PERFORMANCE OF INTERSPECIFIC ARABUSTA COFFEE HYBRIDS FOR YIELD, CUP QUALITY, AND DISEASE RESISTANCE.

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2020

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

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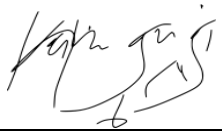
Prof. Eliud Kahiu Ngugi  Date. 22/09/2020

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DEDICATION

To the almighty God, my dear husband Reuben Kipruto, my sons Bowenn and Mutuol

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ABBREVIATIONS

ATC	Agricultural Training Centre
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
CAN	Calcium Ammonium Nitrogen
CGA	Chlorogenic Acids
CIRAD	Centre for International Agricultural Research and Development
CBD	Coffee Berry Disease
CLR	Coffee Leaf Rust
CPR	Coffee Production Recommendation
CQIP	Coffee Quality Improvement Programme
CRI	Coffee Research Institute
CRF	Coffee Research Foundation
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
GA	Genetic Advance
GCV	Genetic Coefficient of Variation
GDP	Gross Domestic Product
G x E	Genotype by Environment
GV	Genotypic Variance
H	Heritability
HPLC	High-Performance Liquid Chromatography
ICO	International Coffee Organization
ISO	Organization for standardization
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significance Difference
MAS	Marker Assisted Selection
NPK	Nitrogen Phosphorus Potassium
NPT	National Performance Trial

PCA Principal Component Analysis

PCR Polymerase Chain Reaction

PCV Phenotypic Coefficient of Variation

PDA Pulsed Diode Array

PV Phenotypic Variance

RAPD Random Amplified Polymorphic

RFLP Restriction Fragment Length Polymorphism

SSR Simple Sequence Repeats

UPGMA Unweighted Pair Group Method with Arithmetic Mean

USDA United States Department of Agriculture

SCA Specialty Coffee Association

TBE Tris Boric Ethylenediaminetetraacetic acid

ABSTRACT

The Kenya annual coffee production has in recent years declined due to different factors such as change of land use and biotic stresses, despite the country being one of the world's producers of high-quality coffee. This study analysed the agronomic potential of Arabusta hybrids developed from *C. arabica* and *C. canephora* for their bean yield, quality, and disease resistance. Nineteen Arabusta genotypes were assessed in two different locations (Siaya ATC and KALRO-Alupe) for growth and bean yield during the second and third years after establishment in the year 2017 and 2018 respectively. The data collected and analysed included plant height, number of bearing primaries, percentage of berries per node, length of longest primary, the total number of berries on the primary, berries per node, laterals, nodes with the highest number of berries, berries per node and yield (g/tree). Genetic variability among the genotypes was determined using 19 SSR markers. Marker Assisted Selection (MAS) using SSR marker SAT 235 was used to identify genotypes with the C*k-1* gene that are CBD resistant. The polymorphism between the Arabusta genotypes and the Arabica coffee varied, with 72% polymorphism calculated among Arabusta genotypes and 46.8% among the Arabica genotypes. The SSR marker SAT 235 was able to identify genotypes that have the CK-1 gene for coffee berry disease resistance.

Genotypes and locations revealed differences that were significant for both growth and yield traits. There was a positive significant correlation between yield and percentage berries per node (r= 0.61), total number of berries on longest primary (r= 0.58), and total number of berries on each node on the longest primary(r=0.60). Arabusta hybrids that had higher yields when compared to other genotypes in Busia were ARH1, ARH4, and ARH5 whereas in Siaya it was ARH4. Significant variation in sensory and bean grade traits showed that Arabusta hybrids gave higher

scores than backcrosses and Robusta. Acidity showed significant positive correlation with (r=0.96), aroma (r=0.84), balance (r=0.85), flavour (r=0.96) and preference (r=0.96). The 100 berry weight showed a positive correlation with the AA bean size indicating that berry weight can be used to predict AA bean size. Genotypes differed significantly in biochemical compounds across the two different environments.

The environmental effects were significant for all the biochemical compounds except for chlorogenic acids, but the G x E effects were not significant. The correlation between the chlorogenic acids and caffeine was significantly positive (r=0.77) but its correlation with lipid oil was significantly negative (r=-0.49) and also with sucrose (r=-0.43). Arabusta hybrids scored over 80 % for the total score in cup quality comparing well with the Arabica coffee and outperforming the Robusta coffee (79.4%) thus qualifying for the specialty market. Arabusta hybrids, backcrosses, and Arabica coffee on average had higher levels of sucrose, oil, and trigonelline when compared to Robusta which was responsible for the improved liquor quality.

This study confirmed that it is possible to transfer genes for high cup quality to Robusta from Arabica coffee through interspecific hybridization. The quantitative traits exhibited a highly significant positive correlation implying that these traits can be utilized in ensuring effectiveness and the efficiency of early selection for yield. The wider genetic variability of the Arabusta hybrids than that of Arabica coffee is key for coffee improvement programmes. All the hybrids and backcrosses did have the CK-1 gene responsible for CBD resistance. The Arabusta hybrids and the backcrosses had a score of > 80% on cup quality and therefore taking into consideration their performance in yield, the high yielding Arabusta hybrids ARH1, ARH4, and ARH5 are recommended for commercial cultivation in the specific environments.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background Information

Agriculture is the backbone of the Kenyan economy contributing majorly to its economy and accounts directly and indirectly for 24.5% and 27% respectively to Gross Domestic Product (GDP) of the country. The agricultural sector contributes to the food security employing over 80% of the rural workforce and also contributes to 65% of the national exports (ICO 2019). Coffee is the fifth most important commodity after diaspora remittances, horticulture, tourism, and tea with foreign earnings of US$230 million. Kenya is endowed with amenable climatic conditions with coffee being cultivated in high altitude ranging from 1500 to 2000 meters above the sea level, favourable rainfall patterns, well-drained red volcanic thus production of high-quality coffee.

During the early years of coffee production before the mid-1930s, the crop was cultivated by the white settlers only, when it was grown on an experimental basis and was restricted to only smallholder coffee production in Kisii and Meru counties. The Swynnerton Plan recommendations allowed the expansion of coffee production in the 1950s (Karanja and Nyoro, 2002). Of the total area under coffee cultivation in 1963/64, only 13,000ha was being utilized by smallholder farmers, and this was enhanced in the 1970s and 1980s when the prices of coffee went up. Land subdivision of large farms also contributed to enhancing the prominence of smallholder production (Karanja and Nyoro, 2002). The production during the 1980s was about 1.7million bags which have reduced to the current 900,000 bags on an annual basis (IC0 2019a).

Coffee is produced in 32 out of the 47 counties in Kenya under a total area of 115,570ha (ICO 2019a). Currently, Arabica coffee is the only species of coffee being produced with an estimated yield of 302kg/ha and 556kg/ha for the smallholder's farms and large estate farms respectively

Coffee production in Kenya is largely supported the smallholder farmers contributing to over 99% of the total production (ICO 2019a). The large coffee plantations around the urban centers have given way to housing developments which have led to coffee production to be increasingly smallholder dominated (Table 1.1). The coffee production in Kenya over the last six years is shown in Figure 1.1.

Table 1.1. Farmer distribution of coffee holding based on land sizes in Kenya

Sector	Size of acreage	Number of farmers	Share of total farmers
Smallholder affiliated	Varies	800,000	99.63%
Estates holdings			
Small estates	5-20 acres	2,400	0.30%
Medium estates	21-50 acres	500	0.06%
Large estates	Over 51 acres	100	0.01%
Total number of coffee farmers		**803,000**	100%

Source ICO, 2019.

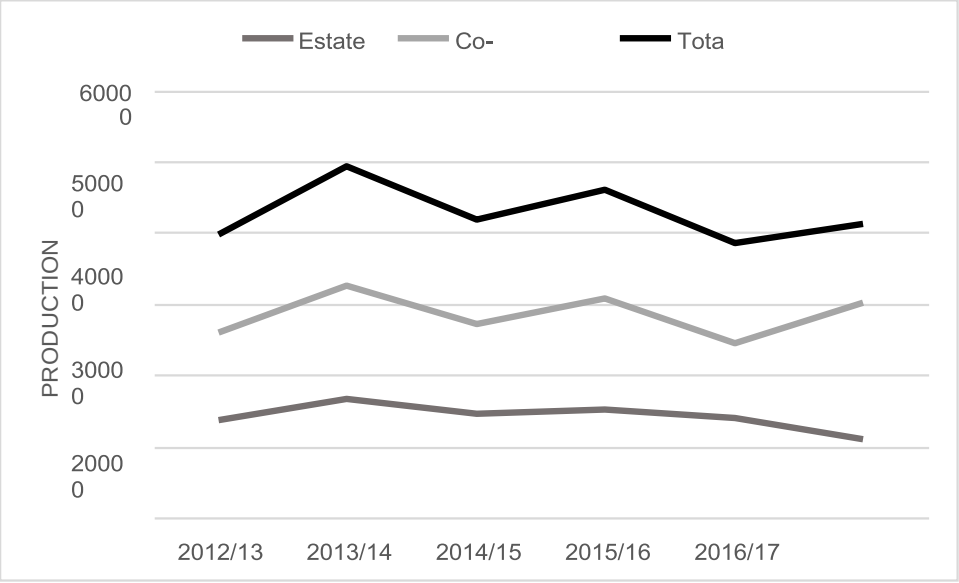


Figure 1.1: Line chart showing coffee production trends for the last six years in Kenya (Source ICO 2019)

The Kenya workforce that is involved in agriculture is about 80%, and of this, 30% are working in the coffee industry. The coffee sector continues to face challenges including, increased cost of production, erratic weather conditions, diseases, and weak structures governing the cooperatives in the marketing of the coffee. As evident from the drop in total coffee beans being exported, coffee production in Kenya has been declining (Condliffe, 2008). Arabica coffee contributes to over 90% of the coffee produced in Kenya. The old cultivars, grown in Kenya are K7, SL28 and SL34 cultivate in areas with adequate rainfall. The more recently released cultivars Ruiru 11 and Batian (released in 2010) which are resistant to the two major coffee diseases (Coffee Leaf Rust and Coffee Berry Disease) are grown in Kenya (Nyoro and Sprey, 1986, Opile and Agwanda, 1993, Gichimu et al., 2012). Batian and Ruiru 11 varieties are high yielding and continually replace the old cultivars on a wider scale within the nation (Van der Vossen, 2001).

1.2 Development of Arabusta coffee

Robusta coffee is more tolerant of major coffee diseases but has inferior cup quality (Bertrand et al., 2003). The aim of coffee improvement programs has been transferring the genes that confer resistance to diseases without affecting adversely the traits of cup quality. The gene transfer has been the main challenge due to the differences in ploidy levels in *C. arabica* and *C. canephora* since *Coffea canephora* is a diploid (2n=22) whereas C*offea arabica* is tetraploid (2n=44) (Lashermes et al., 1997; Ky et al., 2001). is a tetraploid while). Despite the challenge, induced tetraploid *Coffea canephora* were created in Brazil by doubling of chromosomes using colchicine in the 1950s (Capot et al., 1968). Some of the induced tetraploids were also developed in Uganda (Gimase et al., 2015). Induced tetraploid *Coffea canephora* were crossed to *Coffea arabica* to develop the interspecific hybrids (Lashermes et al., 2011), Gimase et al., 2015. The Interspecific hybridization has been applied to introgress resistance for CLR and CBD from *Coffea canephora*

into *Coffea arabica* in various coffee improvement programmes. The hybridization between *C. arabica* and induced tetraploid *C. canephora,* resulted in interspecific hybrid termed as Arabusta developed to improve Robusta cup quality. In Kenya, the interspecific crosses were made from arabica and induced tetraploid Robusta (2n= 4x=44) coffee to introgress the superior cup quality of *C. arabica* into Robusta coffee. Tetraploid Robusta clones which were developed earlier in Uganda including UT2, UT3, UT6, UT7, UT8, UT10, and UT12 were introduced to Kenya. The objective was to introgress genes that confer resistance to CBD and CLR from the UT's into local Arabica varieties as a long term objective of coffee breeding programmes. The tetraploid Robusta clones were then crossed with Arabica coffee varieties (Caturra, SL8, SL34) to obtain interspecific Arabica x Robusta F_1 hybrids (CRF, 1980).

These F1 crosses were evaluated in Ruiru and found to be resistant to CBD (CRF, 1976). The Arabusta hybrids were backcrossed to the Arabica's and further studies revealed that the best results were achieved in UT3, UT6, UT8, and UT10 as they were good sources of genes for resistance to CBD after being subjected to pre-selection test for CBD resistance. The best performing hybrids and backcrosses were selected and established by Coffee Research Foundation in Busia and Siaya for evaluation of their performance at low altitudes. These areas were selected since they are best suited for Robusta production. However, this work was put into abeyance by then the Coffee Research Foundation due to financial constraints. Coffee Research Institute in the year 2012, developed a new strategy to incorporate Robusta research as one of its programmes to avail improved varieties to farmers in the low altitude zones to increase coffee production. It was decided that the research on Arabusta coffee be revived and to assess their performance to determine its commercial viability.

1.3 Statement of the Problem

In Africa, West African countries produce more of Robusta coffee while East African countries produce mainly Arabica coffee except for Uganda which produces Robusta coffee. Coffee is a major export commodity in Kenya, supporting over 800,000 smallholder households (ICO 2019). Kenya has been known to produce one of the best Arabica coffees globally which are blended with other coffees of low quality. Uganda has overtaken Kenya in total production volumes despite its producing Robusta coffee which is low in quality (Waitathu, 2010). Kenya coffee production has been on a decline over several years from a peak of 1.7 million bags in the '80s to 900,000 bags being produced currently (ICO 2019). The production of Arabica coffee has been limited by an increase in diseases and insects, changing weather patterns, and encroachment of land under coffee cultivation for human settlement.

Kenya is seeking to restore and surpass coffee-growing volumes that Arabica coffee has produced by expanding its production areas. Despite the Arabica species producing high-quality coffee, its yield production is low since it is most susceptible to major coffee diseases and insects (Agwanda et al., 1997). Arabica coffee farming and production are further affected negatively by the restriction that it be cultivated at an altitude of about 1400m to 2100m above sea level (CRF, 2011). This has disadvantaged the lower regions around Lake Victoria and the coastal strip that has the potential for Robusta coffee production. The prices of Robusta coffee is lower by 30% when compared to Arabica due to inferior cup quality, however, its production/acreage is higher. Robusta coffee is resistant to major coffee diseases however its cup quality is inferior when compared to Arabica coffee (Bertrand et al., 2003). The coffee market is determined by cup quality which is influenced by the level of biochemical compounds found in the green bean. The unfavorable effects on the cup are normally caused by higher caffeine and chlorogenic content

which is normally found to be high in Robusta coffee, whereas the low levels of sucrose, oil, and trigonelline are known to impart neutral taste in the Robusta (Clifford, 1985; Ky et al., 2001a, b). Arabusta coffee has been developed from crosses of tetraploid Robusta and Arabica coffee to be evaluated in low altitude zones suitable for Robusta production. The performance of this Arabusta coffee in terms of quality and yield is expected to be higher than Robusta.

1.4 Justification

Coffee Research Institute has developed inter-specific hybrids tetraploid (*C. canephora* x *C. Arabica)* that have a high cup quality than Robusta. Liquor quality is a significant feature of coffee and is important in determination of prices in the market (Muschler, 2001; Agwanda et al., 2003; Kathurima et al., 2009). The inherent genetics together with climatic factors contribute majorly to having desirable quality traits of the coffee bean. The levels of the biochemical compounds found in the green bean contribute to the final liquor quality (Buffo and Freire, 2004). The yield and cup quality of a specific genotype are critical in determining its success to be released as a new variety (Agwanda et al., 2003).

Efforts have been made to transfer genes for resistance to CBD and drought tolerance into Arabica coffee through backcrossing (Owuor and Omondi 1992, Gimase et al., 2015). The production of coffee variety that is disease resistant is an economical and sustainable way of controlling CBD (Omondi et al., 2016) reducing the use of chemicals thus ensuring the safety of the environment (Gichuru et al., 2008). The Arabusta hybrids are expected to be superior in cup quality when compared to the Robusta coffee and will fetch better prices in the market. The introduction of Arabusta coffee will assist in realizing higher yields and thus increase the Gross Domestic Product

(GDP). This study, therefore, sets out to investigate the yield and quality potential of these Arabusta hybrids and backcrosses with introgressed CBD resistance.

1.5 Objectives of the study

The broad objective of the study was to improve coffee productivity through the development of high yielding, high quality, and disease-resistant coffee varieties

The specific objectives were:

1. To evaluate Arabusta hybrids and backcross progenies for improved bean yield
2. To characterize Arabusta coffee hybrids and backcross progenies for resistance to Coffee Berry Disease and genetic diversity
3. To determine the bean physical and liquor quality
4. To determine the biochemical composition of beans

1.6 Hypothesis

1. There were no significant differences in the yield performances of the Arabusta hybrids and its backcross
2. There are were no significant variations in resistance to coffee berry disease and the genetic diversity among the Arabusta hybrids and backcross progenies
3. There was no variability on beans physical and liquor quality of the Arabusta hybrids
4. The biochemical composition of beans of the Arabusta hybrids and backcross progenies did not differ significantly

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and distribution of coffee

The center of coffee origin and its genetic diversity for all coffee species produced worldwide is Africa**.** The highlands of South West Ethiopia and the Boma Plateau of Sudan are the primary centers of genetic diversity for the Arabica coffee (Lashermes, 1999). There are *C. arabica* Populations that were discovered in Mt. Imatong and Mt Marsabit found in Sudan and Kenya respectively (Gichimu, 2013, Gimase 2014 et al., 2014b). The tetraploid *Coffea arabica* originated from Ethiopia while other species including *Coffea canephora* have their origin in Central West African countries (Adepoju et al., 2017). During the 18th century, Arabica coffee species were distributed to other parts of the world where they are currently cultivated (Teressa et al., 2010).

It is believed that the natural hybridization led to the creation of *Coffea arabica* L. The hybridization followed by unreduced gamete formation might have occurred between C*offea eugenioides* and *Coffea canephora* or (*Coffea liberica* or *Coffea congensis* (Esayas, 2005). The two distinct botanical varieties of *Coffea arabica* include *Coffea arabica* var. Arabica and *Coffea arabica* var. bourbon (Hue, 2005). Around the 18th century, the typica and bourbon genetic bases originated from Indonesia and Mocha in Yemen respectively (Gichimu and Omondi, 2010a). *Coffea canephora* was discovered in the Democratic Republic of Congo. Robusta coffee is cultivated in Africa, Indonesia, and to some extent in Northern Brazil. Robusta accounts for up to 95% of the *Coffea canephora* that is cultivated globally (Gimase et al., 2014a). Robusta distribution is within hot with humid climatic regions. Countries such as Cote d'Ivoire, France, and Brazil include some of the countries whereby the Robusta gene pool is conserved in *ex-situ*

with Cote d'Ivoire having the largest collection of wild genotypes (Tshilenge et al., 2009, Gomez et al., 2009).

2.2 History of coffee in Kenya

Coffee was an item of trade with Yemen in the fifteenth century and by 1700, the seed from Arabica plants was sourced from the ports of Aden and Mocha. They were grown in Bourbon during the 18th century and by 1817 about 3,000 tons were being produced annually (Mureithi, 2008). The Holy Ghost Fathers of the French Catholic Church brought the Bourbon seeds to mainland Tanzania (notably Bagamoyo and Morogoro) in 1863 and were finally introduced to Kenya through Bura Taita Hills in Kenya during the 1890s. During the same period, the protestant Scottish missionaries were planting the Mocha plants to experiment on them in their respective stations including Kibwezi and Kikuyu (Mureithi 2008). A special variety of coffee named "French Mission" coffee, was developed through over many years of coffee cultivation under different environmental conditions which seemed to have led to its hybridization (Mureithi 2008). Coffee was then planted in restricted to few estates and the natives were not allowed to cultivate it (Thuku et al, 2013)

2.3 Taxonomical classification of coffee

Coffee is classified under the genus and family Rubiaceae. The coffee genus (*Coffea*; Rubiaceae) consists of about one hundred and twenty-four (124) species which naturally occurs in most continents some of which are Africa, Madagascar, and tropical Asia (Davis et al, 2011). From the species found in genus *Coffea* only *Coffea arabica* and *Coffea canephora* are commercially grown (Davis et al., 2006). Although *Coffea liberica* is also cultivated, it is limited to local consumption and is grown on a small scale (Mishra and Slater 2012). Except for Arabica coffee which is an

allotetraploid (2n = 4x = 44), all the other existing species are diploid (2n = 2x = 22) (Charrier and J. Berthaud, 1985). *C. canephora* is widely grown in Africa, Indonesia, and to some extent in Northern Brazil. Cross-pollination studies were conducted on cultivars of *C. arabica* by subjecting them through several cycles of intensive selection using marker genes that were recessive and it was reported that the outcrossing was 7-15%. The degree of self-incompatibility and heterozygosity was high in diploid species in populations has had great consequences for coffee breeding (Charrier and Berthaud, 1985).

2.3.1 Coffea arabica

Species from Ethiopia and Mt. Marsabit were analysed using six enzymes through electrophoretic analysis to study their variations within their natural populations and it was found out the accessions had similar homogenous patterns making it possible to have a description of the electrophoretic type for *C. arabica*. (Berthou and Trouslot, 1977). Reynier et al., (1978) and Louarn (1978) carried out a hierarchical variance analysis that indicated that variations of the genotypes between origins and families for *C. arabica*. Species that are indeed uniform even after several cycles of line selection should be termed variety (or cultivar) (Charrier and J. Berthaud, 1985)

2.3.2 Coffea canephora

The cultivation of *C. canephora* began in the year 1923 with a wider adaptation in different climatic conditions while its initial population was discovered later in 1975 (Berthaud and Guillaumet, 1978). A study on morphological features and floral biology carried out indicated significant variation, with no sub-populations being detected. This was confirmed through an electrophoretic analysis carried out whereby, an analysis of incompatibility points to a

considerable number of S alleles being present within the population (Charrier and J. Berthaud, 1985).

2.4 Interspecific hybridization of coffee

Interspecific hybridization is a significant source of genetic diversity attained through genetic recombination occurring naturally among plant species (Mishra et al., 2018, Ellstrand et al., 1996) The hybridization however is limited to a minor fraction of plant families and genera (Ellstrand et al. 1996). The natural and artificial interspecific hybridization has been reported in the genus *Coffea* (Berthaud, 1976, Owour and Van der Vossen, 1981 Gimase et al., 2015).

C. arabica is an only tetraploid coffee species which is self-compartible whereas the rest of the species are diploids and self-incompatible (Gimase et al., 2015, Gichuru et al., 2008). Origin of Arabica coffee is indicated by its diploid meiotic behavior and that the center of genetic diversity for this species is not found in areas it was distributed to (Carvalho, 1952). Ancestral parents of
C. arabica are believed to be *C. eugenioides* and *C. canephora* (or *C. liberica or C. congensis*) (Narasimhaswamy, 1962). The chromosome pairing of genomes *C. arabica* diploid plants was found to be inferior to interspecific hybrids of *C. canephora* and *C. eugenioides* (Mendes and Bacchi, 1940,). Evidence from studies involving cytogenetic characteristics of coffee and interspecific hybridization among the genus Coffea indicates that all coffee originated from a species with a chromosome number of x = 11.

Natural hybridization between tetraploid and diploid species does occur, with for example C. *canephora* and C. *liberica* (Mahe´ et al. 2007). Timor hybrid is one of the most known spontaneous hybrids that occurred, commonly used hybrid breeding for disease resistance to CBD. The original Timor hybrid was located in Timor highlands within Arabica fields and has been distributed

globally. Lines that have been derived from the Timor hybrids are utilized within improvement programmes of coffee being a source of genes for CBD resistance. Lashermes et al., (2000a) reported that a greater percentage of genetic matter from C. *canephora* is included within Timor Hybrid-derived introgression lines.

Colchicine treatment has been used in developing successful interspecific hybrids from crosses between C. *arabica* and tetraploid *C. canephora* (Gimase et al., 2015). The interspecific hybrids have been utilized in inter genomic recombination and gene introgressions within the coffee improvement programs (Regina et al., 2008). Coffee Breeders have tried to create variability on Arabica coffee by undertaking various hybridization programmes to widen its genetic variation. With new varietal development through hybridization, the morphological variations between and within the existing coffee germplasm need to be determined since the variability within coffee plantations is key in product quality (Hue, 2005, Gichimu and Omondi 2010a).

2.5 Botany of coffee

Coffee species vary from each other on their morphological traits as they range from small to wide robust trees. The tall trees can go up to over 10m in height other being deciduous whereas others are evergreen. The phenotypic variation is wide across the species with variations on leaf size, leaf colour which ranges from yellow, bronze, green, purple-green. The coffee flower has a lobe corolla which is white in colour, a pistil, stamen, and a calyx of five (Kathurima 2013). The coffee tree produces two fertilized beans while others may produce up to five beans from one ovary. The ovules that are fertilized are found within the base of the corolla (Charrier and Eskes, 2004). The maturation of coffee beans after fertilization varies depending on the agricultural practices, cultivars, and environmental factors. *C. arabica* matures early than C. *canephora* taking 6-9

months and 9-11 months respectively (Wintgens, 2004, Kathurima, 2013). The variations are summarized in Table 2.1

2.6 Production and economic importance of coffee

A third of the global population consumes coffee making it one of the most important beverages (Tolessa et al., 2018, Da Matta et al., 2018). The *C. Arabica* L. and *C. canephora* is of economic importance within the genus *Coffea* dominating the global trade (Da Matta and Ramalho, 2006, Davis et al., 2006). Of the total world coffee production, Arbica coffee accounts for 60% of it (ICO, 2019b) and the rest is accounted for by Robusta. Robusta coffee is highly productive however, it is limited by its liquor quality which is low in comparison to Arabica coffee (Da Matta and Ramalho, 2006).

Coffee is a commodity of economic importance in the world being traded second after petroleum and is the largest import product in the United States. The value chain in coffee production is global involving producers, exporters, importers, roasters, retailers, and consumers. 10.2 m ha is under coffee production within in 80 countries in the world. The income that comes from coffee production benefits millions of people in the coffee-growing areas worldwide both directly and indirectly (Lashermes, et al., 2011; Mishra and Slater, 2012). Coffee production is generally determined by considerable environmental factors and the bi-annual bearing which affects yield since a higher crop in a year is always followed by a lower crop the following year.

Table 2.1: Summary of phenotypic variation of coffee species

Trait	Description	Other notes
Stem	Woody	All species
Tree range	Small shrubs to robust trees	Maximum height of 10m
Phenotypes	Wide variation among species	Deciduous to evergreen
Leaves	Range from yellow and dark green to bronze	Some purple green *Coffea Liberica* has the largest leaves
Fruit size	Vary in size ranging from a small pea to a good-sized plum	
Flowers	Consist of a white fine lobe corolla, Calyx of five, stamen and a pistil, Ovary at the base of corolla with two ovules	Produces two beans when fully fertilized
Berry maturity	Depend on agronomic practices, genetics and environmental factors	C. *canephora* 9-11 months C. *arabica* 6-9 months

Adapted from (Kathurima 2013, Charrier and Eskes, 2004, Wintgens, 2004)

2.7 Coffee Berry Disease

2.7.1 Economic importance of coffee Berry Disease (CBD)

Coffee Berry Disease is an important fungal coffee disease of coffee caused by *Colletotrichum kahawae* and can occur within all coffee species (Adepoju et al., 2017). The disease is still confined to Africa but still poses a threat to other coffee-producing continents (Bekele, 2019). Several species or strains of *Colletotrichum* occur on coffee, but only *C. kahawae* (formerly *C. coffeanum)* causes CBD. When CBD first occurred in Kenya, up to 75 to 80% of losses were noted in some of the farms. The CBD infestation occurs during the early stages of cherry development destroying the beans causing difficulties during the processing at the mucilage removal stage since most of

the pulp cannot be removed causing what is known as ‘‘stinkers’’ affecting the quality of the coffee (Hindorf and Omondi, 2010). Once it is not controlled early enough, it progresses during the expansion period of coffee berry-producing mummified berries which cannot be marketed.

2.7.2 Characterization of Coffee Berry Disease

Colletotrichum kahawae infects every stage of coffee cherry development from the flowers to ripe berries whereby the dark sunken lesions spot with symptoms that resemble anthracnose symptoms are formed (Belachew and Teferi 2016, Hindorf and Omondi, 2010). Branches that are dead, mummified berries, and back of twigs are known to harbor the fungi thus becoming the key primary sources of the inoculum. The one-celled, cylindrical, and hyaline conidia of *C. kahawae* germination occurs only when there is free water available and with an optimum temperature within 24 hours after contact with the host (Bekele, 2019). The germ-tubes grow slowly forming dark brown thick-walled appressoria at their tips sticking strongly to the host cuticle and penetrate it through infection pegs 4 to 5 hours later (Belachew and Teferi, 2016).

The fungus produces fruiting bodies forming pink masses of conidia after lesion development. This occurs after an incubation period of about three weeks whereby the black necrotic lesions are developed (Belachew and Teferi, 2016). The fungus produces spores that infect flowers and berry and active lesions which are small dark sunken spots that spread rapidly to the entire berry causing mummification. With favorable climatic conditions, mummified berries are formed and if the berry ripens the anthracnose develops fully causing infection of the bean becoming seed-borne (Hindorf and Omondi, 2010).

2.7.3 Environmental conditions favouring coffee berry disease infection

Favorable weather causes infection to occur that can lead to severe yield loss. Temperature and rainfall (amount and duration), and relative humidity are key environmental factors that determine the occurrence, prevalence, and severity of the disease (Arega et al., 2008; Grima et al., 2008). The losses from CBD infection do vary from one farm to the other with high rainfall, with low night temperature, in combination with high altitude may cause 100% losses. (Bekele 2019, Girma 1995). The optimal temperature for spore germination and lesion development is 22ºC with a temperature range of 10º C to 30ºC. The developing coffee berry is most susceptible in the course of the rapid expansion stage (Hindorf and Omondi, 2010, Girma et al., 2008). CBD infection in the laboratory occurs at a temperature between 17^0C and 22^0C when the atmosphere is completely saturated.

2.8 Breeding for resistance to Coffee Berry Disease

The original objective for coffee improvement in Kenya was to breed for cup quality, yield, and adaptation to different environmental conditions. The varieties K7, SL28, and SL34 were developed based on the objectives stated here but with subsequent CBD outbreak, all the varieties mentioned earlier were found to be susceptible (Hindorf and Omondi, 2010). The development of varieties with CBD resistance was not in the initial breeding programme since the disease was not widespread. As the disease became an epidemic, the breeding for CBD became one of the objectives. This is because the cultural and chemical spray methods that were applied were found not to be economical and sustainable in managing the disease. The strategy was to search for genes that confer CBD resistance and transfer them to the commercial varieties.

From the Inheritance studies that were conducted by Van der vossen and Walyaro in 1980, there was a variation on CBD resistance within the mapping populations developed whereby three major genes on separate loci were revealed (Gichimu et al., 2014, Hindorf and Omondi 2010,). It was found out that Rume Sudan which is a highly resistant cultivar to CBD carries both the dominant R- and the recessive k-gene. The recessive k-gene is linked to the K7 cultivar being moderately CBD resistant. T-locus is found within the Clone 1349/ 269 which is one of the lines of HDT together with Catimor which is a hybrid derivative of HDT with intermediate gene action (Omondi and Hindorf, 2010). These genes have been intensively used in the development of the new CBD resistant varieties (Ruiru 11 and Batian) (Omondi et al., 2016).

2.9 Marker Assisted Selection (MAS)

One generational cycle for Arabica coffee takes up to 8 years and using the conventional test cross approach consumes a lot of time and resources. Using this approach makes it not possible to develop varieties that are disease resistant using within a shorter time. Therefore, the approach using molecular markers assists greatly in selecting desirable traits thus hastening the breeding process. Van Der Vossen and Walyaro (1980) suggested that there is an existence of a locus within the HDT cultivar that confers CBD resistance based on the inheritance studies that were conducted. Three, Randomly Amplified Polymorphic DNA (RAPD) markers were identified by Agwanda et al. (1997) and due to their dominant nature, they have not been used in the breeding programme s for disease resistance. These markers (M62027, M20830, and N18250) are related closely to the T gene.

