrus, and caramel in 2010 while in 2011 it was fruity and caramel. Cr30 was fruity, juicy, tea rose, lemon and grassy in 2010 and fruity, honey and floral in 2011. Cr8, was herbal, nuts, caramel, grassy, course and astringent in 2010 while in 2011 it was described as harsh, spicy and fruity.

Table 18: Mean sensory characteristics of coffee genotypes evaluated at Koru.

Genotypes	Sensory variables													
	Fragrance		Flavour		Aftertaste		Acidity		Body		Balance		Overall	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Cr22	7.45ab	7.63a	7.60bc	7.62a	7.54ecd	7.62ab	7.62c	7.65a	7.55c	7.64a	7.53bcd	7.57ab	7.56cd	7.62a
Cr23	7.49ab	7.54a	7.63b	7.49bc	7.59bc	7.50ab	7.70ab	7.50b	7.62ab	7.62a	7.57ab	7.49b	7.65ab	7.49bc
Cr27	7.53a	7.64a	7.70a	7.61ab	7.66a	7.62ab	7.75a	7.60ab	7.63a	7.66a	7.58a	7.57ab	7.67a	7.64a
Cr30	7.50ab	7.61a	7.64b	7.64a	7.62ab	7.61ab	7.68b	7.67a	7.61ab	7.61a	7.55abc	7.60ab	7.63ab	7.65a
Cr8	7.45ab	7.61a	7.59bc	7.55ab	7.58bcd	7.62ab	7.68b	7.61ab	7.58bc	7.58a	7.53bcd	7.55ab	7.62bc	7.60ab
Ruiru11	7.40b	7.55a	7.52d	7.40c	7.53de	7.51b	7.61c	7.49b	7.55c	7.58a	7.48e	7.49b	7.55cd	7.45c
SL28NS	7.38b	7.60a	7.55cd	7.57ab	7.49e	7.55ab	7.59c	7.61ab	7.55c	7.59a	7.49cde	7.55ab	7.54cd	7.57abc
SL28S	7.39b	7.62a	7.53cd	7.62a	7.54cde	7.64a	7.61c	7.65a	7.59abc	7.68a	7.50de	7.61a	7.54cd	7.67a

Means along a column not sharing a letter are significantly different ($P<0.05$). Means separated by Student-Newman-Keuls (SNK5%) test

Key: SL28NS, SL28Not Sprayed; SL28S, SL28 Sprayed

The mean sensory characteristics of the coffee genotypes evaluated at Machakos for two consecutive years is shown in (Table 19). In 2010 the genotypes did not show significant variations in the sensory attributes except in fragrance, acidity and overall. SL28S and Cr30 scored significantly higher in fragrance than Ruiru 11. The acidity of 30 was significantly higher than that of Ruiru 11 while the overall score of Cr27, Cr30 and SL28S were significantly higher than that of Ruiru 11. In 2010 no significant differences were observed in the senory variabled due to spacing.

In 2011 all the sensory variables among the genotypes showed significant differences (at $P<0.05$) except body. Cr27 differed significantly from Cr22, Cr8, SL28S and SL28NS in fragrance and aftertaste. It also had significantly higher flavour and acidity than Cr22, Cr8, SL28S, SL28NS and Ruiru 11. Cr27 also showed significant differences in balance with Cr22, Cr8, SL28S, SL28NS, and Cr23. Ruiru 11 had significantly higher flavour, balance and overall under space 2 than under space 1.Cr8 showed significantly higher flavour, aftertaste and acidity under space 2. Except in body, Cr22 under space 2 had significantly higher scores in all other sensory attributes. Both SL28S and SL28NS had significantly higher scores in overall under space 2 than under space 1. Cr30 had significantly higher flavour under space 2 than under space 1.

The flavour of Cr22 was described as grassy, floral, fruity and winey in 2010 and in 2011 grassy and woody. Cr23 was describes as toasty, grassy, floral, fruity and citrus in 2010 and slightly floral, burnt and smoky in 2011. In 2010, Cr27 was described as citric, winey, fruity, floral and honey, and in 2011 fruity, juicy, floral,

citrus and complex. Cr30 was described as fruity, grassy, floral and lemon in 2010 and fruity, floral, grassy in 2011. Cr8 was described as citric, floral, and fruity and lemon in 2010 while in 2011, it was described as fruity, herbal, floral and citrus. Ruiru 11 was described as grassy, citric and fruity in 2010 and grassy, burnt, floral, and harsh in 2011. SL28NS was described as grassy and floral in 2010 and smoky, burnt, grassy and floral in 2011. SL28S was floral, citrus, lemon, fruity in 2010 and floral, slightly grassy and fruity in 2011.

Comparison of the performance of the advanced breeding lines together with the check cultivars Ruiru 11 and SL28, at Machakos and Koru for two years are shown in Table 20. In 2010, Cr22 scored significantly higher in all sensory variables at Machakos than at Koru while in 2011 balance was higher at Machakos than at Koru while all the other variables were not significantly different. Cr23 scored significantly higher in aftertaste, acidity and body at Machakos in than at Koru while in 2011 all the variables were significantly higher at Machakos than at Koru except in aftertaste. Fragrance, acidity and body of Cr27 were significantly higher at Machakos than at Koru in 2010 whereas in 2011 all the sensory variables were higher at Machakos than at Koru except overall. Cr30 showed higher scores in all sensory variables at Machakos than at Koru in 2010 while no significant variations were observed in 2011. In 2010, Cr8 scored significantly higher in all sensory variables at Machakos than at Koru except overall whereas in 2011 only the variable balance was significantly different. For Ruiru 11 only fragrance in 2010 and flavour in 2011 showed significant differences between the sites with both being significantly higher at Machakos than at Koru. SL28NS scored significantly higher

in fragrance, aftertaste, acidity and balance ant Machakos than at Koru in 2010 whereas no variations were observed in 2011. SL28S was significantly higher in all variables at Machakos in 2010 while in 2011 the body was significantly higher at Koru than at Machakos.

Table 19: Mean sensory characteristics of coffee genotypes evaluated at Machakos

Genotypes	Sensory variables													
	Fragrance		Flavour		Aftertaste		Acidity		Body		Balance		Overall	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Cr22	7.63ab	7.56b	7.63a	7.51b	7.66a	7.50b	7.72ab	7.53b	7.69a	7.58a	7.55a	7.57b	7.66ab	7.50c
Cr23	7.60ab	7.64ab	7.61a	7.63ab	7.65a	7.61ab	7.76ab	7.64ab	7.67a	7.63a	7.57a	7.58b	7.61ab	7.58abc
Cr27	7.62ab	7.71a	7.65a	7.70a	7.66a	7.74a	7.84a	7.74a	7.70a	7.63a	7.61a	7.70a	7.69a	7.65ab
Cr30	7.67a	7.61ab	7.69a	7.62ab	7.64a	7.63ab	7.81ab	7.64ab	7.67a	7.60a	7.58a	7.61ab	7.69a	7.67a
Cr8	7.61ab	7.57b	7.62a	7.63ab	7.69a	7.60ab	7.76ab	7.66ab	7.71a	7.63a	7.58a	7.63ab	7.60ab	7.64ab
Ruiru11	7.55b	7.61ab	7.56a	7.55b	7.57a	7.60ab	7.67b	7.52b	7.65a	7.58a	7.54a	7.53b	7.57b	7.50c
SL28NS	7.58ab	7.54b	7.60a	7.56b	7.62a	7.55b	7.70ab	7.54b	7.62a	7.63a	7.56a	7.54b	7.60ab	7.55abc
SL28S	7.65a	7.56b	7.65a	7.54b	7.67a	7.52b	7.80ab	7.57b	7.73a	7.59a	7.57a	7.54b	7.69a	7.55bc

Means along a column not sharing a letter are significantly different (P<0.05). Student-Newman-Keuls (SNK5%) test. Key: SL28NS, SL28Not Sprayed SL28S:SL28 Sprayed

Table 20: Comparison of sensory performance of advanced breeding lines together with check cultivars Ruiru 11 and SL28, at Machakos and Koru

Genotypes	Year	Sites	Sensory variables						
			Fragrance	Flavour	Aftertaste	Acidity	Body	Balance	Overall
Cr22	2010	Koru	7.38b	7.55b	7.51b	7.58b	7.52b	7.55a	7.51b
		Machakos	7.63a	7.63a	7.65a	7.72a	7.69a	7.50b	7.65a
	2011	Koru	7.61a	7.55a	7.62a	7.66a	7.58a	7.55b	7.60a
		Machakos	7.57a	7.63a	7.60a	7.61a	7.63a	7.63a	7.64a
Cr23	2010	Koru	7.38a	7.57a	7.56b	7.64b	7.57b	7.52a	7.61a
		Machakos	7.60a	7.61a	7.65a	7.76a	7.67a	7.57a	7.58a
	2011	Koru	7.54b	7.49b	7.50a	7.50b	7.62a	7.49b	7.49b
		Machakos	7.64a	7.63a	7.61a	7.64a	7.63a	7.58a	7.58a
Cr27	2010	Koru	7.46b	7.67a	7.64a	7.72b	7.61b	7.57a	7.63a
		Machakos	7.62a	7.65a	7.66a	7.84a	7.70a	7.61a	7.68a
	2011	Koru	7.64b	7.61b	7.62b	7.60b	7.63b	7.57b	7.65a
		Machakos	7.71a	7.70a	7.74a	7.74a	7.66a	7.70a	7.64a
Cr30	2010	Koru	7.40b	7.57b	7.57a	7.63b	7.58b	7.50b	7.57b
		Machakos	7.67a	7.68a	7.64a	7.81a	7.67a	7.58a	7.68a
	2011	Koru	7.61a	7.64a	7.61a	7.67a	7.61a	7.66a	7.65a
		Machakos	7.61a	7.62a	7.63a	7.64a	7.60a	7.61a	7.67a
Cr8	2010	Koru	7.39b	7.53b	7.55b	7.63b	7.54b	7.49b	7.57a
		Machakos	7.61a	7.63a	7.70a	7.77a	7.73b	7.60a	7.60a
	2011	Koru	7.55a	7.61a	7.62a	7.61a	7.58a	7.55b	7.60a
		Machakos	7.57a	7.63a	7.60a	7.66a	7.63a	7.63a	7.64a
Ruiru 11	2010	Koru	7.40b	7.52a	7.55a	7.58a	7.55a	7.52a	7.54a
		Machakos	7.55a	7.56a	7.56a	7.66a	7.56a	7.54a	7.57a
	2011	Koru	7.55a	7.40b	7.51a	7.49a	7.58a	7.49a	7.45a
		Machakos	7.61a	7.55a	7.60a	7.52a	7.58a	7.53a	7.50a
SL28NS	2010	Koru	7.38b	7.55a	7.49b	7.59b	7.55a	7.50b	7.55a
		Machakos	7.58a	7.60a	7.62a	7.70a	7.62a	7.56a	7.60a
	2011	Koru	7.60a	7.57a	7.55a	7.61a	7.60a	7.55a	7.57a
		Machakos	7.54a	7.56a	7.55a	7.54a	7.63a	7.54a	7.55a
SL28S	2010	Koru	7.39b	7.53b	7.54b	7.61b	7.59b	7.49b	7.54b
		Machakos	7.65a	7.65a	7.67a	7.80a	7.73a	7.57a	7.69a
	2011	Koru	7.62a	7.63a	7.64a	7.65a	7.68a	7.61a	7.67a
		Machakos	7.56a	7.54a	7.52a	7.57a	7.59b	7.54a	7.55a

Means across a genotype for a specific year not sharing a letter between the sites for a specific variable are significantly different (P<0.05). (Student-Newman-Keuls (SNK5%) test) .Key: SL28NS, SL28Not Sprayed; SL28S, SL28 Sprayed

All the genotypes evaluated had a total score of more than 80 points which means they all attained specialty grade. At Koru, Cr27 took the first position in 2010 while in 2011 SL28S was the highest. At Machakos SL28S took the first position in 2010 while Cr27 was the best in 2011. Cr30 was the most adapted by consistently recording high total scores at Koru and Machakos and maintaining position two for the two years at both sites. SL28 sprayed with fungicides recorded better quality than the unsprayed SL28 in absolute terms at both sites.

Table 21: Average performance of each genotype per location.

	Koru				Machakos			
	2010		2011		2010		2011	
Rank	Genotypes	Total score	Genotypes	Total score	Genotypes	Total score	Genotypes	Total score
1	Cr27	83.30a	SL28S	83.50a	SL28S	83.75a	Cr27	83.89a
2	Cr30	82.82b	Cr30	83.39a	Cr30	83.74a	Cr30	83.36b
3	Cr23	82.81b	Cr27	83.34a	Cr27	83.69a	Cr8	83.36b
4	Ruiru 11	82.70b	Cr22	83.35a	Cr8	83.64 a	Cr23	83.29b
5	Cr8	82.69b	Cr8	83.12a	Cr22	83.53a	Ruiru 11	82.911bc
6	SL28S	82.68b	SL28NS	83.04a	Cr23	83.46a	SL28S	82.88bc
7	SL 28NS	82.62b	Cr23	82.58b	SL28NS	83.27ab	SL28NS	82.86bc
8	Cr22	82.55b	Ruiru 11	82.46b	Ruiru 11	83.010b	Cr22	82.71c

Means along a column for the total scores not sharing a letter are significantly different ($P<0.05$). Student-Newman-Keuls (SNK5%) test. **Key**: SL28NS, SL28 Not Sprayed SL28S, SL28 Sprayed

4.4 Discussion

Tatu- Ruiru area receives a bimodal type of rainfall with the first rainy season falling between March and July and second season falling between September and December. Under this kind of rainfall pattern there are two distinct coffee seasons in a year (April to July early crop and October to December, main crop or late crop). Some of the genotypes evaluated at Tatu, Ruiru were found to exhibit variations in the sensory variables in different seasons. The order of sensory performance could also swop. For instance the flavour of Cr30 at Tatu, Ruiru was significantly higher during the early crop than in the main crop in 2008 and vice versa in 2009. Ruiru 11 did not achieve specialty grade during the early crop for the two of analysis. Gichimu and Omondi (2010a) reported that Ruiru 11 by being a compact variety may have a relatively shallow the root system compared to the tall traditional varieties and therefore not suitable for marginal areas with inadequate rainfall. The problem of shallow rooting system have been solved by grafting Ruiru 11 scions on the root stocks of the traditional varieties. However, the Ruiru 11 planted at Tatu Ruiru was not grafted. During the two years of evaluation, Tatu area received annual total rainfall of 902mm and 766mm in 2008 and 2009 respectively. This shows that if the rainfall is less than 1000 mm it would be necessary to support the bearing trees by irrigation. SL28Sprayed evaluated at Tatu, Ruiru did not show any significant differences in the sensory variables due to seasons. This is an indication that it is also possible for the market to repeatedly receive coffee of consistent quality irrespective of the season.

In addition to the genetic background of a cultivar, there are many other factors influencing cup quality characteristics. The environment also has a strong influence on coffee quality. The genotypes were found to perform differently in different geographical location. However, the performance of a genotype in the different locations at times reversed in different years. For example in the year 2010, Cr22 performed better at Machakos than at Koru but in 2011 it was the opposite. Altitude, daily temperature fluctuations, amount and distribution of rainfall and the physical and chemical characteristics of the soil are very important factors (Decasy *et al*., 2003). The role of soil types has been well studied and it is generally agreed that the most acidic coffee quality is grown on rich volcanic soils (Harding *et al*., 1987). Soil nutrients may be inherited from the parent rock or inputs applied externally.

Some studies conducted on coffee trees density tended to come up with findings that higher yields could be obtained from closely planted trees as compared to widely planted trees (Njoroge and Mwakha, 1993; Chanika and Mokono, 2008 Gichimu and Omondi (2010a). Other findings on the same observed that average yields per plant decreased with the increasing plant densities and attributed this to a consequence of coffee plant competition for water, nutrients and mainly for the active photosynthesis radiation from the canopy apex to the base (Gathaara and Kiara 1984, Paulo and Furlani, 2010). Vaast *et al.* (2006) reported that fruit load had a significant effect on beverage quality, with a trend indicating higher preference with decreasing fruit load. May could partially explain the differences in quality observed between the coffees under the two spacing regimes. Agricultural practices such as limiting fruit load, lowering tree stress, better balancing leaf-to-fruit ratios

and favoring slow ripening of coffee berry pulp and adequate bean filling should help produce coffee of higher quality.

The major coffee diseases in Kenya are Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae*, Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix.* SL28 is sucseptible to both CBD and CLR. The CBD pathogen is able to infect flowers and green expanding berries. CLR fungus kills the section of the leaves on which it grows. If the infection is severe this causes premature leaf fall. The trees ability to produce carbohydrates is then reduced. Vegetative growth and berry growth and size are then significantly reduced. Gradual infection by the diseases may explain the reason why the quality of SL28 Not sprayed deteriorated with time at Meru as demonstrated by the total scores. The same was observed at Koru and Machakos whereby SL28 sprayed with fungicides recorded better quality than the unsprayed SL28.

Coffee quality evaluators often associate coffees with specific flavour descriptors. For instance, Ruiru 11 has been described as harsh and astringent just like SL28 has been described as fruity. The flavour descriptors such as sour, winey, sweet, mellow, salt, astringent, bitter and harsh are categorized under tastes. Loss of organic matter in the coffee results in flavours described as woody, aged and grassy among others. Enzymatic activities as the coffee beans develop result in the formation of aroma compounds such as flowery, floral, coffee blossom, tea rose, fruity, citrus, lemon, berry like, herby and leguminous (Lingle 2001). Astrigency is defined as complex sensation accompanied by shrinking drawing or pluckering of the skin or mucosal

surface in the mouth produced by substances such as kaki tannins or soloe tannins International Standard, ISO 5492 (2008). Flavour descriptors encountered in this study are given in Appendix 17. Njoroge *et al*. (1990) conducted a study whose results indicated that Ruiru 11 and SL28 had higher intensities of acidity, fruity and winer, citrus, flavours than Robusta. They also reported that astringency was about the same in Ruiru 11 and SL28 and had more or less equal intensities in most of attributes except winey, fruity, floral, malty, flavours which were very slightly lower in Ruiru 11. Occurrence of astrigency taste and grassy aroma has been associated to processing of immature coffee beans. It is crucial to note that the degree of ripeness of the cherry is judged by the eye and could differ from person to person. This judgment is subjective at time could lead to inclusion of some immature cherries hence the encounter of such flavour descriptors. The flavour descriptors were not localized any particular coffee genotype and fluctuated from time to time. For instant in 2008, Cr23 evaluated at Tatu-Ruiru was described as herbal, lemon and fruity, all characteristic emanating from enzymatic activities as the coffee beans developed. However in 2009, there were no flavour descriptors given for this genotype. Premature ripening of the berries as a result of excessively long dry seasons has been reported to produce immature beans with astringent notes (Van der Vossen, 2009). Variations in the sensory characteristics like were observed at Tatu-Ruiru were also observed across the sites where the genotypes were evaluated. Generally the new advanced breeders' lines were comparable to the commercial varieties used as check cultivars in terms of sensory characteristics and have got specialty potential.

CHAPTER FIVE

5.0 CHARACTERIZATION OF COFFEA ARABICA GENOTYPES GROWN IN KENYA BY DETERMINATION OF THEIR BIOCHEMICAL COMPONENTS.

5.1 Introduction

The green bean has only a faint odor that is not at all reminiscent of coffee aroma. The characteristic flavour of coffee results from a combination of chemical compounds produced by the reactions that occur during roasting of green coffee. However, it contains all of the necessary precursors to generate the coffee flavour. Some of the traits that can be quantified are sugar, caffeine, chlorogenic acids, oil, and trigonelline (Bertrand *et al.*, 2003). Sucrose and trigonelline give rise to appreciated flavour products, including furans, pyrazine, alkyl-pyridines and pyrroles (Ky *et al.*, 2001) and on the other hand, chlorogenic acids and caffeine contribute to bitterness. The levels and biochemical status of these precursors may vary in relation to factors such as species and variety of bean, geographic origin, soil conditions, storage of the beans, duration and temperature of the roasting procedure**.** genetic traits, environmental factors, maturation level, postharvest treatment, and storage (Clifford, 1985). Biochemical analysis of green coffee is preferred to analysis of roasted cofeee beans since compositional changes occur during roasting. However, analysis of the freshly brewed coffee volatiles that linger in the air and reach the human nose could be a direct way to understand the factors that attract people to the pleasant coffee aroma. Ojijo (1993) made a review of some common aroma notes in coffee and their chemical origins. Gas chromatograph mass spectroscopy (GC-MS)

is commonly employed for the analysis of volatile organic compounds in green, roasted beans and the final brewed coffee. The aroma of the brew is different from that of ground coffee although the change in the aroma profile is not caused by the formation of new odorants but by a shift in the concentrations (Grosch, 2001). However no report on the analysis of volatiles in Kenyan coffee varieties is reported. In this part of f the study caffeine, trigonelline, oil, sucrose and total chlorogenic acids were analysed in green coffee samples. In addition, analysis of volatile organic compounds of brewed coffee was explored.

5.2 Materials and methods

The coffee samples generated under subsections 4.2, 4.3 and 4.4 and analysed by sensory method were also subjected to analysis by green coffee biochemical components. Portions of the green coffee samples were placed in small plastic bottles and stored under -80°C. After 24 hours of freezing, the samples were ground in liquid nitrogen using an analytical mill model Q10.

5.2.1 Analysis of moisture content

About 5g of the green coffee powder were weighed in a tarred aluminium dish (about 7.5 cm in diameter and 2.5 cm deep). The samples were dried in an air oven at 103 °C for 16 hours, cooled and reweighed. The loss in weight was calculated as the moisture loss and converted to percent moisture content. The moisture content levels were used to obtain the dry matter of the green coffee samples.

5.2.2 Extraction and quantification of crude oil

Five (5) grams of the dried green coffee powder was weighed accurately in a thimble and dried for 2 h at 100 ºC ± 2 ºC. An empty round bottomed flask was weighed after being dried at 105 ºC for an hour and cooled in a dessicator and recorded. The thimble was placed in the soxhlet extraction apparatus and extraction with hexane done for 8 hours (AOAC, 1995). The extract was evaporated to near dryness using rotavapor and dried for one hour in an oven at 105± 2 ºC. cooled in a desiccator and weighed. Drying and weighing at 30 minutes intervals was continued until the loss in weight between two successive weighing was not more than one milligram. The increase in weight of the extraction flasks was calculated as the crude oil content hereafter refered to as oil.