Gichuru et al. (2008) using SSR markers identified the locus responsible for resistance to CBD by using the mapping population of HDT x SL28 cultivars and which was named Ck-1 located in

segment with a distance of 11 cM. Despite discovering this Ck-1 gene, the authors did not discard the possibility of the existence of another marker that confers CBD resistance even after suggesting that locus was identical to that described by Vander Vossen and Walyaro (1980). The SSR marker- SAT 235 linked to the Ck-1 has been used extensively in coffee breeding programmes within Coffee Research Institute and this marker was confirmed by Gichimu et al (2014b) when evaluating Ruiru 11 sibs. Application of MAS using molecular markers would hasten the breeding process by reducing the linkage drag since the resistant plants can be identified during the early generations thus reducing the breeding time.

2.10 Genetic diversity of coffee

Coffee diversity can be studied in different ways which include biochemical, physiological, molecular markers, and morphological traits (Muvunyi et al., 2017). The molecular and cytological studies that have been carried out show that C. *arabica* was created through the hybridizat ion between two diploid species, C. *canephora*, and C. *eugenioides*, or related ecotypes to these. The low variation among the sub-genomes of C. *arabica*, namely C. *canephora* and C. *eugenioides* indicate that the speciation was recent (Lashermes et al. 1999; Herrera et al. 2002). Furthermore,

C. *arabica* exhibits diploid-like meiotic behavior with bivalent formation despite the low variation among the two constitutive sub-genomes, (Lashermes et al., 2000b). C. *arabica* due to self-compatible and this has caused it to be have a genetic base that is narrow and therefore the breeding programmes have focused on widening the genetic base through transferring traits that desirable (Missio et al., 2009, Van der Vossen 2001). Before the successful production of interspecific hybrids, the different ploidy levels between coffee species with a lack of know-how on genome recombination were the major bottlenecks (Herrera et al. 2002).

For any successful conservation and utilization of existing genetic resources, it is important to understand the genetic variations within the species. Previously characterization of coffee genotypes was based on the morphological and biochemical characteristics. Markers have been developed recently and are currently being utilized in studying the relationships between different coffee genotypes. These markers are limited to, physical space for evaluation, effects caused by the environmental conditions, and the time for phenotypic description which has to be carried out the whole period of plant growth (Weising et al., 2005, Teressa et al., 2010 and Vieira et al., 2010). Using conventional breeding methods, the development of a coffee variety may take up to 25-30 years. The use of markers is an easier way for genetic characterization since there is a more efficient use of resources and time, unlike the conventional system.

The characterization of genetic diversity among coffee genotypes is important for any breeding programmes. The use of markers for the selection of coffee genotypes assists in screening closely related genotypes by grouping them enhancing the breeding process thus increasing the efficiency of the crop improvement in the future (Motta et al., 2014, Missio et al., 2009). The variations in coffee have been carried out using different molecular markers such as RFLP (Lashermes et al., 1999), RAPD (Diniz et al., 2005; Anthony et al., 2002, Silveira et al., 2003), AFLP (Steiger et al., 2002; Anthony et al., 2002) and SSRs (Gimase et al., 2014a; Anthony et al., 2002; Missio et al., 2011, Omingo et al., 2017, Aggarwal et al., 2007). These studies revealed that the cultivated Arabica species has low genetic variation when compared to uncultivated Arabica, Robusta, and Arabusta coffee.

2.11 Quality attributes in coffee

Coffee quality is one of the determinant factors during the selection process in any coffee breeding programme. Genetics and environmental factors affect the final cup quality of a specific coffee genotype. For instance, the Caturra which is known to be dwarf coffee genotype due to the "Cat" gene is highly productive, however, its cup quality is low whereas Maragogype coffee genotypes produce large beans with lower productivity but its demand in the market is very high. Variety Laurina which is a mutant has been found to have the lowest levels of caffeine (0.6 % dm) (Silvarolla et al., 2004).

The quality of coffee has been described by the International Organization for Standardization as "the ability of a set of inherent characteristics of a product, system or process to fulfill the requirement of customers and other interested parties". These characteristics can also be termed "attributes". The description of quality in coffee has changed over time and currently varies at different levels the producer to the consumer levels (Leroy et al., 2006). The definition of quality varies along with quality check and include quantity, quality (bean physical traits and liquor quality) determining the final market price. Consumers may define their qualities depending on the tastes and prices, health effects, the origin of the coffee, and the environments under coffee production. (Leroy et al 2006, Belay et al 2016). The coffee cup quality is often described as drinking or liquor quality (Muschler, 2001).

The final price of coffee in the market is determined by its quality and usefulness (liquor or cup) (Agwanda et al., 2003, Belay et al., 2016). Coffee-producing countries have ensured that the supply quality coffee beans is their priority. Therefore, the production of varieties with high cup quality is considered of importance in most breeding programmes as disease resistance and

increased productivity. (Abadiga, 2010). Various factors affecting the quality of coffee include climate (environmental factors), post-harvesting processes, roasting, growth conditions, and genetics (Ameyu 2017, Schnabel 2017, and Yang et al., 2016).

2.11.1 Organoleptic quality

Organoleptic relates to those attributes that are perceived by human senses in evaluating the quality of foods (Kathurima, 2013). The sensory evaluation is a technique used in analyzing responses of products measured through the percieveness of sight senses, taste, hearing including touch, and analyzing them. (Martens, 1999). The ISO-5492 (2008) describes the various sensory attributes used in coffee whereby flavour is termed as a combination of both senses of smell, taste, and oro- nasal chemesthesis. The gaseous chemical that is emitted after roasting beans are ground and during brewing are considered as the aroma (Lingle, 2001). During brewing both soluble organic and inorganic chemical compounds are extracted from the roast giving the liquid what is termed as taste (Lingle, 1996).

The assessment of the organoleptic quality of coffee by relying on overall sensory evaluation is difficult (Leroy et al., 2006). Hedonic and descriptive methods have been used in evaluating sensory attributes of coffee beans. The hedonic analysis evaluates the consumer preferences by randomly selecting at least 60 assessors on the population characterized by a specific preference that is being sought. In the descriptive analysis, the trained assessors are used to discriminate various coffees using a described profile using specific descriptors based on the acidity, flavour, and aroma of the beverage (Leroy et al., 2006, Van der Vossen, 1985; Agwanda, 1999, Kathurima 2013). Owuor (1988) reported that the results after ranking various cups from different liquors

were similar and therefore using the panel of liquors is more reliable in describing the sensory attributes of various cultivars.

The definition of organoleptic quality could be unreliable since consumer preferences for specific tastes are taken into account during assessment which might not be stable for the roasters and consumers (Wintgens, 2004; Leroy et al., 2006). Immediately coffee is roasted, the smell that emanates from dry ground coffee is referred to as fragrance/aroma. Overall sensory evaluation determines the organoleptic quality of a specific cultivar. (Leroy et al., 2006). The multiple aromatic compounds found in coffee has made the procedure for organoleptic tasting more challenging and complex. Extensive training is required for the panelists who participate in tasting coffee to ensure the reliability of the results obtained during the activity regardless of the approach used (Findlay et al., 2006). Different countries including Kenya, Colombia, and Ethiopia have designed their methodologies in evaluating coffee cup quality (Asfaw, 2008).

Organoleptic quality has been used in the coffee improvement programmes since this acts as the final determinant of prices in the coffee market. Agwanda (1999) analyzed organoleptic attributes to assess their suitability for use during selection for liquor quality improvement and found out that flavour was the most ideal trait in beverage quality selection. He reported that flavour and preference had a high genetic correlation with acidity, aftertaste, and balance and were easy to determine organoleptically when compared to the other organoleptic attributes. Walyaro (1983) and Van der Vossen (1980) reported that overall standard (preference) was highly heritable implying that it is possible to select for high cup quality using this attribute.

2.11.2 Physical quality

Bean size, defined as a grade, is a key factor during the marketing since the price is directly correlated with the coffee bean grade where smaller coffee beans fetch lower prices and vice versa (Leroy et al., 2006). Roasting is preferable when done using beans of the same size. The different bean sizes lead to ununiformed beans during roasting since the smaller beans roast faster tending to burn while the larger beans end up being under roasted affecting the final cup quality (Barel and Jacquet, 1994). International Coffee Organization (ICO, 2001) came up with the Coffee Quality Improvement Programme (CQIP) with the view that beans to be exported have to meet set standards. The standards were exporting coffee beans that were clean without any foreign material whether from coffee or non- coffee origin, without any defects, the physical visual look. Different environmental factors and the genetics of bean influence the final bean shape and size**.** (Dessalegn, 2008). Van der Vossen (1985) reported that the variations within the different Arabica coffee varieties exist in which SL28 produced big beans with an exceptional cup quality when compared to Caturra and Rume Sudan which produce small beans and have low quality. Despite Hibrido de Timor producing bigger beans than SL 28, its cup quality was poor (Mekonen, 2009).

2.12 Biochemical compounds of coffee

The interaction of various biochemical compounds in coffee including caffeine, oil, sucrose, chlorogenic acids, and trigonelline determines the final cup quality of coffee (Aluka et al., 2016). Different factors characterized together such as aroma and taste within the coffee bean are related to the biochemical composition of the bean that affects the final cup quality. The characterization of different coffee varieties within and between species has been conducted by examining

biochemical compounds levels (Ky et al., 2001). These biochemical compounds act as aroma precursors and their correlations can be used in the determination of coffee quality for a specific variety.

2.12.1 Caffeine

Caffeine is a major alkaloid found in different kinds of products consumed including both food and liquids (Mumin et al., 2006; Singh and Sahu, 2006; Najafi et al., 2003). There are over 63 species of plants where this chemical occurs naturally and is mainly in the leaves and fruits/seeds (Mumin et al., 2006). The biological role of caffeine in plants has not been ascertained, although there are some hypotheses that caffeine shields the plant from pests and that it has an allelopathic effect on other seeds affecting their germination (Hollingsworth et al., 2002). Caffeine has a molecular weight of 194.19 g, a melting point at 236°C, and also subliming at 178°C with a ph value varying from 6 to 9 (Mumin et al., 2006; Clarke and Macare, 1985). There are variations on the levels of between and even within species (Silvarolla et al., 2004; Ky et al., 2001).

When compared to other coffee species, Robusta coffee has a higher quantity of caffeine averaging of 2.2%, with Arabica having an average of 1.2% (Franca et al., 2005, Belay, 2010;). Liberica which commercially less important species contains 1.35% of caffeine and 1.72% is found within Arabusat coffee (Clarke and Macarae, 1985). Genetic and environmental factors are the major causes of variations of caffeine content in the coffee beans. Farah et al., (2006) and Clarke and Macarae, (1985 have reported that the roasting of coffee to some extent did cause loss of other compounds but did not reduce the content of caffeine.

Different levels of caffeine content in the coffee bean cause different effects (physiological and psychological) (Zhang et al., 2005; Minamisawa, 2004; Yukawa, 2004). The metabolization of caffeine through demethylation occurs to about 80% of the total administered caffeine (1,3,7-trimethylxanthine) to paraxanthine (1,7- dimethylxanthine). This occurs through the liver *cytochrome* P-450 1A2, whereas almost 16% is converted to theophylline and theobromine (Benowitz et al., 1995). Higher levels of caffeine consumption have been linked to improved human performance in memory, reaction time and also reasoning in terms of visuospatial. However, an increase in the concentration of caffeine can cause heart disease, kidney malfunction, and asthma among other disorders (Belay 2011).

2.12.3 Carbohydrates

Based on the beverage quality, Arabica coffee is preferred by most consumers since it is less bitter with good flavour (Geromel et al, 2008). Over 50% of the coffee bean dry weight is accounted for by the carbohydrates (Wrigley, 1988). Sucrose during roasting degrades faster to form the a hydro-sugars and glyoxal being ley in determining flavour and aroma (De Maria et al., 1995). They react with amino acids through Maillard reaction to form aliphatic acids, hydroxymethylfurfural, other furans, and pyrazine. The decomposition of the sugars including monosaccharides results in the formation of Furan derivatives (Flament and Bessière-Thomas, 2002). The composites roasting is regarded as essential in contributing to the final coffee flavour (Grosch 2001, Ky et al., 2001). Arabica coffee has a sucrose range of 5.1% - 9.4% of dry matter in coffee beans being higher than Robusta having a range between 4% to 7% (Ky et al., 2001; Campa et al., 2005). During roasting, there is the formation of different compounds that result in various flavour types and pigmentation, and the sucrose within the bean is converted to sugars (Flament and Bessière-Thomas, 2002).

2.12.4 Trigonelline

Trigonelline is a nitrogenous compound that is derived from the methylation of the nitrogen atom of nicotinic acid (niacin) and alkaloid with chemical formula $C_7H_7NO_2$ and a 137.138 g/mol molecular weight (Nuhu 2014, Kathurima, 2014). Trigonelline is produced through nicotinic acid methylation using methionine (Anaparti, 2013). Trigonelline which naturally occurs in coffee is also a major source is used in discriminating both different coffee species (Robusta and Arabica) during roasting (Bicho et al., 2011). Various studies have shown that Arabica displays different trigonelline levels in *C. arabica* and *C. canephora* with 0.88% to 1.77% and 0.75% to 1.24% dmb respectively (Ky et al., 2001). Trigonelline is a vitamin B6 derivative with 100% solubility in water having a bitter taste contributing to undue bitterness in coffee (Anaparti, 2013). During roasting, trigonelline degrades to various compounds that include pyrroles and pyridines that affect the flavour through release of nicotinamide and niacin (Clifford, 1985; Ky et al., 2001).

2.12.2 Chlorogenic acids

Chlorogenic acids (CGA) are polyphenols that mostly occur in coffee forming a significant part of antioxidants found in coffee (Svilaas et al., 2004; Wen et al., 2004). CGA which has been analyzed extensively belongs to hydroxycinnamic acids classes comprising caffeic acid (3, 4- hydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric (4- hydroxycinnamic acid), and sinapic acid (3,5-dimethoxy-

4-hydroxycinnamic acid) (Zhu et al., 2006; Manach et al., 2004). The genetics of the coffee variety, maturation of the beans, agricultural practices, environmental factors affect the amounts of the CGA found (Farah et al., 2005b; Clifford, 1985). CGA has a range of 4 to 8.4% and 7 to 14.4% within Arabica and Robusta coffee respectively and intermediate levels within the interspecific

hybrids (Farah et al., 2005a, 2005b). A known almost caffeine-free species is C*offea pseudozanquebariae* has been reported to also have very low CGA levels of 1.2% (Belay 2011). Maillard and Strecker's reaction causes chlorogenic acids to form pigments affecting the taste and flavour of coffee beans, influencing coffee beverage (Belay, 2011, Variyar et al., 2003). A high CGA level is found in low-quality samples.

2.12.5 Lipids/oils

Oil is the key determining factor of the flavour produced by coffee during roasting thus the quantity in the coffee green is an indicator of the performance of a specific variety to cup quality. The most studied part of lipids within the coffee Arabica beans is the fatty acids. Triacylglycerols, sterols, and tocopherols form part of the lipids in coffee and are commonly found within the oils of vegetables (Cheng et al., 2016). The average amount of lipids found in Arabica and Robusta coffee is 15% and 10% respectively. A higher percentage of the total lipids in the green coffee bean is found in the endosperm whereas the rest is found on the outer layer of the beans higher percentage of lipids is within the bean endosperm (Wilson et al., 1997). The quantity of triacylglycerols with fatty acids found in the coffee oil is the same as those found in oils that are (Speer and Kölling- Speer, 2006). Triacylglycerols sterol esters, sterols/triterpene alcohol, hydrocarbons together are the major lipid oil elements whereas minor components are hydrolyzed products of triacylglycerols (Speer and Kölling-Speer, 2006). Better roasts of coffee are found in coffee with high oil content since the oils are expelled to the bean surface preventing further loss of compounds by trapping the aroma. (Kathurima, 2013).

CHAPTER THREE:

PERFORMANCE OF ARABUSTA COFFEE HYBRIDS AND BACKCROSS PROGENIES FOR GROWTH TRAITS AND BEAN YIELD

3.1 Abstract

The development of improved coffee varieties for improved bean yield has been one of the main objectives of coffee breeding. This study aimed at evaluating Arabusta hybrids and its backcrosses for yield performance of the using the morphological traits during the early stages of coffee crop development. Nineteen different coffee genotypes were assessed at Siaya ATC and KALRO- Alupe with the morphological data for both growth and yield traits being recorded during the 2017 and 2018 growing seasons. The traits measured included, number of berries per node, tree height, percentage of berries per node, the total number of laterals the total number of berries, number of bearing nodes, number of bearing primaries, length of the longest primary, number of berries on the highest bearing node, mean of the number of primaries and red ripe cherry. The traits were measured from five trees per plot and their means derived. The results showed t significant differences in growth and yield traits among the coffee genotypes and between the locations. The yield increase from the year 2017 to 2018 was significant ($P \leq 0.05$) by 3452g/tree and 647g/tree at KALRO-Alupe and Siaya ATC respectively. The yield had positive significant correlations with percentage berries per node ($r= 0.61$), berries on the longest primary ($r= 0.58$), berries per node on the longest primary($r=0.60$), berries per node, and nodes with the highest number of berries ($r= 0.55$) respectively. The genotypic coefficient of variation (GCV) values for the morphological traits varied from 6.50 to 39.11%. with broad-sense heritability ranging from 0.15 to 0.59 with bean yield recording heritability of 0.31. The G x E interaction for yield was significant and this showed that there exists variation of genotypes and environments. The best

performing hybrids are recommended for commercial cultivation in the specific environments based on their yield performance subject to evaluation of the bean and liquor quality.

3.2 Introduction

The global production of coffee increased in the year 2018/2019 by 1.6% to 168.77 million bags when compared to the production of year 2017/2018. Out of the total production, 109.41 million bags were exported with an increase of 10.2% when compared to the 2017/2018 total exports. The exports for both Arabica and Robusta coffee were 64% and 36% respectively (ICO 2019b). Coffee in Kenya with foreign earnings of US$230 million comes fifth after diaspora remittance, horticulture, tourism, and tea exports and also supports the livelihoods of approximately 800,000 farmers (ICO 2019a). Arabica coffee is the dominant species cultivated in Kenya covering over 98% of total hectares under coffee production whereas Robusta coffee occupies the rest. Despite Kenya producing high-quality coffee, its production has been declining since the 1980s from about 1.7million bags to current estimate of 900,000 bags being produced annually (Karanja and Nyoro 2002, ICO 2019a). The decrease in production has been caused by an increase of human population within urban areas and agricultural land converted into real estates, increased cost of production in terms of inputs, use of chemicals for pest control, and the changing weather patterns due to climate change.

Interspecific hybrids in Kenya have been developed from crosses of induced tetraploid Robusta and Arabica coffee resulting in a hybrid termed as Arabusta coffee. The aim is to develop a high yielding coffee variety which is not only disease-resistant but has a high cup quality. The variety should also perform better than Robusta coffee and adapt to lowlands which include areas found within the coastal and lake victoria regions. This will in turn lead to increased coffee production

in the country. Coffee varietal development takes up to 30 years to be released and this due to its long productive nature and biennial bearing. These conditions, therefore, cause a greater challenge in releasing the variety with a short period. Identification of genotypes that have growth traits with a positive correlation with yield during the early production years is critical to shortening the breeding cycle. Growth and yield characters' influences yield and this has been reported in other crops (Gichimu and Omondi 2010).

For any successful breeding, it is important to assess the variations during the selection of the phenotypic traits. This will assist in determining the response to selection as a result of the genetic diversity found within the different genotypes. The genotypic parameters (Genetic coefficient of variation-GCV and phenotypic variation of coffee-PCV) have been utilized in the identification of variations found in the genotypes being evaluated (Solomon, 2009). The effectiveness of any selection for genotype performances for quantitative traits is referred to as heritability. For this to be more useful, it needs to be combined with high genetic advance to select the highest performing genotypes for the quantitative and yield characters. Heritability during selection is important factor in maximizing the potential found in specific genotypes during genetic improvement (Getachew et al., 2017). The studies of Ethiopian coffee by Yigzaw, (2005) and Atinafu, (2017) have been able to show that there exist high heritability values on some of the morphological traits. Some of these traits include secondary branches, primary branches, internode length, and the hundred bean Correlation is key in studying relationships within traits under study since the genetic variation among them could be due to genetic effects is key is identifying useful traits during selection (Anim-kwapong and Adomako 2010).

3.3 Materials andMethods

3.3.1 Experimental materials

There were nineteen coffee genotypes evaluated during the study and included Arabusta coffee hybrids, six different backcross derivatives of Arabica to Arabusta hybrids, Congusta, Congensis, Arabusta cultivar, Robusta, *C arabica* (Batian), and *C arabica* (Ruiru 11) (Table 3.2). The Uganda Tetraploids used in generating the interspecific hybrids were sourced from Uganda whereas the Robusta and Arabica genotypes are from Coffee Research Institute- Ruiru. The Arabica genotypes in the study are Kenyan commercial varieties.

3.3.2. Description of the experimental Site

The establishment of the trials was conducted in Siaya ATC (Siaya County) and KALRO Alupe (Busia County) located near the Lake Victoria basin in the low altitude zones suitable for planting Robusta coffee. Siaya lies between 0º 30 N' and 0º 45' E with an altitude that varies from 1,135m to 1,500m above sea level. The soils are moderately acid with moderate low exchangeable acidity. The exchangeable potassium indicates an acute deficiency in soil potash. The exchangeable calcium and magnesium are within the adequate sufficient range. Busia county is also located at 0º 30 N' and 34º 30' SE and the altitude ranges from 1241m to 1343m above sea level. Soils within the site were strongly acid with fairly high exchangeable acidity. The exchangeable calcium and magnesium indicate a deficient supply of these basic macronutrients. The rainfall and temperature patterns for the two locations are described in Table 3.1.

Table 3. 1 Rainfall patterns and temperature ranges for the years 2017 and 2018

	Annual Rainfall (mm)		Temperature range ^{0}C	
Years	**2017**	**2018**	**2017**	**2018**
Siaya	1105	1844	15.0-30.4	14.4-32.4
Busia	1526	1551	17.9-30.8	17.2-33.0

Source (Kenya Meteorological Department

Table 3. 2. Arabusta hybrids, backcross progenies and varieties used for yield and morphological evaluation at Alupe and Siaya

S/no.	Code	Pedigree information	Genotype description
1	ARH1	B11 2415 = CATURRA X B6. 1834 = (SL 28 X UT 6)	Arabusta Hybrid
2	ARH2	B11 2554 = CATURRA X B6. 1834 = (SL 28 X UT 6))	Arabusta Hybrid
3	ARH3	B11 2406 = CATURRA X B6. 1834 = (SL 28 X UT 6)	Arabusta Hybrid
4	ARH4	B11 2407 = CATURRA X B6. 1757 = (SL 34 X UT 6)	Arabusta Hybrid
5	ARH5	B11 2556 =CATURRA X B6. 1757 = (SL 34 X UT 6))	Arabusta Hybrid
6	ARH6	B13 2271 = SL 28 X B6. 1835 = (SL 34 X UT 6)	Arabusta Hybrid
7	ARH7	B14 1140 = SL 28 (SL 34 X UT 8)	Arabusta Hybrid
8	BC01	B13 2400 = SL 34 X B6. 1764 = (SL 34 X UT 6)	Backcross
9	BC02	B13 2567 = SL 28 X B6. 1778 = (SL 28 X UT 6)	Backcross
10	BC03	B13 2286 = SL 28 X B6. 1836 = (SL 28 X UT 6)	Backcross
11	BC04	B13 2617 = SL 34 X B6. 1616 = (SL 34 X UT 6)	Backcross
12	BC05	B13 2806 = SL 34 X B6. 1756 = (SL 34 X UT 6)	Backcross
13	BC06	B14 1108 = SL 28 (SL 28 X UT 8)	Backcross
14	CV1	PL 4 CONGUSTA 161 CRAMER	Congusta
15	CV2	PL 4 CONGENSIS 263 CRAMER	Congensis
16	ARV	PL 4 169, 177, 178 ARABUSTA	Arabusta
17	Robusta	Cultivar	Robusta
18	Ruiru 11	Hybrid	Arabica
19	Batian	Pure line	Arabica

3.3.3 Experimental design

Each plot had five trees of coffee spaced at 3m by 3m. Randomized Complete Block Design with three replications was used when laying down the experiments at the two locations. A guard row of Robusta surrounded the plots within a distance of 4m from the block. The holes were dug to a depth and width of 0.65m by 0.65m respectively and left for one month to dry up. After one month the holes were filled using topsoil mixed with, cattle manure at the rate of 4.3 M^3/Ha and Triple Super Phosphate (TSP) 46% P_2O_5 at a rate of 137.5kg/Ha left for another one month before planting the coffee seedlings for the soil to be compact. The seedlings were propagated at KALRO- CRI through cuttings and established when they were 9 months old. All management practices such as weeding and fertilizer application were carried out per the recommendations described in the CPR, (2016). Calcium Ammonium Nitrate (CAN) 26% N was applied in two splits in the first

two weeks during the two months during the long rains season at a rate of 400 kg/Ha whereas Nitrogen Phosphorus Potassium (NPK) was applied in the first week of the month during the short rainy season at a rate of 375 kg/Ha. Muriate of Potash (MOP) was applied at a rate of 221kg/Ha two months after NPK application. Magmax was applied to Busia farm at a rate of 1268kg/Ha to remove the excess acid. Data on morphological traits were collected and recorded on

3.3.4. Growth parameter measurements

The growth and yield parameters were recorded as described by (Walyaro, 1983). They included; Percentage of berries per node where the bearing nodes with berries, flowers, or flower buds were counted and expressed as a percentage of the total number of nodes on the same tree and this was collected from five trees per plot. The total number of berries on the three longest primaries was counted and the mean was derived. The number of bearing primaries recorded as the total number of primaries carrying berries, flowers, or flower buds. The number of berries per node was obtained as the mean number of berries per node on the selected four primaries. Tree height was recorded as the length from base to the tip of the tree (cm). The total number of laterals was derived by counting all lateral per tree, length of the longest primary, number of berries on the highest bearing node from the longest primaries, number of bearing nodes on the longest primaries, mean of the number of primaries, mean of 100 berry weight (g). All these parameters were measured from five trees per plot and their means derived. The harvesting seasons began from May to July and September to November in 2017 and 2018 where the red ripe cherry was picked from the coffee trees. The red ripe cherry harvested from each tree, weighed, recorded, and bulked per replication. This yield was recorded as the yield of ripe cherry indicated as grams per tree.

3.4 Statistical analysis and determination of genetic parameters

Data from yield and growth characters were subjected to Analysis of Variance (ANOVA) with effects declared at a significance level of 5% using the General Linear Model (GLM) was used (Jansen, 1993). The Genstat statistical software version 2016 was used in the analysis of the data. $Y\hat{} = \beta_0 + \beta_1X_1 + \beta_2X_2 + ... + \beta_kX_k + E_i$

Where,

For each observation $i=1,.....,n.$ where n is the observations of one dependent variable

$Y\hat{} = j^{th}$ observation of the dependent variable

$j= 1,2,....., k$

X = is the observation of the j^{th} independent variable

β = parameters to be estimated

Ei = Distributed normal error

Least Significance Difference (LSD) was used to separate the means (Martin et al., 1978). Separate as well as combined analysis of variance was performed on data from the two locations. The correlation was calculated to show the relationship between growth and yield characters using Pearson's Correlation Coefficient.

Genotypic and phenotypic variances were calculated using the formula by Baye (2002) as follows

i) Genotypic variance, GV= (MSg –MSe)/r, MSg being the mean square of genotypes, MSe = mean square of error, and r = number of replications.

ii) Phenotypic variance, PV= GV + MSe, where GV = genotypic variance and MSe = mean square of error.

Phenotypic and genotypic coefficient of variation was calculated according to Singh, and Chaudhary (1985) as follows:

iii) Phenotypic coefficient of variation, PCV = (PV/X) * 100, where PV = phenotypic variance and X = mean of the character

iv) Genotypic coefficient of variation, GCV = (GV/X) * 100, where GV = genotypic variance and X = mean of the character

v) Broad sense heritability H = GV/PV, where GV = genotypic variance and PV = phenotypic variance (Falconer 1989)

vi) Genetic advance (GA) expected and GA as a percent of the mean assuming selection intensity of superior 5% of the genotypes.

The GA was calculated as suggested by Assefa et al (1999) as follows:

GA = K * (PV/X) *H

GA (as % of the mean) = (GA/X) * 100, where K is a constant (K varies depending on selection intensity if the latter is 20%, stands at 1.40), PV/X is phenotypic standard deviation

X= mean of the trait being assessed

vii) Expected response to selection (Re) was estimated as(2) □□ = □ $\sqrt{\square\square}h2$ Where $i = 1.40$ at 20% selection intensity, V_p = phenotypic variance for a trait, and h^2 = broad-sense heritability for a specific trait (Singh and Chaudhary 1985).

GGE biplot method was used to analyse G x E interaction as described by Yan, 2001. The biplot graphs were derived through a multivariate analysis of yield data by using the Genstat software version 2018 for the genotype and environmental interaction study.

3.5. Results

3.5.1 Growth and yield traits of coffee genotypes

The variations of the growth and yield traits were significantly ($P \leq 0.05$) different among the genotypes based performance on the morphological traits across the two environments in the year

2017 and 2018 (Table 3.3). The genotypes in Busia recorded significantly higher numbers for all the growth and yield traits when compared to those in Siaya except for the number of laterals (Table 3.5). At Siaya, the number of berries on the bearing primaries, number of berries on each node, nodes with the highest number of berries, and the number of total primaries were significantly not different among the coffee genotypes. Genotype, Batian recorded significant ly ($P \leq 0.05$) a high percentage of berries per node in both environments (64.7 and 42.8). There was variation in the total number of berries on the longest primary where genotype ARH4 recorded significantly ($P \leq 0.05$) higher number of berries in Busia (131.4) and Batian recorded the highest in Siaya (61.6) (Table 3.5). The yield was significantly different across the two locations where genotype ARH1 and BC06 recorded higher yields Busia and Siaya respectively (Table 3.5).

The variations of the morphological traits in both locations during the growing seasons of 2018 were significantly different (Table 3.4). The berries on the longest primary were significantly ($P \leq 0.05$) different among the genotypes at Siaya (Table 3.6). Berries per node on the longest primary varied whereas in Siaya genotype ARV recorded the highest number but genotype ARH3 recorded the least. The number of primaries per tree varied among the genotypes where genotype ARH3 recorded significantly ($P \leq 0.05$) higher number (77) whereas Ruiru 11 recorded the lowest number in Busia (59). Genotypes in Siaya recorded higher yield when compared to Busia with genotypes ARH1 and ARH4 being the highest yielding genotypes in Busia and Siaya respectively (Table 3.6).