5.2.3 Extraction of caffeine, trigonelline and total chlorogenic acids (CGA)

Determination of caffeine and trigonelline was done by following the protocols of CIRAD, (2003a) for caffeine and CIRAD (2003b) for trigonelline. For extraction of caffeine, 0.5g of green coffee powder was accurately weighed into a 250 ml flat bottomed flask with a ground neck. Magnesium oxide (Merck) 0.5g and 200ml of distilled water were added. Two pumice stones were put in each flask. Refluxing was done for 25 minutes and the contents left to cool. After cooling filtration was done under vacuum on celite and the filtrate recovered in a 250ml volumetric flask. The volume was topped up to the mark with distilled water. Twenty (20) millilitres of the filtrate was drawn and put into a 100 ml volumetric flask and the volume adjusted to the mark with the mobile phase (details given below). The eluate was filtered through a 0.45µm micro-filter (Chromafil) and analyzed by HPLC. For the

extraction of trigonelline, 0.6 g of the green coffee powder was accurately weighed and put into a 250 ml flat bottomed flask with a ground neck. Magnesium oxide (Merck) 0.2g and 40ml of distilled water were added. Two pumice stones were put in each flask. Refluxing was done for 10 minutes and the contents left to cool. Filtration was done under vacuum through celite and the filtrate recovered in a 50ml volumetric flask. Twenty millilitres (20ml) of the filtrate was drawn and put into a 100 ml volumetric flask and the volume adjusted to the mark with the mobile phase (details given below). The eluate was filtered through a 0.45µm micro-filter (Chromafil) and analyzed by HPLC.

For the extraction of CGA, 0.7g of green coffee powder was weighed into 250 ml conical flask and 40ml of distilled water (Tse, 2005). Refluxing was done for 15 minutes and the contents left to cool. Filtration was done under vacuum through celite and the filtrate recovered in a 50ml volumetric flask. Twenty millilitres (20ml) of the filtrate was drawn and put into a 100 ml volumetric flask and the volume adjusted to the mark with the mobile phase (same as for caffeine and trigonelline). The eluate was filtered through a 0.45µm micro-filter (Chromafil) and analyzed by HPLC.

5.2.4 Analysis of caffeine, trigonelline and total chlorogenic acids

Caffeine, trigonelline and CGA were analysed using HPLC system (KNEUR) equipped with a Supel Co Discovery C-18 column for caffeine and trigonelline and BDS HYPERSIL C-18 column for chlorogenic acids. The detector was Diode Array Detector at three wavelengths, 278nm for caffeine, 266nm for trigonelline and 324nm for CGA. The mobile phase was HPLC grade methanol (PANREAC) 35%,

distilled water 65%, acetic acid (PROLABO) 0.1%, at a flow rate of 1 ml/min under ambient temperature. Caffeine, trigonelline and CGA were identified by comparing the retention times of caffeine standard (99%) (Fischer Scientific), trigonelline standard (Sigma Aldrich) and CGA standard (Acros organics) and their concentrations- calculated from peak areas using calibration equations.

5.2.5 Extraction and analysis of sucrose

Sucrose was extracted from green coffee powder using the method of Osborne and Voogt (1978) with modifications. About 2.5g of the green coffee powder was weighed and put into a round bottomed flask. Extraction was done for one hour in 100mls of 96% ethanol (AR) under reflux. The extract was cooled and filtered through Whatman filter paper number 42 and evaporated to dryness. Sucrose was recovered with 10mls deionised water and 2mls of the extract mixed thoroughly with 2mls Diethyl ether (AR) left to settle and the top layer discarded. This was repeated three times. One milliliter of the clarified extract was mixed with 1ml of acetonitrile and filtered through a 0.45µm micro filter (Chromafil). Sucrose was analysed using a HPLC system (KNEUR) equipped with a Eurospher 100-5 NH_2 column and a refractive index detector. The mobile phase was acetonitrile HPLC grade (SCHARLAU) 75%, and distilled water 25% at a flow rate 1 ml/min under ambient temperature. Identification was done by comparing the retention times of sucrose standard (Fischer Scientific) and sample peak. The concentration was calculated using a calibration equation.

5.2.7 Data analysis

The statistical packages used to analyse the accruing data were Costat, R-statistics, SPSS and XL-STAT 2011. The data was subjected to analysis of variance and multivariate analysis. Principal component analysis, dendrograms and discriminant function analysis were done using XLSTAT 2011.

5.3 Results

5.3.1 Biochemical components of 40 *ex-situ* conserved coffee germplasm alongside two commercial varieties

Analysis of variance showed that the genotypes portrayed significant differences ($P<0.05$) in all the biochemical components sucrose (Appendix 12). The mean biochemical components in forty (40) coffee genotypes under *ex-situ* conservation together with two commercial varieties (K7 and SL34) are shown in Table 22. Hibrido de Timor (HDT) had significantly higher amount of caffeine than all the other genotypes except Angustifola. Dilla Alghe had the lowest amount of caffeine at 0.77% . The levels of trigonelline ranged from 0.50% in DRII to 1.10 % in Ennareta. HDT recorded the lowest level of oil at 10.80% while Gimma Galla Sidamo had the highest at 18.15%. Sucrose content was lowest in Wollamo at 5.10% while HDT had the highest amount at 8.12%. Mokka Cramers had the lowest amount of CGA (6.13%) while Angustifola had the highest (10.97%).

Table 22: Mean biochemical variables (%DWB) in 40 ex-situ conserved genotypes alongside 2 commercial cultivars

Genotypes	Caffeine	Trigonelline	Oil	Sucrose	CGA
Hibrido de Timor	1.68±0.12a	0.68±0.05e-h	10.80±0.28d	8.12±1.01	7.99±0.12d-i
Angustifola	1.59±0.01ab	0.81±0.01b-g	15.40±0.57 abc	5.44±0.66	10.97±0.27a
M63	1.56±0.02bc	0.71±0.01d-h	14.20±1.13bc	5.29±0.13	6.94±0.66ghi
Ennareta	1.56±0.01bc	1.10±0.01a	16.56±0.26abc	7.91±0.25	8.34±0.05c-i
SL4	1.51±0.01bcd	0.70±0.01d-h	15.80±0.28abc	5.97±1.73	8.33±0.37c-i
Plateau Bronze	1.46±0.01cde	0.66±0.04e-h	15.00±0.01bc	6.22±0.72	7.27±0.06e-i
Dilla	1.44±0.01def	0.75±0.09c-h	15.60±0.57abc	6.37±0.31	8.49±0.24d-i
Gimma Galla	1.42±0.05d-g	0.69±0.01d-h	15.85±0.91abc	5.73±1.44	6.37±0.31i
Barbuk Sudan	1.42±0.05d-g	0.95±0.06a-f	16.50±2.12 abc	5.11±0.98	7.54±0.31 d-i
Purpurascens	1.42±0.01d-g	0.75±0.08c-h	15.60±0.57abc	5.81±0.67	7.12±0.22f-i
Gimma Galla Sidamo	1.41±0.01d-g	0.88±0.01a-g	18.15±0.49a	5.82±0.05	10.21±0.21abc
Mokka Cramers	1.40±0.02d-h	0.78±0.04c-h	14.45±0.07bc	5.32±0.17	6.13±0.11i
ZeghieLtana	1.38±0.01e-i	1.00±0.04abc	13.45±1.48bc	7.26±1.53	10.54±0.40ab
Pretoria	1.38±0.01e-i	0.52±0.01h	14.45±0.07bc	6.70±1.06	6.65±0.06hi
G5B	1.35±0.04 e-j	0.74±0.03c-h	15.05±0.64bc	7.34±1.09	6.62±1.77hi
Polysperma	1.34±0.01e-k	0.95±0.12a-f	14.10±0.14bc	6.69±0.34	8.84±0.05b-h
Murta	1.34±0.01 e-k	0.98±0.10a-d	15.00±0.01bc	6.10±0.44	9.14±0.67a-g
Erecta	1.34±0.01e-k	0.90±0.04a-g	15.00±0.01bc	6.20±2.13	7.61±0.06d-i
Mocha	1.33±0.02 e-k	0.97±0.01a-f	15.60±0.57abc	5.87±0.18	9.28±0.16a-f
K7	1.32±0.01 f-k	0.76±0.01c-h	14.25±0.35bc	5.90±0.02	8.43±0.12 d-i
Series C	1.31±0.10 g-l	0.95±0.06a-f	15.45±1.48 abc	7.25±0.05	6.56±0.49i
Wollamo	1.29±0.03 h-l	0.68±0.01e-h	13.65±0.21bc	5.10±0.29	6.76±0.06hi
Series L	1.28±0.15i-m	0.74±0.03c-h	15.30±0.42bc	7.67±1.10	10.80±0.83ab
Blue Mountain	1.28±0.01 i-m	0.91±0.04a-g	16.65±1.20 abc	5.78±1.10	6.47±0.01i
Mocha (Series D)	1.27±0.03 i-n	0.75±0.02c-h	15.65±0.21 abc	6.72±1.69	7.81±0.07d-i
Geisha12	1.26±0.01 i-n	0.75±0.15c-h	13.10±0.14c	7.08±0.08	8.37±0.78 d-i
Geisha 11	1.26±0.01 i-n	0.72±0.01c-h	14.45±0.07bc	6.09±0.93	7.26±0.21e-i
Drought Resistant II	1.24±0.02j-o	0.50±0.01h	16.45±1.48abc	5.77±0.01	9.57±0.17a-d
Dalle	1.24±0.02j-o	0.69±0.01e-h	16.10±1.27abc	5.61±0.57	7.95±0.04 d-i
Drought Resistant 1	1.22±0.01k-o	0.94±0.30a-f	14.90±0.14bc	6.79±0.02	9.45±0.15a-e
G53	1.20±0.01 l-o	0.67±0.01e-h	15.40±0.85abc	5.29±0.10	7.24±0.36e-i
Padang	1.19±0.03 l-o	0.64±0.01h-g	14.95±0.78bc	6.10±1.04	6.89±1.09ghi
Gimma Mbuni	1.17±0.02m-p	0.76±0.06c-h	15.55±1.34abc	6.62±0.07	7.12±0.02f-i
1225VI	1.15±0.17 n-q	0.87±0.02a-g	16.10±0.14abc	6.96±1.34	6.57±0.81hi
F53	1.15±0.03n-q	0.75±0.10c-h	15.10±0.14bc	7.55±1.01	7.87±0.06d-i
Arousi	1.14±0.01 opq	0.88±0.01a-g	14.35±0.21bc	6.33±0.39	6.57±0.01hi
Eritrean Moca	1.13±0.05opq	0.93±0.04a-f	15.35±0.21 abc	7.29±0.25	6.84±0.14hi
H1	1.07±0.04pq	1.06±0.06ab	15.80±1.70 abc	7.89±0.08	8.28±0.37c-i
Tanganyika Drought Resistant	1.07±0.05pq	0.94±0.06a-f	15.65±0.21abc	5.78±1.43	7.16±2.20f-i
Yellow Amarello	1.06±0.05q	0.71±0.09c-h	15.70±0.42 abc	6.03±0.73	7.23±0.16e-i
SL34	0.89±0.05r	0.75±0.10c-h	15.90±0.14abc	6.02±0.42	7.55±0.46d-i
Dilla Alghe	0.77±0.01s	0.73±0.01c-h	15.70±0.42 abc	7.12±0.52	7.58±0.62d-i

Means within a column not sharing a letter are significantly different at P<0.05 Key: DWB-: Dry weight basis; CGA: Total chlorogenic acids. Means separated by Student-Newman-Keuls (SNK5%) test.

Cluster dendrogram constructed using biochemical data of the forty two (42) genotypes was used to estimate diversity amongst them. Results of the cluster analysis are illustrated in Figure 13. The genotypes first separated into two broad clusters which recorded a diversity of about 46%. The first cluster contained 28 genotypes while the second cluster had 14 genotypes as determined by the degree of diversity based on biochemical characteristics. In the second sub-cluster HDT was grouped on its own.

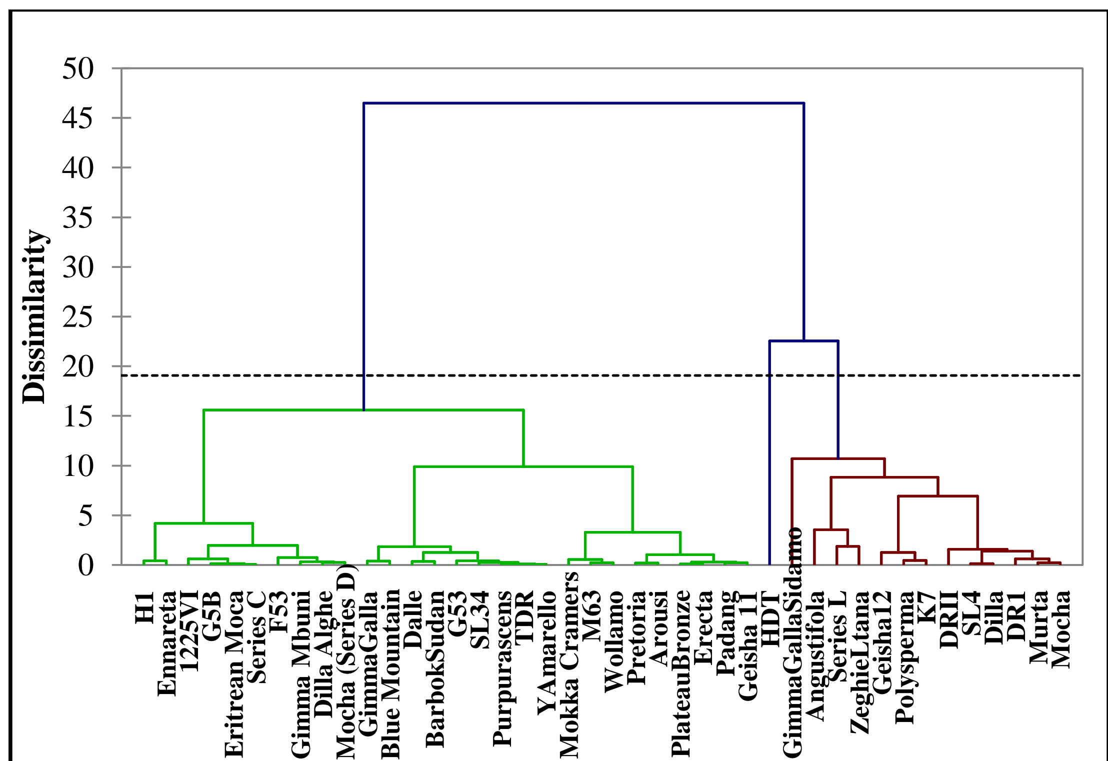


Figure 13:Dendrogram of the 42 coffee genotypes constructed by cluster analysis of of five biochemical variables

5.3.2 Characterization of five advanced breeding lines of Arabica coffee and two commercial coffee cultivars by biochemical components

5.3.2.1 Biochemical components of coffee genotypes evaluated at Tatu-Ruiru

Analysis of variance revealed that the genotypes varied in the biochemical components as show in Appendix 13. The mean levels of caffeine, trigonelline, oil, sucrose and CGA determined in the coffee genotypes evaluated at Tatu-Ruiru in 2008 are shown in Table 23. Significant differences ($P<0.05$) were observed in the levels of caffeine, oil, and sucrose among the genotypes evaluated. The level of caffeine was significantly ($P<0.05$) higher in Ruiru 11 (1.45%) than in SL28S (1.08%). Ruiru 11 had the lowest amount of oil yield (14.55%) which was significantly lower than in Cr23 which had highest amount of oil (16.87%). In terms of sucrose, Cr22 and Cr8 recorded significantly higher levels than all the other genotypes evaluated.

Principal component analysis of five biochemical components anaysed in the genotypes showed that the first two PCs explained 75.09% of the variation observed (Figure 14). Cr23, Cr27, Cr30 and SL28S were placed in the positive side of PC1 while SL28NS; Cr22, Cr8 and Ruiru 11 were placed in the negative side of PC1. Trigonelline, caffeine, oil and CGA contributed most to the variation observed in PC1 while sucrose contributed most to PC2 as shown in Table 24.

Table 23: Mean biochemical components (caffeine, trigonelline, oil, sucrose and CGA % DWB) of coffee genotypes evaluated at Tatu-Ruiru in 2008

	Biochemical components				
Genotypes	Caffeine	Trigonelline	Oil	Sucrose	CGA
Cr22	1.36±0.08ab	1.05±0.04a	16.43±0.25ab	11.04±0.26a	8.67±0.15a
Cr23	1.20±0.03ab	1.24±0.03a	16.87±0.23a	10.01±0.03b	7.59±0.06a
Cr27	1.31±0.01ab	1.30±0.01a	15.94±0.020ab	9.65±0.01bc	7.90±0.03a
Cr30	1.22±0.04ab	1.30±0.04a	16.69±0.30ab	9.16±0.18bc	8.29±0.03a
Cr8	1.31±0.01ab	0.93±a0.01a	15.30±0.64ab	11.50±0.38a	7.65±0.27a
Ruiru 11	1.45±0.02a	1.00±0.06a	14.55±0.33b	8.95±0.25c	8.86±0.18a
SL28NS	1.34±0.04ab	1.25±0.04a	16.41±0.51ab	10.060.45b	8.91±0.12a
SL28S	1.08±0.08b	1.07±0.01a	16.38±0.67ab	9.64±0.61bc	8.13±0.10a

Means within a column not sharing a letter are significantly different at P<0.05. Means separated by Student-Newman-Keuls (SNK5%) test.

Key: SL28S-SL28 Sprayed; SL28NS-SL28 Not Sprayed

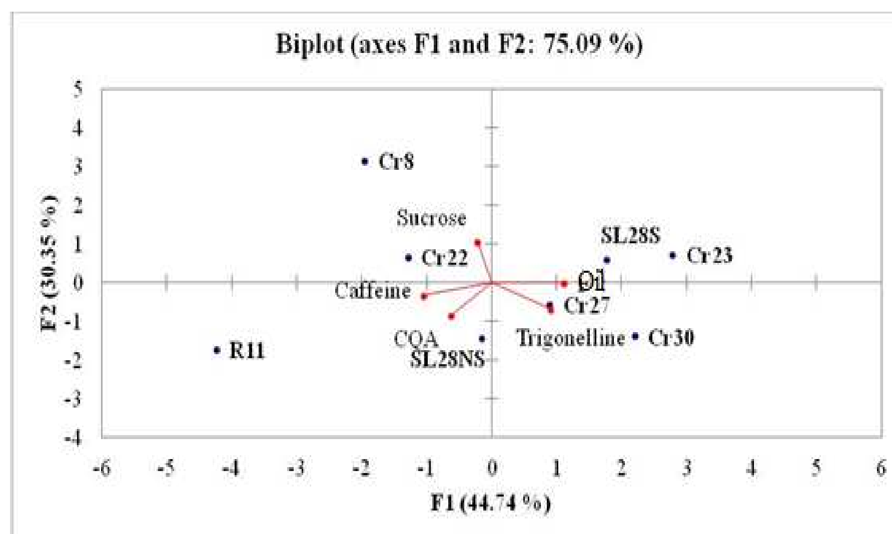


Figure 14: Principle component (PC) analysis plot of first two principle components, illustrating relationship among the eight coffee genotypes evaluated in Tatu- Ruiru in 2008

Key: R11- Ruiru 11, SL28S-SL28 Sprayed; SL28NS-SL28 Not Sprayed

Table 24: The first two principle components (PC) of the five biochemical variables

Variables	PC1	PC2
Caffeine	-0.55	-0.21
Sucrose	-0.12	0.67
CGA	-0.33	-0.55
Oil	0.59	-0.01
Trigonelline	0.48	-0.45
Eigen value	2.24	1.52
Variability (%)	44.74	30.35
Cumulative %	44.74	75.09

5.3.2.2 Biochemical components of coffee genotypes evaluated at Meru

Analysis of variance on the biochemical data of the coffee genotypes evaluated at Meru for three years showed significant variations among the genotypes in caffeine, trigonelline and oil contents (Appendix 14). Some of the the biochemical components fluctuated from year to year.

The mean biochemical components analysed in the genotypes at Meru for three years (2009 to 2011) are shown in Table 25. In 2009 caffeine levels in Cr8 were significantly ($P<0.05$) lower in all the other genotypes except in SL28NS. Cr22 had significantly lower levels (0.67%) of trigonelline than Cr27 (1.22%). Cr30 had significantly higher levels of oil (17.66%) than SL28NS (10.12%). In 2010, Cr23 had significantly higher caffeine level (1.23%) than Cr27 (0.94%). The genotypes Cr22, Cr23, Cr8 and SL28NS had significantly lower CGA than the other genotypes (levels ranged from 10.68% (Cr30) to 9.36% in (Cr23). No significant differences ($P >0.05$) were found in the levels of trigonelline, oil and sucrose in 2010. The results of mean biochemical components analysed in 2011 showed significant differences ($P<0.05$) in the levels of caffeine, trigonelline, oil, and CGA. SL28NS, Cr8 and Cr23 had caffeine and trigonelline levels that were significantly higher ($P<0.05$) than Cr22. Ruiru 11 had the lowest amount of oil (16.74%) while Cr30 had the highest amount (18.76%). Cr8 had the significantly higher amount of CGA (10.52%) than the other genotypes.

Table 25: Mean biochemical components of the genotypes evaluated at Meru for 3 years

Biochemical variables (%DWB)	Years	Genotypes Cr22	Cr23	Cr27	Cr30	Cr8	Ruiru 11	SL28NS
Caffeine	2009	1.10±0.11a	1.04±0.02a	1.06±0.01a	1.10±0.04a	0.84±0.03b	1.17±0.08a	0.95±0.02ab
	2010	1.01±0.16ab	1.23±0.20a	0.94±0.13b	1.07±0.15ab	1.06±0.06ab	1.14±0.13ab	1.09±0.07ab
	2011	1.10±0.15bc	1.22±0.22a	1.20±0.05ab	1.15±0.12abc	1.23±0.16a	1.07±0.05c	1.23±10a
Trigonelline	2009	0.67±0.10c	1.14±0.09ab	1.22±0.18a	0.82±0.07bc	1.03±0.01ab	0.92±0.02abc	0.97±0.01abc
	2010	1.03±0.13a	1.15±0.06a	0.99±0.11a	1.01±0.20a	1.04±0.14a	1.12±0.08a	1.08±0.19a
	2011	1.05±0.11b	1.23±0.19a	1.20±0.06a	1.12±0.10ab	1.15±0.14a	1.05±0.06b	1.19±0.08a
Oil	2009	13.66±0.77ab	16.74±1.01ab	16.82±1.38ab	17.66±2.55a	14.81±4.77ab	14.57±0.15ab	10.12±0.08b
	2010	13.55±1.76a	13.99±2.35a	14.42±1.87a	12.93±1.18a	14.10±2.26a	13.42±2.07a	13.43±2.32a
	2011	18.46±0.57a	18.54±0.32a	18.17±0.50a	18.76±0.58a	18.53±0.76a	16.74±0.46b	18.34±0.38a
Sucrose	2009	10.39±2.04a	10.18±0.21a	9.53±2.62a	8.78±1.14a	11.05±2.46a	10.18±1.07a	8.68±0.38a
	2010	8.49±0.31a	7.30±1.31a	9.00±1.38a	7.89±1.42a	8.22±1.16a	8.40±0.54a	9.07±0.34a
	2011	7.36±0.85a	7.28±0.23a	7.71±0.26a	7.85±0.42a	7.81±0.39a	7.94±0.33a	7.50±0.97a
CGA	2009	11.46±1.43a	11.03±1.57a	9.14±0.75a	10.81±0.37a	10.11±1.23a	10.40±0.84	10.63±0.94a
	2010	9.73±1.42a	9.36±0.16a	10.50±0.90a	10.68±0.71a	9.63±0.87a	10.41±0.71a	9.38±0.46a
	2011	8.99±0.94a	9.10±1.44a	8.88±0.50a	8.51±0.48a	10.52±0.47a	8.77±0.31a	8.57±0.24a

Means within across a row for a specific chemical variable not sharing a letter are significantly different at $P<0.05$**Key**: SL28NS-SL28 Not Sprayed CGA: Chlorogenic acids. Means separated by Student-Newman-Keuls (SNK5%) test.

The combined data of the five biochemical components anaysed in seven coffee genotypes evaluated at Meru for three years was subjected to principal component analysis. The first two PCs explained 66.86% (PC1 40.23% and PC2 26.63%) of the variation observed (). Cr30, Cr23, Cr27 and, SL28NS were placed in the positive side of PC1 while Ruiru 11, Cr8 and Cr22 were placed in the negative side of PC1. Trigoneline and caffeine, contributed most to the variation observed in PC1 while chlorogenic acid contributed most to variations observed in PC2 (Table 26) .

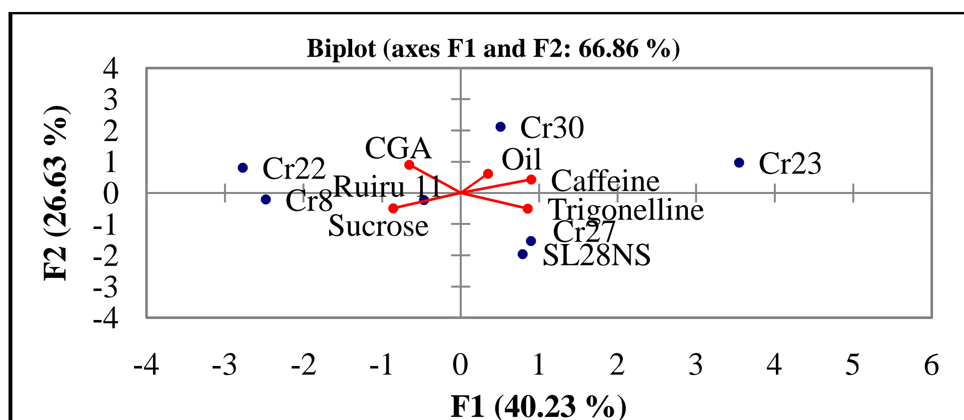


Figure 15: Principle component (PC) analysis plot of first two principle components, illustrating relationship among coffee genotypes evaluated at Meru for 3 years

Table 26: The first two principle components (PC) of the five biochemical variables

Variables	PC1	PC2
Caffeine	0.53	0.31
Trigonelline	0.50	-0.36
Oil	0.20	0.45
Sucrose	-0.51	-0.35
CGA	-0.39	0.66
Eigen value	2.01	1.33
Variability (%)	40.23	26.62
Cumulative %	40.23	66.85

5.3.2.3 Biochemical components of coffee genotypes evaluated at Koru and Machakos for two years

Analysis of variance showed significant differences biochemical components (Appendix 15) among the genotypes. Site was significant for the levels of trigonelline, caffeine and sucrose. Fluctuations in the level of chlorogenic acids, sucrose and oil were observed in the different years. The mean biochemical components determined in coffee genotypes evaluated at Koru and Machakos for two consecutive years are shown in Table 27 .

The genotypes did not show any significant differences in oil and total chlorogenic acids due to site. All the genotypes did not show any significant differences in the caffeine levels in the two years except Cr22 and Ruiru 11. In 2011, Cr22 and Ruiru 11 at Machakos had significantly more caffeine than at Koru. Cr22 at Koru had significantly higher amounts of trigonelline in 2010 than at Machakos while in 2011, Cr23 at Machakos had s significantly higher amounts of trigonelline than at Koru. Sucrose was found to be quite variable in among the genotypes in the two sites. Cr27 at Machakos significantly more sucrose than at Koru in 2010 and 2011. In 2010 Cr30 had significantly more sucrose at Machakos than at Koru. Cr8 had more sucrose at Machakos in 2010 than at Koru. Ruiru 11 had more sucrose in 2010 at Koru and significantly more at Machakos in 2011. SL28NS had significantly higher sucrose at Machakos than at Koru while SL 28S had significantly higher amount of sucrose at Machakos than at Koru.

Table 27: Mean biochemical components (caffeine, trigonelline, oil, sucrose and CGA % DWB) of coffee genotypes analysed in two regions

			Biochemical components				
Genotypes	Year	Sites	Caffeine	Trigonelline	Sucrose	CGA	Oil
Cr22	2010	Koru	1.11±0.06a	1.08±0.06a	9.88±1.94a	11.18±0.91	17.26±0.68
		Machakos	1.01±0.22a	0.97±0.07b	8.52±0.82a	11.13±0.24	18.27±0.01
	2011	Koru	0.98±0.21b	1.06±0.18a	7.29±1.54a	10.18±0.51	14.16±1.43
		Machakos	1.08±0.13a	1.04±0.13a	8.20±0.69a	10.08±1.24	13.80±1.48
Cr23	2010	Koru	1.12±0.22a	1.05±0.01b	10.10±0.48a	9.44±0.02	17.91±1.39
		Machakos	1.10±0.03a	1.27±0.12a	10.09±0.34a	11.15±1.37	18.43±0.77
	2011	Koru	1.03±0.17a	1.10±0.08a	7.25±1.43a	10.41±0.71	13.32±1.07
		Machakos	1.13±0.22a	1.08±0.12a	8.42±1.37a	10.08±0.70	13.67±1.77
Cr27	2010	Koru	0.96±0.15a	1.12±0.16a	9.35±2.04a	11.25±0.96	16.95±0.00
		Machakos	1.16±0.12a	1.00±0.19a	10.24±1.30a	10.91±0.52	16.41±0.15
	2011	Koru	1.08±0.24a	0.92±0.20a	7.77±1.22b	10.12±0.87	13.49±1.49
		Machakos	0.96±0.14a	1.18±0.24a	8.72±1.41a	10.28±0.70	14.38±1.67
Cr30	2010	Koru	1.00±0.14a	0.91±0.28a	7.58±1.12b	11.03±1.91	14.36±1.84
		Machakos	1.22±0.04a	1.13±0.06a	9.69±2.04a	10.86±0.96	14.39±3.52
	2011	Koru	0.90±0.07a	0.97±0.02a	8.99±0.09a	9.75±1.08	16.44±1.06
		Machakos	1.03±0.13a	0.99±0.17a	8.66±1.87a	10.90±0.79	13.24±1.23
Cr8	2010	Koru	0.94±0.29a	0.82±0.07a	7.72±0.83b	10.93±0.93	13.56±1.73
		Machakos	0.99±0.07a	1.32±0.10a	9.87±0.02a	10.17±0.82	13.73±0.63
	2011	Koru	0.80±0.12a	1.14±0.01a	9.63±0.18a	9.91±1.91	14.44±0.38
		Machakos	1.04±0.06a	1.01±0.13a	8.29±0.88a	9.26±0.86	13.73±2.33
Ruiru 11	2010	Koru	1.05±0.30a	0.94±0.20a	7.62±0.87b	10.74±0.18	13.32±1.34
		Machakos	1.08±0.17a	1.16±0.06a	10.48±0.70a	10.99±0.26	15.43±2.10
	2011	Koru	0.86±0.06b	1.00±0.27a	11.69±1.78a	10.12±2.11	18.58±0.70
		Machakos	1.18±0.12a	1.18±0.11a	8.62±0.74b	10.71±0.85	13.15±2.12
SL28NS	2010	Koru	0.97±0.14a	0.83±0.14a	6.77±0.42b	10.64±0.09	12.95±1.69
		Machakos	1.15±0.12a	1.04±0.05a	9.79±0.48a	10.17±0.80	14.85±1.46
	2011	Koru	0.93±0.10a	0.83±0.24a	10.00±0.04a	10.63±0.00	18.12±1.38
		Machakos	1.09±0.14a	1.22±0.19a	9.53±1.17a	9.49±0.42	14.64±2.43
SL28S	2010	Koru	0.93±0.10a	0.83±0.24a	10.00±0.04a	8.03±0.00	18.12±1.38
		Machakos	1.11±0.10a	1.00±0.30a	9.68±1.73a	10.03±1.77	18.04±1.66
	2011	Koru	1.07±0.09a	1.06±0.18a	6.94±0.58b	9.84±1.01	12.81±1.22
		Machakos	0.99±0.07a	1.08±0.05a	8.82±1.19a	10.17±1.01	14.42±2.35

Means across a genotype for a specific year not sharing a letter between the sites for a specific biochemical component are significantly different ($P<0.05$). (Student-Newman-Keuls (SNK5%) test). Key: SL28NS, SL28Not Sprayed; SL28S, SL28 Sprayed

5.3.2.4 Determination of coffee biochemical components by NIR.

5.3.2.4.1 Materials and methods

Thirty four (34) Kenyan Arabica samples were analysed for caffeine, trigonelline, oil, sucrose and CGA using the conventional methods described under sub-section 5.2.2 and 5.2.3. The same samples were also analysed by near infrared spectrophotometer and NIR spectra aquired. Analysis by NIR was done in order to confirm if it was possible to use a calibration equation to predict the level of biochemical component of Arabica coffee from Kenya. NIRS 6500 monochromator (Foss NlRS ystems, Silver Spring, MD) was used to scan reflectance from 400 to 2500 nm at 2 nm intervals, using ring cups (50 mm in diameter) with about 3 g of fine green coffee powder. Data were saved as the average of 32 scans and stored as log (1/R), where R was the reflectance at each wavelength and 1 the reflectance of a standard ceramic reference. Spectra were acquired randomly, each sample was measured twice, and the average spectrum was stored. Statistical analyses were performed using Win-ISI II software (Infrasoft International, Port Matilda, USA). Caffeine, trigonelline, oil and sucrose contents were determined using specific green Arabica coffee calibrations (Davrieux *et al.*, 2004).

5.3.2.4.2 Results

The NIR fingerprints of the 34 samples were projected together with the global NIR fingerprints of Arabica coffee maintained at CIRAD France into a principal component analysis matrix. A two dimension projection showed that Kenyan Arabica coffees fitted well (Figure 16) within the database. The distances of each new spectra when measured from the centre of the CIRAD database

[Mahalanobis(H) distance] showed that the Arabica samples from to the CIRAD Kenya could belong database (H< 3 with an average of 2.0427). Figure 17 shows a typical spectrum of green Arabica coffee drawn using the average spectra of the thirty four smples.

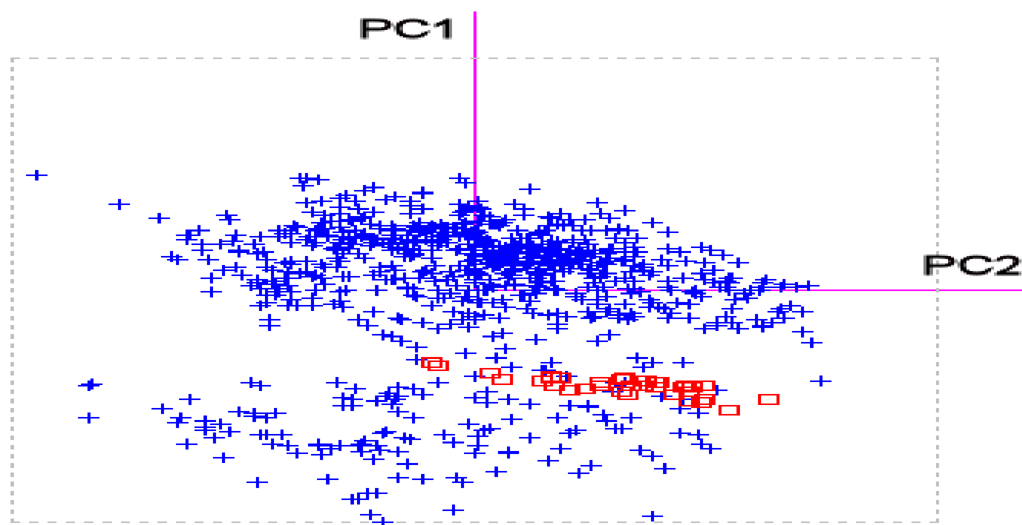


Figure 16 : PCA showing the global Arabica samples in the CIRAD, France database in blue and a projection the green Arabica coffee from Kenya in red

***NIR: Near infrared;** ****Conv.**: Conventional

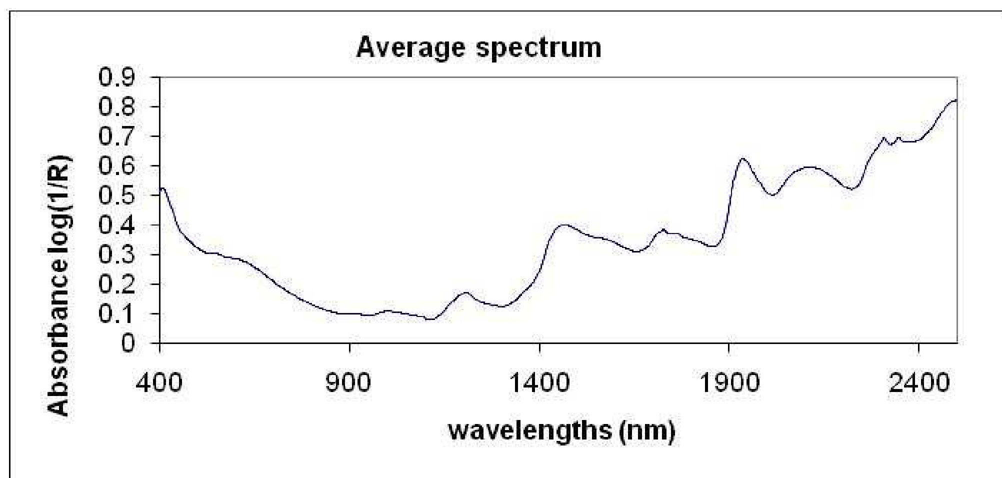


Figure 17: Average NIRS spectrum for 34 Arabica samples

A comparison of the levels of caffeine and oil analysed by conventional methods and NIR is shown in Table 28. A t- test failed to show a significant difference between the means of caffeine and oil levels anaysed by the wet method and that predicted using NIR calibration equation.

Table 28: Comparison of the levels caffeine and oil analysed by conventional method and NIR in eight coffee genotypes

Genotypes	Caffeine- NIR*	Caffeine-Conv.**	Oil-NIR	Oil-Conv.
Cr22	1.34±0.08	1.37±0.13	16.75±0.26	16.43±0.13
Cr23	1.21±0.03	1.13±0.16	16.24±0.24	16.87±0.26
Cr27	1.26±0.01	1.27±0.07	15.58±0.20	15.94±0.58
Cr30	1.23±0.04	1.23±0.03	16.38±0.30	16.69±0.25
Cr8	1.22±0.02	1.28±0.05	15.06±0.64	15.30±0.49
Ruiru 11	1.42±0.01	1.43±0.10	14.59±0.33	14.55±0.40
SL28NS	1.26±0.04	1.30±0.08	16.23±0.51	16.41±1.03
SL28S	1.22±0.09	1.11±0.04	16.62±0.82	16.44±1.00
Sig. (2-tailed)	0.96		0.07	
Correlation	0.78		0.90	

*NIR: Near infrared; **Conv.: **Conventional**

The fact that samples from Kenya could belong to the CIRAD, France database proved that the calibration equation at CIRAD, France could be utilized to predict the biochemical components in Kenyan Arabica samples. NIR absorbance spectra of green coffee powder of ninety (90) Kenyan Arabica coffee samples (Ruiru 11 siblings from Ruiru, Koru and Kitale as listed in Table 9 and processed as explained in subsection 4.2.1.2.) was acquired. The NIR fingerprints were projected into a Principal Component analysis matrix together with global Arabica coffee spectra

maintained by CIRAD, France in their database. A two dimension projection of the NIR fingerprints is shown in Figure 18.

The global fingerprints from CIRAD Arabica coffee database are shown in blue while the fingerprints from Ruiru 11 progenies are shown in red. One Robusta sample projected into the same database is marked in pink. Discriminant function analysis performed on the near infrared spectra revealed that the NIR fingerprints could be used to discriminate different siblings into three groups according to the region where they were grown (Figure 19) which further expounded the significant of site effect in biochemical components of the genotypes. The genotypes from Kitale and Ruiru were placed in the poitive side of PC1 while Koru was placed on its own in the negative side of PC1.

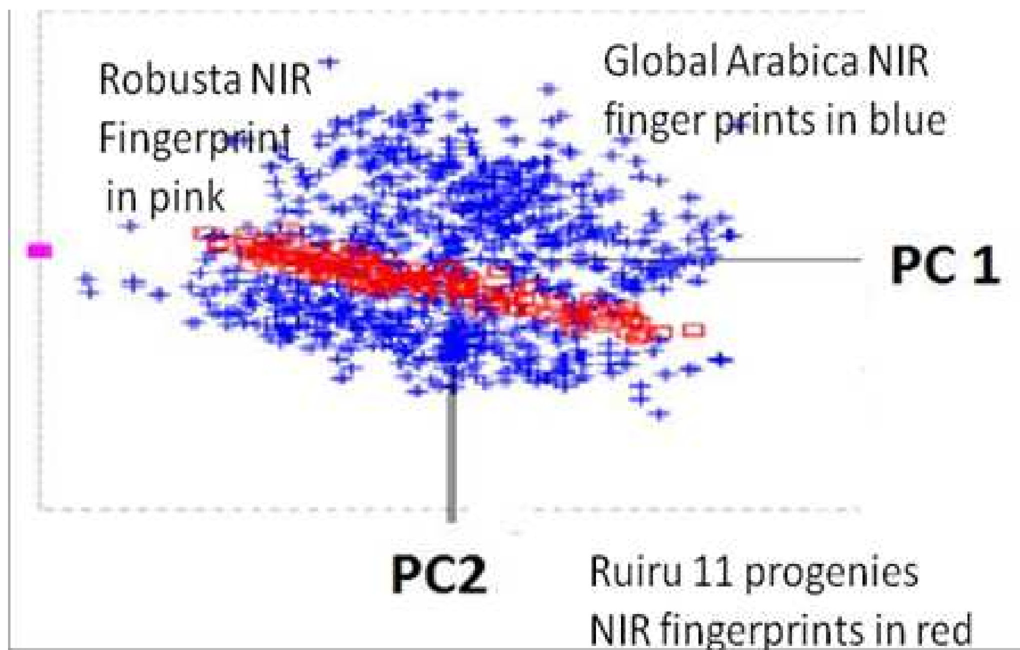


Figure 18: Principal component analysis projection showing the global Arabica NIR finger prints maintained at the CIRAD database, Ruiru 11 progenies and one Robusta sample

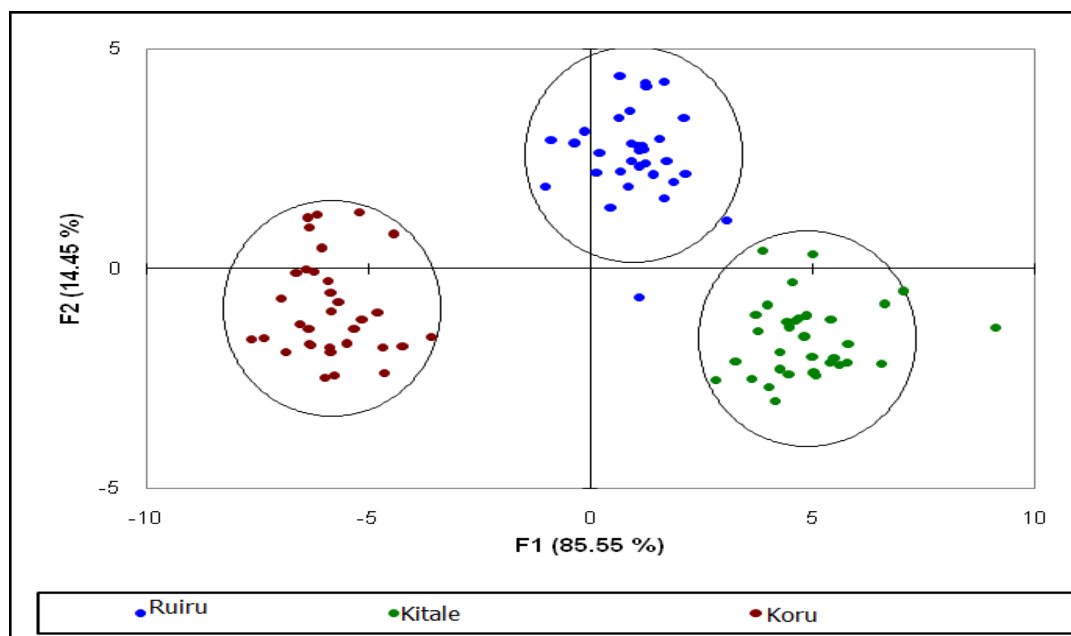


Figure 19: Discriminant factor analysis projection of NIR spectra of the Ruiru 11 progenies showing the coffee grouped according to site.

Analysis of variance on the biochemical data showed the Ruiru 11 progenies evaluated at Kitale, Koru and Ruiru varied significantly in the levels of in the caffeine, trigonelline, oil, CGA and sucrose with site having a significant contribution (Appendix 16). The mean biochemical components are shown in Table 29. The results revealed that the progenies at Kitale had caffeine levels ranging between 1.51-1.61%, those from Ruiru ranged from 1.34-1.59% and in Koru they ranged from 1.22-1.36%. Caffeine levels obtained in the progenies at Koru were significantly lower than in the progenies at Kitale and Ruiru except for CRF-50 and CRF-91. The progenies CRF-03 and CRF-1I evaluated at CRS-Ruiru had significantly (P<0.05) higher amount of trigonelline than at Koru and Kitale.