Table 3. 3. Mean squares for growth and yield traits of coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) in the growing season of 2017

	Busia			Siaya		
	Rep	**Genotype**	**Error**	**Rep**	**Genotype**	**Error**
DF	2	18	36	2	18	36
%BN	177.1	280.9**	103.9	582.0	2527**	91.8
BELP	20406.0	3155**	1004.0	669.5	5541***	172.6
BPR	20.9	31.0	25.4	498.6	278.7	198.7
B/N	13.4	16.8***	4.3	1.3	3.2	1.9
H	29.6	891.1***	150.2	603.3	828.6**	284.7
LAT	7.5	21.1	11.9	10.1	27.5***	92.6
LPR	3.0	12.8***	3.7	7.3	9.8*	4.8
NHB	89.3	53.9***	7.8	22.7	13.8	8.4
NBLP	12.2	25.4***	7.6	29.3	14.8**	5.2
PR	57.8	47.7***	14.8	40.8	32.9	29.4
Yield (g/tree	1526859.0	2190519***	644994.0	1400800.0	751026.0	296087.0

Table 3. 4. Mean squares for growth and yield traits of coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) in the growing season 2018

	Busia			Siaya		
	Rep	**Genotype**	**Error**	**Rep**	**Genotype**	**Error**
DF	2	18	36	2	18	36
%BN	1210.7	244.5	224.4	672.4	265.3	141.1
BELP	1714.2	2820.0	2836.0	3296.0	4837**	1560.0
BPR	786.0	72.0	82.9	2.4	45.3	60.8
B/N	18.1	2.9	3.3	3.7	6.7***	2.0
H	0.5	1290.6***	289.5	165.0	1112.7**	492.6
LAT	12.5	14.7**	6.2	7.5	30.7***	9.6
LPR	48.2	329.8**	123.8	85.2	465.3***	123.9
NHB	81.8	29.7	20.8	29.1	56.5**	21.4
NBLP	125.4	27.8	20.4	49.8	28.2**	12.5
PR	204.5	66.9*	33.8	37.7	50.5	57.6
Yield (g/tree)	405.2	2869.4***	198.6	296.7	2593.9	668.4

Key: *, ** and*** indicates significance at (P<0.05), (P<0.01) and (P<0.001) respectively. % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

Table 3. 5 Growth and yield traits for coffee genotypes taken at KALRO-Alupe (Busia) and Siaya ATC in 2017

Genotypes	%BN		BELP		BPR		B/N		H		LAT		LPR		NHB		NBLP		PR		Yield	
	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si
ARH1	53.4	11.6	122.9	6.8	31.7	14.0	8.6	1.7	153.6	139.9	11.1	11.4	95.4	86.2	17.9	2.4	26.8	23.7	50.6	45.6	3941.1	970.9
ARH2	35.2	23.1	39.0	22.0	18.8	19.4	5.1	3.4	125.0	124.0	11.1	10.4	73.6	78.8	7.9	5.4	20.9	23.0	42.0	46.3	533.3	1008.3
ARH3	47.0	11.6	76.0	6.1	24.8	10.7	7.3	2.2	131.2	120.1	13.9	12.7	71.5	65.1	14.8	3.2	23.1	20.4	45.8	45.3	943.8	316.7
ARH4	46.7	20.7	131.9	27.8	24.2	20.6	11.0	4.4	120.3	124.0	10.5	9.9	84.0	80.0	19.6	8.4	25.6	23.2	45.4	51.0	1593.3	893.3
ARH5	44.8	23.1	91.4	20.4	26.7	14.4	6.9	4.1	114.0	130.4	12.7	6.4	89.3	84.2	13.7	6.3	26.9	23.8	43.3	51.6	2166.0	724.6
ARH6	38.5	17.6	45.2	17.3	22.3	17.6	4.9	2.9	136.3	137.9	8.5	11.9	75.6	90.2	8.7	4.4	22.1	23.4	39.8	46.8	708.3	619.4
ARH7	46.9	10.9	113.0	7.4	27.5	16.4	8.6	2.0	130.4	128.6	9.1	9.4	88.8	98.2	15.6	3.3	27.0	25.9	46.3	49.2	1780.6	1270.8
BC01	43.6	10.8	90.8	8.8	29.8	13.0	8.4	2.4	145.0	152.6	9.8	8.7	94.5	97.0	15.2	3.2	24.6	25.5	46.2	54.2	2041.1	438.9
BC02	35.1	17.7	43.4	9.2	25.0	20.0	4.3	1.9	140.5	137.4	15.1	13.0	93.9	94.0	8.3	3.7	27.0	25.3	45.2	49.2	408.3	666.7
BC03	29.7	13.3	23.3	7.6	25.6	15.3	2.7	2.7	153.8	133.2	9.9	10.8	92.0	87.2	5.9	3.0	25.1	22.3	45.7	43.3	1265.0	290.6
BC04	48.6	21.4	111.4	17.3	24.8	21.6	10.3	2.8	145.9	173.3	3.7	7.0	100.2	112.8	16.2	4.9	22.5	24.6	40.9	51.7	787.5	234.6
BC05	61.4	28.2	115.0	29.4	30.0	19.7	6.6	2.8	159.2	139.7	9.2	11.7	106.9	83.8	13.0	5.2	28.6	22.2	49.0	46.9	1266.7	855.2
BC06	30.3	11.1	51.0	9.6	27.3	16.7	5.1	2.9	114.5	123.7	14.8	15.1	90.2	92.9	9.6	3.0	28.2	27.6	41.9	45.1	2248.9	2168.9
CV1	38.3	21.7	103.9	26.7	24.9	15.6	10.8	4.1	136.4	141.2	9.6	5.9	87.4	88.2	20.0	6.6	23.8	23.3	45.8	50.9	1254.4	748.0
CV2	40.1	11.5	112.4	9.5	27.2	8.3	11.0	2.9	146.7	136.8	7.2	5.6	94.8	86.0	20.3	3.7	25.0	21.1	48.2	46.2	1797.2	148.6
ARV	53.3	31.2	90.5	28.3	30.6	18.2	6.2	3.9	168.0	158.3	10.8	7.6	115.0	110.3	13.3	7.1	25.6	24.4	49.1	45.2	1777.8	422.8
Robusta	37.5	4.6	74.9	5.9	24.1	7.4	7.1	1.4	128.0	155.1	9.6	3.6	97.9	111.9	10.8	2.4	24.7	26.6	53.7	53.1	2445.0	229.2
Ruiru	52.3	26.8	99.5	25.7	22.9	22.9	7.5	3.3	100.0	96.1	11.5	12.9	81.5	69.7	13.8	6.8	24.7	22.7	37.7	43.2	2462.5	1082.3
Batian	64.7	42.8	116.4	61.6	22.7	22.4	6.8	5.3	125.1	134.8	11.5	10.2	85.0	87.7	13.2	9.9	26.6	24.4	41.2	46.3	2284.4	1414.5
LSD	16.9	15.9	52.5	21.8	8.3	13.5	3.4	2.3	20.3	27.9	5.7	4.8	13.3	15.9	4.6	4.8	3.2	3.6	6.4	9	1329.9	901.1
Cv%	6.8	29.2	0.2	32.5	4.1	17.4	11.5	8.8	0.9	4.1	6	7.5	1.1	2.7	15.9	22.4	1.6	2.6	3.9	3.1	17	35.6
Ftest	S	S	S	S	S	NS	S	NS	S	S	NS	S	S	S	S	NS	S	S	S	NS	S	S

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree). S=Significant, NS=Non significant

Bu- Busia Si- Siaya

Table 3. 6. Growth and yield traits taken from coffee genotypes at KALRO-Alupe (Busia) and Siaya ATC in 2018

	%BN		BELP		BNLPR		BPR		B/N		H		LAT		LPR		NHB		PR		Yield	
Genotype	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**	**Bu**	**Si**
ARH1	57.1	53.3	120.1	88.3	17.1	14.3	51.7	54.6	4.0	3.3	205.0	199.0	8.6	12.7	108.0	100.0	12.4	14.5	71.3	67.4	4580.8	2329.2
ARH2	53.8	63.9	79.6	115.3	13.6	16.7	47.3	55.3	3.2	4.4	165.0	177.0	7.9	13.1	89.8	95.2	11.5	15.7	72.8	70.0	1240.0	2004.9
ARH3	61.0	36.9	78.7	32.2	17.3	8.8	58.3	50.0	2.7	1.4	179.0	161.0	10.7	12.8	90.1	85.3	9.9	7.7	77.3	62.1	1245.1	2668.2
ARH4	68.3	68.1	127.0	177.8	19.8	18.6	56.6	53.6	4.4	6.6	155.0	160.0	4.4	9.3	87.9	92.6	14.7	22.5	69.2	69.9	4550.0	8227.8
ARH5	66.3	61.6	140.1	134.0	20.5	17.2	50.3	54.7	4.6	4.8	152.0	170.0	4.9	8.8	96.1	97.0	15.9	18.9	65.3	68.3	3930.1	3677.6
ARH6	58.2	65.2	87.9	102.3	16.9	17.8	46.9	54.7	3.0	4.0	190.0	161.0	6.8	9.8	107.0	104.0	10.6	14.1	64.6	65.7	1422.9	3044.7
ARH7	33.2	54.5	60.2	50.1	11.5	14.7	50.3	49.6	1.7	1.9	174.0	170.0	7.3	10.4	104.0	106.0	8.3	10.4	63.3	62.9	1616.7	1977.1
BC01	56.5	62.6	122.1	125.4	16.4	17.2	51.8	58.6	4.3	4.6	182.0	198.0	8.3	11.1	110.0	108.0	18.8	18.3	66.3	71.9	2778.2	3724.2
BC02	46.8	52.0	79.8	98.4	12.7	13.9	47.4	59.1	3.0	3.7	194.0	192.0	11.4	10.9	106.0	108.0	8.4	11.2	71.6	73.8	2830.8	3167.8
BC03	49.4	56.3	103.5	79.4	13.9	14.6	51.0	55.4	3.6	3.1	200.0	199.0	8.3	13.4	100.0	101.0	10.6	15.8	71.2	67.7	2708.3	2860.8
BC04	51.4	70.7	135.6	139.3	14.9	19.3	48.6	54.4	4.6	4.9	196.0	196.0	5.3	8.0	118.0	117.0	12.3	16.1	65.1	63.4	1459.7	7235.0
BC05	70.8	46.9	166.8	48.2	21.8	13.3	55.2	49.3	5.3	1.7	218.0	185.0	4.7	17.8	109.0	102.0	16.2	9.2	75.8	64.9	2029.2	2115.0
BC06	58.5	66.8	82.9	95.9	19.3	21.1	49.4	57.1	2.5	3.1	153.0	145.0	9.7	14.6	102.0	108.0	8.7	17.5	64.4	66.8	969.5	6613.5
CV1	44.7	61.8	119.3	153.4	12.1	16.4	47.9	58.8	4.4	5.9	176.0	166.0	5.5	5.2	95.5	96.9	16.7	22.2	70.3	71.9	2056.7	2932.5
CV2	62.4	63.6	94.0	125.6	16.6	14.7	50.8	47.0	3.5	5.5	189.0	180.0	3.1	8.3	95.3	92.4	14.0	20.6	70.1	61.7	2597.9	7666.9
ARV	60.6	75.1	176.6	167.6	19.7	21.9	52.1	56.7	5.5	5.8	195.0	196.0	6.8	9.6	123.0	128.0	16.0	20.0	70.9	66.6	2861.7	4261.5
Robusta	55.6	53.1	117.0	116.3	13.9	15.6	37.6	61.0	4.6	4.0	178.0	198.0	5.2	6.6	95.0	132.0	12.7	14.9	68.9	74.3	727.8	6940.3
Ruiru	63.8	72.7	113.3	97.2	19.1	19.8	41.4	48.8	3.8	3.6	138.0	135.0	6.2	10.4	89.2	83.3	14.3	14.6	59.1	62.1	2550.0	4747.8
Batian	50.2	58.6	95.9	63.6	14.9	17.3	44.7	54.8	3.2	2.2	163.0	184.0	6.1	16.2	84.4	99.8	9.2	10.4	62.1	62.2	1848.8	3883.3
LSD	24.8	19.7	88.2	65.4	7.5	5.8	15.1	12.9	3.0	2.3	28.7	36.8	4.1	4.1	18.4	18.4	7.6	7.7	2.6	12.6	1968.9	3577.0
%CV	14.2	9.9	27.2	12.4	15.6	9.8	13.0	0.6	25.7	11.2	0.1	1.7	11.8	5.7	1.6	2.1	16.9	8.0	4.8	2.1	17.2	30.4
Ftest	NS	NS	NS	S	NS	S	NS	NS	NS	S	S	S	S	S	S	S	NS	NS	S	S	S	S

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree), S=Significant NS= Non-Significant.

The differences among the coffee genotypes based on the quantitative traits measured during the years 2017 and 2018 were significant (Table 3.7). In the year 2017, the total number of primaries were not significantly different among the genotypes. In 2018, the traits that were not significant ly different included the percentage of berries per node, total berries on the longest primary, bearing primaries, and the total number of primaries. In 2017, Batian recorded a significantly ($P \leq 0.05$) higher number of percentage berries per node (53.7) when compared to other genotypes, whereas BC06 recorded the least (20.7). In 2017, genotype BC05 recorded a high number of bearing primaries per tree followed closely by genotype ARV whereas Robusta recorded the least. The berries per node varied for both years, genotypes ARH4 and CV1 recording significantly ($P \leq 0.05$) higher number of berries in 2017 and 2018 respectively (Table 3.7).

The average height of the coffee plants varied among the genotypes where Ruiru 11 had shorter plants in both 2017 (98.1) and 2018 (136.7) whereas genotype ARH1 had taller plants in 2018 (Table 3.7). There was a significant ($P \leq 0.05$) difference in the number of laterals produced where genotype BC06 produced more laterals in 2017 and genotype ARH3 produced few laterals in the year 2018. For the total number of primaries, Ruiru11 recorded significantly ($P \leq 0.05$) shorter primaries in 2017, and genotype ARV recorded longer primaries in 2018. The nodes with the highest number of berries varied significantly among the coffee genotypes where genotypes ARH1 and Robusta recorded the least in the year 2017 whereas genotype CV1 recorded the highest in the year 2018. There was variation in yield being high in 2018 and low in 2017. The genotype ARH1 and ARH4 recorded significantly ($P \leq 0.05$) high yield in 2017 (2456 g/tree) and 2018 (6388.9g/tree) respectively (Table 3.7).

Table 3. 7. Growth and yield traits for coffee genotypes taken in the growing season of 2017 and 2018

	%BN		BELP		BPR		B/N		H		LAT		LPR		NHB		PR		Yield	
Genotypes	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
ARH1	32.5	55.2	64.8	104.2	22.8	53.1	5.1	3.7	146.8	201.9	11.3	10.6	90.8	104.2	2.4	13.5	48.1	69.4	2456	3455
ARH2	29.1	58.9	30.5	97.5	19.1	51.3	4.2	3.8	124.5	170.7	10.7	10.5	76.2	92.5	5.4	13.6	44.2	71.4	770.8	1622.4
ARH3	29.3	49	41.1	55.4	17.8	54.2	4.7	2.1	125.7	170.1	13.3	11.7	68.3	87.7	3.2	8.8	45.6	69.7	630.2	1956.6
ARH4	33.7	68.2	79.9	152.4	22.4	55.1	7.7	5.5	122.2	157.5	10.2	6.9	82	90.2	8.4	18.6	48.2	69.6	1243.3	6388.9
ARH5	34	63.9	55.9	137.1	20.5	52.5	5.5	4.7	122.2	160.9	9.5	6.8	86.8	96.6	6.3	17.4	47.5	66.8	1445.3	3803.9
ARH6	28.1	61.7	31.2	95.1	19.9	50.8	3.9	3.5	137.1	175.2	10.2	8.3	82.9	105.4	4.4	12.3	43.3	65.1	663.9	2233.8
ARH7	28.9	43.9	60.2	55.2	22	49.9	5.3	1.8	129.5	171.9	9.2	8.9	93.5	105.3	3.3	9.4	47.8	63.1	1525.7	1796.9
BC01	27.2	59.5	49.8	123.8	21.4	55.2	5.4	4.5	148.8	190	9.3	9.7	95.7	109.2	3.2	18.6	50.2	69.1	1240	3251.2
BC02	26.4	49.4	26.3	89.1	22.5	53.3	3.1	3.3	139	192.9	14.1	11.2	93.9	107.1	3.7	9.8	47.2	72.7	537.5	2999.3
BC03	21.5	52.9	15.5	91.4	20.4	53.2	2.7	3.3	143.5	199.5	10.3	10.9	89.6	100.3	3	13.2	44.5	69.4	777.8	2784.6
BC04	35	61.1	64.3	137.5	23.2	51.5	6.6	4.8	159.6	196	5.3	6.7	106.5	117.3	4.9	14.2	46.3	64.3	511	4347.4
BC05	44.8	58.8	72.2	107.5	24.8	52.3	4.7	3.5	149.5	201.5	10.4	11.3	95.4	105.6	5.2	12.7	47.9	70.4	1060.9	2072.1
BC06	20.7	62.7	30.3	89.4	22	53.3	4	2.8	119.1	149	15	12.1	91.6	105.3	3	13.1	43.5	65.6	2208.9	3791.5
CV1	30	53.3	65.3	136.4	20.3	53.3	7.5	5.2	138.8	171.3	7.7	5.3	87.8	96.2	6.6	19.4	48.3	71.1	1001.2	2494.6
CV2	25.8	63	61	109.8	17.8	48.9	6.9	4.5	141.8	184	6.4	5.7	90.4	93.8	3.7	17.3	47.2	65.9	972.9	5132.4
ARV	42.2	67.9	59.4	172.1	24.4	54.4	5.1	5.7	163.1	195.1	9.2	8.2	112.6	125.2	7.1	18	47.1	68.7	1100.3	3561.6
Robusta	21.1	54.3	40.4	116.6	15.8	49.3	4.2	4.3	141.5	187.7	6.6	5.9	104.9	113.4	2.4	13.8	53.4	71.6	1337.1	3834.1
Ruiru	39.6	68.2	62.6	105.3	22.9	45.1	5.4	3.7	98.1	136.7	12.2	8.3	75.6	86.3	6.8	14.4	40.4	60.6	1772.4	3648.9
Batian	53.7	54.4	89	79.8	22.6	49.7	6.1	2.7	129.9	173.2	10.8	11.1	86.3	92.1	9.9	9.8	43.8	62.2	1849.5	2866
LSD	11.3	17	31.4	5.1	8	10.3	2.4	20	17.4	22.5	3.7	3.2	10.9	12.7	2	5.6	5.6	7.9	885.2	2203.7
%CV	4	5.6	4.2	57.88	6.2	6.2	6.2	9.9	1.8	0.9	3.3	7.5	1	1.3	12.4	3.9	1.5	2.8	6.5	14
Ftest	S	NS	S	NS	S	NS	S	S	S	S	S	S	S	S	S	S	NS	NS	S	S

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree

There was a variation on the average performance of the genotypes across the two environments for all the traits during the two years except for the total number of berries on the primaries (Table 3.8). The percentage of berries per node varied within the genotypes where genotype ARV recorded a significantly ($P \leq 0.05$) higher number of berries (55) whereas BC03 recorded a significantly ($P \leq 0.05$) low number (37.2). The berries on the longest primaries also varied within genotypes where genotype ARV recorded a significant ($P \leq 0.05$) higher number of berries (15.8) when compared to other genotypes followed closely by genotype BCO5 and Batian which recorded 14.9 berries. The total number of berries on the primaries varied among the genotypes. Genotype ARV recorded a significantly ($P \leq 0.05$) higher number of berries whereas genotypes BC03 and ARH3 both recorded significantly ($P \leq 0.05$) low number (Table 3.8).

There was a variation on the berries per node with genotypes CV1 and ARH4 recording significantly ($P \leq 0.05$) higher number of berries each (13.5, 13.2) whereas genotype BC02 recorded significantly ($P \leq 0.05$) low number (6.4). Height varied among the coffee genotypes where ARV was significantly ($P \leq 0.05$) taller whereas cultivar Ruiru 11 had significantly ($P \leq 0.05$) shorter plants (117.4). The total number of laterals per genotype recorded a significant ($P \leq 0.05$) difference with BC06 recording a higher number and genotypes BC04 and CV2 both recorded fewer laterals (Table 3.8). The length of the primaries ranged varied among the genotypes where genotype ARV had significantly ($P \leq 0.05$) longer primaries but genotype ARH3 had shorter primaries. The total number of primaries was significantly ($P \leq 0.05$) different among the genotypes with Robusta recording the highest number of primaries but Ruiru 11 recorded the least. Yield ranged varied among the genotypes where genotype ARH4 recorded a significantly ($P \leq 0.05$) high yield of (3816g/tree) but ARH2 was the lowest in yield (1196g/tree) (Table 3.8).

Table 3. 8. Growth and yield traits for coffee genotypes taken at KALRO-Alupe(Busia) and Siaya ATC during 2017 and 2018 growing seasons

Genotypes	%BN	BELP	BNLPR	BPR	B/N	H	LAT	LPR	NHB	PR	Yield (g/tree)	
ARH1	43.9	84.5	12.0	38.0	9.3	174.3	10.9	97.5	3.0	58.7	2955.5	
ARH2	44.0	64.0	10.8	35.2	8.9	147.6	10.6	84.3	4.6	57.8	1196.6	
ARH3	39.1	48.2	9.8	36.0	6.8	147.9	12.5	78.0	2.6	57.7	1293.4	
ARH4	51.0	116.1	13.8	38.7	13.2	139.8	8.5	86.1	5.3	58.9	3816.1	
ARH5	49.0	96.5	13.8	36.5	11.4	141.5	8.2	91.7	5.5	57.1	2624.6	
ARH6	44.9	63.2	11.9	35.4	8.1	156.2	9.3	94.1	4.0	54.2	1448.8	
ARH7	36.4	57.7	10.4	36.0	7.3	150.7	9.0	99.4	2.5	55.4	1661.3	
BC01	43.4	86.8	11.8	38.3	12.0	169.4	9.5	102.5	3.9	59.7	2245.6	
BC02	37.9	57.7	10.1	37.9	6.4	166.0	12.6	100.5	3.5	59.9	1768.4	
BC03	37.2	53.5	9.7	36.8	7.9	171.5	10.6	95.0	3.2	57.0	1781.2	
BC04	48.0	100.9	12.6	37.3	9.6	177.8	6.0	111.9	4.8	55.3	2429.2	
BC05	51.8	89.8	14.9	38.5	8.7	175.5	10.8	100.5	4.8	59.2	1566.5	
BC06	41.7	59.9	12.9	37.6	8.5	134.0	13.6	98.4	2.9	54.6	3000.2	
CV1	41.6	100.8	10.6	36.8	13.5	155.0	6.5	92.0	4.2	59.7	1747.9	
CV2	44.4	85.4	10.9	33.3	12.1	162.9	6.0	92.1	4.1	56.6	3052.7	
ARV	55.0	115.7	15.8	39.4	11.5	179.1	8.7	118.9	6.4	57.9	2330.9	
Robusta	37.7	78.5	10.0	32.5	9.0	164.6	6.3	109.1	4.1	62.5	2585.6	
Ruiru 11	53.9	83.9	14.5	34.0	9.9	117.4	10.2	81.0	5.3	50.5	2710.7	
Batian	54.1	84.4	14.9	36.2	8.0	151.6	11.0	89.2	6.3	53.0	2357.8	
LSD	10.4	32.2	1.1	6.6	3.0	14.0	2.4	8.0	1.5	4.8	1176.6	
%CV	3.4	9.0	3.9	4.4	5.2	0.9	5.2	1.1	4.1	2.1	10.2	
Ftest	S	S	S	NS	S	S	S	S	S	S	S	

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) = Length of longest primaries, NHB= number of berries on the highest bearing node, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

The performance of genotypes significantly ($P \leq 0.05$) different for yield. The high yielding genotype in Busia was followed by genotypes ARH4 and ARH5. The high yielding genotypes in Siaya were ARH4, BC06, CV2, and BC04. Yield production in Siaya was higher when in comparison to Busia. (Figure 3.1). Those genotypes that performed poorly in Busia and Siaya were ARH2 and ARH3 respectively.

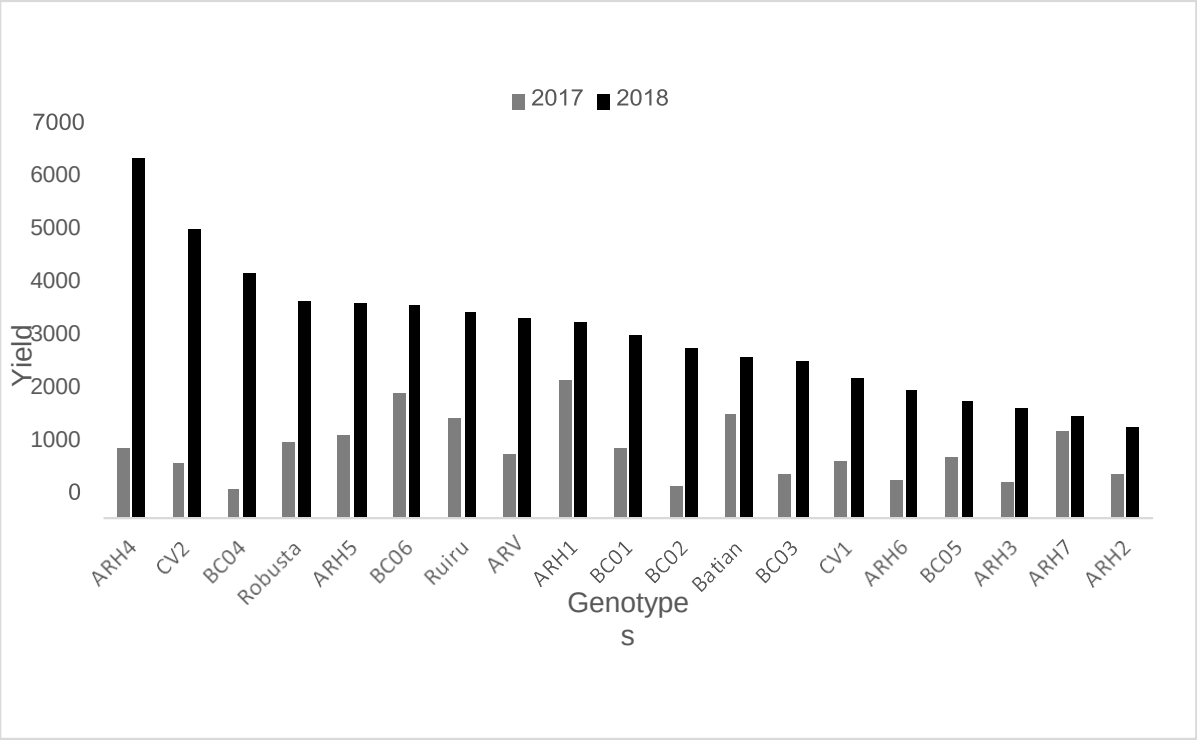


Figure 3. 1. Performance of the coffee genotypes across the two locations (Busia and Siaya) over the two years

There were variations on performance at KALRO-Alupe and Siaya ATC based on morphological traits in 2017 and 2018. There were no significant differences between the two locations in each year for height, longest primaries, and the total number of carrying a high number of berries. There percentage berries per node performed significantly ($P \leq 0.05$) different in Siaya ATC and recorded a high number of berries in the year 2018 (60.2) and KALRO-Alupe recorded a significant ($P \leq 0.05$) high number for the year 2017 (44.6) (Table 3.9).

The variations on the berries on the longest primary were also significantly ($P \leq 0.05$) different in the year 2017 across the two locations where KALRO-Alupe recorded the highest number of berries (86.9) (Table 3.9). Both locations recorded significantly ($P \leq 0.05$) a high number of berries on the longest primary in the year 2018 when compared to the year 2017. The site at KALRO- Alupe recorded a significantly ($P \leq 0.05$) high number of berries on the longest primary in the year

2017 and there was no significant (P≤ 0.05) difference in the year 2018. The total number of primaries varied across the two locations where Siaya ATC recorded a significantly (P≤ 0.05) high number of primaries between the two locations in the year 2017. Yield varied within the two years where Siaya ATC recorded a significantly higher yield for the year 2018 (4214g/tree) whereas KALRO-Alupe recorded a high yield for the year 2017 (1668.7g/tree) (Table 3.9).

Table 3. 9. Growth and yield traits for KALRO-Alupe and Siaya ATC in the year 2017 and 2018

		Morphological traits										
Environment	**Year**	**%BN**	**BELP**	**BNLPR**	**BPR**	**B/N**	**H**	**LAT**	**LPR**	**NHB**	**PR**	**Yield (g/tree)**
KALRO-Alup	2017	44.6	86.9	11.3	25.8	7.2	135.5	8.5	90.4	4.8	45.2	1668.7
Siaya ATC	2017	18.9	18.3	4.5	16.5	3	136.2	9.7	89.7	4.5	48	763.4
KALRO-Alup	2018	56.2	110.6	16.4	49.4	12.7	178.9	9.3	100.5	3.8	68.4	2316
Siaya ATC	2018	60.2	105.8	16.5	54.4	15.5	177.4	11	103	3.9	67	4214.6
	LSD	4.79	14.77	1.365	3.021	1.356	6.42	1.107	3.692	0.697	2.21	539.9
	%CV	3.4	9	1	4.4	5.2	0.9	5.2	1.1	4.1	2.1	10.2
	F test	S	S	S	S	S	NS	S	NS	NS	S	S

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) = Length of longest primaries, NHB= number of berries on the highest bearing node, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

3.5.2 Genotype by environment interaction

The genotypic component of variance revealed that all the traits showed significant differences except for the percentage of berries per node and the number of primaries. There were significant ($P \leq 0.05$) differences within the environments for bearing primaries, number of laterals, nodes with the highest number of berries, and yield. The G x E interaction showed significant ($P \leq 0.05$) differences in the total number of laterals and yield (Table 3.10).

Table 3. 10. Mean squares for growth and yield traits of 19 coffee genotypes evaluated at Siaya ATC and KALRO-Alupe in the growing season of 2017 to 2018

Source	Rep	Gen (G)	Envt (E)	G x E	Error
Df	2	18	1	18	74
%BN	401.4	284.3NS	446.2NS	225.5NS	217.9
BELP	5898	5517**	641NS	2140NS	2531
BNLPR	49.71	35.53*	0.11NS	20.53NS	19.37
BPR	398.52	37.81	699.23**	79.46NS	80.45
B/N	5.593	7.037***	0.352NS	2.638NS	3.071
H	91.1	2062.5***	67.8NS	340.9NS	382.5
LAT	17.078	30.249***	479.29***	15.263*	7.773
LPR	65.3	646.8***	169.3NS	148.2NS	122.3
NHB	11.56	66.63***	225.5**	19.64NS	23.39
PR	135.06	72.71NS	54.75NS	44.64NS	47.4
Yield (g/tree)	7916932	856210000000000**	102735936***	8147762***	3669426

Key: *, **, *** and represent significance at ($P<0.05$), ($P<0.01$) and ($P<0.001$) respectively. NS=non-significant, % BN=percentage bearing nodes, BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) = Length of longest primaries, NHB= number of berries on the highest bearing node, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

The biplot (Fig 3.2) shows genotypes ranked based on the average yield and stability. The distance from the center of the concentric circle to the genotype determines the ideal genotype. The most ideal cultivar should be at the center of the concentric lines. Genotype, ARH4 was closer to the concentric center and was therefore the most ideal genotype when compared to other cultivars. The yield scatter plot (figure 3.3) was grouped into five (5) different sectors. 62.38% of the variability was explained by PC1. The genotypes which won in the different sectors were ARH1, ARH4, ARH2, BC04, and BC06. Most of the genotypes are distant from the origin meaning that they were highly responsive to the environment effect. Both environments had long vectors from the origin but the angle between Busia and Siaya was larger than 90 degrees implying that these environments discriminated against the genotypes and that G x E is large. The environments were not correlated to each other since the angle between the two environments was more than 90 degrees. The best performing genotypes in Busia and Siaya were ARH1 and ARH4 respectively.

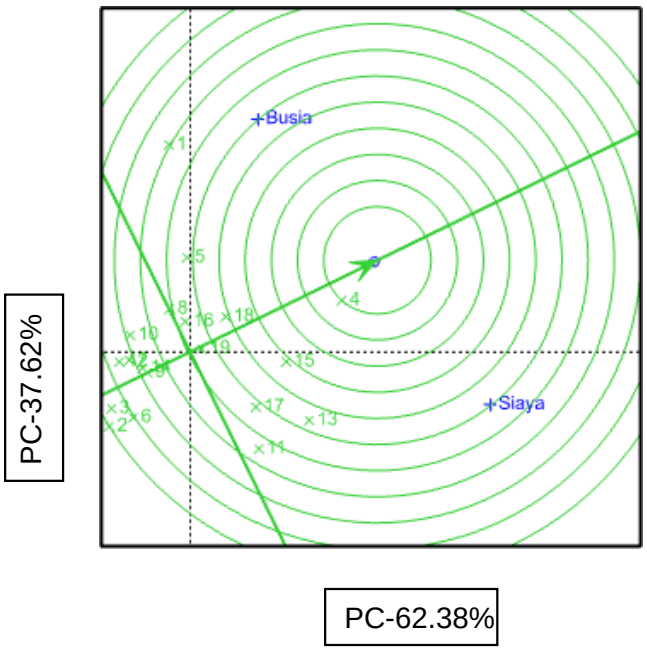


Figure 3. 2. GGE biplot showing comparisons for the ideal genotype

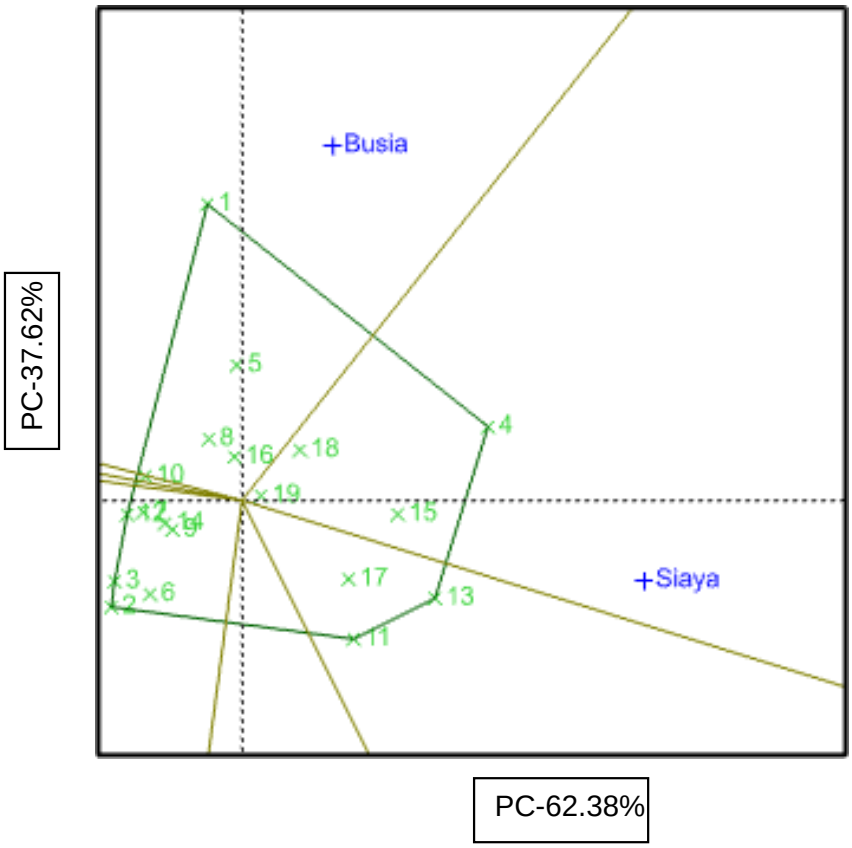


Figure 3. 3 Scatter plot showing the best performing genotypes in each environment

The point (0) of the ranking plot (Figure 3.4) is the average of all the environments. The plot also shows a line from the origin to the mean environment that measures the stability of the genotype. Any genotype above the origin performs higher and any genotype close to the line is stable. Hence, the high yielding genotype was ARH4 and the low yielding was ARH2 The most stable genotype was Ruiru 11 whereas the most unstable genotype was ARH1. Both environments were highly discriminative and less representative of an ideal environment since they were far away from the origin (Figure 3.4).

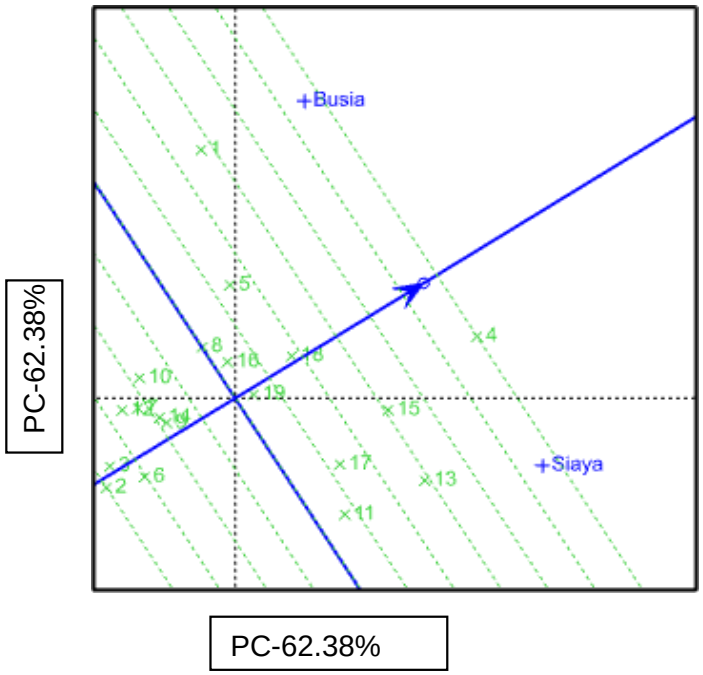


Figure 3. 4. GGE biplot showing the average yield and stability of the genotypes using ranking plot

3.5.3 : Correlation between the growth and yield traits

Table 3.11, shows the correlation coefficients for the morphological traits derived from combined mean analysis from both locations. There were significant positive correlations between percentage berries per node had with berries on the longest primary berries(r=0.69), berries per node on the longest primary (r=0.90), berries per node (r=0.61), nodes with the highest number of berries and yield (r=0.61). The correlation between the berries on the longest primary with berries per node (r=0.96), nodes with the highest number of berries, and yield (r=0.58) were significant ly positive. Longest primaries showed positive correlations to nodes with the highest number of berries (r=0.09), the total number of primaries (r=0.17), and yield (r=0.002) although they were not significant (Table 3.11). Height showed a significant positive correlation with the total number of primaries and was negatively correlated to nodes with the highest number of berries.

Table 3. 11. Pearson's correlation analysis for growth and yield traits for coffee genotypes Siaya ATC and KALRO- Alupe

	%BN	BELP	BNLPR	%BPR	B/N	B	H	LAT	LPR	NHB	PR	Yield(g/tree)
%BN	-	0.698***	0.901***	-0.071	0.651**	0.31	-0.318	-0.329	0.014	0.645**	-0.24	0.612**
BELP		-	0.607**	0.275	0.969***	0.22	0.12	-0.6052**	0.372	0.861***	0.202	0.585**
BNLPR			-	0.022	0.465*	0.086	-0.341	-0.121	0.177	0.486*	-0.331	0.484*
BPR				-	0.233	-0.41	0.337	0.25	0.266	0.239	0.621**	0.037
B_N					-	0.268	0.128	-0.6906**	0.266	0.89***	0.279	0.601**
H							-	0.093	0.612**	-0.057	0.466*	-0.149
LAT								-	-0.086	-0.6477**	0.074	-0.49968*
LPR									-	0.094	0.18	0.002
NHB										-	0.172	0.5538*
PR											-	-0.1049
Yield												-

*** indicates significance at p≤ 0.001; ** indicates significance at p≤ 0.01 and * indicates significance at p≤ 0.05

Key: % BN= percentage bearing nodes BELP=berries on the longest primary, BNLPR= bearing nodes on longest primary, BPR= percentage bearing primaries, B/N= berries per node, H= height (cm), LAT=laterals, LPR=longest primary (cm), NHB= node with highest berries, and PR=Number of primaries and yield (g/tree).

3.5.4 : Estimate of genotypic and phenotypic parameters of combined Analysis of Variance for the quantitative characters.

The genotypic and phenotypic variances estimated indicated that there were variations within the morphological characters measured among the coffee genotypes. (Table 3.12). There were varied GCV values recorded from the morphological traits measured being high in berries on the longest primary (31.01). Those traits that scored low GCV values included percentage berries per node, the total number of berries found in the longest primary, and length of the longest primary. The PCV values varied within the morphological traits with a range between 11.01 and 70.51%. Yield (g/tree) scored the highest PCV value (70.51%) followed closely by the total number of berries on the longest primary and berries on each node. The traits that scored the lowest PCV values include, number of primaries, berries on the longest primary and longest primary (Table 3.12).

Yield recorded a higher value for Genetic advance (699) whereas the least value was recorded from the berries found on each node together with the longest primary. There was a variation on percentage mean of GA (GAM) among the traits with yield (g/tree) where it recorded 21.1% whereas 0.81% was the least and was recorded from percentage berries per node (Table 3.12). Broad sense heritability varied among the traits measures with a range of The values for the broad sense 0.08 to 0.59% being recorded. The traits recorded heritability values of more than 0.50 were the length of the longest primary and height (Table 3.12). The traits that scored a low broad sense heritability (<0.30) included percentage berries per node (0.08), number of primaries (0.15), berries per node on the longest primary (0.21), and the total number of berries on the longest primary (0.21) (Table 3.12). Response to selection also varied with yield recording the highest response.

Table 3. 12. The estimate of genotypic and phenotypic parameters from combined Analysis of Variance of growth and yield traits.

Morphological trait	GCV (%)	PCV (%)	H	GA	GA (% of mean)	Re
%BN	7.61	26.48	0.08	0.47	0.81	4.60
BELP	31.01	55.89	0.31	14.57	13.46	34.89
BNLPR	13.58	29.99	0.21	0.43	2.58	1.20
BPR	11.5	15.68	0.22	0.54	1.04	3.97
B/N	29.79	54.3	0.3	0.48	12.42	1.20
H	13.28	17.23	0.59	4.40	2.47	24.61
LAT	30.58	43.65	0.49	1.17	13.09	2.85
LPR	12.99	16.93	0.59	2.40	2.36	13.75
NHB	28.07	44.32	0.4	1.56	11.03	4.12
PR	4.29	11.03	0.15	0.83	1.22	3.02
Yield (g/tree)	39.11	70.51	0.31	699.31	21.42	1328.15

Key: % BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

3.6 Discussion

There was a variation among the morphological traits for the nineteen different coffee genotypes. Variability among genotypes, for specific traits, offers opportunities for coffee of improvement through the crossing and selecting highly performing genotypes. The variations within the growth and yield traits are similar to those reported by Gichimu and Omondi (2010). They observed significant variations among the phenotypic traits measured. Olika et al., (2011) reported significant variations among the phenotypic characters including the length of the primaries, the

total number of bearing nodes, plant height, yield, number of bearing nodes, and the total number of berries on each node. An efficient selection can be attained by considering the performance of various growth and yield characters for instance percentage of bearing nodes, the total number of berries produced per node, and the percentage of primaries that are bearing (Gichimu and Omondi, 2010). Getachew et al., (2017) also reported significant variations among the morphological traits.

In the growing season of 2017 the coffee genotypes in Busia gave higher yields than those in Siaya and also produced the highest yielding genotypes (ARH1 and Ruiru 11) whereas, in the year 2018, coffee genotypes in Siaya had high yields than those at Busia. During the growing season of 2017, the genotypes that had high yield were ARH1 whereas in 2018 genotype ARH4 recorded the highest yield (Table 3.6). On overall performance over the two years in both locations, ARH4 was the highest yielding and most ideal genotype when compared to the other genotypes. There were significant effects of environment for the total number of berries on each primary, the total number of laterals per tree, number of nodes with higher berry count, and yield (g/tree). The differences could be attributed to the G x E interaction which is a manifestation of how different genotypes respond to different environmental conditions rainfall patterns, the soil type, relative humidity, and temperature across the two locations.

A comparison of the genotypes in the two locations indicated the occurrence of interactions (G x E). The interaction was significant for the number of laterals and yield. Tefera, (2018) and Maeza et al., (2011) reported G x E interactions for coffee yield grown in different environments. Gene action and influence of the environment make yield a more complex trait since yield each genotype in a specific environment is affected by the main effects of the genotype and environment interaction as a result of G x E (Marjanović-Jeromela et al., 2011)**.** The interaction implies that phenotypic performance of a specific genotype is not equal in different environments as the

genotype performs better in one environment and also performs poorly in the other environment. The environments differ from each other due to the soil type, the rainfall pattern and distribution , temperature, and relative humidity. These factors are critical in influencing the stability of genotypes over the various environments and even their expression. About 80% of the variation in yield is caused by environmental factors, 10% by genotypic factors, and another 10% due to the G x E Interaction (Yan, 2001). The changing and unpredictable environmental conditions affect genotypic stability depending on the unpredictable variation mechanisms (Kang, 2002). The difference in performances among genotypes across different environments is due to the effects caused by environmental factors. The presence of G x E interaction indicates that there is a need to develop genotypes that adapt well and are stable to a particular environment to optimize the genetic gain (Cullis et al., 2014).

There were significant positive correlations found within the different growth and yield characters. The correlation of yield with percentage berries on each node, number of berries on the longest primary, total number of berries on each node, and nodes having a high number of berries was significantly positive (Table 3.10). There were also significant positive correlations between percentage berries per node with berries on the longest primary (r=0.69), nodes with the highest number of berries (r=0.64), and yield (r=0.61). Gichimu and Omondi (2010). Olika et al., (2011) and Tefera, (2018) reported significant correlations for most of the quantitative traits. The percentage of berries on each node, the number of berries within the longest primary, berries on each node had a significant positive correlation with yield (Table 3.10). The traits that showed positive correlations with yield are important during selection for yield indirectly. This will lead to an increase in selection efficiency during crop development reducing the time taken for variety development. Evaluation during early crop development allows the elimination of undesired

genotypes early enough maximizing on those that are superior in performance. Those traits that showed negative correlations with most of the traits can hamper the indirect selection process.

High GCV and PCV values were recorded on the total number of berries on the longest primary, the total number of berries on each node, laterals, nodes carrying the highest number of berries, 100 berry weight, and yield. The longest primary, plant height recorded medium PCV and GCV values (Table 3.11). A high mean percentage of GA was recorded from the total number of berries within the longer primaries (13.46), laterals (13.09), nodes carrying a high number of berries (11.03) and yield (g/tree) (21.42), and the same results were also observed by Olika et al., (2011) and Bayetta (2007). The low to moderate genetic advance for most of the morphological characters were also reported by Malua and Pandiagan (2018) This indicates that the environmental variation washing and it affected the expression of the various traits. Genetic advance (GA) is used in quantifying the additive and non-additive gene action for polygenic trait expression. High GA indicates additive gene action whereas low GA indicates non-additive gene action. The low GCV values could have been as a result of natural selection due to the varying environmental conditions. The minimal environmental influence led to a narrow gap between GCV and PCV values for bearing primaries, height, and longest primaries (Getachew et al., 2017)

There was high heritability of more than 50% on height (59) and the number of longest primary (59). The traits with medium heritability yield (31), berries on the longest primary (31), total number of berries on the primary berries per node (21), laterals (49), nodes with the highest number of berries, berries per node were Percentage berries per node, and number of primaries had low heritability values (Table 3.11). High broad-sense heritability was reported by Kebede and Bellachew (2005) on all traits and do not agree to the findings of the study. Bekisa and Ayono (2016) reported low heritability height. Getachew et al., (2013) reported moderate heritability on

all the traits he measured whereas Tefera (2018) reported various percentages of heritability for morphological traits measured.

Heritability values of more than 50% are considered high, with values that lie between 20 and 50% considered moderate and values less than 20% are considered low (Verma and Agarwal 1982). When more genes control the expression of a specific trait, the heritability becomes loss, and thus making the selection process more complex (Sousa et al., 2019). The utilization of genetic advance and heritability enhances an effective selection since it results in the gains from a given selection . The combination of genetic advance and heritability becomes an important tool determining the traits to be used during the selection process. Genetic advance is calculated using heritability and through GA, it is possible to determine point of gain for a specific character of interest (Dyulgerova and Valcheva 2014). The total number of berries found on the longest primary and yield had high GA, high selection response and they also showed positive correlations with each other. The traits that not only show high heritability and GA but also exhibit significant positive correlations enhances the efficiency for yield selection. The expression on the reliability of a phenotype is predicted through measuring heritability as a guide to its breeding value (Tazeen et al., 2009).

The genetic variation has been found to exist among the coffee genotypes evaluated across the two environments. The significant correlations among quantitative characters imply that these traits can be utilized in ensuring effectiveness and the efficiency of the selection process. The traits including the number of longest primary, the total number of berries found on the longest primary, and yield exhibited high heritability and also recorded high selection response and GA meaning that these traits can be made use of in timely selection for yield. Significant G x E interactions for yield indicate that the performance of the genotypes vary from one environment to another as the environments are not similar. From the results, the Arabusta hybrids yielded higher when

compared to other genotypes. In Busia, the high yielding genotypes were ARH1, ARH4, and ARH5 whereas in Siaya the high yielding genotype ARH4. Therefore, these hybrids are recommended for commercial cultivation in the specific environments based on their yield performance subject to evaluation of the bean and liquor quality.

CHAPTER FOUR:

CHARACTERIZATION OF ARABUSTA COFFEE HYBRIDS AND BACKCROSS PROGENIES FOR RESISTANCE TO COFFEE BERRY DISEASE AND GENETIC DIVERSITY

4.1 Abstract

Coffee Berry Disease is a major coffee disease in Kenya causing losses of up to 75% if not controlled. The aims of study were (i) To identify Arabusta genotypes and the backcrosses that have the C*k-1* gene responsible for Coffee Berry Disease resistance using SAT 235 marker and

(ii) To determine the genetic diversity of the coffee genotypes using SSR markers. The SAT 235 marker was used in assessing the presence of the C*k-1* in the coffee genotypes evaluated. Those genotypes which showed a similar banding pattern to the HDT were classified as resistant whereas those with the same banding patterns to susceptible genotype SL28 were classified as susceptible. Nineteen SSR markers were used in estimating the genetic diversity of 18 coffee genotypes. The calculated range of the diversity index was 0.26 to 0.93 among the coffee genotypes. The polymorphism between the Arabusta genotypes and the Arabica coffee varied, with high polymorphism(72%) calculated among Arabusta genotypes and 46.8% within the Arabica genotypes. The results indicated that it is possible to select for CBD resistance using the molecular markers. The variations among the coffee genotypes indicated the possibility of improving the coffee genotypes through hybridization programmes in developing new coffee varieties.

4.2 Introduction

Coffee Berry Disease *(Colletotrichum kahawae)* is an important fungal disease of coffee and can occur within all species of coffee. Several species or strains of *Colletotrichum* occur on coffee, but only *C. kahawae* (formerly *C. coffeanum)* causes coffee berry disease and could cause losses of up to 80% (Gichimu and Phiri, 2010 and Adepoju et al., 2017). This disease has been of major concern in Kenya and is confined within the African continent (Bekele 2019). The traditional cultivars being cultivated in Kenya include K 7 which is grown in low altitude areas, SL 28, and SL 34 for medium to high altitude zones (Mwangi, 1983). Unfortunately, these varieties are susceptible to CBD (Omondi et al., 2001). A breeding programme at the Coffee Research Institute (CRI) was initiated and implemented that led to the release of two new improved varieties (Batian and Ruiru 11. The current breeding programme focuses on developing other varieties that are disease resistant to different Agro-Ecological Zones in Kenya.

Evaluation for CBD resistance was carried out using 11 different mapping populations of Arabica coffee which revealed three major genes on separate loci (Van der Vossen and Walyaro 1980, Gichimu et al., 2014b, Omondi and Hindorf 2010,). Van der Vossen and Walyaro (1980) found out that the Rume Sudan which carried both R and K genes that are dominant and recessive respectively making it highly resistant. K7 which expresses moderate resistance carries the recessive K-gene while the T causing an intermediate gene action is found in Clone 1349/ 269 of the variety Hibrido de Timor (HDT) and its hybrid derivative Catimor (Hindorf and Omondi, 2010). The T and R genes have an intermediate action whereas the recessive K gene confers partial resistance to CBD. Using F2 plants (cv Catimor × cv SL28), Gichuru (2007) identified an SSR marker SAT 235 linked to CBD resistance at the T locus and was able to map on an introgressed

C. canephora fragment carrying the *Ck -1* gene which is located in a segment of 11 cM and was

confirmed by Gichimu et al, (2014b) when the screening of the Ruiru 11 sibs for CBD resistance. The three genes are being employed in coffee breeding programs in developing CBD resistant coffee varieties.

Coffee Berry Disease evaluation has always been carried out by inoculating the coffee seedlings with CBD inoculum and the scoring taken on a scale of 1 to 12, a score of 1 being highly resistant and a score of 12 being most susceptible. The process of inoculating seedlings developed by Van der Vossen et al. (1976) has always been used in the CBD screening and is effective however, it takes a long time for a coffee plant to produce seed for this specific test. The use of Marker Assisted Selection(MAS) enables early identification of coffee genotypes for CBD resistance and thus reduces the selection period since resistant plants can be identified early before producing any crop.

Studying genetic diversity is key in the characterization of genotypes to understand their variations which is useful during crop improvement. Evaluation of genotypes unravels those genotypes that relate closely to each other, increasing efficiency in coffee improvement programmes (Missio et al., 2009). Genetic markers provide good insights to questions related to phylogenetic relationships, key evolutionary processes such as gene flow, mating systems, and population size than ecologically important traits without non-genetic variance that often makes other trait analyses uncertain (Lu et al., 1997). Various molecular markers have been used in determining the genetic variations of coffee genotypes and they include RFLP (Lashermes et al., 1999), RAPD (Diniz et al., 2005;), AFLP (Steiger et al., 2002; Anthony et al., 2002) and SSRs (Combes et al., 2000, Gimase et al., 2014a). These markers have shown that there is a low genetic variation in the *Coffea arabica* when compared with *Coffea canephora*.

Simple Sequence Repeat (SSR) marker is a selectively neutral genetic marker that comprises smaller DNA fragments that flank the SSR region (Grivet et al., 2003). Microsatellite utilizat ion in studying the variabilities among different genotypes for specific traits has been successful (Sousa et al., 2017, Omingo et al., 2017 and Teressa et al., 2010).

4.3 Materials andMethods

4.3.1 Experimental materials

The materials have been defined in chapter 3 section 3.3.1

4.3.2 Molecular screening for CBD resistance and characterization using SSR markers

4.3.2.1 DNA extraction

Three buffer stocks were prepared for use during the extraction process. Buffer A contained 6.8gSorbitol, 20mls of Tris-HCl (Estoque a 1M), 0.19g EDTA, and Água MilliQ. Buffer B had 11.69g of NaCl, 2.42g Tris-HCl,1.86g EDTA, and Água MilliQ. Since buffer B was viscose, its preparation was maintained by agitation and heating. The extraction buffer was prepared using buffer A, buffer B, Sarcosyl 5%, Bissulfito de Sódio [final] de1%, Active charcoal [final] de 0,1% and PVP [final] de 2%. The extraction buffer was put into a becker and agitated to a temperature of 65^0C using a heater agitator. Clean leaves were picked from the growing tips from branches of the genotypes being studied for DNA extraction. DNA extraction was done using Diniz et al. (2005) method with minor modifications as described using mixed alkyl trimethylammonium bromide, instead of cetyltrimethylammonium bromide.

The leaves were ground using liquid nitrogen and put into 2 µl tubes after which the extraction buffer was added covering the leaf tissue and the mixture was shaken. The tubes containing the mixture were placed in a water bath at a temperature of 65^0C for 40 minutes' agitation being carried

out after every 10 minutes. The tubes were removed and kept at room temperature to cool. 1 µl of chloroform: isoamyl was added into each tube at a ratio of 24:1. Only the upper part of the suspension was transferred into a 1.5 µl tube using tips and 1 µl of isopropanol was added to the suspension. The samples were then stored at a temperature of -20^{0}C for 2 to 3 hours after which they were centrifuged at 14000rpm for 20 minutes and the pellets (DNA) were extracted. The sample was then left to dry for 15 minutes. The DNA was dissolved in 200 µl of TE, and 2ml of RNase (10mg/ml) added. The sample was placed into a water tab at 37 ^{o}C for 30 minutes for incubation and DNA suspended. DNA was precipitated using 35µl of 5M NaCl and 200 µl of cold isopropanol. The DNA was then stored at a temperature of -20^{0}C for 2 to 3 hours and centrifuged at 14000 rpm for 20 minutes. The suspension was discarded by inverting the tubes ensuring that the DNA pellet remained inside the tube. 500 µl of 70% ethanol was added into the tube for washing and second washing repeated using 95% ethanol and the sample was dried for 15 minutes at room temperature. The DNA was dissolved using 200 µl of TE for quantification.

4.3.2.2 Quantification of DNA

0.7 g of Agarose 70 ml 0.5X was weighed in Tris Boric Ethylene-diaminetetraacetic acid. The mixture was heated at short intervals of 15 to 30 seconds inside a microwave until it was clear with occasional shaking. To retain the exact weight which could have been reduced by evaporation caused by heating, distilled was added to retain the initial volume and then left to cool to about 55°C. The gel when still viscous was poured into a mini electrophoresis unit (MUPID) and combs fixed and allowed to cool. The combs were then removed with 0.5X TBE buffer and poured to the unit covering the gel. The standard DNA set was lambda DNA/EcoR1 +Hind111 marker 500 µg/ml. The lambda contents were heated at 65°C for 10 min then immediately cooled on ice for five minutes. 10 µl of lambda and 12 µl of sample DNA were loaded into the agarose gel and left

to run for 45 minutes at 50V and this was followed by gel staining using 1 mg/ml Ethidium Bromide for 20 minutes.

4.3.2.3 PCR amplification and screening for CBD resistance

Nineteen SSR primers were used to amplify the DNA of 18 coffee genotypes. PCR reactions were performed in a final volume of 25 µl containing 2.5 µl of 10X PCR buffer (16 mM MgCl2, Dongsheng), 1.0 µl of MgCl2 (25 mM, Dongsheng), 10 ng (10 ng/µl) of genomic DNA template, 5.4 µl of double-distilled water, 3.75 µl of dNTPs (500 µM, Eurogentec), 0.3 µl of Taq DNA polymerase (5U/µl, Dongsheng).1.0 µl each of forward and reverse Primer (10 µM, Eurogentec). The Eugene thermocycler (TECHNE, UK) was used to conduct the amplification of the DNA using an SSR amplification programme. An initial one cycle of initial denaturation began at 94°C for 5 min followed denaturation of 35 cycles at 94°C for 45s and primer annealing at 55°C for and finally elongation at 72°C for 90 s. The final extension was carried out at 72°C for 10 min and a final hold at 4°.

The genotypes under study were screened for the presence or absence of the T gene in their genome by amplifying with T gene marker SAT 235. For marker-assisted selection, alleles were scored based on known resistant T gene locus. A ladder was added with the first load to confirm the allele sizes in the genotypes. The plants that amplified the T gene across the genotypes were identified and recorded as containing the T gene that confers resistance to CBD in coffee whereas those missing the T gene were recorded as susceptible.

Table 4. 1. Oligonucleotide primer sequences of microsatellites (SSR) locus

Locus	Forward primer	Reverse primer
Sat11	ACCCGAAAGAAAGAACCAA	CCACACAACTCTCCTCATTC
Sat32	AACTCTCCATTCCCGCATTC	CTGGGTTTTCTGTGTTCTCG
Sat207	GAAGCCGTTTCAAGCC	CAATCTCTTTCCGATGCTCT
Sat227	TGCTTGGTATCCTCACATTCA	ATCCAATGGAGTGTGTTGCT
Sat235	TCGTTCTGTCATTAAATCGTCAA	GCAAATCATGAAAATAGTTGGTG
Sat240	TGCACCCTTCAAGATACATTCA	GGTAAATCACCGAGCATCCA
Sat255	AAAACCACACAACTCTCCTCA	GGGAAAGGGAGAAAAGCTC
Sat283	GCACACACCCATACTCTCTT	GTGTGTGATTGTGTGTGAGAG
Sat254	ATGTTCTTCGCTTCGCTAAC	AAGTGTGGGAGTGTCTGCAT
Sat229	GGCTCGAGATATCTGTTTAG	TTTAATGGGCATAGGGTCC
M24	TTCTAAGTTGTTAAACGAGACGCTTA	TTCCTCCATGCCCATATTG
Sat172	ACGCAGGTGGTAGAAGAATG	TCAAAGCAGTAGTAGCGGATG
Sat262	CTGCGAGGAGGAGTTAAAGATACCAC	GCCGGGAGTCTAGGGTTCTGTG
M3	ATTCTCTCCCCCTCTCTG	TGTGTGCGCGTTTTCTTG
M2	AGTGGTAAAAGCCGTTGGTG	GCGGTTGTTGTTGGTGAGTTGAA
M27	AGGAGGGAGGTGTGGGTGAAG	AGGGGAGTGGATAAGAAGG
M29	GACCATTACATTTCACACAC	GCATTTTGTTGCACACACTGTA
M25	CCCTCCCTGCCAGAAAGAAAGC	AACCACCGTCCTTTTTCCTCG
M47	TGATGGACAGGAGTTGATGG	TGCCAATCTACCTACCCCTT

4.4 Data analysis

The scoring of SSR amplified bands was in the form of a binary matrix with the presence of the band was scored one (1) and absence (0) (Cruz 2008). The number of alleles per primer, accession, and null alleles (alleles with no amplification product in one or more accessions) was analysed. The degree of polymorphism percentage was derived by dividing the total number of polymorphic bands to the number of amplified bands per group (Missio et al 2009). The Dice Coefficient Similarity Index was used to generate the genetic distance similarity matrices. The cluster dendrograms and Principal Component Analysis (PCA) were calculated using the Unweighted Pair Group Method based on Arithmetic Averages (UPGMA) (Gichimu et al., 2014) to estimate the genetic diversity within the coffee genotypes using XLSTAT software version 2018. The PCA

of allele frequencies was performed using pairwise comparison of accessions to derive a multi-dimensional scatter plot of individuals (Mohammadi and Prasanna 2003). PCA was calculated using a correlation matrix to get the correlation among quantitative traits by converting them into uncorrelated traits called PCs. Eigen-vectors and values produced by the PCs were used respectively to measure the relative discriminative power of the axes and their associated characters thus creating the variation among the variables measured.

4.5 Results

4.5.1 Marker Assisted selection- Occurrence of the T gene

The introgressed alleles of SAT 235 conferring the C*k* -1 gene which is carried by the HDT genotype were present in the Arabusta hybrids, backcrosses, UT6, and Robusta as shown by the arrow in Plate 4.1. The C*k* -1 gene was however absent in Caturra, SL28, and SL34 which are genotypes known to be susceptible to coffee berry disease. The average polymorphism for the SSR markers was 71% with an average of 2.2 for polymorphic alleles (Table 4.1).

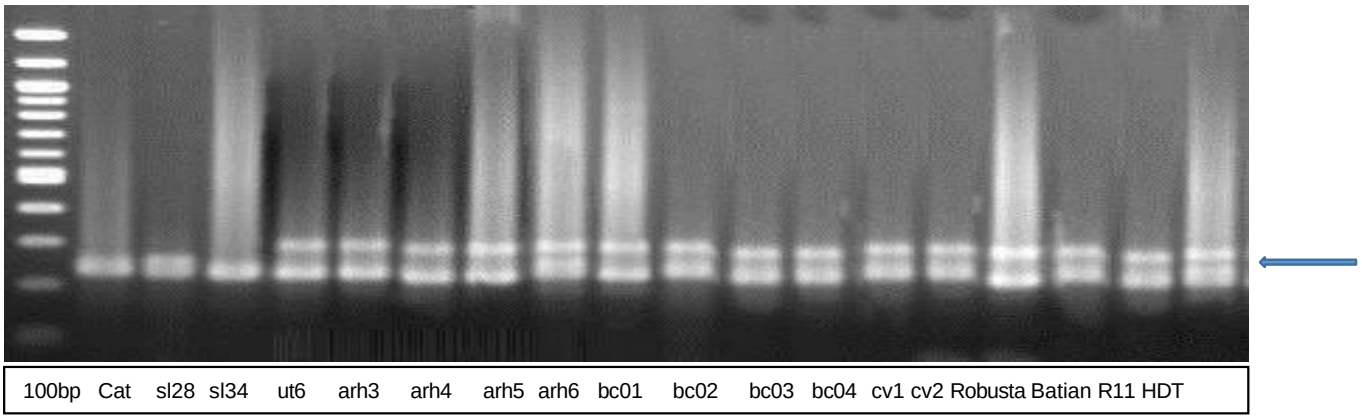


plate 4. 1 The banding patterns of the Arabusta hybrids, commercial Arabica varieties , and their parents. The arrow shows the SAT 235 introgressed allele.