The levels of sucrose in the progenies at Koru ranged between 8.99-10.40%, while at Kitale the levels ranged from 10.12-11.15% and at CRS, Ruiru the levels ranged from 9.91-10.91%. The siblings CRF-03, CRF-05, CRF-23, CRF-41, CRF-50, CRF-123 and CRF-131 had significantly higher (P<0.05) levels of sucrose at Kitale than at Koru. CRF-03 had the highest amount of sucrose 10.40% while CRF-50 had the lowest amount 9.07%. At Koru oil levels in the progenies ranged from 13.38% to 15.54%, at CRS, Ruiru they ranged from 12.25%-14.21% and at Kitale from 11.88% to 12.97%. The amount of oil in the siblings grown at Koru were significantly higher than at CRS-Ruiru ecxept for CRF-11, CRF-41 and CRF-91. At Kitale CRF-03, CRF-50 and, CRF-111, had significantly lower total chlorogenic acids than at Koru and CRS, Ruiru

Table 29: Mean biochemical components (caffeine, trigonelline, oil, sucrose and CGA % DWB) of the Ruiru 11 progenies in three regions

Genotype	Site	Biochemical components				
		Caffeine	Trigonelline	Oil	Sucrose	CGA
CRF-03	Koru	1.35±0.03b	1.22±0.03b	15.08±0.65a	9.14±0.15b	8.99±0.16a
	Kitale	1.53±0.03a	1.18±0.02b	11.88±0.42b	11.15±0.11a	8.26±0.13b
	Ruiru	1.51±0.05a	1.27±0.04a	13.17±0.93b	9.91±0.93b	8.90±0.26a
CRF-05	Koru	1.26±0.05b	1.23±0.05a	15.34±0.74a	9.21±0.38c	8.41±0.06a
	Kitale	1.55±0.04a	1.18±0.03a	12.70±0.59c	10.85±0.26a	8.41±0.27a
	Ruiru	1.45±0.09a	1.25±0.03a	13.46±0.26b	10.07±0.22b	8.52±0.20a
CRF-11	Koru	1.22±0.02c	1.22±0.02b	13.38±0.39a	10.40±0.22a	8.48±0.22a
	Kitale	1.58±0.03a	1.19±0.03b	12.17±0.67a	10.93±0.48a	8.35±0.13a
	Ruiru	1.39±0.06b	1.30±0.03a	12.94±1.11a	10.87±1.40a	8.71±0.12a
CRF-23	Koru	1.36±0.05b	1.13±0.05a	14.47±0.28a	9.27±0.28b	8.61±0.09a
	Kitale	1.52±0.03a	1.16±0.01a	12.33±0.81b	10.44±0.48a	7.97±0.38a
	Ruiru	1.59±0.06a	1.22±0.03a	13.19±0.67ab	9.96±0.49ab	8.49±0.25a
CRF-41	Koru	1.27±0.05c	1.18±0.05a	13.51±0.38a	9.88±0.15b	8.48±0.25a
	Kitale	1.61±0.03a	1.17±0.03a	12.01±1.30a	10.61±0.16a	8.07±0.07a
	Ruiru	1.37±0.03b	1.26±0.06a	12.70±0.92a	10.83±0.22a	8.54±0.23a
CRF-50	Koru	1.27±0.10b	1.13±0.06a	14.53±0.16a	9.07±0.01b	8.93±0.01a
	Kitale	1.59±0.05a	1.17±0.04a	12.97±0.81b	10.41±0.49a	8.35±0.09b
	Ruiru	1.44±0.02ab	1.26±0.00a	13.31±0.32b	10.28±0.23a	8.86±0.18a
CRF-91	Koru	1.25±0.05b	1.22±0.09a	14.22±0.86a	9.71±0.83a	8.60±0.23a
	Kitale	1.59±0.05a	1.15±0.04a	12.94± 0.71a	10.12±0.15a	8.29±0.04a
	Ruiru	1.34±0.05b	1.19±0.15a	12.25±0.58a	10.91±0.75a	8.40±0.13a
CRF-111	Koru	1.32±0.05b	1.19±0.03a	14.25±0.22a	9.40±0.20a	8.91±0.23a
	Kitale	1.51±0.07a	1.20±0.02a	11.91±0.68b	10.81±0.09a	8.03±0.27b
	Ruiru	1.56±0.16a	1.25±0.04a	13.09±1.07ab	9.84±1.04a	8.84±0.24a
CRF-123	Koru	1.28±0.01b	1.23±0.03a	14.50±0.35a	9.50±0.10b	8.87±0.12a
	Kitale	1.59±0.04a	1.18±0.02a	12.70±0.72c	10.28±0.17a	8.28±0.02a
	Ruiru	1.44±0.07a	1.29±0.01a	14.21±1.14b	9.96±0.98ab	8.55±0.16a
CRF-131	Koru	1.26±0.06b	1.21±0.03a	14.73±0.69a	9.25±0.02c	8.52±0.16a
	Kitale	1.51±0.01a	1.19±0.03a	12.55±0.53b	10.36±0.56b	8.16±0.09a
	Ruiru	1.31±0.03a	1.28±0.04a	12.72±0.11b	10.84±0.04a	8.57±0.09a

Means of a genotype in the three sites for a particular biochemical component not sharing a letter are significantly different at $P<0.05$ Student-Newman-Keuls (SNK5%) test

Key: CGA-Total chlorogenic acids

5.3.3 Discussion

Caffeine is probably the most frequently ingested pharmacologically active substance in the world. The stimulating effect of coffee has been accredited to the caffeine content hence making it an important constituent of coffee. It has also been implicated in the defense mechanism of the coffee plant against pathogens (Franca *et al.,* 2005). The positive information about coffee and health does not gain wide publicity and does not yet appear to counteract the effects of the adverse publicity which has seen the demand for decaffeinated coffee increase. In the EU countries decaffeinated coffee' means a maximum caffeine concentration of 0.1% related to the dry mass while in the US, it means less than 3% of the amount initially present in the beans (Heilmann, 2001). Decaffeinated coffee and constitutes about 10% of the world coffee consumption (Silvarolla *et al.,* 2004). However, there is no data to support any relationship between caffeine and coffee quality but coffee hybrids with low caffeine content (0.2%) have had little impact on the commercial markets due to poor quality (Clifford, 1985). Assessment of caffeine content variability is vital to identify genotypes with low or high caffeine content as may be demanded by different market niches. The caffeine content of coffee beans is genotypically defined in a quantitative, polygenic manner, and is also influenced by exogenous factors (Pearl *et al.,* 2004).

Genotypes in this study were diverse for green bean caffeine. Among the *ex-situ* conserved genotypes, Dilla Alghe had the lowest content of caffeine (0.77%) while the highest value was observed in HDT (1.68%). Several studies have reported coffees with low caffeine content Carvalho *et al*., 1965; Mazzafera and Calvalho,

1992; Anthony *et al*., 1993; Campa *et al*., 2005; Nagai *et al.,* 2008). Most of the materials evaluated were not suitable for commercial exploitation because of the poor quality and bitter taste of the resulting beverage and the low productivity of the trees. In this astudy, Dilla Alghe was among the genotypes which attained the specialty score by getting 80.71 points. Though this amount is higher and does not meet the decaffeinated coffee definition (Heilmann, 2001) coffee cultivars combining high cup quality with low caffeine content may provide a better and presumably also a less expensive alternative to meet the demand for coffees with low caffeine levels.

Caffeine content variability was also observed among the advanced breeding lines and the check cultivars SL28 and Ruiru 11. Apart from the effect of genotype, levels were found to fluctuate in the years of evaluation. Cr30 had the lowest amount of caffeine (0.84%) among the genotypes evaluated at Meru in 2009 while Ruiru 11 had the highest amount (1.17%). In 2010, Cr27 had the lowest amount of caffeine (0.94%) the while Cr23 had the highest amount (1.23%). Ruiru 11 had the lowest amount of caffeine in 2011 (1.07%) while SL28NS had the highest amount (1.23%). Similar fluctuations were observed in the advanced breeding lines and the check cultivars SL28 and Ruiru 11 at Koru and Machakos where they were evaluated for two years (2010 and 2011). Commercially cultivated coffee plants have been reported to contain substantial quantities of caffeine. Even with the fluctuations observed among the advanced breeding lines and the check cultivars, the levels of caffeine agreed with most of the documented values in Arabica coffee : Clifford (1985) 0.80–1.70% dwb;Wintgens, (2004) 0.70-2.20 % dwb; Bertrand (2003) 1.26

to 1.37% dwb. However, the caffeine levels reported by Silvarolla *et al*. (2000) were lower than the lowest obtained in this study (0.42%) and higher than was obtained in any genotype in this study (2.90%)

Trigonelline which is a pyridine alkaloid is an important component of the coffee bean which acts as a reservoir of nicotinic acid in plants. In *C. arabica*, the levels of trigonelline have been reported by several authors. These include : 1.52% to 2.9% Mazzafera, (1991), 1% - 1.94% Martin *et al.* (1998) and 0.88% - 1.77% by Ky *et al*. (2001) all in DWB. Green bean trigonelline content showed variations among the coffee genotypes evaluated. Among the *ex-situ* conserved genotypes, trigonelline levels ranged from 0.50% in DRII to 1.10% in Ennareta. Trigonelline content variability was also observed among the advanced breeding lines and the check cultivars SL28 and Ruiru 11. The levels varied among the genotypes evaluated and year of evaluation. At Mariene- Meru in 2009, Cr22 had the lowest trigonelline content 0.67% while Cr27. Cr27 had the lowest trigonelline amount (0.99%) while Cr23 had the highest amount. In 2011, Ruiru 11 and Cr22 had the lowest amount of trigonelline (1.05%) while Cr23 had the highest amount (1.23%). The genotypes evaluated at Machakos (in 2010 and 2011) portrayed variations in the trigonelline levels. In 2010, Cr8 had 1.32% which was the highest while Cr22 had the lowest amount at 0.97%. Cr30 had the lowest amount of trigonelline (0.99%) in 2011 while SL28NS had the highest amount (1.22%). Apart from having the lowest content of trigonelline, DRII also had the lowest score in flavour (6.86) while Ennareta scored 7.79 points. Similarly, Cr22 evaluated at Meru in 2009 had the lowest score in flavour 7.40 while Cr27 had the highest 7.55. Trigonelline is known to contribute to

the formation of the appreciated coffee flavour and the higher trigonelline contents could partially explain the better flavour observed.

Chlorogenic acids (CGA) are phenolic compounds commonly found in green coffee beans. CGA are of great interest because of their possible positive impact on human health (Willcox *et al*., 2004; Le Corre *et al.*, 2005). They also play important role in plants defence system (Kawano *et al*., 2004) especially when bacterial and fungal pathogens invasions (Waldron *et al*., 1996) were confirmed. Genotypes in this study were significantly different in green bean chlorogenic acids contents study, the lowest level of CGA in the genotypes under *ex-situ* conservation was in Mokka Cramers at 6.13% while Angustifola had the highest at 10.97%. CGA content variability was also observed among the advanced breeding lines and the check cultivars SL28 and Ruiru 11. In 2009, the genotypes evaluated at Mariene-Meru showed Cr27 having the lowest amount of CGA (9.14%) while Cr22 had the highest amount (11.46%). In 2010 the levels ranged from 9.30% (Cr23) to 10.81% (Cr30) while in 2011, Cr8 had lowest amount of CGA (8.51%) while Cr30 had the highest amount (10.53%). Similar behaviour was observed in the genotypes at Machakos and Koru where evaluations were done for two years (2010 and 2011). The values reported in this study agree with those reported by other researchers on Arabica coffee except those reported by Dessalegn (2005) among forty two (42) Ethiopian accessions (2.34 to 4.67 %(DWB), which were on the lower side.

Mokka Cramers which had the lowest amount of CGA was among the best genotypes in the sensory characteristics while Angustifola which had the highest

amount was among the genotypes that were rated lower in sensory characteristics. Cr22 which had the highest CGA content in 2009 (evaluated at Mariene-Meru) had its flavour described as harsh while Cr27 which had the lowest amount was described as floral, lemon, fruity and citrus. This could partially be explained by the fact that CGA, contributes to coffee drink bitterness. However in some instances high CGA levels did not translate to any negative impact. For example though Cr30 evaluated at Mariene-Meru had the highest amount of CGA the flavour was described floral, honey, caramelly, tea rose, citrus lemon.

Coffee oil a component of the coffee lipids is an important component of coffee although most of the oil is lost with the grounds during the preparation of the coffee brew (Folstar, 1985). Some components of the coffee lipids have been implicated in raising the serum total cholesterol (Petracco, 2001). However; consumption of moderate quantities of either espresso or filtered brews has no effect on total cholesterol. The genotypes conserved *ex-situ* were found to be diverse in the levels of oil. HDT had lowest amount of oil at 10.12% while Gimma Galla Sidamo had the highest 18.15%.amount of oil. The oil content variability was also observed among the advanced breeding lines and the check cultivars SL28 and Ruiru 11. At Meru, oil yield was lowest in SL28NS (10.12%) in 2009; while the highest levels were observed in Cr30 (17.66%). In 2010, oil levels ranged from 14.42% (Cr27) to 12.93% (Cr30) and in 2011, Ruiru 11 had the lowest amount oil (16.74%) while Cr30 had the highest amount (18.76%). At Koru, SL28NS had the highest amount of oil in 2010 18.12% while Ruiru 11 had the lowest amount in that year 14.44%. In 2011, SL28S had the lowest amount of oil (12.81%) while Cr30 had the highest

amount (14.36%). At Machakos, Cr30 had the lowest oil content in 2010, at 14.39%while Cr8 had 19.91% oil content. Ruiru 11 had the lowest amount of oil in 2011 (13.15%) and Cr27 had the highest amount (14.38%). Several authors have reported oil levels in green coffee. Speer and Kolling-Speer (2001) reported average of green Arabica at 15% whilst in Robusta about 10% dwb. Bertrand *et al. (*2006), found levels ranging from 14.07%-15.47% in a traditional cultivar 'Caturra grown under different elevations in Central America. Oil in coffee can be extracted using various solvents such as diethyl ether, petroleum ether, n-hexane and a mixture of diethyl ether and n-hexane. Due to this the results may not be comparable because variable amounts of other more polar and non-lipid substances, such as caffeine, may be extracted, according to the solvent used. However in this study, n-hexane was used throughout the analysis.

Roasting is an essential step in the formation of various types of 'flavour' compounds. The content and nature of sugars in the green coffee beans is important in the development of flavour and pigmentation during roasting. Sucrose the main contributor of reducing sugars which are implicated in Maillard reactions occurring during the roasting process (Grosch, 2001). The acidity of coffee brews has always been recognized as an important attribute of their sensory quality. Kenyan coffee beans are well known for their well developed acidic character which is often described as `fine acidity. Some acids are found in the green bean such phosphoric acid, citric acid, and cholrogenic acid. Acetic acid is formed as a result due to the breakdown of carbohydrates either during the fermentation process in wet-processed coffee or during coffee roasting (Balzer, 2001). Sucrose is the major free sugar whose

quantity varies according to cultivar, state of maturity, processing and storage conditions (Clifford, 1985). The genotypes evaluated for green bean sucrose content showed variability. Among the *ex-situ* conserved coffee genotypes the value of sucrose was lowest in the genotype Wollamo (5.10%) and highest HDT (8.12%). Sucrose content variability was also observed among the advanced breeding lines and the check cultivars SL28 and Ruiru 11. The variations were observed to vary with site and year of evaluation. The same genotype could show different levels of sucrose in different years when evaluated in a single site. For example at Mariene-Meru in 2009, Cr8 had 11.05% sucrose. In 2010, the same genotype had 8.22% and 7.81% sucrose in 2011. A particular genotype could vary in sucrose levels when evaluated at different sites. Ruiru 11 evaluated at Koru-Kericho, Machakos and Mariene- Meru in 2010, had sucrose levels at 9.63%, 10.48% and 8.40% in the three sites respectively. In literature values for sucrose are reported in the range of 2% to 5% for Robusta beans and 5% to 8.5% for Arabicas (Clifford, 1985). Varnam and Sutherland (1994) reported sucrose ranges of 6 - 8.3 % (DMB) for Arabica and 3.3 - 4.1% (DMB) Robusta coffee. In other studies, Camp *et al*. (2001) showed sucrose contents varying between species from 3.8% to 10.7% dmb. Green bean sucrose reported by Ky *et al*. (2001) varied from 7.4-11.1%. Levels of green bean sucrose in Ethiopian coffee accessions were reported by Dessalegn (2005) as ranging from 5.30% to 8.98% DMB. Bertrand *et al*. (2006) analysed 'Caturra grown under different elevations in Central America and reported sucrose levels ranging from 7.03% to 8.13%. The effects of shade on sucrose and reducing sugar (glucose and fructose) contents studied in fresh and dry coffee beans showed a significant reduction in sucrose content and to an increase in reducing sugars (Geromel *et al.,*

2008). However the genotypes in this study were all under full sun. Sucrose content may increase with degree of ripening. Mazzafera, (1999) reported immature black and immature-green Brazilian beans, had sucrose levels lower than normal beans. Ky *et al*. (2001) reported that the higher sucrose contents in Arabica green bean could partially explain its better cup quality. The values obtained in this study compares with those reported by other researchers although no direct relationship was found between the values in green bean and the sensory characteristics.

General observations using the biochemical data generated on the genotypes evaluated at Tatu –Ruiru, Cr23, Cr27 and Cr30 showed similarities to SL28S while Cr22 and Cr8 showed similarities to Ruiru 11. In Meru, Cr30, Cr23 and Cr27 showed similarities to SL28NS while Cr8 and Cr22 showed similarity to Ruiru 11. At Koru, Cr8, Cr27 showed similarities to, Ruiru 11 and SL28NS while Cr22, Cr23 and Cr30 showed similarities to SL28S. At Machakos, the genotypes Cr23, Cr27 and Cr30 showed similarities to Ruiru 11 and SL28NS while Cr8, and Cr22 showed similarities SL28S. This showed that in terms of the biochemical components, the five advanced breeding lines showed similarity to the check cultivars Ruiru 11 and SL28.

Near-infrared spectroscopy (NIRS) has been proposed as a fast and nondestructive method for predicting chemical and physical properties in complex compositions like agricultural, horticultural and food products (Velasco *et al.,* 2004). In coffee, NIRS has been used successfully to predict the biochemical content of green beans (Guyot *et al*., 1993) and to authenticate coffee varieties (Downey and Boussion,

1996; Bertrand *et al*., 2005). A predictive model based on NIRS was used to determine the levels of caffeine, trigonelline, oil, sucrose and CGA in Ruiru 11 progenies grown in three different regions. The levels obtained were comparable to those reported in literature. Using the spectral fingerprints it was possible to separate the different progenies by the regions where they were grown underscoring the significance of environment when evaluating coffee genotypes. Since breeders have to do many evaluations before a new variety is released, investing in a technology like NIR could be necessary. This method does not require a large quantity of sample and several constituents can be analysed at the same time provided that calibration for each of them has been previously done. In addition, many standard techniques involve the destruction of the test sample, which could be a handicap in the case of valuable and scarce materials. NIR is non-destructive and after analysis the sample is intact and can be used for other analysis or other purpose.

5.3.4 Evaluation of the diversity of coffee by volatile organic components

5.3.4.1 Materials and methods

5.3.4.1.1 Sample choice and brew preparation

The coffee genotypes described in subsection 4.4.1.2 established at Machakos and processed in 2010 were selected for the volatile organic components analysis. Wet processing of ripe cherries, roasting of green coffee was done as described in subsection 4.4.1.3. These genotypes were selected because they were representative of coffees which did not show much variation in the sensory characteristics. Eleven (11) grams of roasted ground coffee was accurately weighed into a cup; boiled deionized water was gently added to the cup until full taking care not to spill over while filling the cup (The protocol followed is similar to that of brewing coffee for sensory analysis). The brewed coffee was allowed to cool to room temperature before further analysis. The brewed coffee was filtered under a vacuum through a whattman filter paper (No. 42) and stored at 4°C while awaiting solid phase extraction (SPE).

5.3.4.1.2 SPE and GC-MS analysis of brewed coffee

Cartridge conditioning was done by passing through 10 ml of methanol and 10 ml distilled water at a flow rate of 1ml/min, ensuring the cartridge did not dry out. Brewed coffee (10 ml) was passed through two preconditioned 1000mg/6ml strata C18- SPE (phenomenex) cartridges at a flow rate of approximately 2ml/min in a vacuum manifold. Ten (10) ml of distilled water was run through to wash away sugars and any other interfering matrices. The cartridges were dried and a stream of nitrogen at high pressure blown through. One cartridge was eluted with 10ml of

dichloromethane (DCM) while the other was eluted with 10ml hexane at a flow rate of 1ml/min followed by further pre-concentration to 1ml under a stream of nitrogen gas in a water bath at room temperature. Both eluents were injected into the GC-MS to determine the solvent that eluted a higher number of compounds. In order to determine the ideal volume of coffee brew to use for the extraction, brewed coffee volumes 10, 20, 30, 40 and 50 ml were passed through pre-conditioned 1000mg/6ml strata C18-E SPE (phenomenex) cartridges at a flow rate of approximately 2ml/min in a manifold. Ten (10) ml of distilled water was run through each cartridge, dried and eluted with 10 ml of dichloromethane at a flow rate of 1ml/min followed by further pre-concentration to 1ml under a stream of nitrogen gas at room temperature. Prior to GC-MS analysis, the eluent obtained was spiked with 100µl of 400ppm of benzophenone (internal standard).

5.3.4.1.3 Chromatographic conditions

GC-MS analyses were performed in a Konic HRGC 400B Gas Chromatograph coupled to a Konic MSQ12 quadruple mass spectrometer. One (1) µl of each extracts were injected into the split less mode in a TechnoKroma TRB5 (Cross-linked 5% phenyl-95% methyl siloxane) capillary column (15m × 0.25mm i.d × 0.1µm film thickness). Helium was used as the carrier gas at a flow rate of 1ml/min. The injection temperature was maintained at 200^{0}c, while the oven temperature was kept at 60°c and programmed to rise at 4°c/min to 150°c and finally to 240°c at a rate of 6°c/min. Mass spectra were recorded in the electron ionization mode at 70 eV scanning from 35-450m/z range. The ion source and transfer line temperature were maintained at 200°c and 250°c respectively.