Table 4. 2. Amplification products generated by SSR primers

Primer	Number of alleles	Polymorphic alleles	% polymorphism
Sat254	3	3	100
Sat 235	3	3	100
Sat11	3	3	100
Sat32	4	4	100
Sat207	4	4	100
Sat227	3	3	100
Sat240	4	3	100
Sat255	4	4	100
Sat283	3	1	33
M24	3	3	100
Sat229	2	2	100
Sat 172	1	0	0
Sat262	3	2	66
M47	2	1	50
M3	1	0	0
M2	5	5	100
M29	2	0	0
M27	1	0	0
M25	2	2	100
Total	53	43	
Range	1-5	1-5	33-100
Average	2.7	2.2	71

The amplification of the SSR markers varied among the Arabica and Arabusta coffee genotypes. The Arabica genotypes recorded total polymorphism of 46.8% with an average of 38.9% per genotype (Table 4.2). The Arabusta genotypes which include the hybrids and backcrosses recorded a total polymorphism of 72% with an average of 58.7% per genotype. Eight (Sat 262, Sat 172 Sat 254, Sat 283, M47, M3, M29, M27) and six (Sat 172, Sat 229, M24, M3, M29, M27) primers were not able to amplify the Arabica and Arabusta genotypes respectively (Table 4.2).

Table 4. 3. Amplification of SSR markers between the Arabica coffee varieties and Arabusta genotypes

Primer	Arabica			Arabusta		
	Total	P	%P	Total	P	%P
Sat 262	3	0	0	3	2	66.7
Sat 172	1	0	0	1	0	0
Sat 254	2	0	0	3	3	100
Sat 255	2	1	50	4	4	100
Sat 227	2	2	100	3	2	66.7
Sat 207	3	2	66.7	4	4	100
Sat 11	2	1	50	3	3	100
Sat 32	4	4	100	3	3	100
Sat 235	3	2	66.7	3	3	100
Sat 229	2	1	50	2	0	0
Sat 283	2	0	0	3	2	66.7
Sat 240	3	2	66.7	3	2	66.7
M 24	3	3	100	2	0	0
M 47	2	0	0	2	1	50
M 25	2	1	50	2	2	100
M 3	1	0	0	1	0	0
M 2	5	2	40	5	5	100
M 29	2	0	0	2	0	0
M 27	1	0	0	1	0	0
Total	45	21	46.8	50	36	72
Range	1-5	1-4	50-100	1-5	1-5	50-100
Average	2.3	1.1	38.9	2.6	1.9	58.7

Key; P- Polymorphism %P-Polymorphism

The highest values for genetic similarity distances/maximum similarity were obtained between genotypes SL34 vs SL28 followed closely by ARH3 vs ARH4 with 0.92 (Table 4.4). The lowest values for genetic similarity distance/minimum similarity were recorded between Ruiru11 and SL28 followed closely by Ruiru11 and SL34.

The cluster analysis using dendrogram grouped the coffee genotypes into four main distinct clusters (Figure 4.1, Figure 4.2) Cluster one comprised of SL34, SL28, and Caturra genotypes,

cluster two comprised of Arabusta hybrids and backcrosses. Cluster three included genotypes CV1, CV2, ARV, UT6, and Robusta, and cluster four included Ruiru and Batian coffee genotypes (Figure 4.2). High similarity occurred between clusters 1 and 2 with the lowest similarity was experienced between clusters 1 and 4 (Figure 4.1). The variance within class varies among the clusters being high in cluster two, followed by cluster three, cluster one, and finally, cluster four had the minimal within-class variance.

PCA classified the 18 genotypes into four main groups using principal components of 21.32% and 19.12% for F1 and F2 respectively contributing 40.65% of the variation. The Arabusta hybrids were grouped with SL8, SL34, and Caturra. Genotypes CV1, CV2, Robusta, UT6, and ARV whereas Ruiru11 and Batian were clustered in the same cluster (Fig 4.3).

Table 4. 4. Matrix of genetic distance (Dice coefficient of similarity) between 18 genotypes based on 19 SSR primers

	Caturra	SL34	SL28	ARH3	ARH4	ARH5	ARH6	BC02	BC03	BC04	BC05	CV1	CV2	Robusta	UT6	ARV	Ruiru 11	Batian
Caturra	1	0.85	0.86	0.7	0.68	0.67	0.63	0.77	0.71	0.7	0.68	0.55	0.52	0.56	0.59	0.56	0.44	0.51
SL34		1	0.93	0.67	0.68	0.71	0.63	0.7	0.68	0.7	0.71	0.55	0.48	0.67	0.66	0.63	0.28	0.41
SL28			1	0.75	0.69	0.72	0.6	0.71	0.72	0.68	0.69	0.49	0.42	0.61	0.63	0.58	0.26	0.39
ARH3				1	0.92	0.74	0.67	0.77	0.81	0.64	0.71	0.52	0.53	0.6	0.69	0.63	0.36	0.33
ARH4					1	0.72	0.64	0.75	0.79	0.61	0.69	0.53	0.54	0.61	0.63	0.64	0.37	0.34
ARH5						1	0.83	0.7	0.79	0.67	0.64	0.5	0.51	0.59	0.62	0.63	0.3	0.33
ARH6							1	0.78	0.72	0.74	0.61	0.46	0.51	0.59	0.55	0.59	0.36	0.39
BC02								1	0.85	0.76	0.74	0.52	0.53	0.6	0.66	0.67	0.36	0.38
BC03									1	0.74	0.79	0.49	0.54	0.54	0.63	0.61	0.37	0.39
BC04										1	0.89	0.6	0.64	0.7	0.66	0.67	0.33	0.44
BC05											1	0.61	0.66	0.68	0.76	0.71	0.29	0.36
CV1												1	0.85	0.74	0.76	0.74	0.42	0.5
CV2													1	0.68	0.7	0.68	0.38	0.34
Robusta														1	0.89	0.87	0.31	0.33
UT6															1	0.95	0.3	0.33
ARV																1	0.31	0.33
Ruiru 11																	1	0.86
Batian																		1

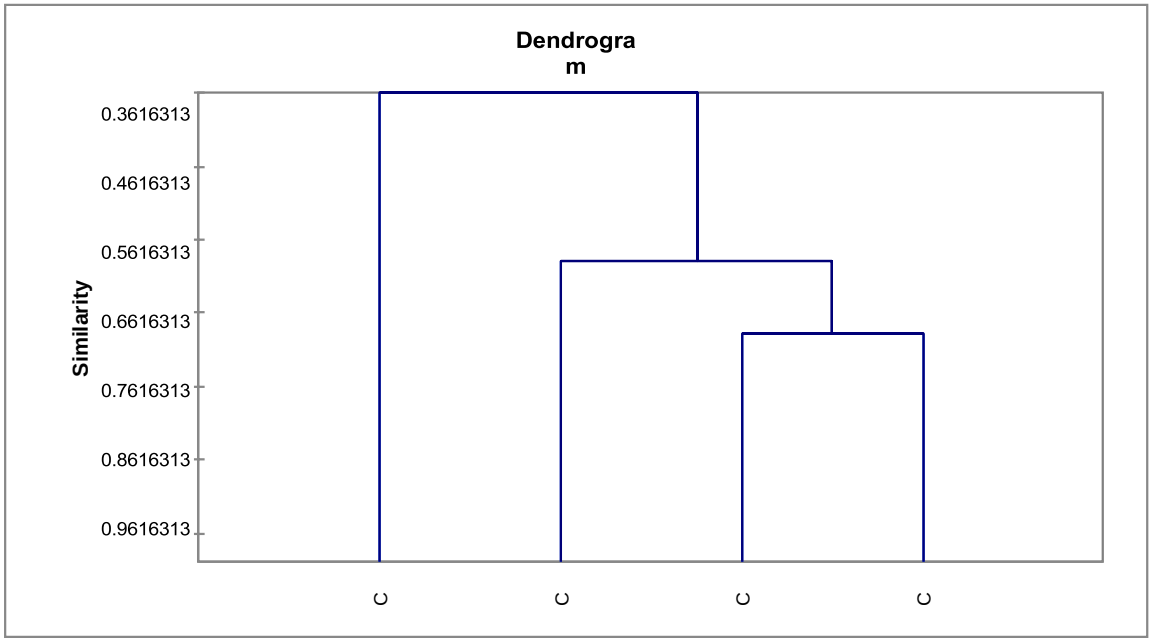


Figure 4. 1. Relationship between clusters as generated using UPGMA among coffee genotypes

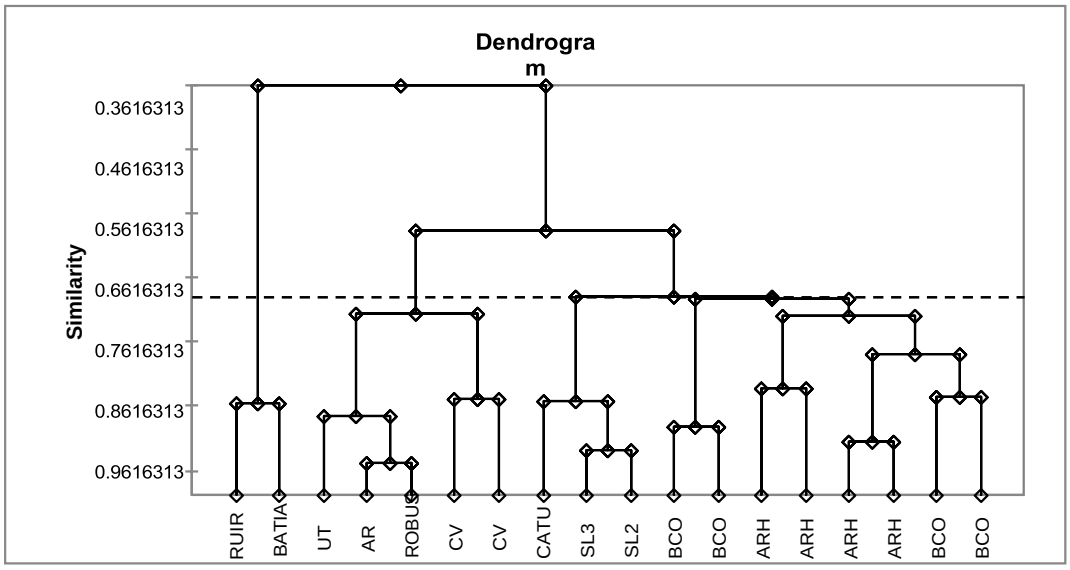


Figure 4. 2. Dendrogram by clusteranalysis showing the relationship among coffee genotypes

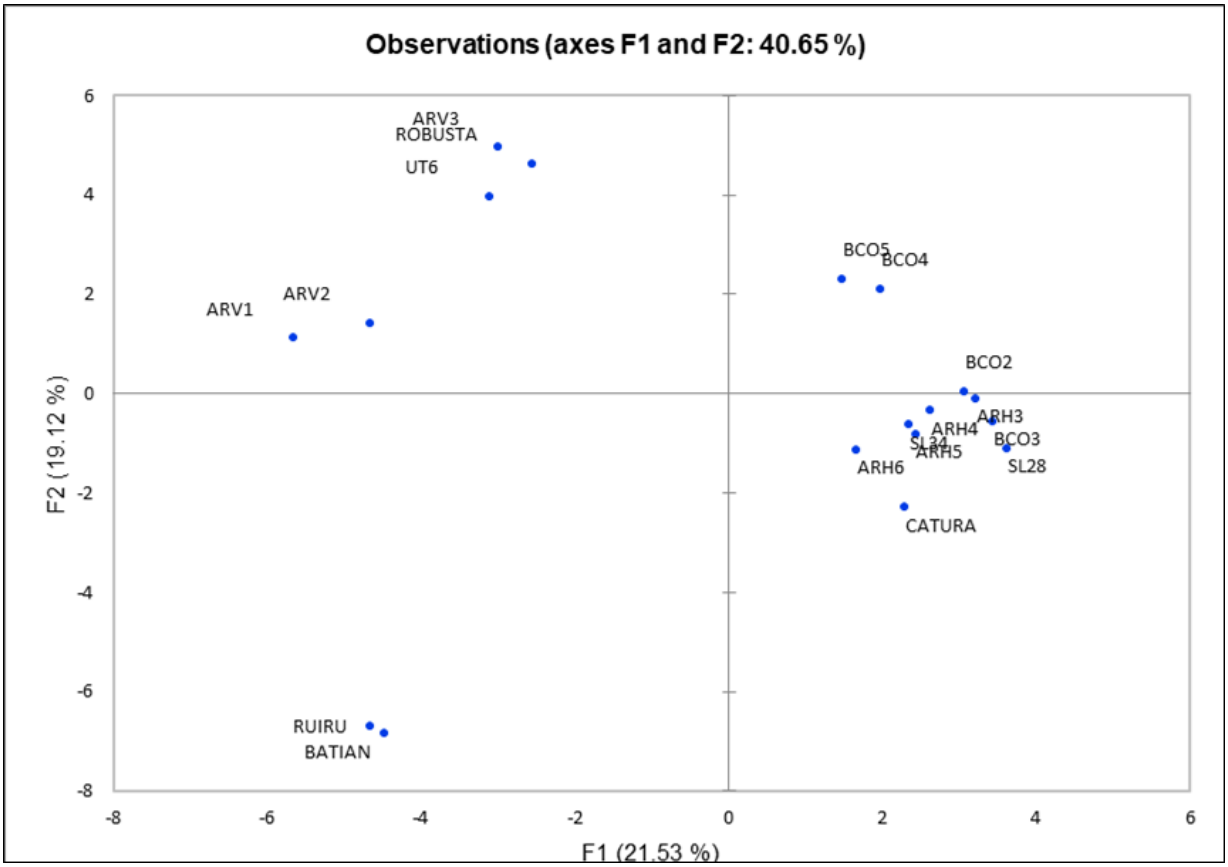


Figure 4. 3. Principal Component grouping chart for the eighteen (18) coffee genotypes

4.6 Discussion

Fifteen out of the eighteen genotypes used were found to carry the C*k* -1 gene which was identified using the SAT 235 molecular marker. The C*k* -1 gene is responsible for the resistance of coffee genotypes to Coffee Berry Disease and this marker was identified by Gichuru et al., (2008). The C*k* -1 gene occurrence was confirmed by Gichimu et al., (2014b) when evaluating the Ruiru 11 sibs. The Arabusta coffee was found to carry the C*k* -1 gene including the newly improved Arabica coffee varieties (Ruiru 11 and Batian). The susceptible varieties (SL28, SL34, and Caturra) did not carry the C*k* -1 gene. The SAT 235 marker has enabled the utilization of MAS in identifying

genotypes that are resistant to CBD that have the C*k* -1 gene. From previous studies done, Ruiru 11 hybrids with this gene have shown complete resistance to CBD hence the genotypes with the gene are believed to be resistant.

From the 18 coffee genotypes evaluated using 19 SSR primers, 53 alleles were amplified out of which 43 were polymorphic with the total number of amplified alleles per primer varying from 1.0 to 5.0. The average number of alleles produced by the nineteen primers was 2.7 with a mean of 71% polymorphism. Omingo et al., (2017) using 13 primers reported a mean of 3.8 whereas Gimase et al., (2014a) reported that 77% of the primers used amplified the alleles respectively. Polymorphism explains the extent of variation within genotypes. The occurrence of differences in terms of alleles number and percentage polymorphism is mainly attributed by sample size, nature of genotypes analyzed, the number of SSR primers employed, and their genome coverage.

The polymorphism between the Arabusta genotypes and the Arabica coffee varied, with high polymorphism(72%) calculated among Arabusta coffee and 46.8% among the Arabica genotypes. The Arabica genotypes expressed low genetic diversity when compared to the Arabusta genotypes. The low polymorphism on Arabica coffee has been reported by Motta et al., (2014). Capucho et al., (2009) and Pestana et al., (2015) reported 10% polymorphism on Arabica coffee. Vieira et al., (2010) reported low polymorphism in Arabica coffee. The narrow genetic base which caused low polymorphism among the Arabica genotypes is expected since Arabica coffee is autogamous being self-pollinated. The high polymorphism among the Arabusta coffee indicates wider genetic variation which is key for a successful breeding programme and this may be attributed to the allogamy of *C. canephora* species which is one of the parents, resulting to the wider variability.

PC1 and PC2 of 21.52% and 19.12% respectively contributed to a larger variation among the coffee genotypes. Pure line Arabica coffee (SL34, SL28, and Caturra) were clustered together with the Arabusta hybrids whereas the Batian and Ruiru 11 were separately clustered. The parental background of the Arabusta hybrids included SL28, SL34, and Caturra thus explaining why they were clustered together with Arabusta hybrids and backcrosses. PCA is an important statistical tool through which correlations among genotypes can be assessed using the geometric distance among the individuals and is more accurate (Mohammadi and Prasanna, 2003). PCA explains the variance-covariance structure through linear combinations of original variables (Johnson and Wichern, 1988). Traits having the largest absolute values closer to the unity within the first principal component (PC1) clustering highly when compared to lower absolute values near zero (Chahal and Gosal 2002).

The cluster analysis grouped the genotypes into four different clusters (Figure 4.2) where Batian and Ruiru 11 genotypes were clustered together. This could be as a result of having the same parental background used during the hybridization programme when developing these two varieties. The Arabusta hybrids clustered together with the Arabusta backcrosses since they had similar parents that were used during the crossing. The Caturra, SL28, and SL34 genotypes are pure line Arabica coffee and were grouped and this is a result of the genotypes being selected through single plant selection and not as a result of hybridization. Omingo et al., (2017) and Gimase et al., (2014a) reported a similar clustering behavior among the different coffee species. The similarity index at the accession level fell in the range of 0.26 to 0.93 indicating a high level of polymorphism among the genotypes. Mission et al., (2009b) and Gimase et al., (2014a) reported different levels of polymorphism within the Arabica, Arabusta, and Robusta coffee they evaluated.

Genotypes SL28 and SL34 had a very high percentage of similarity (0.93) whereas Batian and Ruiru 11 also exhibited a closer relationship (0.86) (Table 4.4). In general, the 18 coffee genotypes clustered based on the source of genetic origin or genetic constitution. The cluster dendrogram represents the most probable genetic relationship between the different coffee genotypes.

Dice coefficient of similarity was used to generate a similarity matrix to understand the polymorphism levels among the various coffee genotypes. The genetic distance is the extent of gene differences (allelic variation) between species or populations that can be measured using genetic differences calculated within species or populations by use of sequence or allele frequencies (Nei, 1987). Based on the genetic distance, the similarity matrix was calculated to establish genetic diversity and variability differences (Aluka, 2013). The extent of sample distribution, areas of sampling and plant features and characteristics, breeding performance, and time of generation are core factors in determining genetic variation in a species. Therefore, the analysis of genetic diversity is a core factor for continued crop improvement and conservation (Omingo et al., 2017). The use of SSR marker made it possible to identify those genotypes with C*k* -1 gene that are resistant to CBD. SSR molecular marker systems can be employed for assessment of the genetic diversity of coffee genotype. The Arabusta hybrids and their backcross derivatives had higher levels of variations when compared to Arabica coffee. The variations are key in selecting diverse genotypes to be used in breeding when developing new varieties to widen the genetic base.

CHAPTER FIVE:

BEAN PHYSICAL AND LIQUOR QUALITY OF ARABUSTA COFFEE HYBRIDS AND BACKCROSS PROGENIES

5.1 Abstract

Robusta coffee is a high yielding coffee species and its growth is vigorous when compared to Arabica coffee however, it fetches lower market prices due to the inferior cup quality known to have. This study aimed to characterize the bean physical features and sensory traits of Arabusta hybrids and its backcross derivatives. Nineteen (19) coffee genotypes were evaluated at Siaya ATC and KALRO-Alupe. The beans harvested during the growing season of 2018 were subjected to bean grading and sensory analysis and the data was collected on various bean grades and liquor quality performance. The beans were graded into seven different grades using the pneumatic separator. A panel of five judges was involved in the cupping procedure to assess the flavour, aroma, body, acidity, preference, balance, and aftertaste of the roasted coffee beans using the Specialty Coffee Association (SCA) method. From the results, the variations on the traits measured were significant from the two different locations. Bases on bean grade analysis, the Arabusta hybrids produced large beans when compared to the Robusta coffee and the backcrosses. For AA bean grade, genotypes ARH4 recorded 21.2% at KALRO-Alupe whereas, in Siaya ATC, genotypes ARH6 recorded 38% and ARH1 recorded 36.3%. The cultivar SL28 which is an Arabica coffee variety was used as a standard in measuring the sensory traits recording the highest total score of 85.8%, followed closely by Batian with 83.4% and Arabusta hybrid-ARH7 was third with 82.6%. The correlations between the sensory traits were highly significant. Acidity showed positive correlation with aftertaste(r=0.96), aroma (r=0.84), balance (r=0.85), flavour(r=0.96) and preference (r=0.96). The sensory traits showed negative correlations with AA, PB, and C bean

grade sizes. The 100 bean weight and AA bean size showed a positive significant correlation(r=0.74) and this indicates that the prediction of AA bean size can be determined using the 100 bean weight. All the Arabusta hybrids evaluated outperformed Robusta in cup quality and bean size scoring over 80% on total score thus qualifying for the specialty market.

5.2 Introduction

Coffee and tea are the common beverages consumed all over the world with a total consumption of 148 million cups being consumed annually since the two drinks are the most preferred globally (ICO 2018a). The liquor quality of Robusta coffee is inferior whereas that of Arabica coffee is superior and therefore most of the time, coffee from Arabica species has always been blended at a ratio of 50:50 with Robusta coffee to increase its quality and formation of the crema (Folmer et al., 2017, Dias et al., 2018 and Liu et al., 2019). Cup quality has been used in determining the prices of coffee in the market thus a critical trait in the selection of coffee varieties and is also known as the liquor quality (Muschler, 2001, Kathurima 2013, Curzi et al., 2014). The coffee cup quality is a key factor in countries that produce and export coffee including Kenya. The quality of coffee is a significant factor within coffee value chain from the breeding program, producers which are the farmers, marketers, and consumers. The uptake of coffee by the consumers depends on their preferences which include the liquor quality, country of origin, and biochemical characteristics (Fridell, 2014). The G x E interaction affects the final cup quality and in the marketing aspect, the country of origin of the coffee is indicated always in the packaging labels (William et al., 2014, Cheng et al., 2016).

In coffee breeding, selection for cup quality is key and regarded with high importance as yield and resistance to coffee diseases. The market price for Robusta is low when compared to Arabica due

to its inferior cup quality. Since Robusta coffee has a higher growth vigour when compared to Arabica, its production per unit area is always high, and also its tolerance to pests gives it an added advantage to outperform the Arabica coffee (Mendes et al., 2001). However, the pricing of coffee in the market gives Arabica an advantage to Robusta due to liquor quality (ICO 2018b). In Arabica coffee, the aroma and flavour generated make the coffee brew to be dense and rich giving it a good body (Ewa and Grazyna, 2006). While Arabica coffee gives an intense aroma in the brew, when combined with Robusta the quality changes since the Robusta will add an astringent bitter taste. (Bicchi et al., 1995)

Sensory analysis although it is less objective than when using instruments, it is easy to carry out in determining cup quality. Determination of cup quality traits such as body, balance, flavor, and aftertaste has always been carried out through liquoring and takes the shortest time possible (Ewa and Grazyna, 2006). The expression of different sensory traits occurs during roasting whereby, different compounds are emitted which can be determined during the sensory analysis which includes the aroma and flavour (Gichimu and Omondi, 2010). It has been difficult over time to try to use other methods other than sensory evaluation to determine the cup quality (Sanz et al., 2002). Hence, different coffee genotypes have been evaluated over time for their performance on cup quality suing the sensory evaluation methods. Different tasters have been trained on liquoring processes for organoleptic tests for determining cup-quality performances and this has enhanced the selection efficiency in breeding programs (Van der Vossen, 1985; Agwanda, 1999 and Walyaro, 1983). Owuor (1988) reported that during the ranking of different coffee varieties, the panel was able to give scores that showed similarities and this implied that any panel could be relied upon in tasting coffee

The quality of coffee is determined by both the sensory and bean grade characteristics. The bean grades have also been used in determining the market prices whereby, beans with larger sizes have an advantage of fetching high prices as compared to smaller beans. The bean physical traits vary also in terms of shape and colour. Beans of the same size and shape allows uniform roasting which affects the final taste of the coffee. The colour of beans has been used in sorting out the defective beans which could be a result of genetics, insect damage or processing which affects the uniformity obtained when roasting affecting the cup quality negatively (Batista & Chalfoun, 2014; Illy & Viani, 2005). During roasting, small beans without uniformity roast faster than larger and uniform beans and this affects the cup quality (Barel and Jacquet, 1994). The coffee beans' characteristics vary depending on the varieties country of the origin where it is cultivated. The efficiency obtained from sensory evaluation depends on the panel used during the process since a trained panel will be more objective and will give reliable results from generating enough data than using an individual thus improved selection (Hampson et al., 2000). Different coffee genotypes were assessed for sensory and bean physical attributes assessment to characterize their performance in terms of quality.

5.3 Materials and Methods

The materials and methods for this section are as presented in chapter 3, section 3.3.1.

5.3.1 Bean processing and grading

After harvest, the coffee cherries underwent primary wet processing to remove the pulp and mucilage followed by drying the parchment as recommended by Kathurima et al, (2010). The beans were dried under the sun to reduce the moisture content of 10.5 to 11 % moisture content (Mburu 2004). The parchment was dehulled to remove the coffee husks using a hand operating machine and the one kilogram of beans from specific genotypes was taken for grading. The beans

were graded into seven different grades namely AA, AB, PB, C, E, TT, and T bean grades using a bean grading machine a hand-operated machine. The grading of the beans was based on bean size, shape, and density as described by Gichimu et al., (2013). The density was determined by using a pneumatic separator (Sortex, London, England), to separate light beans from AA and AB bean grades. The light beans obtained from the pneumatic separator are termed as TT beans. The grading machine separates the beans based on sizes as follows, AA- Beans retained by 7.15mm screen, AB- Beans retained by 5.95mm screen, PB- Beans retained by a piano wire screen with 4.43mm spaces, C- Beans retained by a piano wire screen with 2.90 mm spaces, T- small broken beans which could not be retained by any of screens, E- Beans retained by a piano wire with 8.3 mm space. Beans from each grade were weighed in grams and its percentage obtained based on the 1 kg of beans initially used.

5.3.2 Sensory evaluation of coffee

The AA and AB beans obtained during grading for each coffee genotypes were used in the sensory evaluation. The beans were first weighed before roasting to determine the degree of roasting when weighing after the roasting process is complete. The roasting of the coffee beans was carried out using a probate laboratory roaster after which the roasted beans were left out to cool down for 8 hours before liquoring. The beans were then ground and measured to achieve a weight of 8.25 grams for each cup. Each sample representing each coffee genotype were placed inside five cups (Kathurima et al., 2009). 150 ml of hot water was added into each cup. The organoleptic evaluation for the different sensory traits was carried out using a panel of five judges who have been trained and have qualified for cupping using the procedures described by Lingle (2001). The Specialty Coffee Association(SCA) have described the different descriptors measured during

sensory evaluation which include flavour, body, balance, acidity, body, aftertastes, fragrance/aroma, flavour, aftertaste, and preference.

Uniformity, sweetness, and clean cup were also scored and given a maximum score of 10 by adding two points per cup. The scores were added to the scores obtained from seven sensory traits to achieve a total score of 100. From this, it was then possible to analyse and evaluate genotypic performance in terms of liquor quality for each genotype. Scores from each liqourer were used as a replication thus a total of five replications during sensory evaluation.

5.4 Statistical Analysis

The data generated from the sensory evaluation were subjected to Analysis of Variance (ANOVA) using the GLM and their effects declared at 5% using the using GENSTAT statistical software version 18 The General Linear Model (GLM) was used (Jansen, 1993).

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + E_i$$

Where,

For each observation $i=1,\ldots..,n.$ where n is the observations of one dependent variable

$\hat{Y} = j^{th}$ observation of the dependent variable

$j= 1,2,\ldots....., k$

X = is the observation of the j^{th} independent variable

β = parameters to be estimated

Ei = Distributed normal error

LSD was used to separate means and the effects declared at 5% (Martin et al., 1975) Separate as well as combined analysis of variance was performed on data from the two locations. Cluster analysis was derived using an unweighted pair-group method with arithmetic average (UPGMA) to create a dendrogram based on Euclidean distances (Hue et al, 2000). The cluster analysis was

derived using the XLSTAT version 2019. (Pearson's chi-square was used to test the similarit ies among clusters. GENSTAT statistical software was used to compute correlation and to show the relationship between bean grades and sensory traits using the Pearson Correlation Coefficient. The Principle Component Analysis of the sensory characteristics were plotted based on the important principle components (Lattin et al., 2002) using XLSTAT statistical software, version 2012.

5.5 Results

5.5.1 Bean grading

There were significant (P≤ 0.05) differences in all the bean grade traits measured among the different coffee genotypes established in the different locations.

Table 5. 1. Mean squares for bean traits of coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) in the growing season of 2018

	Busia			**Siaya**		
	Rep	**Genotype**	**Error**	**Rep**	**Genotype**	**Error**
DF	2	19	38	2	19	38
E	0.37	14.35***	1.70	0.12	27.22***	0.89
AA	13.90	333.15**	47.90	50.21	575.64***	51.19
AB	337.50	310.7**	136.00	59.09	434.65***	57.87
PB	6.40	17.86**	6.70	0.83	9.99***	3.16
C	29.39	78.68***	14.21	25.40	154.19***	16.91
T	1.66	1.63***	0.27	0.03	0.82***	0.16
TT	55.17	177.50**	80.20	20.46	100.29***	11.61
100 BW	4.35	10.48*	4.82	0.62	20.47***	2.70

Key: *, **, *** represent significant at (P<0.05), (P<0.01), (P<0.001) and non-significant respectively. **:** AA-% of beans retained by 7.15mm screen, AB- % of beans retained by 5.95mm screen, TT-5 of beans separated from grades AA and AB by density, PB- % of beans retained by a piano wire screen with 4.43mm spaces, C= % of beans retained by a piano wire screen with 2.90 mm spaces, T- % of very small beans and broken bits that cannot be retained by all the above screens and E- % of beans retained by a piano wire screen of the coffee bean grading machine with 8.3 mm space.100 BW(g)- 100 bean weight in grams.

Genotype, BC04 recorded a significantly (P≤ 0.05) higher percentage of E grade in Busia (4) when compared to other genotypes whereas genotype CV2 in Siaya did not record any beans in this category. The AA grade value was significantly (P≤ 0.05) higher in Batian (44.7) followed closely by genotype ARH6 (38) in Siaya (Table 5.1). The percentage AB grade varied from among the genotypes ARH4 recorded the highest whereas genotype ARH7 recorded the least. Genotype ARH6 recorded a significantly (P≤ 0.05) high percentage of PB grade in Busia when compared to the other genotypes in both locations. The quantity of C grade was high in genotype CV2 in Siaya and significantly (P≤ 0.05) lower in genotypes BC02 and genotype BC03 in Siaya. (Table 5.1). Robusta recorded a higher percentage of T beans in Siaya and genotype ARH7 recorded a significantly (P≤ 0.05) high percentage of TT beans in Busia followed closely by genotype BC03 in Siaya. The 100 bean weight varied across the locations where genotype ARV recorded a higher percentage in Siaya (21) whereas genotypes BC04 and ARV (20, 21) both recorded also a high percentage in Busia.