5.3.4.1.4 Compound identification

Identification of the compounds in this study entirely relied on the matching of the mass spectrometric fragmentation pattern corresponding to the various peaks in the samples. Total ion chromatogram with those present in the National Institute of Science and Technology (NIST) mass spectral library. Library searches were done using the Automatic Mass Spectral Deconvolution and Identification **S**ystem (AMDIS). Integration was done automatically for the individual peaks. In determining the best library hit the match factors were taken into consideration. The minimum user set match factor was set at 50 units below that of the internal standard (benzophenone).

5.3.4.1.5 Data analysis

In the GC-MS analysis, each eluent was injected twice, and the peak areas for compounds identified were compared with those of the internal standard for semi quantification. The formula shown below was used to calculate the individual concentrations in ppm.

$Concn_{ci} = (Concn_{is} \times PA_{ci}/PA_{is}) \times CF$

Where: $Concn_{ci}$ = Concentration of Compound of interest

$Concn_{is}$ = Concentration of internal standard

PA_{ci} = Peak area of compound of interest

PA_{is} = Peak area of compound of internal standard

CF = Concentration Factor

(Harvey, 2000).

5.3.4.2 Results

5.3.4.2.1 Solid phase extraction optimization

Table 30 shows the compounds identified from the dichloromethane and hexane eluents and their corresponding concentrations. DCM was found to be the most appropriate eluting solvent as it eluted the greatest number of compounds from the cartridge. Comparison of concentration of compounds eluted with dichloromethane with varying sample volumes are shown in Figure 20.

Table 30: Identity of the compounds used in the optimization of SPE

	Compounds	Concentration in Hexane eluent	Concentration in DCM eluent
1	2,6-dimethyl pyrazine	Nd	84.4
2	5-methyl-2-furancarboxyaldehyde	26.8	53.3
3	2-acetoxymethylfuran	9.37	17.2
4	2-acetylpyrrole	Nd	10.5
5	Maltol	Nd	25.6
6	2,6-dihydroxy acetophenone	20.6	60.3
7	4-hydroxy-2-methylacetophenone	8.8	11.4
8	4-ethyl catechol	Nd	12.5

nd: not detected

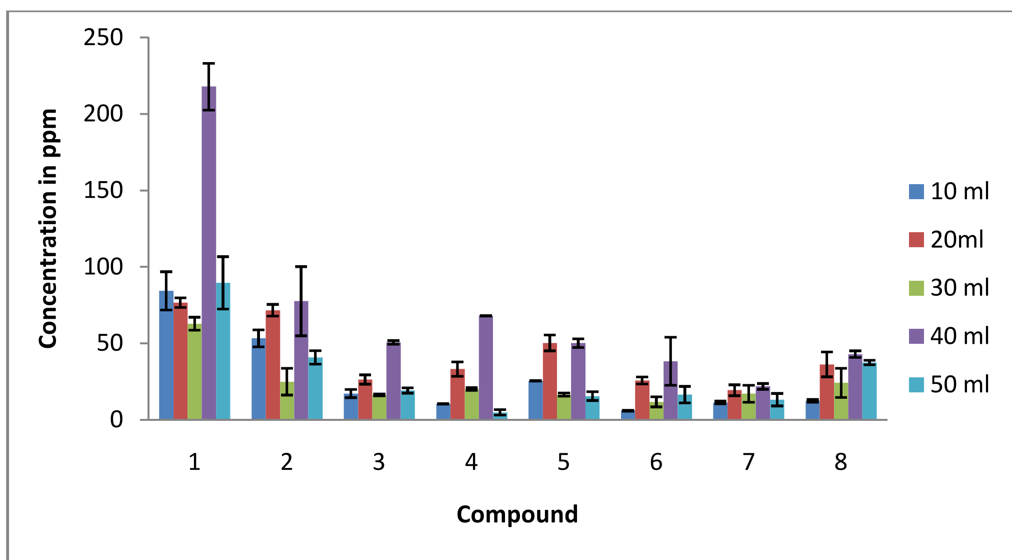


Figure 20: Comparison of concentration of compounds identified in optimization and eluted with DCM at varying sample volumes

5.3.4.2.2 Organic compounds in brewed coffee.

Chromatographic analysis of the eluents obtained by solid phase extraction from the various coffee genotypes of brewed coffee enabled the identification of 18 different compounds. Table 31 shows the volatile components identified in the SPE extract of coffee beverages, along with their apparent concentrations. Among the eighteen (18) volatile components identified, three (3) were pyrazines, two (2) pyrroles, two (2) furans, four (4) alcohols, one (1) aldehyde, four (4) ketones, one (1) carboxylic acid and one (1) compound not grouped. Five (5) compounds were not found in the NIST library. There were observable differences in the chromatographic profiles obtained in the coffee genotypes. The total number of volatile organic compounds in the brews of Cr30, Cr22, Cr23, Cr8, Ruiru11, Cr27, and SL28 were 13, 13, 11, 14, 13 and 14 respectively. The compounds 2,6-dimethyl pyrazine, 1-methyl-1H-Pyrrole-2-

carboxaldehyde, 5-methyl-1H-pyrole-2-carboxyaldehyde, 2-furanmethanol acetate, 5-methyl-2-furancarboxyaldehyde, Maltol, 4-Ethylcatechol, 2-Methoxy-4-vinylphenol, 2,6-Dihydroxyacetophenone and ionone were identified in the brews of all the genotypes. Typical gas chromatograms of SPE eluent of Ruiru 11 and SL28 are shown in Figure 21 and Figure 22 and compound identities in Table 32 and Table 33 and respectively.

Table 31: Identification and apparent concentration (ppm) of compounds present in the various coffee genotypes.

Chemical compound	Coffee genotypes							Match factor
	Cr30	Cr22	Cr23	Cr8	Ruiru 11	Cr27	SL28S	
Pyrazines								
2-Ethyl-5-methylpyrazine	-	-	-	-	326.8	-	-	768
2,6-dimethyl pyrazine	1175.5	1147.9	868.5	1031.1	976.1	859.4	1571.5	785
2-Acetyl-3-methylpyrazine	-	-	-	-	480.5	443	665.9	793
Pyrroles								
1-methyl-1H-Pyrrole-2-carboxaldehyde	156.2	268.3	212.3	246.4	252.6	222.4	330.6	762
5-methyl-1H-pyrole-2-carboxyaldehyde	56. 9	90.4	86.6	140	82	102.4	107	826
Furans								
2-furanmethanol acetate	356.1	334.4	212.3	312.8	288.1	297.2	478.3	803
5-methyl-2-furancarboxyaldehyde	568.7	668.8	608.7	558.3	451.4	499.2	567.9	834
Alcohols								
Maltol	479.4	500.9	432.6	560.7	678.4	478.2	508.7	786
5-Isopropenyl-2-methyl-2-cyclohexen-1-ol	-	-	-	-	-	-	174.9	
4-Ethylcatechol	372.5	281.2	270.1	366.7	367.5	264.5	304.8	793
2-Methoxy-4-vinylphenol	527.4	409.5	413.2	487	600.7	425.3	383	932
Ketones								
2,6-Dihydroxyacetophenone	369.5	240.9	314.5	270.1	400.6	339.3	367. 6	797
Ionone	200.3	149.2	167.5	130.2	154.6	81.1	182.1	769
4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)- 3-Bute	183	172.8	-	-	145	-	191.8	774
3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one	196.6	117.1	-	165.5	157.6	115.5	116.8	785
Aldehyde								
2-Hydroxy-4-methylbenzaldehyde		242						789
Carboxylic acid								
Oxiniacic Acid	-	-	-	-	-	-	235.5	793
Others								
1-[[(1,1-imethylethyl)imino]methyl]-Piperidine	105	-	-	-	-	115.9	-	
*****Not found in the library**								
1	-	-	-	-	219.1	-	-	
2	-			11.9	71.5		-	
3	-	-	138.4	-	284.3			
4	182.8	217.1	164.5			214.6		
5			146.2					

Key

*****Not found in the library**

SL28S- SL28 sprayed

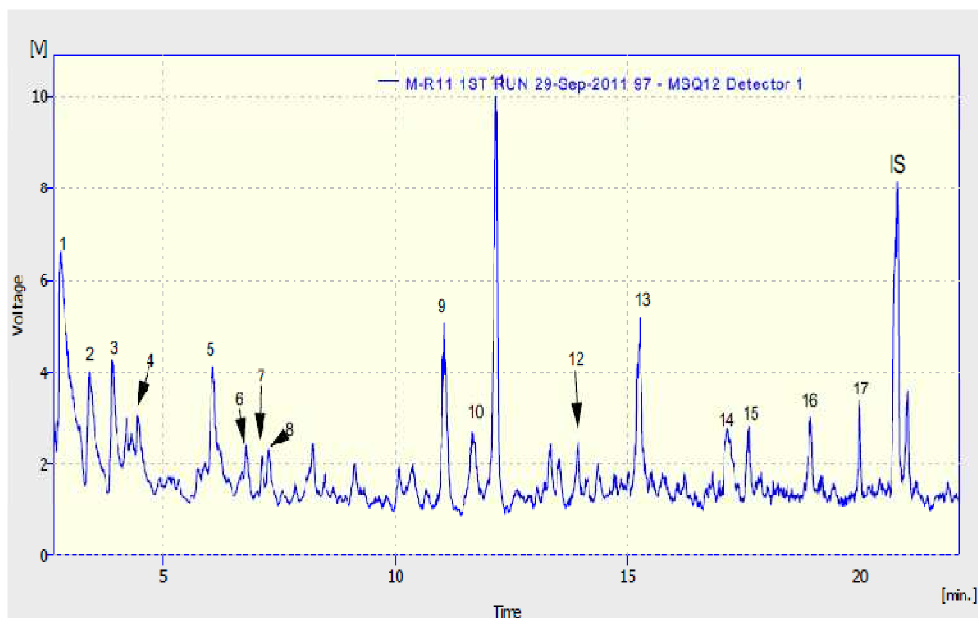


Figure 21:Typical gas chromatogram of SPE eluent of Ruiru 11 brew
Key: 1-23 are peaks of volatile compounds while IS is internal standard

Table 32: Identity of compounds extracted from the brew of Ruiru 11

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	2-ethyl-5-methylpyrazine
5	Maltol
6	2-Acetyl-3-methylpyrazine
7	1-methyl-1H-Pyrrole-2-carboxaldehyde
8	5-methyl-1H-pyrole-2-carboxyaldehyde
9	2,6-Dihydroxyacetophenone
10	1-[[(1,1-imethylethyl)imino]methyl]-Piperidine
11	2-Methoxy-4-vinylphenol
12	***
13	4-Ethylcatechol
14	***
15	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
16	***
17	Ionone

*****Not found in the library**

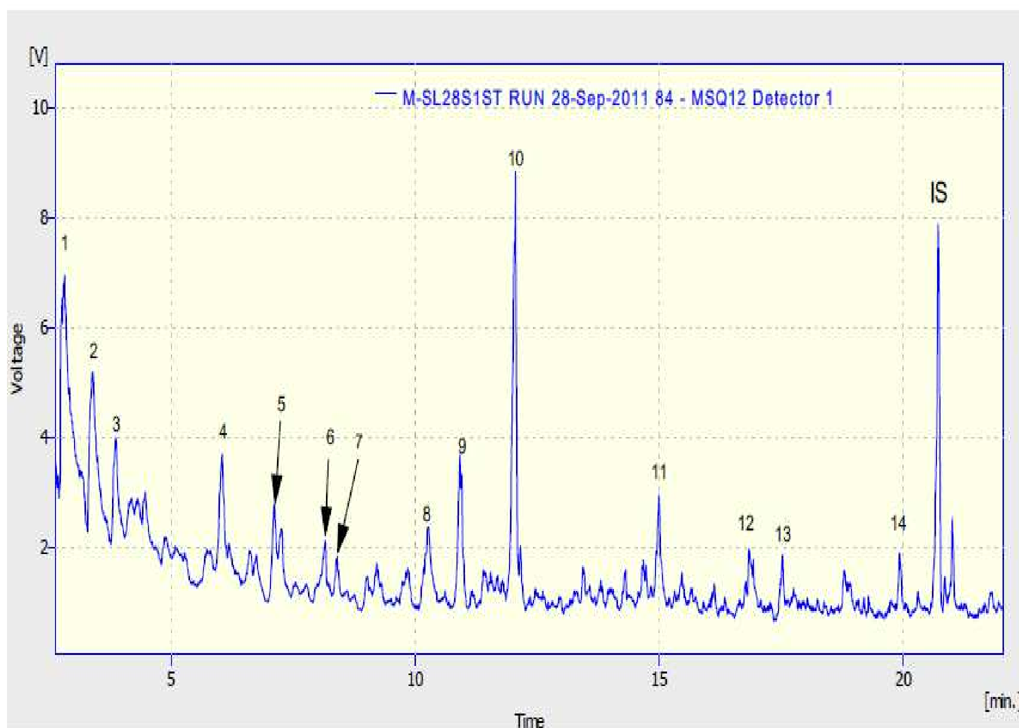


Figure 22: Typical gas chromatogram of SPE eluent of SL28 brew
Key: 1-14 are peaks of volatile compounds while IS is internal standard

Table 33: Identity of compounds extracted from the brew of SL28

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	Maltol
5	2-Acetyl-3-methylpyrazine
6	1-methyl-1H-Pyrrole-2-carboxaldehyde
7	5-methyl-1H-pyrole-2-carboxyaldehyde
8	Oxiniacic acid
9	2,6-Dihydroxyacetophenone
10	2-Methoxy-4-vinylphenol
11	4-Ethylcatechol ***
12	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
13	4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-3-Buten-2-one
14	ionone

***Not found in the library

5.3.4.3 Discussion

The characterization of coffee aroma is a challenging task because many of the important odorants are present in trace amounts and/or are unstable. Factors such as the degree of roasting have been found to influence the composition of potent odorants in Arabica coffee and the aroma is reported to change immediately after grinding (Mayer *et al*., 2000). The concentrations of some aroma compounds in coffee have been reported to be affected by coffee origin (Akiyama *et al*., 2003). During the roasting of coffee, many substances are formed due to reactions at high temperatures. These can contribute to the taste and aroma. One of the substances formed is 5-methyl-2-furancarboxyaldehyde and its concentration in commercially available roasted coffee is in the range of 0.3–1.9 mg/g (Murkovic and Bornik 2007). This compound was found to be present in all the analysed samples. In foods, hydroxyl methyl furfural (HMF) can be formed by different pathways mainly via dehydratisation of hexoses in the presence (Maillard reaction) or absence (caramelisation) of amines to 3-deoxy-2-hexosuloses that can further react to HMF (Antal *et al.,* 1990). The compound, 5-methyl-2-furancarboxyaldehyde has a spicy, candy and slightly caramel odor (Arctander, 1969). The results showed the presence of 4 ethyl catechol in six coffee genotypes and has been found to be generated exclusively upon thermal breakdown of caffeic acid moieties, similar compounds have been investigated such as catechol has been primarily formed by degradation of caffeoylquinic acids from both parts of the molecule, the caffeic acid and the quinic acid moiety, as well as from Maillard-type reactions from carbohydrates and amino acids (Muller, 2006). The alcohol 2-methoxy-4-vinylphenol (4-vinylGuaiacol) was

found to be present in all the varieties but in different concentrations. This chemical has been found to be formed during the coffee roasting process. Ralph *et al.* (2003) proposed 2 mechanisms for the formation of this compound which are based on based on two connected reaction channels. One channel, termed the "low activation energy" channel, consists of ester hydrolysis of 5-O-Ferulyquinic acid followed by decarboxylation of the ferulic acid to form 4-vinylguaiacol, and finally polymerization at the vinyl group to form partly insoluble polymers (coffee melanoidins). The second "high activation energy" channel opens up once the beans have reached higher temperatures. It leads to formation of guaiacol, via oxidation of 4-vinylguaiacol, and subsequently to phenol and other phenolic volatile organic compounds. This compound (2-Methoxy-4-vinylphenol) is associated with a smoky/phenolic odour and has been found to be present in medium roast Arabica coffee blends from Colombia (Mayer *et al.,* 2000). The compound 4-Ethylguaiacol has a smoky and burnt material flavour (Winter *et al*., 1976). It has been found that when 5-methyl-2-furancarboxaldehyde, 4-vinylguaiacol, furfural and furfuryl formate appear in higher amounts, the overall quality of the Arabica coffee is increased (Ribeiro *et al.,* 2009). Three (3) different pyrazines were identified in the brewed coffee extracts, with 2, 6 dimethyl pyrazine being found in all the samples analysed. Pyrolysis of amino acids, especially in the presence of carbohydrates, gives rise to pyrazines that contribute to the "roasted" aromas of various food products including coffee (Rowe, 1998) coffee is no exception. Pyrazine derivatives are formed by Maillard reactions, Strecker degradation and pyrolysis of hydroxyl amino acids and are considered as natural perfuming of foods (Baltes and Bochmann, 1987). The compound, 2-furanmethanol acetate was found to be present

in six of the cultivars with an exception of Cr23. This compound has been found to be presented in roasted Brazillian coffee. It has bee also been found that when compounds such as 2-furanmethanol acetate, 3-methylthiophen, 2-ethyl-3, 6-dimethylpyrazine and 1-(2-furanyl)-2-butanone are more abundant, the overall quality of the product drops (Ribeiro *et al.,* 2009). The volatile groups reported in this study (pyrazines, Pyrrole, furans, alcohols, aldehyde, ketone and carboxylic acid) were very few compared to what has been reported in the literature (Grosch, 2001).

Solid-Phase Micro-Extraction (SPME) is known to be a simple rapid and sensitive sampling method for liquid and gaseous volatile samples (Akiyama *et al*., 2003). However, these were not available during the analysis and hence the use of SPE. This could maybe explain the few compounds obtained compared to what has been reported in the literature (Grosch, 2001).

CHAPTER SIX

6.0 ASSESSMENT OF THE LEVEL OF ASSOCIATION AMONG CUP QUALITY VARIABLES AND BIOCHEMICAL COMPOUNDS.

6.1 Methodology

Beverage sensory characteristics; flavour, acidity, aftertaste, body, balance and overall scores reported in section 5.2.3 and green bean biochemical components caffeine, trigonelline, oil, sucrose and CGA reported in section 5.3.4 were correlated. The computer programme IBM SPSS Statistic 19 was used to perform stastistical correlation analysis using Pearson Correlation Coeffficients.

6.2 Results

6.2.1 Correlation coefficients between sensory and biochemical variables of the ex-situ conserved coffee germplasm.

There were positive significant correlations between all the sensory characteristics (Flavour, overall and aftertaste showed significant ($P<0.05$) correlations with trigonelline (Table 34). Chlorogenic acid had a negative correlation with all the sensory variables although only the correlation with overall was significant. Oil had a significant negative correlation with sucrose and recorded negative correlations with all the sensory variables except flavour. Caffeine showed negative correlations with all sensory variables except fragrance although it was not significant.

Table 34: Correlation coefficients between sensory and biochemical variables of the *ex-situ* conserved accessions and two commercial cultivars

Variables	Trigonelline										
Oil	0.112	Oil									
CGA	0.116	0.008	CGA								
Sucrose	0.243	-0.336*	0.149	Sucrose							
Caffeine	0.097	-0.214	0.279	0.002	Caffeine						
Overall	0.344*	-0.067	-0.351*	0.076	-0.148	Overall					
Balance	0.234	-0.056	-0.201	0.089	-0.127	0.870**	Balance				
Body	0.245	-0.138	-0.168	0.244	-0.101	0.819**	0.836**	Body			
Acidity	0.236	-0.074	-0.213	-0.002	-0.091	0 .881**	0.848**	0.821**	Acidity		
Aftertaste	0.359*	-0.119	-0.29	-0.009	-0.112	0.913**	0.822**	0.776**	0.883**	Aftertaste	
Flavour	0.363*	0.014	-0.283	-0.053	-0.078	0.925**	0.807**	0.799**	0.906**	0.931**	Flavour
Fragrance	0.023	-0.045	-0.198	0.151	0.177	0.403*	0.332*	0.327*	0.333*	0.448**	0.431**

Key
**Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level
CGA- Chlorogenic acid

6.2.2 Correlation coefficients between sensory and biochemical variables of advanced breeding lines together with check cultivars evaluated at Tatu-Ruiru

The correlation coefficients between sensory and biochemical variables of coffee evaluated at Tatu- Ruiru in the year 2008 are shown in Table 35. Significant ($P<0.05$) correlations were observed between all the sensory variables with each other except fragrance. Trigonelline showed significant positive correlation ($P<0.05$) with body. Oil showed positive significant correlations with all the sensory variables except with body and fragrance. Though not significant, chlorogenic acid was negatively correlated with all the sensory and biochemical components except

caffeine. Caffeine was negatively correlated with all the sensory parameters while sucrose showed a positive correlation although it was not significant (P>0.05).

Table 35: Correlation coefficients between sensory and biochemical variables of genotypes evaluated at Tatu- Ruiru

Variables	Trigonellin										
Oil	0.644	Oil									
CGA	-0.059	-0.204	CGA								
Sucrose	-0.466	0.053	-0.292	Sucrose							
Caffeine	-0.254	-0.641	0.534	0.092	Caffeine						
Overall	0.590	0.913**	-0.287	0.262	-0.567	Overall					
Balance	0.631	0.815*	-0.594	0.13	-0.678	0.894**	Balance				
Body	0.744*	0.643	-0.484	0.135	-0.389	0.797*	0.859**	Body			
Acidity	0.389	0.795*	-0.471	0.441	-0.641	0.876**	0.851**	0.775*	Acidity		
Aftertaste	0.431	0.798*	-0.447	0.298	-0.699	0.931**	0.929**	0.754*	0.907**	Aftertaste	
Flavour	0.311	0.775*	-0.458	0.493	-0.645	0.924**	0.837**	0.738*	0.930**	0.939**	Flavour
Fragrance	-0.155	0.217	-0.546	0.675	-0.362	0.446	0.545	0.448	0.721*	0.675	0.658

key

**. Correlation significant at the 0.01 level

*. Correlation significant at the 0.05 level

6.2.3 Correlation coefficients between mean sensory and biochemical variables of Ruiru progenies evaluated in Ruiru, Koru and Kitale.

Trigonelline showed significant positive correlations with all the sensory variables and with CGA (Table 36). Green bean oil content showed negative and significant correlations (p<0.01) with all cup quality attributes and biochemical attributes except trigonelline. chlorogenic acid had a negative correlation with all the sensory variables and biochemical variables except with sucrose and caffeine showing significant

correlation (p<0.01). There were highly significant (p<0.01) positive correlations between all the sensory characteristics.