The variations on the performance of the genotypes in the different locations were significant for the bean grades. For the E bean grade, genotype BC04 recorded a significantly (P≤ 0.05) higher percentage (2.4) with genotypes BC03 and CV1 having recorded the lower value (0.2). The percentage of AA bean grade varied significantly among the coffee genotypes with ARV recording a higher percentage (38.4) whereas CV2 recorded the lowest of (Table 5.2). The bean grade AB was significantly high in Robusta and genotype ARH4. For the PB bean grade, there were significant (P≤ 0.05) differences among coffee genotype whereby, BC05 recorded a higher percentage when compared to other genotypes, and genotype ARV recorded the lowest (Table 5.3). The percentage of C grade was also significantly (P≤ 0.05) different from genotype CV2 recording the highest (23.1%) and Robusta recorded the lowest (1.1%). There was a variation on

T bean grade among the different coffee genotypes where genotype BCO3 had a significantly ($P \leq 0.05$) high percentage whereas genotypes ARH4 and ARV recorded the lowest. The TT bean grade varied among the coffee genotypes, genotype BC03 scored the highest (20.3%) and Robusta scored the lowest (1%). 100 bean weight varied significantly among the genotypes where genotype ARV recorded significantly ($P \leq 0.05$) heavy beans (21) followed closely by genotype BC04 and genotype CV2 scored light beans (13.7) (Table 5.3). There were significant ($P<0.05$) differences recorded for the bean grade characteristics among the genotypes. The environmental variation was significant for the AA, C, T, and the TT bean grades. The G x E interaction was highly ($P<0.05$) significant for all the bean grade traits except for the AB grade (Table 5.3).

Table 5. 2. Bean grade traits for coffee genotypes at KALRO- Alupe (Busia) and Siaya ATC (Siaya)

	% E		% AA		%AB		% PB		% C		% T		% TT		100 BW(g)	
Genotypes	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si
ARH1	0.3	0.8	11.9	30.3	62.6	59.7	2.9	2.2	6.2	2.0	0.8	0.4	15.4	4.6	19.0	18.5
ARH4	0.7	0.1	21.2	7.7	67.8	82.2	2.5	4.6	4.1	4.5	0.5	0.1	3.1	0.9	16.7	15.5
ARH5	1.2	1.4	16.5	16.1	65.8	50.1	1.8	4.4	12.5	24.0	0.4	1.4	1.8	2.7	16.9	13.3
ARH6	1.4	2.1	10.7	38.0	61.2	53.2	7.2	1.1	15.1	2.7	0.8	0.2	3.6	2.7	16.3	17.2
ARH7	0.8	0.2	7.0	26.2	39.5	62.3	2.2	4.0	13.5	5.4	2.4	0.5	34.7	1.5	16.1	15.5
BC01	0.3	0.5	8.6	8.4	66.4	65.7	2.7	6.1	15.3	17.9	1.4	0.6	5.4	0.9	15.7	13.5
BC02	2.1	2.2	11.0	35.8	56.3	55.9	4.8	0.8	15.4	3.4	0.5	1.2	10.0	0.6	14.7	18.0
BC03	0.2	0.2	6.7	15.8	62.6	51.3	1.4	4.3	11.2	1.6	3.2	0.4	14.7	26.3	14.2	18.3
BC04	4.0	0.8	32.1	24.9	46.3	69.4	1.7	0.8	6.5	1.6	1.3	0.5	8.0	2.0	20.1	21.2
BC05	1.7	1.3	20.3	24.2	55.8	59.7	3.2	6.4	7.0	4.9	0.6	0.4	11.4	3.0	16.1	15.8
BC06	0.6	0.8	19.2	33.4	72.2	58.2	1.0	1.9	5.3	3.8	0.5	0.5	1.2	1.5	16.6	16.2
CV1	0.3	0.1	6.8	2.8	73.1	75.3	2.3	3.3	5.9	7.7	0.4	0.4	11.3	10.4	15.4	13.3
CV2	0.7	0.0	3.8	0.3	57.0	62.9	2.4	4.1	21.0	25.2	1.2	1.1	14.0	6.5	16.0	11.4
ARV	2.3	1.4	42.0	34.8	47.0	58.2	0.4	0.7	1.4	0.8	0.5	0.1	6.4	4.0	21.1	21.0
Robusta	0.5	0.1	13.9	9.6	62.5	61.0	0.7	4.1	14.6	17.4	1.0	2.5	6.8	6.3	14.5	14.7
Ruiru	1.9	0.8	28.9	36.7	59.5	56.6	2.8	1.1	2.2	3.1	0.6	0.7	4.1	1.0	17.4	15.5
Batian	0.9	1.8	17.7	44.7	65.8	44.8	1.6	2.5	7.3	3.3	0.8	1.8	6.0	1.2	15.1	16.8
LSD	2.2	1.6	19.3	11.8	19.4	12.6	4.4	3.0	6.2	6.8	0.9	0.7	14.8	5.6	3.6	2.8
%CV	8.6	5.2	7.0	6.5	6.3	2.9	19.3	7.5	13.2	16.6	30.3	5.8	18.9	24.9	3.6	1.1
Ftest	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Key:% AA-% of beans retained by 7.15mm screen, %AB- % of beans retained by 5.95mm screen, %TT-5 of beans separated from grades AA and AB by density, %PB- % of beans retained by a piano wire screen with 4.43mm spaces, % C-% of beans retained by a piano wire screen with 2.90 mm spaces, %T- % of very small beans and broken bits that cannot be retained by all the above screens and %E- % of beans retained by a piano wire screen of the coffee bean grading machine with 8.3 mm space.100 BW(g)- 100 bean weight in gram. Bu- Busia, Si- Siaya

Table 5. 3. Bean grade traits of coffee genotypes at KALRO-Alupe (Busia) and Siaya ATC

Genotype	%E	%AA	%AB	%PB	%C	%T	%TT	100 BW
ARH1	0.6	21.1	61.2	2.5	4.1	0.6	10.0	18.7
ARH4	0.4	14.5	75.0	3.6	4.3	0.3	2.0	16.1
ARH5	1.3	16.3	57.9	3.1	18.3	0.9	2.2	15.1
ARH6	1.7	24.4	57.2	4.2	8.9	0.5	3.1	16.8
ARH7	0.5	16.6	50.9	3.1	9.4	1.5	18.1	15.8
BC01	0.4	8.5	66.0	4.4	16.6	1.0	3.1	14.6
BC02	2.1	23.4	56.1	2.8	9.4	0.9	5.3	16.3
BC03	0.2	11.3	57.0	2.9	6.4	1.8	20.5	16.3
BC04	2.4	28.5	57.9	1.3	4.0	0.9	5.0	20.7
BC05	1.5	22.3	57.8	4.8	6.0	0.5	7.2	15.9
BC06	0.7	26.3	65.2	1.4	4.6	0.5	1.3	16.4
CV1	0.2	4.8	74.2	2.8	6.8	0.4	10.8	14.4
CV2	0.4	2.0	59.9	3.2	23.1	1.1	10.2	13.7
ARV	1.8	38.4	52.6	0.6	1.1	0.3	5.2	21.0
Robusta	0.3	11.7	76.7	0.9	8.5	0.8	1.0	14.6
Ruiru 11	1.3	32.8	58.0	1.9	2.6	0.7	2.6	16.5
Batian	1.3	31.2	55.3	2.1	5.3	1.3	3.6	15.9
LSD	1.3	8.0	11.5	2.6	4.6	0.6	7.8	2.2
% CV	3.4	5.5	3.6	13	7.4	16.4	14.4	1.6
F Test	S	S	S	S	S	S	S	S

Key: % AA-% of beans retained by 7.15mm screen, %AB- % of beans retained by 5.95mm scre en, %TT-5 of beans separated from grades AA and AB by density, %PB- % of beans retained by a piano wire screen with 4.43mm spaces, % C-% of beans retained by a piano wire screen with 2.90 mm spaces, %T- % of very small beans and broken bits that cannot be retained by all the above screens and %E- % of beans retained by a piano wire screen of the coffee bean grading machine with 8.3 mm space.100 BW(g)- 100 bean weight in grams

There were significant ($P \leq 0.05$) differences in all the % pea berry traits measured among the different coffee genotypes across the two locations. In Busia, on average the Arabusta hybrids recorded a significantly higher percentage of the pea-berries when compared to the backcrosses

and Arabica coffee. Robusta recorded the highest percentage (72%) followed closely by Robusta (72%). In Siaya, on average the Arabusta hybrids recorded significantly ($P \leq 0.05$) high percentage followed by genotype ARV whereas CV2 recorded the least (8.2%) (Table 5.3). For combined mean analysis on average the Arabusta hybrids still recorded higher percentages of the peaberries when compared to Arabica coffee.

Table 5. 4. Separate and combined % pea berry beans coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia)

	% pea berries in 200 beans		
Genotypes	Busia	Siaya	Combined means
ARH1	62.0	33.3	47.7
ARH4	43.3	35.2	39.3
ARH5	46.7	35.8	41.3
ARH6	52.2	35.7	43.9
ARH7	28.3	22.3	25.3
BC01	21.5	51.7	36.6
BC02	28.5	16.0	22.3
BC03	68.0	42.5	55.3
BC04	52.3	34.8	43.6
BC05	41.5	19.2	30.3
BC06	20.2	25.3	22.8
CV1	9.7	11.3	10.5
CV2	68.8	8.2	38.5
ARV	51.7	44.5	48.1
Robusta	72.7	29.7	51.2
Ruiru	25.2	15.2	20.2
Batian	32.0	13.0	22.5
LSD	11.2	29.0	7.1
%CV	1.4	3.2	1.9
Ftest	S	S	S

Table 5. 5. Mean squares for bean grade traits of 19 coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia)

	Rep	Gen (G)	Envt (E)	G x E	Error
DF	2	17	1	17	78
E	0.11	19.644***	0.24NS	21.923***	1.28
AA	52.58	612.8***	1344.12***	295.99***	48.58
AB	179.20	489.1***	28.7NS	256.3NS	100.00
PB	5.41	12.816**	1.606NS	15.042***	4.98
C	13.99	181.01***	167.37**	51.86***	16.21
T	0.70	1.2682***	2.4053**	1.177***	0.24
TT	33.95	169.65***	664.21***	108.13**	45.80
100 BW	2.72	21.76***	1.68NS	9.191**	3.72

Key: *, **, *** and NS represent significant at (n), (P<0.001), (P<0.0001) and non-significant respectively. **:** AA-% of beans retained by 7.15mm screen, AB- % of beans retained by 5.95mm screen, TT-5 of beans separated from grades AA and AB by density, PB- % of beans retained by a piano wire screen with 4.43mm spaces, C= % of beans retained by a piano wire screen with 2.90 mm spaces, T- % of very small beans and broken bits that cannot be retained by all the above screens and E- % of beans retained by a piano wire screen of the coffee bean grading machine with 8.3 mm space.100 BW(g)- 100 bean weight in grams.

5.5.2 Sensory performance

The variations on the sensory traits were significant among the coffee genotypes across the two locations except for acidity in Busia and balance in Siaya (Table 5.6).

Table 5. 6. Mean squares for sensory traits of coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) in the growing season of 2018

	Busia			Siaya		
	Rep	Genotype	Error	Rep	Genotype	Error
DF	4	18	72	4	18	72
Aroma	0.04	0.16****	0.04	1.37	0.31**	0.12
Flavour	0.32	0.36***	0.04	0.50	0.39***	0.13
Aftertaste	0.63	0.20***	0.04	0.53	0.34***	0.11
Acidity	0.41	0.4	0.07	0.72	0.49***	0.11
Body	0.24	0.07***	0.09	0.79	0.18**	0.09
Balance	0.13	0.21***	0.04	0.93	0.26	0.22
Preference	0.22	0.35***	0.05	0.36	0.45***	0.11
Total score	0.58	0.25***	0.05	10.12	15.161***	3.57

Genotype, SL28 recorded a higher value of aroma in Siaya (8.2) whereas Robusta recorded a significantly ($P<0.05$) lower value (Table 5.7). Flavour varied among the coffee genotypes and SL28 recorded high values in both locations whereas CV1 recorded the least (6.7) in Siaya (Table 5.7). Genotype, SL8 recorded significantly ($P<0.05$) higher values for aftertaste in Siaya and Busia whereas Batian followed closely in Siaya. SL 28 recorded higher values for acidity in both locations whereas Robusta and BC01 both recorded the least values of in Siaya. Genotype, ARH5 recorded a higher value for the body in Busia after SL28. Arabusta hybrids and their backcross progenies scored a total score of over 80% in Busia (Table 5.7). Genotype, SL28, recorded significantly ($P<0.05$) higher values for total scores at 85.9 and 86.2 at Busia and Siaya respectively whereas genotypes that scored low were ARV and Robusta in Siaya and Busia respectively (Table 5.7).

There were significant variations from sensory characteristics obtained through combined mean analysis for the two locations where the experiments were conducted (Table 5.8). The most outstanding genotype was SL28 which recorded significantly ($P\leq 0.05$) higher values on the sensory attributes except on acidity among the coffee genotypes (Table 5.5). Lower values for aroma (6.9) and body (7.2) were recorded from Robusta coffee. Batian high value for acidity (7.7) whereas genotype, CV1 recorded significant ($P\leq 0.05$) lower values on flavour, aftertaste, and acidity. ARH4, BC04, BC01, and BCO6 recorded significantly ($P\leq 0.05$) low values for balance Genotypes, CV1, CV2, and Robusta recorded total score values of less than 80% (Table 5.8).

Table 5. 7. Sensory traits for coffee genotypes at KALRO-Alupe and Siaya ATC

Genotypes	Aroma		Flavour		Aftertaste		Acidity		Body		Balance		Preference		Total score	
	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si
ARH1	7.5	7.0	7.4	7.0	7.6	7.1	7.4	7.2	7.7	7.5	7.4	7.2	7.4	7.1	82.4	80.1
ARH4	7.4	7.5	7.2	7.0	7.4	6.9	7.2	7.0	7.6	7.4	7.3	7.0	7.4	7.0	81.5	79.8
ARH5	7.8	7.3	7.5	7.2	7.7	7.1	7.5	7.1	7.8	7.5	7.6	7.1	7.6	7.2	83.5	80.5
ARH6	7.5	7.4	7.3	7.3	7.4	7.3	7.4	7.4	7.5	7.6	7.6	7.4	7.5	7.4	82.2	81.8
ARH7	7.6	7.6	7.5	7.4	7.7	7.2	7.6	7.4	7.7	7.7	7.7	7.3	7.7	7.4	83.5	82.0
BC01	7.4	7.4	7.2	6.9	7.3	6.8	7.3	6.9	7.6	7.4	7.4	7.0	7.4	6.9	81.6	79.3
BC02	7.5	7.5	7.3	7.2	7.3	7.1	7.3	7.0	7.5	7.5	7.2	7.2	7.3	7.1	81.4	80.6
BC03	7.8	7.7	7.1	7.3	7.3	7.3	7.3	7.5	7.5	7.5	7.2	7.4	7.4	7.4	81.6	82.3
BC04	7.6	7.5	7.4	7.2	7.5	7.2	7.5	7.4	7.5	7.5	7.4	7.1	7.4	7.2	82.3	81.2
BC05	7.7	7.4	7.6	7.3	7.6	7.1	7.6	7.4	7.7	7.5	7.8	7.4	7.7	7.4	83.7	81.6
BC06	7.6	7.4	7.4	7.0	7.4	6.8	7.5	6.8	7.6	7.5	7.4	7.0	7.4	6.9	82.3	79.0
CV1	7.6	7.1	7.2	6.7	7.4	6.6	7.2	6.7	7.6	7.5	7.4	7.3	7.3	6.6	81.7	78.2
CV2	7.4	7.2	7.2	6.8	7.4	6.9	7.2	6.9	7.5	7.5	7.4	7.0	7.3	6.7	81.4	78.7
ARV	7.4	7.6	7.4	7.3	7.5	7.3	7.4	7.4	7.7	7.5	7.4	7.4	7.4	7.4	82.2	82.0
Robusta	6.8	6.9	7.2	7.0	7.1	7.1	7.0	6.9	7.2	7.5	7.0	7.0	7.1	7.0	79.5	79.1
Ruiru 11	7.7	7.2	7.3	7.0	7.3	7.0	7.5	7.2	7.5	7.5	7.3	7.4	7.4	7.3	82.0	80.6
Batian	7.6	7.9	7.5	7.9	7.3	7.9	7.4	8.1	7.6	7.5	7.2	7.2	7.2	7.2	81.8	83.8
SL28	8.1	8.2	7.9	8.2	8.0	8.1	7.8	8.2	7.9	7.5	7.9	7.9	8.3	7.9	85.9	86.2
LSD	0.3	0.4	0.3	0.5	0.3	0.4	0.3	0.4	0.4	7.5	0.3	0.6	0.3	0.4	1.5	2.4
%CV	0.7	3.6	1.8	2.3	2.5	2.4	2.0	2.7	1.6	7.5	1.1	3.1	1.5	1.9	0.7	0.9
Ftest	S	S	S	S	S	S	S	S	NS	7.5	S	S	S	S	S	S

Key: Bu- Busia Si- Siaya

Table 5. 8. Bean sensory traits of coffee genotypes at KALRO-Alupe (Busia) and Siaya ATC

Genotypes	Aroma	Flavour	Aftertaste	Acidity	Body	Balance	Preference	Total score
ARH1	7.2	7.2	7.3	7.3	7.6	7.3	7.2	81.1
ARH4	7.5	7.1	7.1	7.1	7.5	7.2	7.2	80.7
ARH5	7.5	7.4	7.4	7.3	7.6	7.3	7.4	81.9
ARH6	7.4	7.3	7.3	7.4	7.5	7.5	7.4	81.8
ARH7	7.6	7.4	7.4	7.5	7.7	7.5	7.5	82.6
BC01	7.4	7	7.1	7.1	7.5	7.2	7.1	80.4
BC02	7.5	7.3	7.2	7.1	7.5	7.2	7.2	81
BC03	7.7	7.2	7.3	7.4	7.6	7.3	7.4	81.9
BC04	7.5	7.3	7.3	7.4	7.5	7.2	7.3	81.5
BC05	7.5	7.5	7.3	7.5	7.6	7.6	7.5	82.5
BC06	7.5	7.2	7.1	7.1	7.3	7.2	7.1	80.5
CV1	7.3	6.9	7	6.9	7.4	7.3	6.9	79.7
CV2	7.3	7	7.1	7	7.3	7.2	7	79.9
ARV	7.5	7.3	7.4	7.4	7.6	7.4	7.4	82
Robusta	6.9	7.1	7.1	7	7.2	7	7.1	79.4
Ruiru	7.3	7.1	7.1	7.2	7.5	7.4	7.4	81
Batian	7.7	7.7	7.6	7.7	7.7	7.5	7.5	83.4
SL28	8.3	8.2	7.9	7.2	8	8.1	8.1	85.8
LSD	0.3	0.3	0.3	0.3	0.3	0.4	0.3	1.5
%CV	1.7	0.9	0.9	1.1	1.6	1	0.8	0.2
Ftest	S	S	S	S	S	S	S	S

The results indicated that there existed a variation among the coffee genotypes for all the sensory attributes except for balance only. The environmental variations were significant for all the sensory traits. The G x E interaction was not significant for all the sensory traits measured (Table 5.9). Preference scored a higher maximum score (8.1), whereas acidity scored the least (6.9). The highest-rated sensory attribute was Body with a higher mean (7.53) when compared to other genotypes followed closely by Aroma with a score whereas flavour and Aftertaste had the lowest mean. Acidity and preference had a wider variance range whereas Body had the least (0.53) (Table 5.10).

Table 5. 9. Mean squares for sensory traits of 17 coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia*)*

Source	Rep	Gen (G)	Envt (E)	G x E	Error
Df	4	17	1	17	140
Aroma	0.598	0.3514***	0.73472**	0.12296NS	0.096
Flavour	0.152	0.6629***	2.6889***	0.0793NS	0.102
Aftertaste	0.151	0.4416***	7.4014***	0.0911NS	0.106
Acidity	0.213	0.7609***	2.6281***	0.1524NS	0.113
Body	0.536	0.1769***	0.6183***	0.0926NS	0.102
Balance	0.202	0.3225NS	2.4019***	0.1402NS	0.159
Preference	1.218	21.18***	134.421***	4.525NS	2.882

Key: *, **, *** and NS represent significant at (P<0.05), (P<0.01), (P<0.001) and non-significant respectively.

Table 5. 10. Variability of the sensory attributes for the 20 coffee genotypes

Attributes	Minimum	Maximum	Mean	Variance range	Standard Error
Aroma	7.23	8.00	7.48	0.78	0.09
Flavour	6.93	8.00	7.28	1.08	0.10
Aftertaste	6.98	7.88	7.28	0.90	0.10
Acidity	6.90	8.08	7.31	1.18	0.10
Body	7.30	7.83	7.53	0.53	0.10
Balance	7.15	7.85	7.34	0.70	0.13
Preference	6.93	8.10	7.32	1.18	0.09

The PCA was able to discriminate the scores of the various variables measured based on their correlation. PC1 and PC2 explained 10.34% and 80.11% of the total variation respectively which was sufficient to discriminate the sensory attributes. Balance and aroma are closely related whereas preference, aftertaste, body, and flavour correlated closely to each other (Figure 5.1). Acidity was highly discriminated against and did not relate closely to the rest of the attributes. The genotypes that grouped in the same were similar in the performance sensory attributes. The genotypes (Batian, ARH6, ARH7, ARH5, BCO5, and BC03) were grouped on the right upper side of the quadrant and affected by acidity, after taste and preference. Robusta performed poor in sensory attributes and is located on the far left side (Figure 5.2)

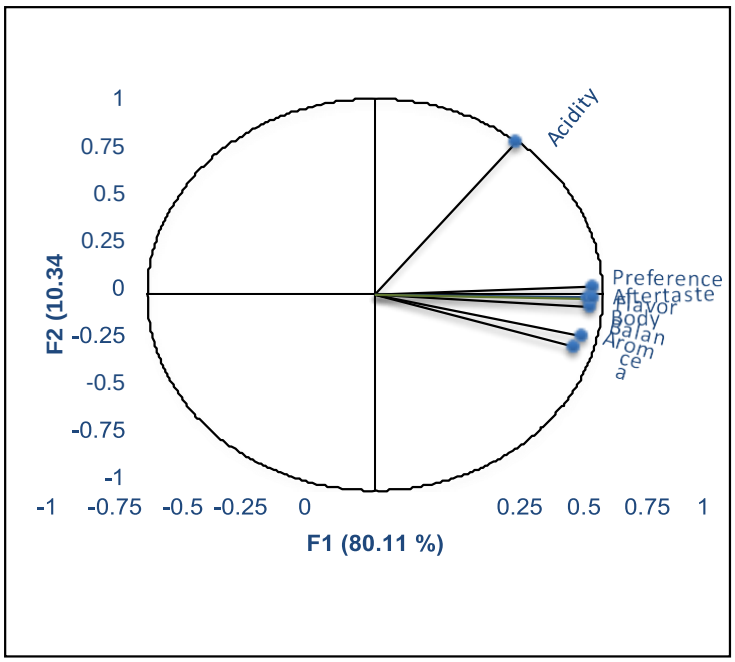


Figure 5. 1. Principal Component Analysis plot for the seven sensory traits

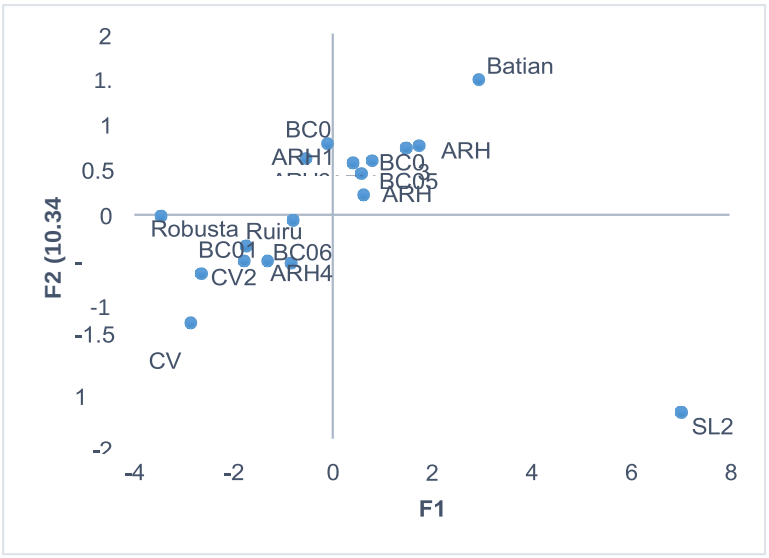


Figure 5. 2. Principal Component Analysis Plot representing the variations of the coffee genotypes regarding the sensory traits.

The cluster dendrogram shows the variation among the seven different sensory characteristics. The diagram is divided into four different clusters by a continuous broken line as shown in figure 5.3

The similarity index was used in generating the four different clusters. The first class had only one genotype SL28 whereas class two had eight genotypes, ARH6, ARH5, ARV, BC03, BC04, Batian, ARH7, and BC05 (Figure 5.3). Class three included genotypes, BC01, BC02, ARH4, BC06, Ruiru 11, ARH1 whereas class four comprised of three genotypes namely, Robusta, CV2, and CV1. The diversity of the coffee genotypes within classes was 20.53 % whereas between classes diversity was 79.47%.

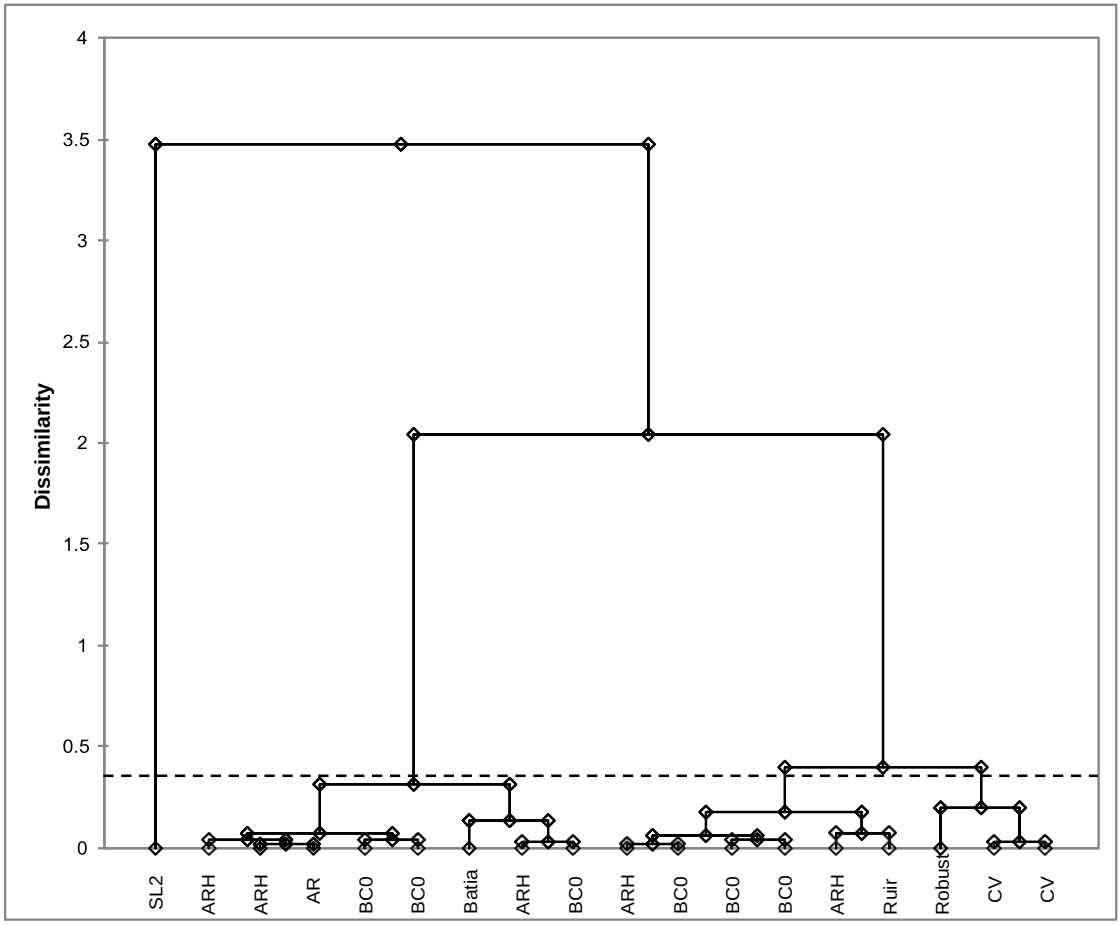


Figure 5. 3. Dendrogram showing the diversity between the coffee genotypes based on the sensory traits

5.5.3 : Correlation between the sensory and bean grade traits

The correlation among the different sensory traits for the genotypes at both Siaya ATC and KALRO-Alupe are shown in Table 5.11. The correlations observed were significant for the sensory attributes measured. The correlation between acidity with aftertaste(r=0.96), Aroma (r=0.84), balance (r=0.85), body, flavour (r=0.96), preference (0.96) was significantly positive. Aroma had a positive significant correlation to flavour (r=0.95). Balance exhibited a positive significant correlation with body, flavour, and preference. AAbean grade had a positive significant correlation with 100 bean weight (r=0.74). The correlation between AB, PB, and the C grade was negative to all sensory attributes (Table 5.11). Figure 5.4 shows the performances of the Arabusta hybrids in comparison to their parent's performances derived from Robusta and Arabica coffee. For the AA bean grade, the hybrids outperformed Robusta and this was also the case for the total score on quality where Arabusta hybrids outperformed the Robusta.

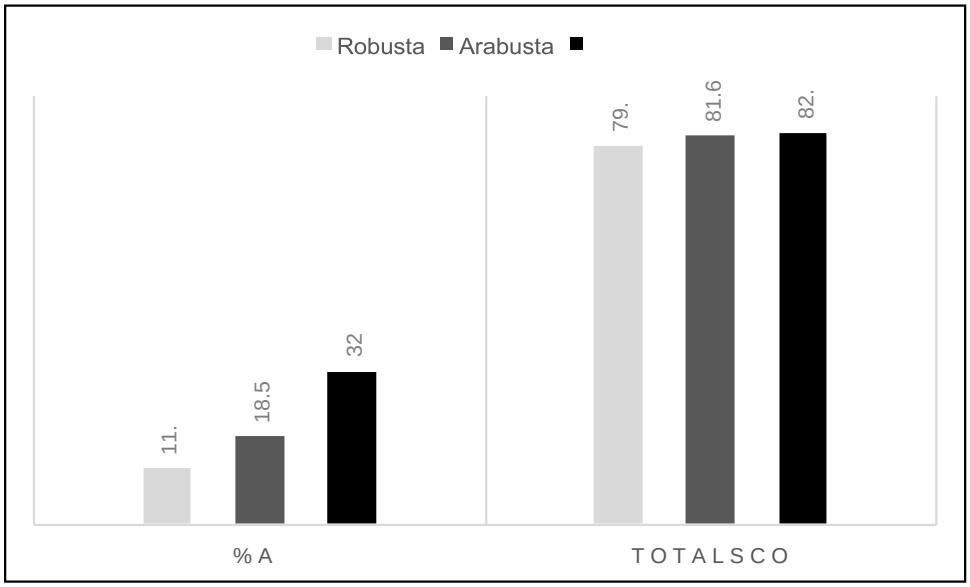


Figure 5. 4. The performances of Robusta, hybrids, and Arabica in terms of bean grade and total score of cup quality

Table 5. 11. Pearson's correlation analysis for sensory and bean grade traits for coffee genotypes KALRO- Alupe and Siaya ATC.

	Acidity	Aftertaste	Aroma	Balance	Body	Flavour	Preference	AA	AB	BW
Acidity	-	0.96***	0.84***	0.85***	0.87***	0.96***	0.96***	0.43	-0.38	0.39
Aftertaste		-	0.82***	0.79***	0.89***	0.95***	0.93***	0.36	-0.4	0.39
Aroma			-	0.66**	0.71***	0.86***	0.85***	0.21	-0.24	0.19
Balance				-	0.80***	0.83***	0.88***	0.27	-0.22	0.15
Body					-	0.84***	0.91***	0.34	-0.37	0.34
Flavour						-	0.93***	0.41	-0.32	0.29
Preference							-	0.37	-0.35	0.3
AA								-	-0.52*	0.74***
AB									-	-0.4
BW										-

Key; *, ** and *** indicates significance at (P<0.05), (P<0.01), (P<0.001) respectively. AA-% of beans retained by 7.15mm screen, AB-% of beans retained by 5.95mm screen, TT-5 of beans separated from grades AA and AB by density, PB- % of beans retained by a piano wire screen with 4.43mm spaces, C-% of beans retained by a piano wire screen with 2.90 mm spaces, T- % of very small beans and broken bits that cannot be retained by all the above screens and E- % of beans retained by a piano wire screen of the coffee bean grading machine with 8.3 mm space.100 BW(g)- 100 bean weight in grams BW- 100 bean weight

5.6 Discussion

The results showed that from the two locations, ATC Siaya and KALRO-Alupe, the coffee genotypes had significant variations for both bean's physical and sensory characteristics. The cherry harvested and processed from genotypes ARH2 and ARH3 were found to be poorly formed. This indicates that there was poor fertility since most of the cherry were floats. The studies from Teffera, (2018) Abrar et al., (2014) Gimase et al., (2014b) and Gichimu et al., (2012) have shown that there were significant differences for organoleptic and bean size traits for different coffee genotypes evaluated. Leroy et al., (2006) reported that the fertility rate for the F_1 hybrids have been low and in coffee, the fertility of the ovule will determine the number of seeds generated (Louarn, 1992) The quantification of fertility rates in coffee can be described based on percentage fully formed beans in the coffee cherries. The bean physical traits including the structure and size are majorly influenced by both the genotypic and environmental factors (Wintgens, 2004).

The AA beans fetch premium prices in the Kenyan market and therefore it is important to select for coffee genotypes that have a higher percentage of this grade with high cup quality to maximize the returns from coffee sales. The best performing Arabusta hybrid on AA bean grade at Busia was genotype ARH4 with 21.2% and genotype ARH6 in Siaya which recorded 38% (Table 5.2). The results showed that on average the Arabusta hybrids had higher proportions of %AA beans when compared to Robusta across the two locations. The percentage AA bean across the environments varied being high in coffee genotypes from Siaya and low in Busia. The actual percentage of pea berries was high within the Arabusta coffee hybrids when compared to the Arabica coffee beans. In Busia, the percentage of the pea berries was significantly high than Siaya and this could be attributed to the different environmental factors across the two locations. Pea berries mostly

develop as a result of the coffee berry-producing one bean instead of two beans. The beans are round in shape and this allows uniform roasting of the bean, unlike the normal beans which have one side flat thus improved quality since there is the concentration of flavour in the bean (Kenneth, 2003). With the flavour concentration, they have superior taste and are therefore sold in specialty markets. (Alemseged, 2018). With Arabusta coffee having a high percentage of pea berries it is possible to sell in special markets to earn premium prices when compared to the normal beans. The inherent attributes of the coffee green beans are determined by several factors which include genetics, environmental conditions, post-harvest handling, and processing (Tolessa et al., 2017 and Worku et al., 2018). Gichimu et al., (2012), Omondi, (2008), and Kathurima et al., (2014) significant differences on the bean physical traits.

There were significant variations on the bean grades from the two locations and this could be a result of the rainfall patterns during the year. Reliable and well-distributed rainfall are critical during the expansion of the coffee cherry since with good rainfall the coffee forms larger bean sizes unlike during long prolonged drought season. Adugnaw et al., (2015) has suggested that during the development of the coffee bean, various physiological processes take place which affects the bean formation. The availability of sufficient moisture and the turgor pressure combined during fruit expansion leads to the development of beans which are larger (Wrigley, 1988). Photosynthesis is also an important factor in bean development since with increased leaf potential photosynthesis will be maximized and therefore the beans will be well filled generating larger beans (Agwanda et al., 2003). The significant effects of the environments for AA, AB, PB, and 100 bean weight suggest that the environments were not similar and the G X E interactions

calculated indicated that the genotypes are not stable in the two locations for the bean physical characteristics.

There were significant variations for the organoleptic traits measured including aroma, flavour, aftertaste, acidity, preference except for balance across the two environments. Various factors contribute to the final cup quality. These factors vary from the field to consumption that affects the liquor quality. They include G x E effects, agronomic practices in the field, post-harvest procedures, roasting intensity, brew preparation processes, and the preference by the consumers (Hameed et al., 2018, Kathurima 2013). The consumer preference contributes largely to the determination of market prices since the consumers are specific on the brew quality determined by the performances of different coffee genotypes (Kahiu and Aluka, 2016). The environmental factors are not limited to rainfall patterns, soils, temperature, altitude, and humidity together the plant genetics contributes largely to the variations on the sensory performance (Decasy et al.,2003). The time taken for the cherry to mature and ripen affects the quality of the cup and these are much dependent on the environmental influence. The variations measured indicate that there is a possibility of selecting for better performing coffee genotypes for a high cup quality across the different environments.

Batian and genotype SL28 which are Arabica coffee as well as commercial varieties outperformed Arabusta and Robusta coffee in terms of cup quality. The Arabusta hybrids and the backcrosses performed better in quality in comparison to Robusta. With the SL28 genotype being used as a standard in determining cup quality of coffee in Kenya, the performance of the Arabusta hybrids by scoring an average of 82% on total score outperforming the Robusta, therefore, confirms that the tested hybrids have a great potential for improved cup quality. The performance of the hybrids

and backcrosses confirms also that there was introgression of cup quality from Arabica to Robusta coffee (Leroy et al., 2006). It has been reported that coffee lines introgressed from Arabica have shown to have a high cup/beverage quality (Moreno et al., 1995). From the results, the Arabusta hybrids pass for the specialty market due to their exemplary performance of scoring over 80% as well-defined by Lingle, (2001).

The G x E showed no significant variations on the sensory attributes and this implies that the coffee genotypes were stable for these traits across the two environments despite the environmental variations (Table 5.5). The best six performing genotypes included SL28, Batian, ARH7, BC05, ARH5, ARH6, and BC03. Gichimu et al., (2012) and Tefera (2018) reported there were significant differences for G X E on the sensory traits which were not the case in this study. The results from the PCA analysis shows that there were variations among the coffee genotypes for the cup quality. SL28 on its own whereas genotypes ARH5, ARH6, BC03, BC04, BC05 and Batian were grouped in the same cluster (Figure 5.3). The clustering of genotypes with Batian implies that their cup quality was high. The genotypes that clustered in the same group were differentiated by the sensory characteristics including the acidy and aftertaste. All the Arabusta hybrids except genotypes ARH1 and ARH4 grouped differently from the commercial Arabica variety Ruiru 11 meaning that their performance is greater than the latter in terms of cup quality which was in agreement with the total scores recorded.

There was significant correlation between acidity and aftertaste (r=0.96), aroma (r=0.84), balance (r=0.85), flavour(r=0.96) and preference (r=0.96) (Table 5.11). Balance also exhibited positive correlations that were significant to and body (r=0.80), flavour (0.83) ad preference (0.88). For an efficient and effective breeding program, it is important to consider the correlation effects in

selection to identify traits that have a significant positive correlation to be utilized in selecting genotypes with the preferred quality. However, AB bean grade traits showed negative correlations with acidity (r=-0.38), aftertaste (r=-0.36), aroma (-0.24), balance (r=-0.34), flavour (-0.42) and preference (-0.52). The AA bean and 100 bean weight had positive correlations (0.74). It was found out that despite coffee genotypes recording a high percentage of larger beans at Siaya ATC, their cup quality was lower than the genotypes at KALRO- Alupe and vice versa. Tessema et al., (2011) reported significant and positive correlations among sensory attributes and Kathurima et al., (2009) reported the negative correlations between the sensory and bean attributes.

The PCA results imply that those traits which showed positive significant correlations could be used during selection and they include aroma, after taste, and preference. The negative correlations between bean grades and sensory imply that the bean grades cannot be used as indicators for the cup quality improvement during breeding. The outcome of the results also indicates that selection of sensory and bean grade traits cannot be carried out simultaneously. This also implies that the bean grade traits are more influenced by the environment which affects the berry expansion due to rainfall reliability and distribution and also photosynthesis which is dependent on the availability of the sun (Agwanda et al., 2003). The genetics of a specific genotype to a greater extent determines the biochemical compounds in the green bean which in turn affects the beverage quality in terms of sensory performance (Wintgens, 2004). The significant correlation between AA bean grade and 100 berry weight indicates that the weight of the beans is directly proportional to the bean size.

Preference was correlated positively and significantly with all the sensory traits and therefore can be used in discriminating genotypes for quality due to the significant correlation as described by Tessema et al., (2011). Flavour is a major trait that influence what the consumer prefers affecting

their choice of beverage (Cantergiani et al. 1999; Marin et al. 2008) since flavour combines both acidity, body, and aroma determining the overall cup performance. Flavour had a positive significant correlation with all sensory attributes (Table 5.7). Flavour is easy to describe organoleptically and can be easily used as a single trait in differentiating various genotypes for cup quality during selection (Yigzaw ,2005 and Agwanda et al., 2003). From this study, the results imply that the introgression of bean grade and cup quality from Arabica coffee was successful. Robusta coffee generally is known to produce smaller beans when compared to Arabica coffee and the results showed that the hybrids scored high for AA bean sizes when compared to Robusta coffee. Arabusta hybrids also performed better than Robusta in terms of cup quality and their performances were comparable to Arabica coffee. The hybridization confirmed that it is possible to improve the bean's physical and quality traits of Robusta coffee.

The difference in performances for the bean sizes and cup quality confirmed existence of genetic variation among genotypes of coffee evaluated. Interspecific hybrids performed better than Robusta by scoring over 80% on the total score. With interspecific hybridization, it is possible to improve the quality for both bean size and liquor of coffee genotypes. The positive significant correlations between the sensory traits confirmed that sensory traits can be used in the selection of coffee genotypes for cup quality.

CHAPTER SIX:

BIOCHEMICAL COMPOSITION OF BEANS FOR ARABUSTA HYBRIDS AND BACKCROSS PROGENIES

6.1 Abstract

Caffeine and chlorogenic acids in coffee beans are known to cause bitterness and astringency of the beverage thus lowering the cup quality. The study aimed at evaluating biochemical compounds found in Arabusta coffee hybrids. Green beans were harvested in the growing season of 2018 from KALRO-Alupe (Busia) and Siaya ATC and analysed for sucrose, oil, trigonelline, caffeine , and chlorogenic acids using the HPLC and Soxhlet method. There were significant differences within the genotypes for these biochemical compounds across the two different environments. The G x E interaction effect was not significant for all the biochemical compounds, though the environmental effects were significant except for chlorogenic acids. Caffeine, sucrose, oil, and trigonelline levels were significantly high for genotypes evaluated in Siaya but not for those in Busia. Chlorogenic acids had a positive significant correlation with caffeine (r=0.77) but were significantly negatively correlated with lipid oil (r=-0.49) and sucrose (r=-0.43). Coffee oil indicated a positive significant correlation with sucrose (r=0.81) and Trigonelline (r=0.49). The Principal Component Analysis (PCA) that differentiated the genotypes based on the levels of biochemical compounds indicate d high genetic variation among the genotypes. Arabusta hybrids exceeded Robusta coffee in performance for all the biochemical compounds which imply that the introgression of quality genes from Arabica coffee was successful. The best performing hybrids in combination with cup quality and yield are recommended for commercial cultivation.

6.2 Introduction

Coffee is normally traded as green coffee before it is roasted for consumption. Green coffee beans contain various chemical composites that are complex in structures interacting at all stages of coffee growth determining the final cup quality (Kathurima et al., 2010, Gichimu et al., 2014a). Factors that affect the quality and biochemical compounds present in coffee are altitude, genetics, shade, harvesting period, and processing practices (Tolessa et al., 2018, Duarte et al., 2010 and Worku et al., 2018). The flavour and aroma of roasted coffee depend on the metabolites that accumulate within the coffee bean after roasting acting as precursors. (George et al., 2008). These attributes depend s also on roasting degree and presence of defects in coffee beans. (Franca et al., 2005). Maillard reactions and caramelization which occur during roasting influences the interaction of the chemical composition of the green bean which is responsible for the aroma that develops during roasting (Liu et al., 2019).

The major biochemical compounds which compose of chlorogenic acids, caffeine, trigonelline sucrose, and oil have been used for discrimination of coffee varieties within and across species (Clifford et al., 1989; Ky et al., 2001b). The presence of these compounds helps to discriminate between the different coffee genotypes making them key factors in the determination of organoleptic cup quality (Aluka et al., 2016). Characterization of varieties for the analysis of the biochemical composites is crucial in developing coffee genotypes with the desired quality. The presence of trigonelline sugars and oils could have a positive influence on liquor quality whereas chlorogenic acids and caffeine could be unfavorable (Kathurima et al.,2010).

Caffeine exists in different plant species normally found in different parts of a plant which include the fruits, seed, and even the leaves. Coffee, cocoa beans, and tea leaves are the known major sources of caffeine (Mumin et al., 2006). Caffeine, which is partially accountable for the bitterness in coffee is one of the highest occurring purines in green coffee (Farah, 2012). The levels of caffeine vary between and even within species (Silvarolla et al., 2004; Ky et al., 2001b). Robusta generally has a higher value of caffeine (2.2%) whereas Arabica has an average value of 1.2% ranging from 0.6 to 1.9 %(Belay et al., 2008, Franca et al., 2005). The less commercialized species of Liberica and Arabusta have 1.35 and 1.72% caffeine content respectively (Clarke and Macarae, 1985).

Sucrose is a major occurring sugar in the coffee beans with *Coffea arabica* reported having a range of 5% to 9.5% and *Coffea canephora* 4% to 7% in (Ky et al., 2001). During roasting, reducing sugars that are involved in Maillard reactions are from sucrose (Grosch, 2001, Kathurima 2013, Ky et al., 2001b) and is a precursor affecting the aroma and taste of the coffee beverage (Maria et al., 2017, Farah, 2012). Trigonelline is a nicotinic acid (pyridinium-3-carboxylic acid) produced through methylation using methionine (Anaparti, 2013). The levels of trigonelline in *C. arabica* range between 0.88% to 1.77% and in *C. canephora* it ranges between 0.75% to 1.24% (Ky et al., 2001). Trigonelline has a low bitter taste when compared to caffeine and is known to be a vitamin B6 derivative being 100% water-soluble (Anaparti, 2013, Gichimu et al., 2014a).

Triacylglycerols and fatty acids are major coffee oils in the green bean found in equal proportion as those of oils found in vegetables (Speer & Kölling-Speer, 2006). The coffee oil levels of green Arabica coffee averages 15%, whereas it is 10% for Robusta coffee (Gichimu et al., 2014a). Of the total lipids found in the coffee bean, oil makes 20%. Oil contributes to the viscosity of the

coffee beverage affecting the aroma of beans during roasting (Buffo and Freire, 2004). The amount of chlorogenic acids (CGA) varies with the species with *C. Arabica* (4 to 8.4%) and *C. Canephora* (7 to 14.4%) It has been found out that some of the hybrids have medium levels of the biochemical compounds (Farah et al., 2005a, 2005b). Chlorogenic acids are critical in pigment formation and also affect the taste, and flavour of beans, acidity and define cup quality and preference of the brew (Gichimu et al., 2014a, Variyar et al., 2003).

The performance of both sensory and the biochemical attributes of coffee beans determines the liquor quality (Farah et al., 2006). Biochemical compounds act as aroma and flavour precursors affecting the quality of the coffee beverage (Cheng at el., 2016). Assessment of the diversity of biochemical attributes is key to the development of coffee varieties. Determining the elements that influence coffee quality remains an important area of study in coffee breeding. This is because the biochemical components in coffee influence the organoleptic properties that contribute to the final cup quality, and this is key in determining its market value and use. The aim was to assess the biochemical content of the Arabusta hybrids and their backcrosses in two separate locations in Western Kenya with the focus of selecting the best performing genotypes for the breeding programme.

6.3 Materials and methods

The materials and methods have been described in Chapter 3, Section 3.3.

6.3.1 Extraction and quantification of crude oil

Two (2) grams of the dried green coffee powder from the green coffee bean was weighed and dried for 1 h at 105 ºC ± 2 ºC. Extraction was carried out after adding 100 mm of hexane to the coffee powder which was then in the soxhlet extraction apparatus (AOAC, 1995). Rota vapour was used to dry the extract and placing it an oven at105± 2 ºC to complete the drying process. The extract was cooled and then weighed to get the final weight after evaporation. The drying process continued for another two hours weighing being undertaken at a 30-minute interval until there was no more than one-milligram loss between successive weighing. Crude oil content was then calculated as the increase in weight of the extraction flasks (Kathurima, 2013).

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

Caffeine, trigonelline, and chlorogenic acid levels were determined using the protocols as provided by CIRAD, (2003a and CIRAD (2003b) with slight modifications as described below.

For caffeine extraction, 0.2g of green coffee powder, 0.5g Magnesium oxide (Merck), and 200ml of distilled water were put into a 250 ml Erlenmeyer flask. Refluxing of the content took 25 minutes before cooling and this was enhanced by using two pumice stones during refluxing. Filtration was carried out after cooling under vacuum on celite and the filtrate was placed into a 250ml volumetric flask. Twenty-five (25) milliliters of the filtrate were drawn and put into a 100 ml volumetric flask and the volume adjusted to the mark with the mobile phase consisting of 35% v/v methanol, 65% v/v distilled water, and 0.1% v/v glacial acetic acid. A 0.45µm micro-filter (Chromafil) was used in filtering the eluate and analysis was carried out using the High- Performance Liquid Chromatography (HPLC) system equipped with a pulsed diode array detector (PDA).

For trigonelline extraction, 0.2 g of the green coffee powder, 0.2gm Magnesium oxide (Merck), and 40ml of distilled water was added into a 250 ml Erlenmeyer flask. Pumice stones were put inside and refluxing of the content carried out for 20 minutes and left to cool, after which the filtration under vacuum was conducted using celite. 25 ml of the filtrate was then drawn and adjusted to the mobile phase then filtered through a 0.45µm micro-filter (Chromafil) and analysis done using the HPLC.

For chlorogenic acids extraction, 0.2g of green coffee powder and 40ml of distilled water was added into a 250 ml Erlenmeyer flask (Tse, 2005). Refluxing was done and the filtrate recovered and the volume adjusted to the mark with the mobile phase (same as for caffeine and trigonelline). A 0.45µm micro-filter (Chromafil) was used to filter the eluate and analyzed by HPLC (Knauer) (Kathurima, 2013).

6.3.3 Analysis of caffeine, trigonelline, and total chlorogenic acids

HPLC system (Knauer) equipped with a Super Co Discovery C-18 column was used to analyse caffeine and trigonelline and the BDS HYPERSIL C-18 column was used to analyse chlorogenic acids. Diode Array Detector was used to detect the three wavelengths, at 278nm for caffeine, 266nm for trigonelline, and 324nm for CGA. HPLC grade methanol (PANREAC) 35% was used as the mobile phase, distilled water 65%, acetic acid (PROLAB) 0.1%, at a flow rate of 1 ml/min under ambient temperature. The retention times of the trigonelline standard (Sigma Aldrich), CGA standard (Acros organics), and caffeine standard (99%) (Fischer Scientific) were used to calculate trigonelline, CGA, and caffeine quantities respectively. Calibration equations were used to calculate using the peak area of the slope (Kathurima, 2013).

6.3.4 Extraction and analysis of sucrose

The extraction and analysis of sucrose were carried out according to the method of Osborne and Voogt (1978) used by Kathurima, (2013). 0.2g of the green coffee powder was added to 100mls of 96% ethanol under reflux. The extract was evaporated to dryness after filtering it using the Whatman filter paper number 42. Recovery of sucrose was done using 10mls deionized water and 2mls of the extract mixed with 2mls Diethyl ether (AR) and the top layer was discarded after settling. The process was repeated three times and 1ml of acetonitrile was added to 1ml of the extract. Filtering was conducted using the 0.45µm microfilter. HPLC system (Kna uer) equipped with a Eurospher 100-5 NH2 column and a refractive index detector was used to analyse sucrose. Acetonitrile HPLC grade (SCHARLAU) 75%, and distilled water 25% was used as the mobile phase at a flow rate of 1 ml/min. The sucrose standard (Fischer Scientific) was used in quantifying the sucrose level through comparison of the retention peak of standards and sample peak the sucrose level calculated using the calibration equation.

6.4 Statistical analysis

The biochemical data were subjected to Analysis of Variance (ANOVA) and effects declared significant at 5% using GENSTAT statistical software version 18. The General Linear Model (GLM) was used (Jansen, 1993).

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + E_i$$

Where,

For each observation $i = 1, \ldots, n$. where n is the observations of one dependent variable

$\hat{Y} = j^{th}$ observation of the dependent variable

$j = 1, 2, \ldots, k$

X = is the observation of the j^{th} independent variable β

= parameters to be estimated

Ei = Distributed normal error

Least Significance Difference was used to separate means (Martin et al., 1975) Separate as well as combined analysis of variance was performed on data from the two locations. The correlation was derived from the Pearson Correlation Coefficient using the GENSTAT statistical software to show the correlation between bean grades and sensory traits using the. The Principle Component Analysis of the sensory characteristics were plotted based on the important principle components (Lattin et al., 2002) using XLSTAT statistical software, version 2012. XLSTAT version 2012 was used to carry out cluster analysis on biochemical data using the unweighted pair-group method with arithmetic average (UPGMA) to create a dendrogram based on Euclidean distances (Hue et al, 2000).

6.5 Results

6.5.1 Biochemical composition

There was a significant difference for the biochemical components which included oil, chlorogenic acid, trigonelline, caffeine, and sugars among the coffee genotypes established at Busia and Siaya (Table 6.1).

Table 6. 1. Mean squares for biochemical attributes of coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) in the growing season of 2018

	Busia			Siaya		
	Rep	**Genotype**	**Error**	**Rep**	**Genotype**	**Error**
DF	2	19	38	2	19	38
Chlorogenic acids	0.90	7.03***	2.20	0.22	3.88**	1.28
Oil	0.04	12.34***	2.62	3.40	11.30***	2.47
Sucrose	0.43	3.24***	0.78	0.01	4.88***	0.99
Trigonelline	0.02	0.11***	0.04	0.05	0.04	0.04
Caffeine	0.05	0.42***	0.09	0.03	0.47***	0.07

Oil content varied among the genotypes, SL28 recorded a significantly higher percentage of oil (19.1) followed closely by genotype Batian (18.6) in Siaya but, genotype CV1 in Busia recorded the least percentage (10.1) (Table 6.2). There was a significant difference within the coffee genotypes in the composition of chlorogenic acids where Robusta and genotype CV1 recorded the highest percentage in Busia but genotype ARH2 recorded the least in Siaya (Table 6.2). The variation of trigonelline was significant within three genotypes (ARH4, ARH7, and BC01) recording the least levels but Batian recorded the highest (1.7) in Busia. The caffeine content varied significantly with Robusta coffee recording the highest in Siaya and Busia (2.6) and genotype ARH7 the least in Busia (1.2) (Table 6.2).

There was variation among the coffee genotypes for the biochemical components recorded. Chlorogenic acid varied among the genotypes with Robusta and genotype CV2 recording significantly higher percentage (11.2), whereas genotype BC03 recorded the lowest (Table 6.3). There were significant differences in caffeine levels where Robusta recorded a higher percent followed closely by genotypes CV1 and CV2 whereas genotypes SL28 and ARH7 recorded the least. The percentage of oil recorded differed among the genotypes with genotype SL28 having a significantly higher percentage (17.8) when compared to other genotypes followed closely by

Batian (17) and genotype ARH4 (12.3) recorded the least. There was a significant difference in the sucrose levels among the genotypes and genotype SL28 had the highest percentage whereas genotypes ARH4 and CV2 (5.4) recorded the lowest percentage. Trigonelline levels varied among the genotypes, where the Batian genotype recorded the highest percentage (1.5) whereas four genotypes (AHR4, BC01, BC02, ad Robusta) recorded the lowest (Table 6.3).

Table 6. 2. Mean of biochemical components of green bean for coffee genotypes from KALRO-Alupe (Busia) and Siaya ATC during 2018 season

	Oil		Chlorogenic acid		Trigonelline		Caffeine		Sucrose	
Genotype	**Busia**	**Siaya**	**Busia**	**Siaya**	**Busia**	**Siaya**	**Busia**	**Siaya**	**Busia**	**Siaya**
ARH1	15	15.8	8.7	8.7	1.1	1.2	1.6	1.7	7.2	8.2
ARH2	13.9	15.2	7.7	7	0.9	1.3	1.4	1.3	5.7	8.6
ARH3	16.6	16.7	8.7	7.9	1.3	1.3	1.3	1.6	8.6	8
ARH4	11.8	12.8	9.3	8.9	0.9	1.1	1.9	2.1	4.8	6.1
ARH5	12.8	14.4	8.4	9.8	1	1.3	1.7	2.1	5.6	6.7
ARH6	15	15.8	8	9.6	1	1.3	1.5	1.6	7.2	9.1
ARH7	12	16.6	8	7.7	0.9	1.3	1.2	1.5	6.4	8.8
BC01	11.8	12.5	9.2	9.4	0.9	1.1	1.9	2.1	6	5.6
BC02	12.3	17.2	9.8	8.1	1	1.1	1.9	1.4	6.7	8.1
BC03	15.3	16.1	6.4	7.9	1.1	1.4	1.6	1.7	7.4	8.7
BC04	12.2	15.1	10.8	10.3	1	1.4	2	2.5	6.4	7.8
BC05	12.8	15.6	9	8.1	1.1	1.3	1.6	1.7	6.8	9
BC06	16.6	17	7.4	8.3	1.3	1.2	1.4	1.8	7.3	7.6
CV1	10.1	12.2	10.3	10.7	1	1.4	2.1	2.4	5.4	5.6
CV2	10.3	12.9	12.1	10.4	1.1	1.4	2.4	2.3	5.6	5.2
ARV	13.1	15.2	10.6	8.3	1.2	1	2	1.9	7	8.1
Robusta	11.6	13.3	12.4	10	1	1	2.6	2.6	5.6	6.3
Ruiru	13.2	16.2	8.3	8.3	1.2	1.3	1.4	1.6	7.6	7.9
Batian	15.5	18.6	8.8	6.6	1.7	1.3	1.6	1.4	7.6	8.6
SL28	16.5	19.1	8.3	7.8	1.2	1.3	1.3	1.3	8.4	9.2
LSD	2.67	2.59	2.45	1.87	0.312.8	0.3	0.5	0.42	1.46	1.6
%CV	0.3	2.7	2.3	1.2	0.31	3.8	3	2	2.2	0.3
Ftest	S	S	S	S	S	NS	S	S	S	S

Table 6. 3. The combined mean of biochemical components of green beans for coffee genotypes from KALRO-Alupe (Busia) and Siaya ATC during 2018 season

Genotype	CGA	Caffeine	Oil	Sucrose	Trigonelline
ARH1	8.7	1.6	15.4	7.7	1.1
ARH2	7.3	1.4	14.5	7.2	1.1
ARH3	8.3	1.5	16.7	8.3	1.3
ARH4	9.1	2.0	12.3	5.4	1.0
ARH5	9.1	1.9	13.6	6.1	1.2
ARH6	8.8	1.5	15.4	8.1	1.2
ARH7	7.9	1.3	14.3	7.6	1.1
BC01	9.3	2.0	12.1	5.8	1.0
BC02	9.0	1.7	14.7	7.4	1.0
BC03	7.1	1.6	15.7	8.0	1.3
BC04	10.5	2.2	13.7	7.1	1.2
BC05	8.6	1.7	14.2	7.9	1.2
BC06	7.8	1.6	16.8	7.5	1.2
CV1	10.5	2.3	11.2	5.5	1.2
CV2	11.2	2.3	11.6	5.4	1.2
ARV	9.5	1.9	14.2	7.6	1.1
Robusta	11.2	2.6	12.5	5.9	1.0
Ruiru	8.3	1.5	14.7	7.7	1.2
Batian	7.7	1.5	17.0	8.1	1.5
SL28	8.1	1.3	17.8	8.8	1.3
LSD	**1.5**	**0.3**	**1.8**	**1.1**	**0.2**
%CV	**1.5**	**0.4**	**1.4**	**1.2**	**3.2**
Ftest	**S**	**S**	**S**	**S**	**S**

6.5.2 Genotype by Environment interactions

There were significant ($P \leq 0.05$) differences among the genotypes for all the biochemical attributes (chlorogenic acid, caffeine, oil, sucrose, and oil) (Table 6.4). The environmental effect was significant for the biochemical attributes except for the chlorogenic acids. There was no G x E interaction for the biochemical attributes recorded (Table 6.4). Chlorogenic acids scored high in Busia than in Siaya but there were no significant differences (Figures 6.1, a-e). Siaya recorded significantly higher caffeine, sucrose, oil, and trigonelline content than Busia. The biochemical compound means were either placed above or below the median. The interquartile range differed among the environments for each attribute measured indicating the variation recorded (Figures 6.1, a-e).

Table 6. 4. Mean squares for biochemical components of green bean for coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) during 2018 season

Source	Rep	Gen (G)	Envt (E)	G x E	Error
Df	2	19	1	19	9
Chlorogenic acid	0.744	8.591*	4.994	2.316	1.704
Caffeine	0.002	0.81***	0.382*	0.081	0.079
Oil	1.566	21.01***	120.453***	2.631	2.524
Sucrose	0.271	6.796***	29.46***	1.321	0.865
Trigonelline	0.056	0.088**	0.935***	0.065	0.036

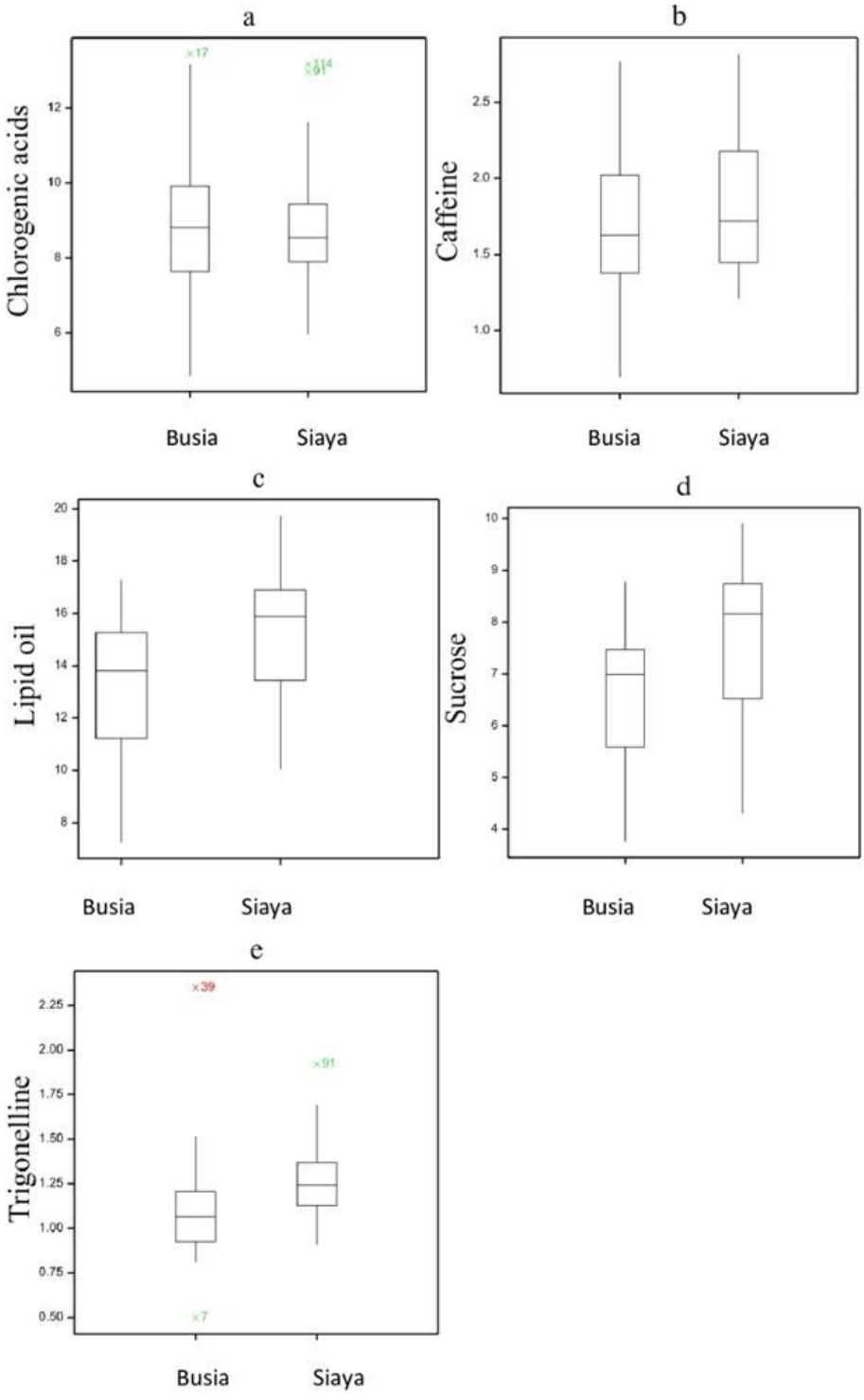


Figure 6. 1 (a-e). Description of the biochemical attributes in the two environments (1 - Busia, 2- Siaya).