Table 36: Correlation coefficients between sensory and biochemical variables of coffee Ruiru 11 progenies

Variables	Trigonelline										
Oil	0.094	Oil									
CGA	0.382*	0.636**	CGA								
Sucrose	0.128	-0.911**	-0.561**	Sucrose							
Caffeine	-0.165	-0.663**	-0.472**	0.476**	Caffeine						
Overall	0.450*	-0.579**	-0.195	0.673**	0.201	Overall					
Balance	0.423*	-0.529**	-0.172	0.624**	0.178	0.981**	Balance				
Body	0.392*	-0.562**	-0.193	0.660**	0.218	0.950**	0.942**	Body			
Acidity	0.442*	-0.548**	-0.18	0.621**	0.241	0.976**	0.974**	0.957**	Acidity		
Aftertaste	0.371*	-0.637**	-0.284	0.717**	0.255	0.956**	0.928**	0.909**	0.953**	Aftertaste	
Flavour	0.435*	-0.571**	-0.173	0.655**	0.217	0.980**	0.960**	0.939**	0.973**	0.960**	Flavour
Fragrance	0.384*	-0.609**	-0.221	0.727**	0.173	0.897**	0.875**	0.836**	0.861**	0.882**	0.886**

Key
**. Correlation significant at the 0.01 level
*. Correlation significant at the 0.05 level

6.3 Discussion

Results of this study showed some and significant correlations among some of the sensory attributes. Sucrose in some instances showed positive correlations with all sensory variables. Similarly, trigonelline showed significant positive correlation (P<0.05) with all sensory variables. The caffeine content of green beans showed negative and statistically significant correlations with all cup quality attributes for ex-situ conserved germplasm, advanced breeders lines alongside check cultivars and

the Ruiru11 progenies. Trigonelline negatively correlated with caffeine, i.e. high caffeine values were accompanied by low trigonelline values and vice versa, indicating a close but competing linkage of the two pathways (Baumann, 2006).

Franca *et al*. (2005) analysed green beans for caffeine and found, the highest and lowest caffeine levels to be the highest and lowest quality samples, respectively. There was a negative significant correlation observed between chlorogenic acid in the sensory and biochemical correlations of the ex-situ conserved germplasm. Similarly, chlorogenic acid showed significant negative correlations with sucrose and caffeine in the correlation analysis of the ten Ruiru 11 progenies evaluated at Koru, Ruiru and Kitale. With these kind of correlations, selection for high sucrose, high trigonelline and low caffeine content and better cup quality would seem possible in the coffee genotypes.

Van der Vossen (1985) recommended overall standard as the best cup quality selection trait due to its high heritability. On the other hand, based on correlation, repeatability and sensitivity analyses, Agwanda (1999) recommended flavour rating as the best selection criterion for genetic improvement of cup quality in Arabica coffee. However this study showed that all the sensory variables analysed in this study using a trained panel of tasters were important in determining the overall quality of a coffee.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Coffee genetic resources will have little value unless efficiently conserved, evaluated and properly utilized. Efficient utilization as well as conservation depends on the availability of reliable genetic diversity information because it is the basis for genetic improvement. The first step objective involved molecular analysis of selected coffee germplasm using RAPD and microsatellites. Molecular analysis separated the twenty four coffee genotypes into three main clusters. The first cluster had one genotype, *Coffea eugenioides*. The second cluster was dominated by non-introgressed Arabica genotypes while, Ruiru 11, HDT, Catimor and C. canephora (Robusta) were clustered in the third cluster. As would be expected, different accessions of HDT derivatives have different levels of introgressed *C. canephora* genome (Lashermes *et al*., 2000; Silveira *et al*., 2003) and could perhaps explain the close relationship observed between HDT, Ruiru 11 and Catimor Line 90 to Robusta. Hybrid varieties have revolutionized crop production, including cross- and self-pollinated species.

Walyaro (1983) stated that organoleptic evaluation of coffee could be considered more akin to the consumers' preference as it is the consumer in the end who finally judges beverage quality. The second objective involved sensory analysis of selected coffee germplasm. In some past studies, some of the *in situ* conserved genotypes maintained at CRF have been evaluated for disease resistance, yield, and quality (Walyaro, 1983). However the consumer preferences are continually changing.

Specialty buyers are looking for unique and differentiated products (Hide, 2009). These conserved genetic resources should therefore be properly conserved in order to utilize them for genetic improvement of sensory coffee quality in the future. It should be noted that the ex-situ conserved genotypes at CRS museum plots are more than two hundred (200) accessions. The kind of diversity presented could be exploited with the aim of recommending some accessions for commercial cultivation. For example, Ennareta, Blue Mountain and Geisha 11 which were clustered with non- introgressed Arabica genotypes in the molecular studies also revealed high beverage quality.

The cultivar Ruiru 11 is a composite F1 hybrid between lines of the variety Catimor, (as the female parent), and male selections most of which have HDT in their pedigree. This could probably explain the wide range of diversity observed between HDT and its derivatives (Catimor Line 90, Ruiru 11 line 5) analysed in this study. Introgression of some *C. canephora* genomic fragments into *C. arabica* varieties may affect their beverage quality (Bertrand *et al.*, 2003). However, introgression of disease and pest resistances from related species is a common breeding practice in many crops without necessarily resulting in permanent loss of quality (Van der Vossen, 2009). High variations between Ruiru 11 siblings in this study concurred with the report of Ojijo (1993) who reported that the composite Ruiru 11 cultivar present significant variability in terms of beverage quality. Considering that Ruiru 11 variety was released as a composite cultivar, further selection within the progenies (about 60) for beverage quality would be desirable considering that some progenies could do well in some environments than others. Over and again, doubts

have been raised from the specialty cycles as to the suitability of newly bred coffees such as Ruiru 11 for the gourmet markets (Van Der Vossen, 2009). In Central America, most buyers prefer traditional varieties (Bourbon, Caturra, Catuai, Pacamara) over the newer varieties derived from the HDT (Bertrand *et al*., 2006). The sensory method applied in this study (Lingle, 2001) enabled the positioning of the Ruiru 11 siblings in the specialty scale. It was evident that Ruiru 11 cultivar attained specialty grade (80 points and above) and has potential to compete as specialty coffee.

In the molecular analysis, the advanced breeding lines (Cr8, Cr22, Cr23, Cr27 and Cr30) clustered mainly with the non-introgressed Arabica coffee genotypes. The backcrossing and selfing at various selection stages involved in their development of could have affected the amount of Robusta coffee genome passed on to the next generation. Knowing the sensory characteristics of the new upcoming coffee varieties alongside the known existing varieties is important for rolling them the out to the coffee industry. Comparison of the performance of the advanced breeding lines together with the check cultivars Ruiru 11 and SL28, at Ruiru, Meru, Machakos and Koru was necessitated by the fact that environment is very important in the adaptation of the coffee. Diversity was observed in some of the genotypes due to season, year of evaluation and the site where they were grown. However, their sensory characteristics were similar to those of the check cultivars SL28 and Ruiru 11.

Knowledge of correlations among different characteristics is fundamental to designing an effective breeding programme for any crop, especially for perennial crops like coffee. The complexity of the chemical composition of the coffee bean has so far defied any analytical method of producing a quantitative chemical profile of green or roasted mild Arabica coffees, which correlates well with the beverage quality as determined by cup tasting (Avelino *et al*., 2005; Bertrand *et al*.,2006). Clifford and OhioKpehai (1983) reported a correlation between the coffee astrigency to chlorogenic acids. Immature beans, those that come from immature fruits, contribute to beverage astringency. Though some genotypes in this study were found to contain high levels of total chlorogenic acids for example Cr30 evaluated at Meru the flavour of the coffee was reported to be good with descriptors such as floral fruity honey, caramel among others being used to describe it. In this study only total chlorogenic acids were analysed without looking into the different specific fractions of the acid. Moreira *et al.* (2001) associated individual contents of chlorogenic acid with bad coffee. Farah *et al.* (2006) found 3, 4-dicaffeoylquinic acid levels in green coffee correlating strongly with high quality. The fact that coffees with high total chlorogenic acids had equally good flavour underscores the importance of analyzing specific chlorogenic acid fractions in coffee.

Similarly no direct link was found between the sensory characteristics and oil content. Avelino *et al.* (2005) showed that altitude was positively linked to oil content. Bertrand *et al*. (2008) demonstrated that determining the fatty acid composition of the coffee bean is an effective tool for distinguishing varieties. Based on these findings Villarreal *et al.* (2009) conducted a study to validate the study of

Bertrand *et al*. (2008) and found that environmental temperature during the development of coffee beans had an outstanding influence on their fatty acid composition more than the effect of genotype differences. However, the fact that some correlations between cup quality and chemical attributes were observed in several instances indicates that chemical analysis of green beans may be used as an additional tool for coffee quality evaluation.

RECOMMENDATIONS

1. The divergence of HDT derivatives like was shown in this study is of importance in breeding programmes. Ruiru 11 (HDT derivatives) siblings comprise of 60 lines and more intensive molecular analysis could help in characterizing them and consequently selecting elite lines.
2. The diversity observed in the *ex situ* conserved genotypes and advanced breeders lines in terms of sensory characteristics can be exploited and release some of them as commercial varieties for the specialty market.
3. The levels of caffeine, trigonelline, sucrose, and total chlorogenic acids were similar to those reported for other Arabica coffees. However, diversity of the genotypes by specific fatty acids contents and chlorogenic acid fractions needs to be explored in future studies.
4. Organoleptic procedures will continue to be the backbone of coffee quality assessment. However, it will be of immense advantage for coffee breeders to incorporate biochemical components analysis as a complementary method of evaluating coffee genotypes. This is because the additional information on the coffee genotypes may reveal further diversity and potential for eventual exploitation

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APPENDICES

Appendix 1: DNA Extraction buffers

(Before use, the buffers were kept for 20-30min at 62 °C).

(i) Extraction buffer*

NaCl 8.77g

Matab 2% (2g, added just before extraction) (Mixed Alkyltri-methylammonium Bromide)

Sarcosil 3% (9.5ml of 5% solution) (N-Lauroyl-Sarcosine)

Sodium bisulphite 1% (1g, added just before extraction)

Tris HCl 0.20M (20ml of 1 M, pH=8.0)

EDTA 40mM (1.49g)

*- The solution was viscous. It was dissolved at 40°C and stored at 4°C

(ii) Lysis buffer

Sorbitol 0.35M (6.38g)

Tris-HCl 0.20M (20ml of 1 M, pH=8.0)

EDTA 40mM (1.49g)

PVP 2% (2g) (polyvinyl pyrrolidone, added just before extraction)

Volume up to 100ml with distilled water

(iii)EDTA 0.5M pH 8 at 25°C (1L)

EDTA 186g

NaOH 20g

Add distilled water, dissolve, adjust pH and adjust final volume to 2L

(iv)Formamide Blue (for loading in denaturing acrylamide gels)

Formamide 98% 49ml

EDTA 10mM 186mg

Bromophenol Blue 125mg

Xylene cyanol a pinch

(v)TAE 50X (1L)

Tris 242g

Glacial acetic acid 57.1ml

EDTA 0.5M pH 8 100ml

make volume to 1 L

TBE 10X (2L) (Tris Boric acid EDTA)

Tris 216g

Boric acid 110g

EDTA 0.5M pH 8 80ml

Distilled water top to 2L

TE (Tris –EDTA buffer)

1ml of Tris HCl 1M pH=8

200µl of EDTA 0.5 M pH=8, volume make to 100ml

<u>dDNPs 5mM</u>

dATP 100mM 50µl

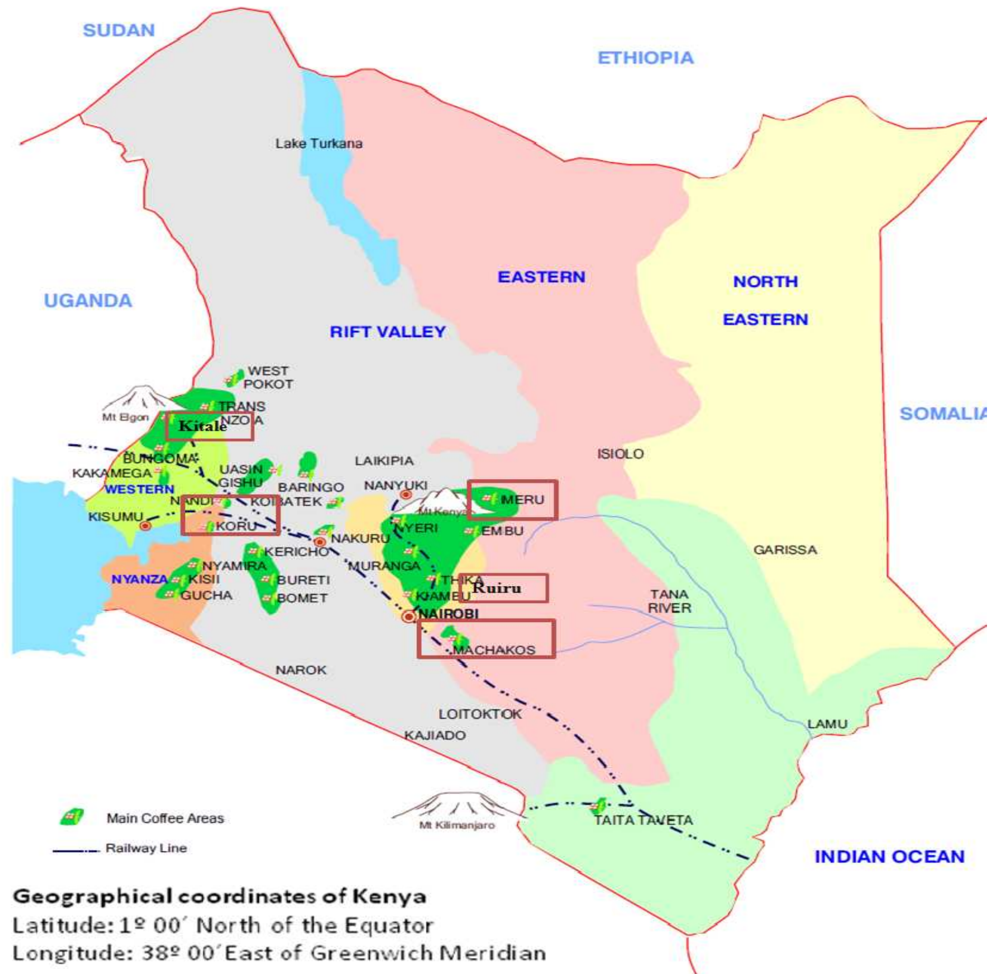
dGTP 100mM 50µl

dTTP 100mM 50µl

dCTP 100mM 50µl

Added double distilled water to make 1000µl

Appendix 2: **Geographical regions (in red boxes) where coffee samples were collected**



Appendix 3: Sensory evaluation scoring form

Classification:
6.00 - Good 7.00 - Very Good 8.00 - Excellent 9.00 - Outstanding
6.25 7.25 8.25 9.25
6.50 7.50 8.50 9.50
6.75 7.75 8.75 9.75

Name:
Date: Table: Session:

Sample # | Roast level | Fragance/Aroma Total: | Flavor Total: | Acidity: Total: | Body Total: | Uniformity Total: | Clean Cup: Total: | Overall Total: | Total Score

6 7 8 9 10

Dry Quality: Crust | Aftertaste Total: | Intensity High Low | Intensity High Low | Balance Total: | Sweetness Total: | Defects (subtract) Taint=2 Fault=4 # of cups X Intensity =

Notes: Final Score

Appendix 4: ANOVA table for sensory data for *ex-situ* conserved genotypes together with two commercial varieties

Fragrance

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	4.2959	0.7159	4.1383	0.0006 ***
GENOTYPES	41	9.0994	0.2219	1.2827	0.1294 ns

Flavour

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	3.0051	0.5008	3.920	0.0009 ***
GENOTYPES	41	19.106	0.4660	3.648	0.001 ***

Aftertaste

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	5.9013	0.983	8.214	0.001 ***
GENOTYPES	41	16.8001	0.4097	3.422	0.001 ***

Acidity

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	2.484	0.4141	3.451	0.0027 **
GENOTYPES	41	19.800	0.4829	4.0250	0.001 ***

Body

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	1.2908	0.2151	1.4880	0.1827 ns
GENOTYPES	41	10.588	0.2582	1.7862	0.0040 **

Balance

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	4.1836	0.6972	3.418	0.0029 **
GENOTYPES	41	16.249	0.396	.9431	0.0011 **

Overall

Source of variation	df	Sum of Squares	Mean Square	F	P
Reps	6	6.0221	1.0036	6.5258	0.001 ***
GENOTYPES	41	22.6471	0.5523	3.5914	0.001 ***

Appendix 5: ANOVA table for sensory data of Ruiru 11 siblings

Fragrance

Source of variation	DF	SS	MS	F	P
Genotype	9	0.1705	0.0189	1.1801	0.3249 ns
Source	2	0.1000	0.05003	3.1169	0.0518 ns
Genotype * Source	18	1.0789	0.05993	3.7336	0.0001 ***
Error	58	0.9311	0.0160		
Total	87	2.3107			

Favour

Source of variation	DF	SS	MS	F	P
Genotype	9	0.2340	0.0260	0.8793	0.5488 ns
Source	2	0.2947	0.1473	4.9832	0.0101 *
Genotype * Source	18	0.8570	0.0476	1.6101	0.0877 ns
Error	58	1.7151	0.0295		
Total	87	3.1136			

Aftertaste

Source of variation	DF	SS	MS	F	P
Genotype	9	0.2578	0.0286	1.0058	0.4460 ns
Source	2	0.1932	0.0966	3.3917	0.0405 *
Genotype * Source	18	1.2436	0.0690	2.4253	0.0057 **
Error	58	1.6522	0.0284		
Total	87	3.3597			

Acidity

Source of variation	DF	SS	MS	F	P
Genotype	9	9.8598	1.0955	0.9060	0.5263 ns
Source	2	1.4253	0.7126	0.5893	0.5580 ns
Genotype * Source	18	16.6453	0.9247	0.7647	0.7302 ns
Error	58	70.1326	1.2091		
Total	87	98.0745			

Body

Source of variation	DF	SS	MS	F	P
Genotype	9	0.1032	0.0114	0.5689	0.8168 ns
Source	2	0.4157	0.2078	10.3120	0.0001 ***
Genotype * Source	18	0.3212	0.0178	0.8854	0.5971 ns
Error	58	1.1692	0.0201		
Total	87	2.01478			

Balance

Source of variation	DF	SS	MS	F	P
Genotype	9	0.2548	0.0283	1.1264	0.3594 ns
Source	2	0.2545	0.1272	5.060	0.0094 **
Genotype * Source	18	0.8465	0.0470	1.8704	0.0377 *
Error	58	1.4583	0.0251		
Total	87	2.8290			

Overall

Source of variation	DF	SS	MS	F	P
Genotype	9	0.5010	0.0556	2.3711	0.0236 *
Source	2	0.9415	0.4707	20.0490	0.0001 ***
Genotype * Source	18	1.2022	0.0667	2.8444	0.0014 **
Error	58	1.3384	0.0234		
Total	87	3.9962			

Appendix 6: **ANOVA table for sensory data of five advanced breeding lines and two commercial cultivars evaluated at Tatu–Ruiru in 2008**

Source	Dependent Variable	df	Mean Square	Sum of Squares	F	Sig.
Season	Fragrance/aroma	1	2.917	2.917	12.343	.001
	Flavour	1	.434	.434	2.538	.112
	Aftertaste	1	2.002	2.002	10.156	.002
	Acidity	1	1.429	1.429	6.328	.012
	Body	1	2.002	2.002	11.109	.001
	Balance	1	.329	.329	1.429	.233
	Overall	1	.467	.467	2.164	.142
	Total score	1	65.018	65.018	9.432	.002
Genotypes	Fragrance/aroma	7	.375	2.622	1.585	.139
	Flavour	7	.936	6.550	5.473	.000
	Aftertaste	7	.775	5.425	3.931	.000
	Acidity	7	1.240	8.683	5.492	.000
	Body	7	.977	6.838	5.420	.000
	Balance	7	1.270	8.893	5.524	.000
	Overall	7	1.445	10.114	6.699	.000
	Total score	7	42.905	300.335	6.224	.000
Season * Genotypes	Fragrance/aroma	7	.129	.903	.546	.799
	Flavour	7	.220	1.540	1.287	.256
	Aftertaste	7	.219	1.531	1.109	.357
	Acidity	7	.427	2.992	1.893	.070
	Body	7	.276	1.932	1.532	.156
	Balance	7	.434	3.037	1.886	.072
	Overall	7	.328	2.294	1.520	.160
	Total score	7	11.564	80.949	1.678	.114
Error	Fragrance/aroma	292	.236	69.000		
	Flavour	292	.171	49.929		
	Aftertaste	292	.197	57.571		
	Acidity	292	.226	65.952		
	Body	292	.180	52.631		
	Balance	292	.230	67.155		
	Overall	292	.216	62.976		
	Total score	292	6.893	2012.790		
Total	Fragrance/aroma	307	76.997			
	Flavour	307	58.652			
	Aftertaste	307	67.500			
	Acidity	307	78.886			
	Body	307	64.255			
	Balance	307	78.562			
	Overall	307	75.214			
	Total score	307	2478.254			

Appendix 7 :ANOVA table for sensory data of five advanced breeding lines and two commercial cultivars evaluated at Tatu–Ruiru in 2009

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotypes	Fragrance	7	2.434	.348	4.258	.000
	Flavour	7	8.380	1.197	15.974	.000
	Aftertaste	7	7.730	1.104	15.677	.000
	Acidity	7	9.609	1.373	16.547	.000
	Body	7	6.092	.870	14.630	.000
	Balance	7	6.049	.864	11.673	.000
	Overall	7	7.186	1.027	13.374	.000
	Total score	7	310.172	44.310	21.372	.000
Season	Fragrance	1	.517	.517	6.335	.012
	Flavour	1	3.099	3.099	41.349	.000
	Aftertaste	1	2.077	2.077	29.489	.000
	Acidity	1	1.888	1.888	22.760	.000
	Body	1	.490	.490	8.229	.004
	Balance	1	.332	.332	4.490	.035
	Overall	1	1.072	1.072	13.969	.000
	Total score	1	57.860	57.860	27.907	.000
Genotypes * Season	Fragrance	7	.470	.067	.822	.570
	Flavour	7	1.221	.174	2.328	.026
	Aftertaste	7	1.549	.221	3.141	.003
	Acidity	7	1.495	.214	2.574	.014
	Body	7	2.160	.309	5.187	.000
	Balance	7	.857	.122	1.654	.121
	Overall	7	1.198	.171	2.230	.032
	Total score	7	54.515	7.788	3.756	.001
Error	Fragrance	257	20.987	.082		
	Flavour	257	19.260	.075		
	Aftertaste	257	18.103	.070		
	Acidity	257	21.321	.083		
	Body	257	15.287	.059		
	Balance	257	19.024	.074		
	Overall	257	19.726	.077		
	Total score	257	532.839	2.073		
Total	Fragrance	272	24.160			
	Flavour	272	33.264			
	Aftertaste	272	30.374			
	Acidity	272	34.086			
	Body	272	23.086			
	Balance	272	26.485			
	Overall	272	29.771			
	Total score	272	963.316			

Appendix 8: ANOVA table for sensory data of genotypes evaluated in Meru 2009

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
GENOTYPE	FRAGRANCE	6	.327	.055	4.252	.004
	FLAVOUR	6	.887	.148	8.655	.000
	AFTERTASTE	6	.935	.156	8.320	.000
	ACIDITY	6	1.657	.276	10.913	.000
	BODY	6	.725	.121	6.499	.000
	BALANCE	6	.818	.136	11.671	.000
	OVERALL	6	1.271	.212	15.490	.000
	TOTAL	6	43.866	7.311	12.418	.000
SPACING	FRAGRANCE	1	.074	.074	5.751	.024
	FLAVOUR	1	.412	.412	24.150	.000
	AFTERTASTE	1	.188	.188	10.051	.004
	ACIDITY	1	.439	.439	17.362	.000
	BODY	1	.227	.227	12.206	.002
	BALANCE	1	.205	.205	17.540	.000
	OVERALL	1	.267	.267	19.508	.000
	TOTAL	1	12.888	12.888	21.890	.000
GENOTYPE * SPACING	FRAGRANCE	6		.019	1.488	.221
	FLAVOUR	6	.114	.026	1.551	.201
	AFTERTASTE	6	.159	.031	1.658	.171
	ACIDITY	6	.186	.033	1.315	.286
	BODY	6	.200	.030	1.632	.178
	BALANCE	6	.182	.029	2.521	.047
	OVERALL	6	.177	.031	2.294	.065
	TOTAL	6	.188	1.373	2.331	.062
Error	FRAGRANCE	26	8.235	.013		
	FLAVOUR	26	.333	.017		
	AFTERTASTE	26	.444	.019		
	ACIDITY	26	.487	.025		
	BODY	26	.658	.019		
	BALANCE	26	.484	.012		
	OVERALL	26	.304	.014		
	TOTAL	26	.355	.589		
Total	FRAGRANCE	39	15.307			
	FLAVOUR	39	.801			
	AFTERTASTE	39	1.782			
	ACIDITY	39	1.684			
	BODY	39	2.784			
	BALANCE	39	1.535			
	OVERALL	39	1.392			
	TOTAL	39	1.961			

Appendix 9: ANOVA table for sensory data of genotypes evaluated in Meru 2010

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotypes	Fragrance/aroma	6	1.368	.228	3.038	.007
	Flavour	6	1.322	.220	5.563	.000
	Aftertaste	6	.822	.137	4.035	.001
	Acidity	6	.624	.104	3.114	.006
	Body	6	.422	.070	2.173	.046
	Balance	6	.783	.131	4.826	.000
	Overall	6	.822	.137	3.726	.001
	Total Score	6	38.361	6.394	8.574	.000
Space	Fragrance/aroma	1	.216	.216	2.879	.091
	Flavour	1	.420	.420	10.597	.001
	Aftertaste	1	1.131	1.131	33.301	.000
	Acidity	1	.579	.579	17.323	.000
	Body	1	.350	.350	10.805	.001
	Balance	1	.257	.257	9.505	.002
	Overall	1	.589	.589	16.022	.000
	Total Score	1	23.072	23.072	30.939	.000
Genotypes * Space	Fragrance/aroma	6	.481	.080	1.067	.383
	Flavour	6	.768	.128	3.230	.004
	Aftertaste	6	.657	.109	3.223	.005
	Acidity	6	.779	.130	3.888	.001
	Body	6	.333	.056	1.714	.118
	Balance	6	.506	.084	3.116	.006
	Overall	6	.331	.055	1.498	.179
	Total Score	6	21.474	3.579	4.800	.000
Error	Fragrance/aroma	266	19.961	.075		
	Flavour	266	10.539	.040		
	Aftertaste	266	9.034	.034		
	Acidity	266	8.884	.033		
	Body	266	8.616	.032		
	Balance	266	7.196	.027		
	Overall	266	9.784	.037		
	Total Score	266	198.360	.746		
Total	Fragrance/aroma	279	22.106			
	Flavour	279	13.132			
	Aftertaste	279	11.803			
	Acidity	279	10.943			
	Body	279	9.746			
	Balance	279	8.778			
	Overall	279	11.602			
	Total Score	279	284.892			

Appendix 10: ANOVA table for sensory data of genotypes evaluated in Meru 2011

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
GENOTYPE	FRAGRANCE	.047	6	.008	1.150	.362
	FLAVOUR	.234	6	.039	1.565	.197
	AFTERTASTE	.093	6	.016	1.103	.387
	ACIDITY	.183	6	.031	1.583	.192
	BODY	.016	6	.003	.568	.752
	BALANCE	.112	6	.019	1.068	.407
	OVERALL	.167	6	.028	1.188	.343
	TOTAL	4.793	6	.799	1.388	.257
SPACING	FRAGRANCE	.003	1	.003	.383	.542
	FLAVOUR	.002	1	.002	.075	.786
	AFTERTASTE	.022	1	.022	1.589	.219
	ACIDITY	.001	1	.001	.070	.794
	BODY	7.827E-5	1	7.827E-5	.016	.899
	BALANCE	.002	1	.002	.127	.724
	OVERALL	.000	1	.000	.012	.912
	TOTAL	.016	1	.016	.027	.871
GENOTYPE * SPACING	FRAGRANCE	.082	6	.014	2.012	.100
	FLAVOUR	.319	6	.053	2.132	.084
	AFTERTASTE	.167	6	.028	1.980	.105
	ACIDITY	.292	6	.049	2.524	.046
	BODY	.060	6	.010	2.108	.087
	BALANCE	.123	6	.020	1.171	.352
	OVERALL	.359	6	.060	2.562	.044
	TOTAL	8.080	6	1.347	2.340	.061
Error	FRAGRANCE	.176	26	.007		
	FLAVOUR	.649	26	.025		
	AFTERTASTE	.366	26	.014		
	ACIDITY	.502	26	.019		
	BODY	.124	26	.005		
	BALANCE	.453	26	.017		
	OVERALL	.608	26	.023		
	TOTAL	14.963	26	.575		
Corrected Total	FRAGRANCE	.326	39			
	FLAVOUR	1.314	39			
	AFTERTASTE	.667	39			
	ACIDITY	1.044	39			
	BODY	.220	39			
	BALANCE	.736	39			
	OVERALL	1.187	39			
	TOTAL	30.029	39			

Appendix 11: ANOVA table for sensory data of genotypes evaluated in Koru and Machakos

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotypes	Fragrance/aroma	7	1.308	.187	4.104	.000
	Flavour	7	2.979	.426	12.001	.000
	Aftertaste	7	2.205	.315	8.332	.000
	Acidity	7	3.237	.462	10.664	.000
	Body	7	.555	.079	2.528	.014
	Balance	7	1.290	.184	8.501	.000
	Overall	7	2.734	.391	12.328	.000
	Total Score	7	84.712	12.102	18.588	.000
Space	Fragrance/aroma	1	.275	.275	6.038	.014
	Flavour	1	1.253	1.253	35.343	.000
	Aftertaste	1	1.873	1.873	49.529	.000
	Acidity	1	1.255	1.255	28.934	.000
	Body	1	.128	.128	4.096	.043
	Balance	1	.353	.353	16.276	.000
	Overall	1	.822	.822	25.953	.000
	Total Score	1	37.624	37.624	57.788	.000
site	Fragrance/aroma	1	2.316	2.316	50.884	.000
	Flavour	1	.383	.383	10.798	.001
	Aftertaste	1	.730	.730	19.300	.000
	Acidity	1	1.148	1.148	26.472	.000
	Body	1	.514	.514	16.401	.000
	Balance	1	.503	.503	23.188	.000
	Overall	1	.134	.134	4.215	.040
	Total Score	1	32.982	32.982	50.658	.000
Genotypes * Space	Fragrance/aroma	7	.409	.058	1.283	.255
	Flavour	7	.805	.115	3.242	.002
	Aftertaste	7	.629	.090	2.377	.020
	Acidity	7	.563	.080	1.854	.074
	Body	7	.146	.021	.664	.703
	Balance	7	.282	.040	1.854	.073
	Overall	7	.424	.061	1.913	.064
	Total Score	7	13.759	1.966	3.019	.004
Genotypes * site	Fragrance/aroma	7	.132	.019	.414	.894
	Flavour	7	.567	.081	2.283	.026
	Aftertaste	7	.345	.049	1.304	.245
	Acidity	7	.453	.065	1.492	.166
	Body	7	.230	.033	1.050	.394
	Balance	7	.228	.033	1.501	.163
	Overall	7	.088	.013	.396	.905
	Total Score	7	7.239	1.034	1.588	.135
Error	Fragrance/aroma	1454	66.189	.046		
	Flavour	1454	51.564	.035		
	Aftertaste	1454	54.983	.038		
	Acidity	1454	63.061	.043		
	Body	1454	45.566	.031		
	Balance	1454	31.533	.022		
	Overall	1454	46.060	.032		
	Total Score	1454	946.655	.651		
Total	Fragrance/aroma	1517	78.696			
	Flavour	1517	61.584			
	Aftertaste	1517	65.208			
	Acidity	1517	78.707			
	Body	1517	50.534			
	Balance	1517	36.503			
	Overall	1517	54.454			
	Total Score	1517	1279.045			

Appendix 12: Anova table for biochemical analysis data of 40 ex situ conserved and 2 commercial varieties

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotypes	Trigonelline	41	1.483	.036	5.662	0.000
	Oil	41	110.952	2.706	4.349	0.000
	sucrose	41	51.939	1.267	1.548	0.081
	Chlorogenic acid	41	118.392	2.888	4.575	0.000
	caffeine	41	2.630	0.064	29.499	0.000
Error	Trigonelline	42	0.268	0.006		
	Oil	42	26.132	0.622		
	sucrose	42	34.365	0.818		
	Chlorogenic acid	42	26.510	0.631		
	caffeine	42	0.091	0.002		
Total	Trigonelline	83	1.751			
	Oil	83	137.084			
	sucrose	83	86.304			
	Chlorogenic acid	83	144.902			
	caffeine	83	2.722			

Appendix 13: Anova table for biochemical analysis of advanced breeding lines and 2 commercial varieties analysed at Tatu in 2008

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotype	caffeine	7	0.102	0.015	6.548	0.001
	trigonelline	7	0.021	0.003	2.581	0.055
	Oil	7	11.939	1.706	9.186	0.000
	sucrose	7	3.670	0.524	4.079	0.009
	Chlorogenic acid	7	1.152	0.165	8.156	0.000
Error	caffeine	16	0.035	0.002		
	trigonelline	16	0.019	0.001		
	Oil	16	2.971	0.186		
	sucrose	16	2.057	0.129		
	Chlorogenic acid	16	0.323	0.020		
Total	caffeine	23	0.137			
	trigonelline	23	0.040			
	Oil	23	14.910			
	sucrose	23	5.727			
	Chlorogenic acid	23	1.475			

Appendix 14: ANOVA table for biochemica data of genotypes evaluated at Meru for three years

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotype	Caffeine	6	.059	.010	3.949	.008
	Oil	6	33.052	5.509	1.647	.184
	Trigonelline	6	.280	.047	13.103	.000
	Sucrose	6	7.484	1.247	1.266	.314
	CQA	6	2.065	.344	.732	.629
Year	Caffeine	2	.132	.066	26.417	.000
	Oil	2	153.730	76.865	22.983	.000
	Trigonelline	2	.214	.107	30.009	.000
	Sucrose	2	26.027	13.014	13.213	.000
	CQA	2	15.267	7.633	16.231	.000
Genotype * Year	Caffeine	12	.241	.020	8.067	.000
	Oil	12	53.956	4.496	1.344	.267
	Trigonelline	12	.245	.020	5.746	.000
	Sucrose	12	23.060	1.922	1.951	.087
	CQA	12	13.894	1.158	2.462	.034
Error	Caffeine	21	.052	.002		
	Oil	21	70.234	3.344		
	Trigonelline	21	.075	.004		
	Sucrose	21	20.683	.985		
	CQA	21	9.876	.470		
Total	Caffeine	41	.484			
	Oil	41	310.972			
	Trigonelline	41	.813			
	Sucrose	41	77.255			
	CQA	41	41.102			

Appendix 15: ANOVA table for biochemica data of genotypes evaluated at Koru and Machakos for two years

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotype	Trigonelline	7	.125	.018	1.867	.108
	Caffeine	7	.145	.021	2.492	.037
	Chlorogenic acid	7	6.163	.880	2.401	.043
	Sucrose	7	2.502	.357	.442	.868
	Oil	7	29.260	4.180	1.881	.106
Site	Trigonelline	1	.145	.145	15.146	.000
	Caffeine%	1	.263	.263	31.642	.000
	Chlorogenic acid	1	.623	.623	1.699	.202
	Sucrose	1	4.192	4.192	5.183	.030
	Oil	1	.925	.925	0.416	.523
Genotype * Site	Trigonelline	7	.118	.017	1.757	.131
	Caffeine	7	.139	.020	2.382	.044
	Chlorogenic acid	7	9.041	1.292	3.522	.006
	Sucrose	7	3.377	.482	0.596	.754
	Oil	7	13.604	1.943	0.875	.537
Error	Trigonelline	32	.306	.010		
	Caffeine	32	.266	.008		
	Chlorogenic acid	32	11.736	.367		
	Sucrose	32	25.880	.809		
	Oil	32	71.115	2.222		
Total	Trigonelline	63	1.009			
	Caffeine	63	1.053			
	Chlorogenic acid	63	41.917			
	Sucrose	63	110.956			
	Oil	63	336.832			

Appendix 16: Anova table showing effect of site and genotypes on Ruiru 11 progenies biochemical variables

Source	Dependent Variable	df	Sum of Squares	Mean Square	F	Sig.
Genotypes	caffeine	9	.117	.013	4.066	.000
	trigonelline	9	.046	.005	2.420	.021
	Oil	9	11.567	1.285	2.547	.015
	sucrose	9	5.936	.660	2.362	.024
	Chlorogenic acid	9	1.336	.148	4.258	.000
source	caffeine	2	1.144	.572	178.882	.000
	trigonelline	2	.105	.052	24.658	.000
	Oil	2	61.967	30.983	61.405	.000
	sucrose	2	20.702	10.351	37.072	.000
	Chlorogenic acid	2	3.892	1.946	55.825	.000
Genotypes * source	caffeine	18	.195	.011	3.391	.000
	trigonelline	18	.038	.002	.999	.474
	Oil	18	10.692	.594	1.177	.309
	sucrose	18	7.185	.399	1.430	.152
	Chlorogenic acid	18	1.336	.074	2.129	.015
Error	caffeine	59	.189	.003		
	trigonelline	59	.125	.002		
	Oil	59	29.770	.505		
	sucrose	59	16.474	.279		
	Chlorogenic acid	59	2.057	.035		
Total	caffeine	88	1.642			
	trigonelline	88	.313			
	Oil	88	113.892			
	sucrose	88	50.132			
	Chlorogenic acid		8.652			

Appendix 17: Coffee aroma compounds as described by International Coffee Organization (ICO).

Burnt/Smokey: This odour and flavour descriptor is similar to that found in burnt food. The odour is associated with smoke produced when burning wood. This descriptor is frequently used to indicate the degree of roast commonly found by tasters in dark-roasted or oven-roasted coffees.

Chocolate-like: This aroma descriptor is reminiscent of the aroma and flavour of cocoa powder and chocolate (including dark chocolate and milk chocolate). It is an aroma that is sometimes referred to as sweet.

Caramel: This aroma descriptor is reminiscent of the odour and flavour produced when caramelizing sugar without burning it. Tasters should be cautioned not to use this attribute to describe a burning note.

Floral: This aroma descriptor is similar to the fragrance of flowers. It is associated with the slight scent of different types of flowers including honeysuckle, jasmine, dandelion and nettles. It is mainly found when an intense fruity or green aroma is perceived but rarely found having a high intensity by itself.

Fruity/Citrus: This aroma is reminiscent of the odour and taste of fruit. The natural aroma of berries is highly associated with this attribute. The perception of high acidity in some coffees is correlated with the citrus characteristic. Tasters should be cautioned not to use this attribute to describe the aroma of unripe or overripe fruit.

Grassy/Green/Herbal: This aroma descriptor includes three terms which are associated with odours reminiscent of a freshly mowed lawn, fresh green grass or herbs, green foliage, green beans or unripe fruit.

Rubber-like: This odour descriptor is characteristic of the smell of hot tyres, rubber bands and rubber stoppers. It is not considered a negative attribute but has a characteristic strong note highly recognisable in some coffees.

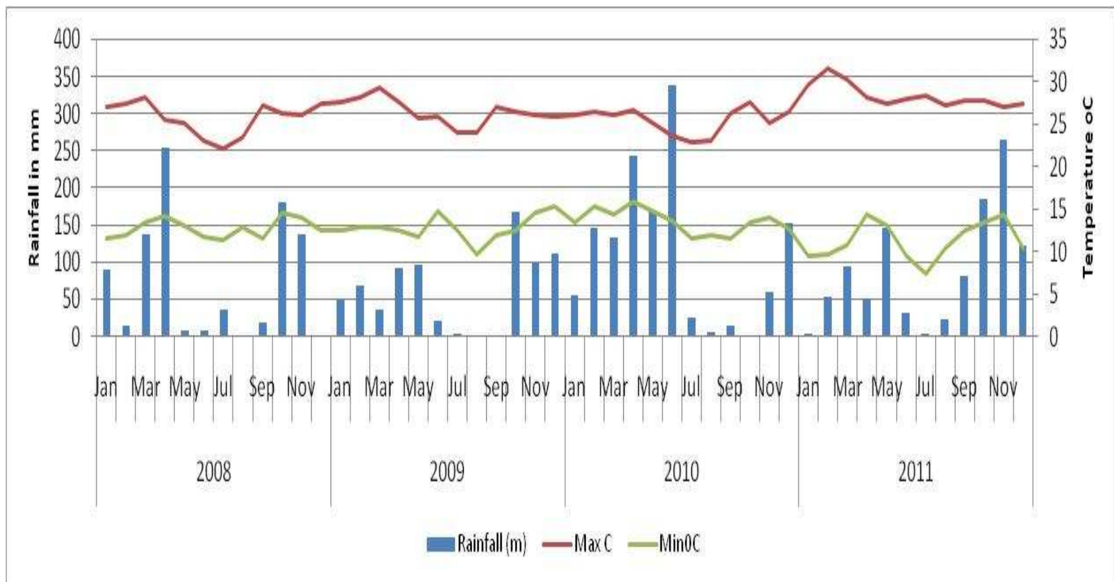
Spicy: This aroma descriptor is typical of the odour of sweet spices such as cloves, cinnamon and allspice. Tasters are cautioned not to use this term to describe the aroma of savoury spices such as pepper, oregano and Indian spices.

Winey: This terms is used to describe the combined sensation of smell, taste and mouthfeel experiences when drinking wine. It is generally perceived when a strong acidic or fruity note is found. Tasters should be cautioned not to apply this term to a sour or fermented flavour.

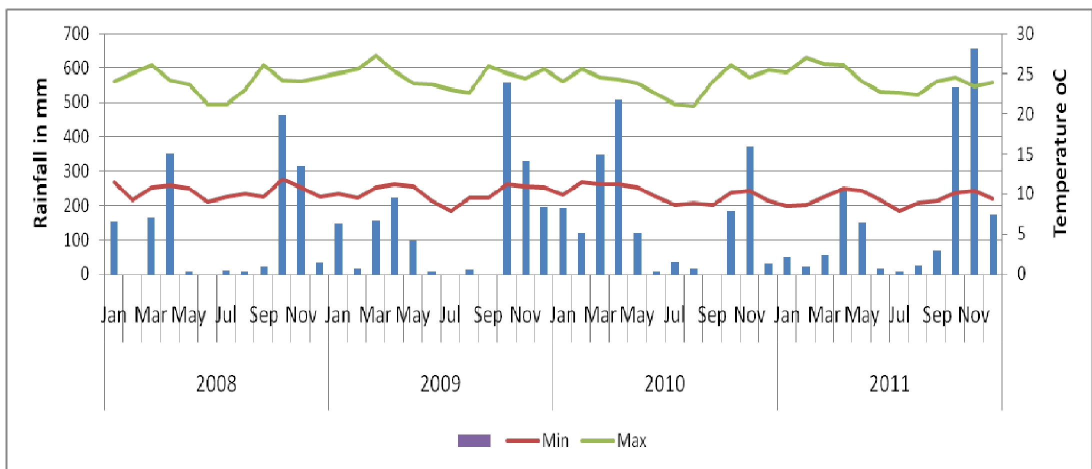
Astringency: This attribute is characteristic of an after-taste sensation consistent with a dry feeling in the mouth, undesirable in coffee.

Bitterness: A primary taste characterised by the solution of caffeine, quinine and certain alkaloids. This taste is considered desirable up to a certain level and is affected by the degree of roast brewing procedures.

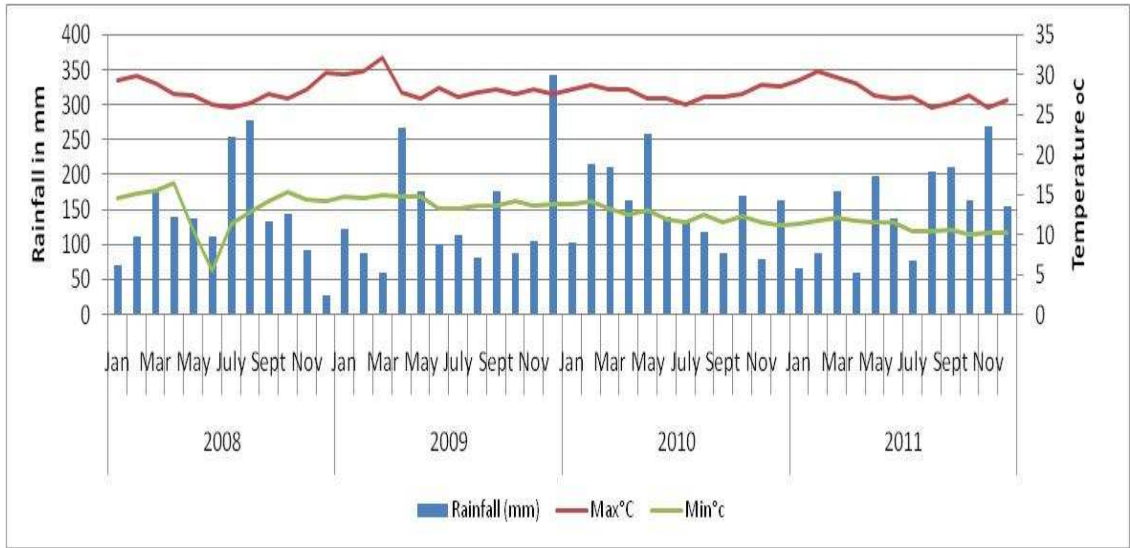
Appendix 18: Weather patterns in the regions of the study



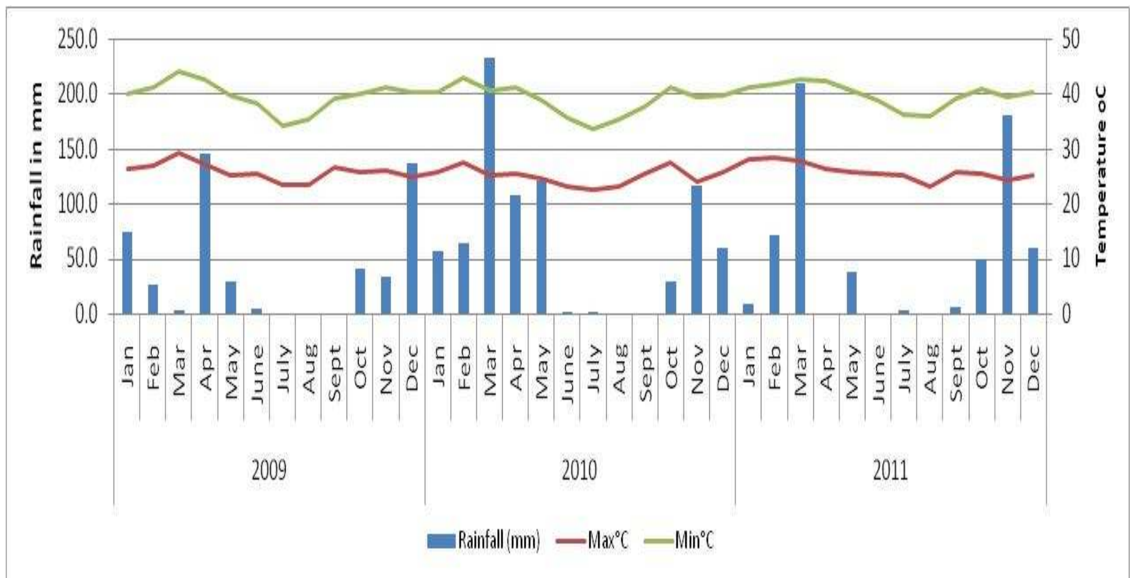
A:Temperature and rainfall patterns at Ruiru from 2008 to 2011



B: Temperature and rainfall at patterns in Mariene -Meru from 2009 to 2011

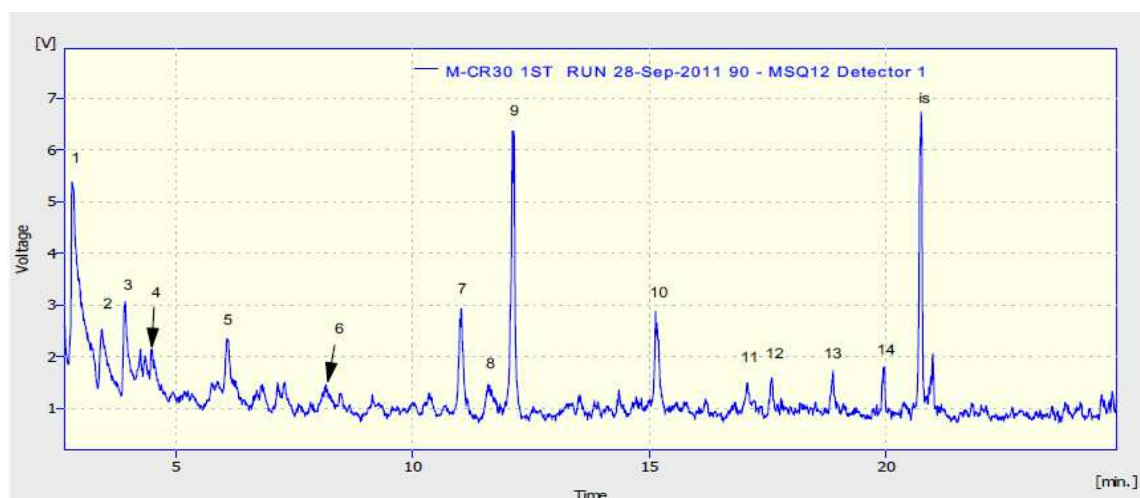


C: Temperature and rainfall patterns in Koru from 2008 to 2011



D: Temperature and rainfall patterns at Machakos from 2009 to 2011

Appendix 19: Gas chromatograms of brewed coffee eluents from various coffee genotypes

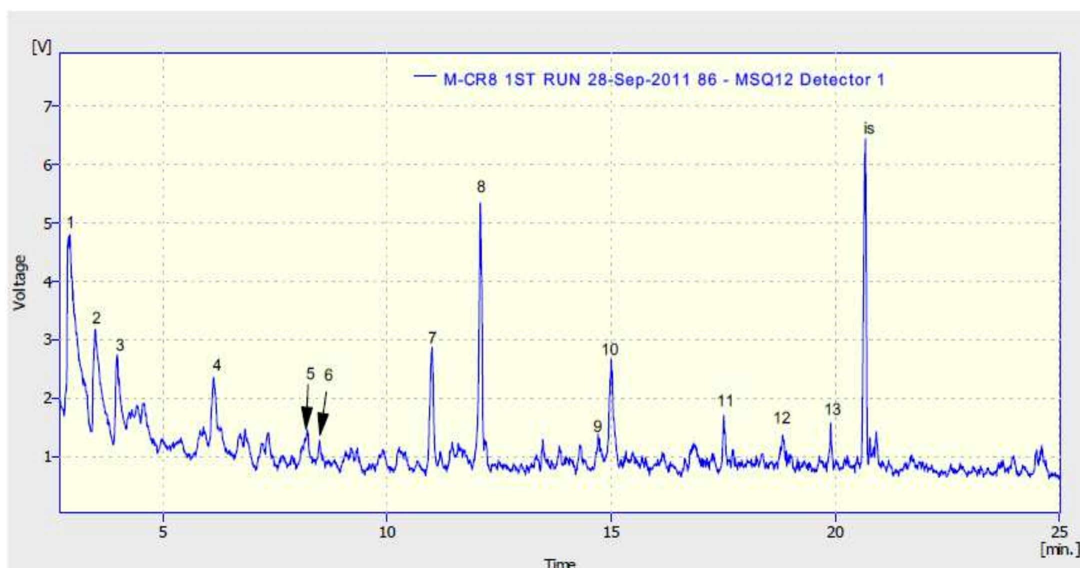


Typical gas chromatogram of SPE eluent of the brew of Cr30
Key: 1-12 are peaks of volatile compounds while IS is internal standard

Identity of compounds present in the brew of Cr30

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	1-methyl-1H-Pyrrole-2-carboxaldehyde
5	Maltol
6	5-methyl-1H-pyrole-2-carboxyaldehyde
7	2,6-Dihydroxyacetophenone
8	1-[[(1,1-imethylethyl)imino]methyl]-Piperidine
9	2-Methoxy-4-vinylphenol
10	4-Ethylcatechol
11	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
12	4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-3-Buten-2-one
13	***
14	Ionone

Key ***Not found in the library

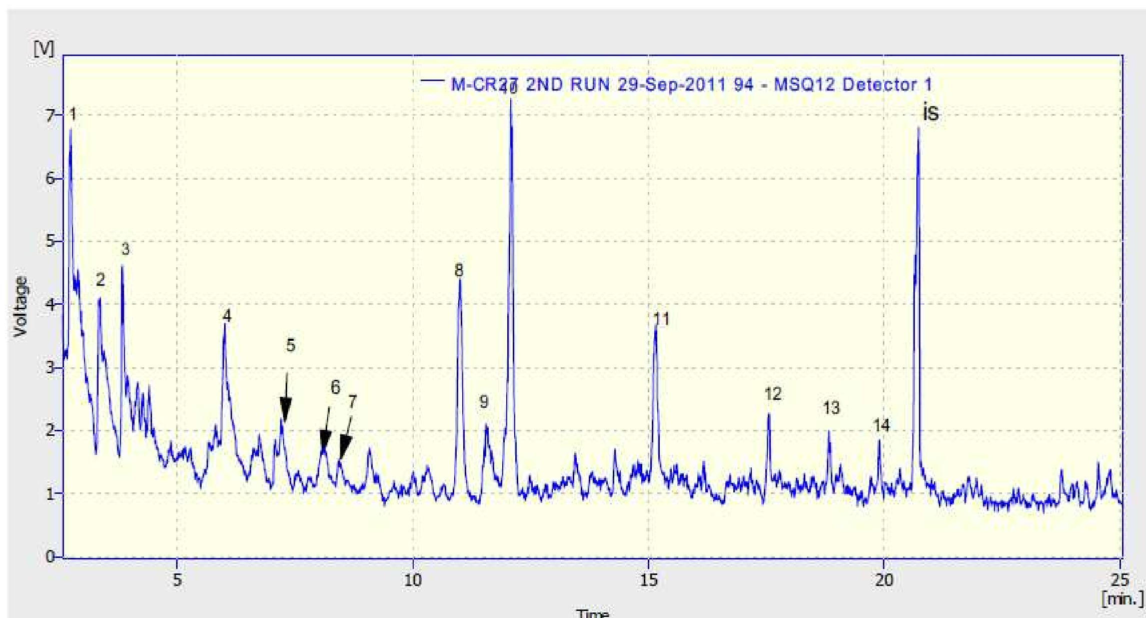


Typical gas chromatogram of SPE eluent of the brew of Cr8
Key: 1-13 are peaks of volatile compounds while IS is internal standard

Identity of compounds present in the brew of Cr8

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	Maltol
5	1-methyl-1H-Pyrrole-2-carboxaldehyde
6	5-methyl-1H-pyrole-2-carboxyaldehyde
7	2,6-Dihydroxyacetophenone
8	2-Methoxy-4-vinylphenol
9	***
10	4-Ethylcatechol
11	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
12	***
13	Ionone
14	

Key
*****Not found in the library**

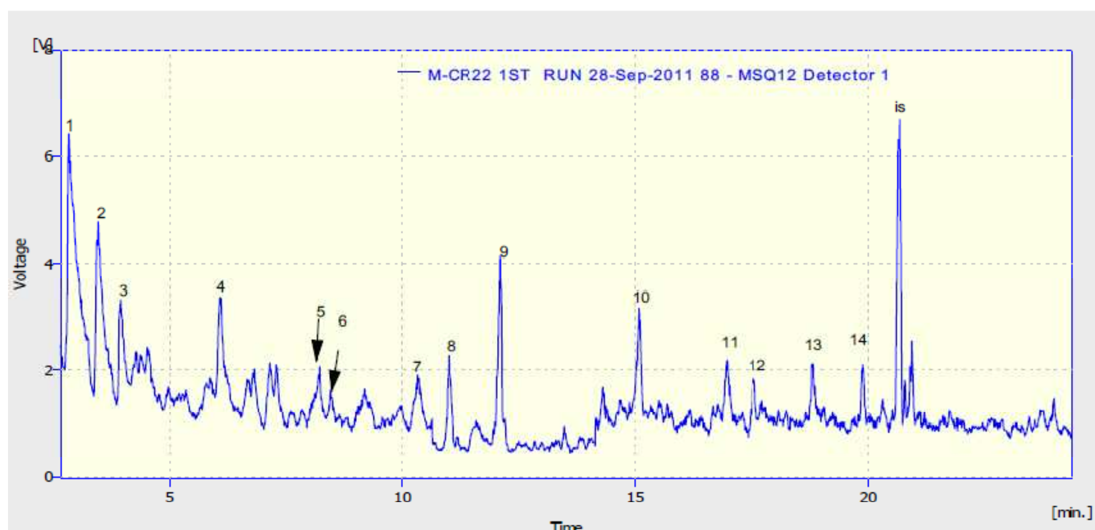


Typical gas chromatogram of SPE eluent of the brew of Cr27
Key: 1-14 are peaks of volatile compounds while IS is internal standard

Identity of compounds present in the brew of Cr27

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	Maltol
5	2-Acetyl-3-methylpyrazine
6	1-methyl-1H-Pyrrole-2-carboxaldehyde
7	5-methyl-1H-pyrole-2-carboxyaldehyde
8	2,6-Dihydroxyacetophenone
9	1-[[(1,1-imethylethyl)imino]methyl]-Piperidine
10	2-Methoxy-4-vinylphenol ***
11	4-Ethylcatechol
12	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
13	***
14	Ionone

Key ***Not found in the library



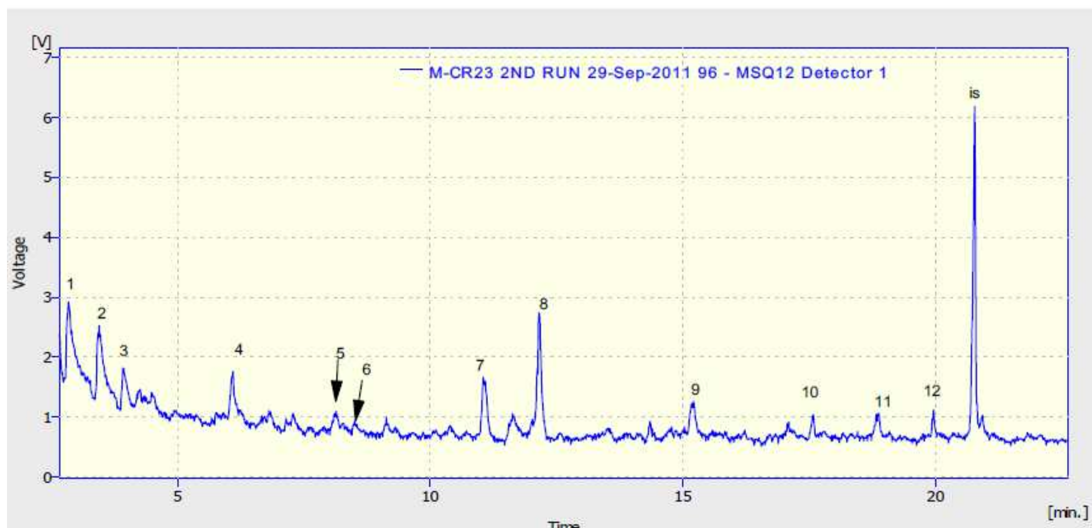
Typical gas chromatogram of SPE eluent of the brew of Cr22
Key: 1-14 are peaks of volatile compounds while IS is internal standard

Identity of compounds present in the brew of Cr22

Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	Maltol
5	1-methyl-1H-Pyrrole-2-carboxaldehyde
6	5-methyl-1H-pyrole-2-carboxyaldehyde
7	2-hydroxy-4-methylbenzaldehyde
8	2,6-Dihydroxyacetophenone
9	2-Methoxy-4-vinylphenol
10	4-Ethylcatechol
11	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
12	4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-3-Buten-2-one
13	***
14	Ionone

Key

*****Not found in the library**



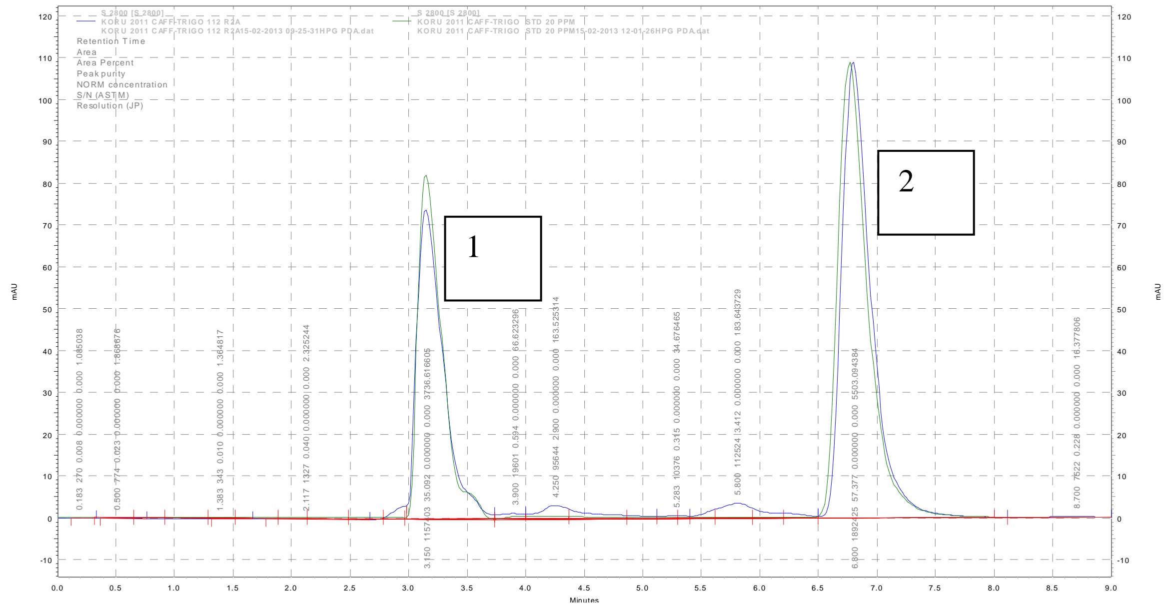
Typical gas chromatograms of SPE eluent of Cr23 brew
Key: 1-12 are peaks of volatile compounds while IS is internal standard

Identity of compounds present in the brew of Cr23

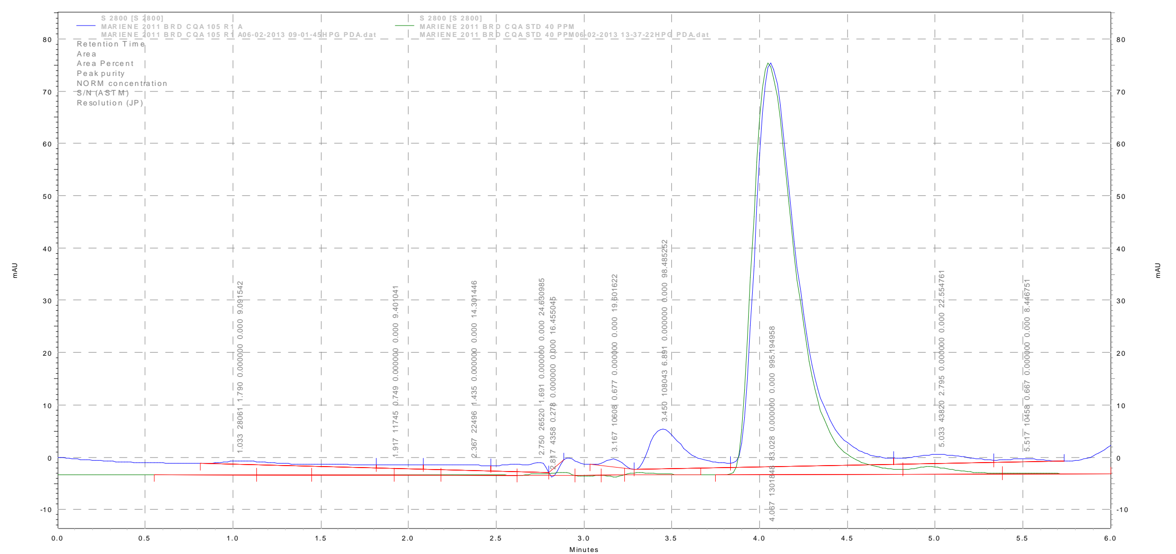
Peak Number	Identity
1	2,6-dimethyl pyrazine
2	5-methyl-2-furancarboxyaldehyde
3	2-furanmethanol acetate
4	Maltol
5	1-methyl-1H-Pyrrole-2-carboxaldehyde
6	5-methyl-1H-pyrole-2-carboxyaldehyde
7	2,6-Dihydroxyacetophenone
8	2-Methoxy-4-vinylphenol
9	4-Ethylcatechol
10	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one
11	4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)- 3-Buten-2-one
12	***
13	Ionone

Key

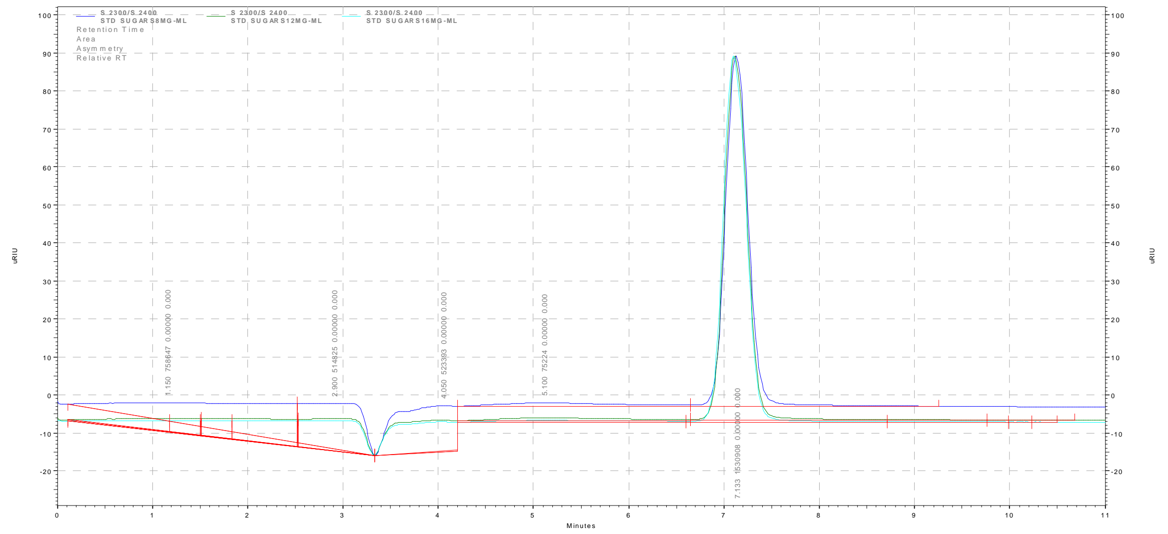
*****Not found in the library**



Trigonelline (1) and caffeine (2) sample and standard peaks overlaid



Chlorogenic acid sample and standard peaks overlaid



Sucrose sample and standard peaks overlaid