The performance across the two locations showed a significant variation on the biochemical composition between the Arabusta hybrids, backcrosses, Robusta, and Arabica coffee evaluated (Figure 6.2). Arabica coffee had a higher composition of sucrose (8.2), Trigonelline (1.3), and oil (16.5) followed closely by the Arabusta coffee hybrids. The caffeine and chlorogenic acid levels also varied where Robusta coffee recorded higher percentages (2.6, 11.2) whereas the Arabica coffee recorded the least (Figure 6.2).

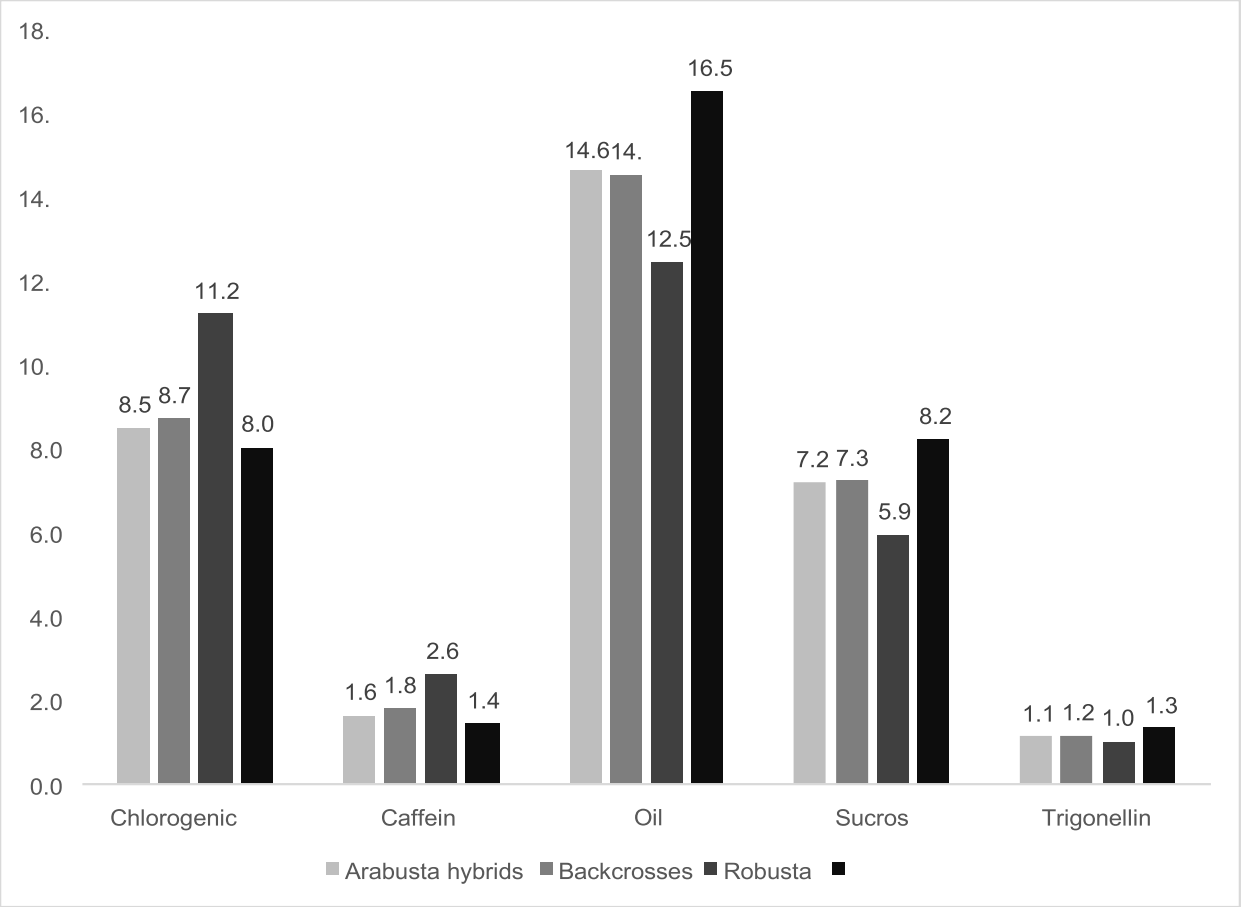


Figure 6. 2. Biochemical composition for the Arabusta coffee hybrids, Backcrosses, Arabica and Robusta coffee

6.5.3 Correlation

Chlorogenic acid and caffeine showed a negative significant correlation with oil and sucrose but had a positive significant correlation with trigonelline (Table 6.5). Coffee oil showed positively significant correlations with sucrose (r=0.81) and trigonelline (r=0.48). Trigonelline correlated positively with all the biochemical components showing significant variations with oil (r=0.48) and sucrose (r=0.43) (Table 6.5). The Principal Component Analysis (PCA) discriminated against the biochemical components as shown in Figure 6.3. The PC1 and PC2 explained variation of

74.76 % and 15.35% respectively for the biochemical attributes. Oils, sucrose, and trigonelline recorded high positive values whereas caffeine and trigonelline had low values (Figure 6.4). Genotypes, SL28, BC03, and ARH3 were grouped closely together based on oil and sucrose whereas Batian was discriminated solely based on trigonelline levels. Robusta, CV1, and CV2 were grouped based on chlorogenic acid and caffeine levels (Figure 6.4).

Table 6. 5. Correlation coefficients for the different biochemical attributes of coffee green beans for coffee genotypes in Busia and Siaya for the year 2018

	Chlorogenic acid	Caffeine	oil	Sucrose	Trigonelline
Chlorogenic acid	-	0.7741***	-0.4893***	-0.4302***	0.0519
Caffeine		-	-0.4926***	-0.4833***	0.1019
Oil			-	0.8119***	0.4889***
Sucrose				-	0.4354***
Trigonelline					-

Key: *, ** and *** indicates significance at (P<0.05), (P<0.01) and (P<0.001)respectively.

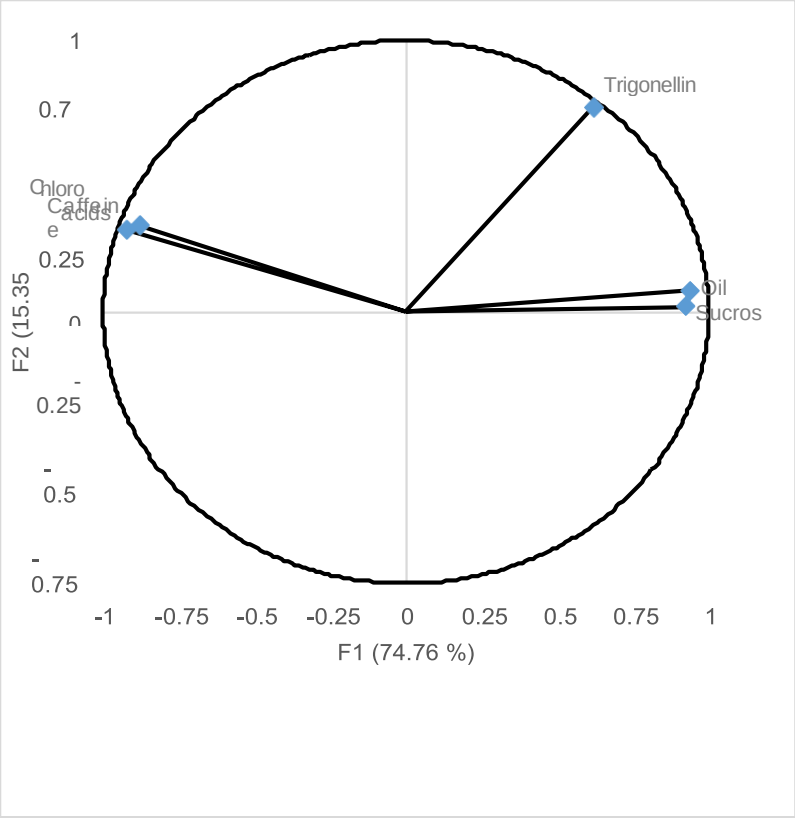


Figure 6. 3. Principal component analysis for biochemical compounds

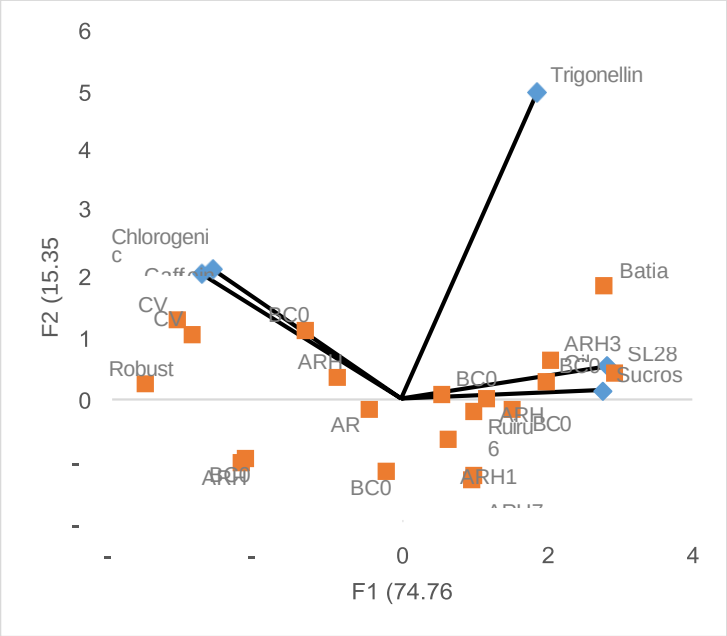


Figure 6. 4. Principal component analysis describing the twenty coffee genotypes based on the biochemical compounds

Coffee oil had a wider mean-variance (6.6) among the coffee genotypes (Table 6.6). Trigonelline had the least variance range of 0.5. Chlorogenic acids were second invariance range after oil followed closely by sucrose (Table 6.6). Oil recorded a higher mean of 14.4, whereas trigonelline recoded a lower mean of 1.2. The standard deviation ranged from 0.1 to 1.9 oil recording the highest. Three clusters of coffee genotypes were generated based on their biochemical composition. The broken lines truncate the diagram into three clusters generated using the similarity index. The first cluster contained genotypes, BC04, Robusta, CV1, CV2, ATH5, ARH4, and BC01 (Figure 6.5). Cluster two, comprised of genotypes ARH2, BC05, ARH7, Ruiru11, ARH1, ARH6, BC02, and ARV whereas the third cluster included genotypes BC03, SL28, ARH3, BC06, and Batian. The diversity of genotypes within and between classes was 21.94% and 78.06% respectively (Figure 6.5).

Table 6. 6. Variability of the biochemical composition of green bean for coffee genotypes sown in Busia and Siaya for the year 2018.

Variable	Minimum	Maximum	Mean	Variance range	Standard deviation
Chlorogenic acids	7.1	11.2	8.9	4.1	1.2
Caffeine	1.3	2.6	1.8	1.3	0.4
Oil	11.2	17.8	14.4	6.6	1.9
Sucrose	5.4	8.8	7.2	3.4	1.1
Trigonelline	1	1.5	1.2	0.5	0.1

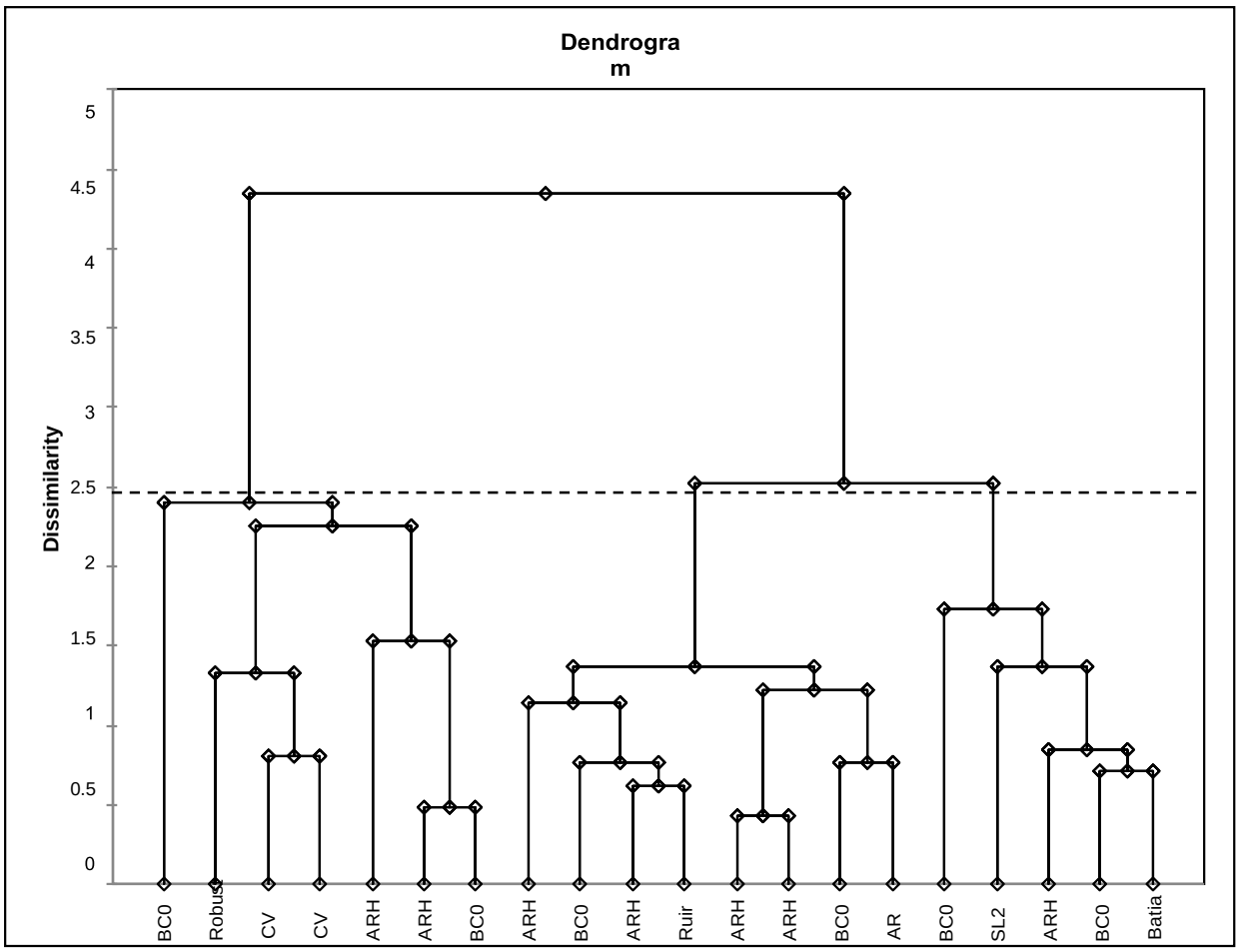


Figure 6. 5. Dendrogram showing the variability of coffee genotypes based on the biochemical composition

6.6 Discussion

There were variations on biochemical composition among the coffee genotypes evaluated within and between the two different locations (Busia and Siaya). The differences showed a high genetic variation within the genotypes that led to different performances in terms of the lipid oils, sucrose, caffeine, chlorogenic acids, and the trigonelline. The SL28 and Batian varieties recorded high sucrose, trigonelline, and lipid oils values which were expected since they are of Arabica coffee origin. Caffeine, trigonelline, sucrose, and oil levels were high for genotypes established in Busia

when compared to those in the Siaya location. Gichimu et al., (2014a), Gimase et al (2014b), and Kathurima et al., (2011) reported significant variations for the biochemical composition of coffee evaluated in different environments in Kenya. Tolessa et al., (2019) evaluated biochemical compounds in coffee using different processing methods and reported ranges of biochemical compounds in Arabica coffee and found significant variations among the coffee genotypes. This implies that it is possible to improve selection efficiency for biochemical composition within the breeding programme. The better performance of Arabusta hybrids when compared to Robusta and the backcrosses indicates that these traits were introgressed successfully from the Arabica coffee. The differences are because biochemical composition within coffee genotypes varies depending on the species, the maturation time, and less important due to environmental factors and agricultural practices (Farah et al., 2005b, Belay 2011).

In this study, Robusta coffee had higher CGA values of 11.2% whereas Arabusta hybrids had 7.3 to 9.1%, backcrosses had 7.1 to 10.5% whereas Arabica coffee scored 7.7 to 9.1%. The ranges are within those that were reported by Bicho et al., (2013b) and Upadhyay & Mohan Rao, (2013) who reported Robusta had 7.0 to 14.4% except for Arabica which had a high of 9.1% against the reported (4.0 to 8.4%). This could be a result of the G x E effect. The chlorogenic acid content in coffee is influenced more by genetics with Robusta species produces high levels of CGA than the Arabica species which negatively affects cup quality since the chlorogenic acids add to the astringency or unwanted bitterness. Arabica coffee on the other hand has high cup quality since its chlorogenic acid levels are low allowing the balance of the coffee brew. The chlorogenic acid levels of the Arabusta coffee hybrids were comparable to those of Arabica coffee. This indicates that the interspecific hybridization was positive resulting in lowered chlorogenic levels.

Chlorogenic acids are phenolic compounds that protect plant cells against oxidation, UV irradiation, and pest infection (Niggeweg et al., 2004, Peterson et al., 2005). When coffee is roasted the chlorogenic acids degrade forming lactones, caffeic acid, and phenols through Maillard and Strecker's reactions. These then contribute to the final acidity and bitterness of the cup which in turn affects the flavour and aroma which is responsible for the variation on liqour quality in Robusta and Arabica coffee brew (Upadhyay & Mohan Rao, 2013).

Caffeine levels varied from 1.3 to 1.5% in Arabica coffee, 1.3 to 2% in Arabusta hybrids, 1.6 to 2.2% in backcrosses, and 2.6% in Robusta. The caffeine levels vary within species being high in *C canephora* and low in *C arabica*. Robusta coffee has higher levels of caffeine when compared to the Arabica coffee causing unwanted bitterness and acidity of the cup. Arabusta coffee had intermediate levels of caffeine due to hybridization. For the total caffeine produced, 94% is due to the genetic effects and the rest is a result of environmental factors (Montagnon, 2000). For caffeine production, as the coffee berry matures its levels increase (Cheng et al., 2016). *C. canephora* was recently sequenced for caffeine and it was found out that caffeine evolved separately (Cheng et al., 2016). The differences on accumulation of caffeine in Robusta and Arabica coffee is dependent on expression of CcDXMT transcript which is high in *C. canephora* whereas the expression of CaXMT1, CaMXMT1, and CaDXMT2 transcripts was low in *C. arabica* explaining the lower levels of caffeine in Arabica (Kumar et al., 2015 and Perrois et al., 2015). Adding to this, the hybridization of *C.arabica* to reduced *C. eugenioides* sub-genome contributed also to low caffeine levels in Arabica (Perrois et al., 2015).

Trigonelline levels varied among the genotypes of the different species with Arabica coffee having recorded a range of 1.2 to 1.5%, Robusta (1%), Arabusta backcrosses 1 to 1.3% and Arabusta

hybrids ranged from 1.0 to 1.3%. Trigonelline is a derivative of pyridine formed during roasting, producing a water-soluble vitamin B bioavailable in coffee brew than any other natural source and is key in determining the flavour of the brew (Trugo,2003). Bicho et al., (2013a) carried out a study to discriminate the different levels of biochemical compounds and reported higher levels of trigonelline in Arabica when compared to Robusta. Trigonelline determines the cup quality of coffee since it's an aroma precursor contributing to the desired flavour in the coffee brew. During crop improvement, those genotypes that have higher levels of trigonelline together with increased oils and sucrose levels are selected for improved cup quality.

Sucrose levels ranged from 7.7% to 8.8% for Arabica, Robusta (5.9%), backcrosses 5.8 to 8%, and Arabusta hybrids. 5.4 to 8.3%. The sucrose levels are within the ranges reported by Tran et al., (2016) who reported ranges of 7.4 to 11.1% in Arabica and 4.05 to 7.05% for Robusta coffee. The difference in the sucrose content is due to the genotypic effect. Robusta species naturally have lower sugar levels than the Arabica species. The levels calculated within the backcrosses and the Arabusta hybrids are due to the interspecific hybridization between the two species which led to increased sugar levels among the different genotypes. Temperature is also known to affect sugar levels whereby, coffee that is grown in low altitude areas where temperatures are high usually record increased sugar levels when compared to those grown in high altitude areas with low temperatures. Joet et al., (2009) studied sucrose synthesis within the coffee plant and reported that during the early berry development at the perisperm-endosperm transition, the sucrose levels remained constant. During the endosperm development, the sucrose levels increased until when the berry stated ripening after which it slowed down for Robusta whereas the accumulation in Arabica was continuous throughout the fruit ripening. The total amount of sucrose found in the green coffee bean is more than 90 % with glucose and fructose contributing to about 0.5% (Knopp

et al., 2006 and Ky et al., 2001a). Sucrose is an aroma precursor contributing immensely to cup quality. Sucrose degrades during the roasting forming volatile and non-volatile compounds which, pyrazine, furans and hydroxy remethyl furfural during the Maillard reactions (Grosch, 2001).

Coffee oil improves the coffee flavour when roasting by adding to the final texture and mouthfeel as they carry fat-soluble vitamins (Oestreich-Janzen, 2010). The levels varied from 14.7 to 17.8% for Arabica coffee, 12.3 to 16.7 % in Arabusta hybrids, 12.1 to 16.8% for backcrosses whereas for Robusta it was 12.5%. Gichimu et al., (2014) and Gimase et al., (2014b) reported ranges of 13.4 to 15.25% in Arabusta coffee, 12.5 to 18.4% fin Arabica coffee, and 13.4% in Robusta coffee. Odeny et al., (2016) reported an oil content of 15.79-18.99% for Arabica coffee genotypes. Environmental and genetic factors affect the final oil content in the coffee bean. For environmental factors such as shade and altitude, with increased shade and higher altitude there is a rise in oil content in the green bean as reported by Odeny et al., (2014) and Avelino et al., (2005).

Simkin et al., (2006), carried out gene profiling for the five oleosin genes (OLE1-1, OLE-2, OLE- 3, OLE-4, and OLE-5) that encode the oil storage proteins in *C arabica* and *C. canephora* species at different stages of ripening. OLE-1 and OLE-2, together with OLE-4 genes were predominant during the early development of berries in Arabica and Robusta. For Robusta coffee OLE-3 and OLE-5 genes were reported in all berry development stages except during the pin berry stage in early development where there was no expression of all the five genes. (Cheng et al., 2016). This implies that storage of oil in Arabica coffee start early of berry development unlike the Robusta coffee and this also explains why the oil concentration levels are high in Arabica than in Robusta.

There were no G x E interactions for the biochemical attributes measured in the study indicating that the genotypes were stable for the two environments and can be selected independently in each location. The effects of the environments were significant for all the biochemical attributes except for chlorogenic acid. Kathurima et al., (2010) and Gichimu et al., (2014a) evaluated Ruiru 11 hybrids and sibs in different environments and reported significant environmental and G x E effects for oil, sucrose, chlorogenic acids, caffeine, and trigonelline The differences between their results and those of this study could be attributed to the fact that they evaluated their genotypes within environments located in higher altitude. G x E interactions are key to breeders when making decisions during the selection process in varietal development since the focus is on developing a stable variety for a specific trait of interest.

Significant G x E effects have been considered as a major factor in delaying breeding achievements within a shorter period since the genotypes need to be stable to a specific trait to select for specific environments (Agwanda et al., 2003). The lack of environmental effect on chlorogenic acids could be an indication that their synthesis is largely influenced by the genotypic effects than environmental effects since *C. canephora* produces chlorogenic acids when compared to *C arabica* species. The biochemical composition is dependent on the species, environmental factors including temperature, rainfall, soils, and humidity which are responsible or the variations created (Rodrigues et al.,2009). the genotypes in this study were evaluated in low altitude areas.

Chlorogenic acids showed positive correlations with caffeine whereas the correlation with oil and sucrose was significantly negative. Genotypes including Robusta, CV1, and CV2 had high caffeine and chlorogenic acid levels (11.2, 10.5,11.2), and also low sucrose and oil levels when compared to the other genotypes. This affected their performances in terms of quality by negatively affecting

their aroma, flavour and their preference thus scoring low in cup quality. Trigonelline showed a positive correlation with all the other components measured with a significant correlation with oil (r=0.49) and sucrose (0.44).

On the other hand, the Arabica genotype (Batian and SL28), together with ARH1, ARH6, ARH7, and BCO3, had high levels of sucrose, oil, and trigonelline and low chlorogenic acids and caffeine levels. The above-named genotypes in turn had a good balance, aroma, flavour and preference scoring high for total score on cup quality. Odeny et al., (2016) evaluated coffee under shade in different environments and found a positive correlation of oil to sucrose and trigonelline. The findings of Caporaso et al., (2018) agree to the findings of the study where he reported a positive correlation of trigonelline with sucrose and also a correlation that was negative between caffeine and sucrose. This implies that when the chlorogenic acids and caffeine levels are high the quality will be low and vice versa.

There were significant differences in all the biochemical attributes between the environments except for chlorogenic acids. Sugars, oils, caffeine, and trigonelline levels were high in Siaya when compared to Busia. But despite these high levels, the average total score on cup quality was lower than in Busia. This indicates that caffeine is key in defining the final quality of coffee when compared to the other attributes. Caffeine and chlorogenic acids affect the cup quality by lowering the quality levels due to the bitter and acidic effects on the cup whereas the coffee oils and sucrose are direct precursors for aroma and flavour. The negative correlations between the sucrose, oils with caffeine, and chlorogenic acids indicate a close but competing linkage between the two pathways (Baumann, 2006). The negative correlation implies that selection for increased levels of oil and sucrose levels will lead to lowered levels of chlorogenic acids and caffeine that

negatively affect the cup quality. The positive correlations between the trigonelline on one hand and oil and sugars on the other hand indicated that trigonelline can be used for direct selection for sucrose and oil to increase the quality of the cup. To improve on the selection efficiency for high cup quality, the focus should be geared toward those attributes that contribute positively to an improved cup which includes sucrose, trigonelline, and oils.

The genotypes were discriminated based on their scores for the five biochemical components and this indicated that there was variation among the genotypes. PCA was able to explain the percent variability of each attribute that results in the total variation. The genotypes SL28, ARH3, Batian, and BC03 were grouped based on sucrose and oil levels whereas Batian stood out solely indicating that it was also differentiated by the trigonelline levels since it scored the highest for this attribute. The chlorogenic acids and caffeine grouped genotypes CV1, CV2, and Robusta in the same cluster which scored low for the sucrose, oil, and trigonelline. The PCA and the cluster dendrogram illustrates that the genetic variation among the coffee genotypes that were evaluated. Three different clusters of genotypes were generated using the UPGMA based on their biochemical composition. The different groupings of the genotypes indicated that it is possible to differentiate genotype based on their genetic distances using the biochemical data available.

Robusta coffee has undesirable attributes including high caffeine levels making it less competitive in the market when compared to Arabica due to its astringency feel and bitterness. Arabica has high levels of trigonelline, sucrose, and oil which contributes to the improved flavour and aroma with reduced bitterness in coffee. It is therefore imperative to select for coffee genotypes that have high levels of oil, sucrose, and trigonelline to satisfy the market needs. The Arabusta hybrids on average scored high for oil, sucrose, and trigonelline, and low for chlorogenic acids and caffeine

when compared to Robusta. Some of the hybrids compared well for with Arabica coffee meaning that the interspecific hybridization was successful in passing on of high-quality traits from Arabica to Robusta.

The biochemical composition varied between the Arabusta hybrids, Robusta coffee, backcrosses, and Arabica coffee. The Arabusta hybrids performed better than Robusta for oil, sucrose, trigonelline, caffeine, and chlorogenic acids with some of them (ARH1 and ARH6) comparing well with Arabica genotypes. This indicated that the genes responsible for these biochemical compositions (sucrose, oil, and trigonelline) were successfully introgressed to Arabusta coffee and this led to a decrease in caffeine and chlorogenic acids calculated in Robusta coffee. The environmental effects played a key role in differentiating the biochemical attributes among the coffee genotypes across the two locations. The correlation of the biochemical attributes indicated that it is possible to use oil and sucrose for selection due to its positive relationship to improved cup quality since it would lead in indirect selection for low levels of caffeine and chlorogenic acids.

7.0 GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATION

7.1 General discussion

The variations calculated during the study for both growth and yield traits indicate there exists a genetic variation which is key for an efficient selection programme among the coffee genotypes. The results showed a higher number of total bearing primaries, number of berries found on longest primaries, the total number of berries on each node on the longest primary, berries per node, laterals lead to increased yield. The performance of coffee cultivars in yield and cup quality is important in the development of a coffee variety in coffee breeding programmes. The coffee plant takes up to six to eight years per cycle and this means it would take several years (25-30 years) to breed for a coffee variety. Maximizing the use of the growth and yield traits during the early years of the crop development would reduce the time required to release variety. The use of the quantitative traits for yield selection is key to improving the selection efficiency during crop improvement.

The genetic variation within the coffee genotypes can be confirmed by the polymorphism results where the Arabusta hybrids showed a high polymorphism rate (72%) when compared to the Arabica (46%) implying that the Arabica genotypes have a narrow genetic base. Interspecific hybridization has been used in creating the genetic variabilities for coffee improvement through genetic. The wide genetic variation in Arabusta coffee is contributed largely by the interspecific hybridization between induced Robusta tetraploid and Arabica, whereas Arabica had a low genetic variation due to its autogamous nature. The genetic variability is required during coffee improvement programmes.

Liquor quality and the biochemical composition varied among the different genotypes of coffee evaluated. The Arabica and Arabusta genotypes scored more than 80% on total score thus qualifying for the specialty market. Arabica coffee had higher levels of oil, sucrose, and trigonelline with intermediate levels reported in Arabusta coffee. The inherent attributes of the coffee green beans are determined by several factors which include genetics, environmental conditions, post-harvest handling, and processing. The chemical attributes contribute significant ly to the cup quality for instance oil and sucrose contribute immensely to both the aroma and flavour of the beverage.

In the eighteen coffee genotypes evaluated for CBD resistance with SSR marker SAT 235, the introgressed genotypes (Arabusta hybrids, backcrosses, Robusta, CV1, ARV, Batian, and Ruiru 11) and Robusta was found to have the C*k* -1 gene indicating that they have resistance to the CBD The susceptible genotypes SL28, SL34, and Caturra did not have the gene. Screening of plants for disease resistance using markers is possible during any growth stage of a plant since it directly identifies the genes of interest which can be differentiated with ease unlike using the phenotypic evaluation employing quantitative traits.

There was significant G x E interaction on bean yield but the biochemical attributes and the sensory traits did not show any interaction. This implies that the coffee genotypes are not stable for bean yield across the two environments and selection needs to be location-specific but are stable for biochemical attributes. The environmental effects for all the traits measured were significant indicating that the environments were not similar thus the differences in performance. The presence of significant G x E interaction implies that genetic variations for bean yield exist among the genotypes.

7.2 Conclusions

The coffee genotypes in the study revealed significant variations on growth traits, yield, cup quality, bean grades, CBD resistance, and biochemical attributes. This signified the variations found within coffee and this is key in improving selection efficiency during breeding. The morphological traits are utilized in improving yield selection during the early growth stages in coffee by shortening of breeding time which normally takes five to six years to record its yield potential. Arabusta ARH4 was the high yielding genotype across the two environments when compared to the other genotypes and was also the most ideal genotype. Arabusta hybrids ARH1, ARH4, and ARH5 had higher yields with an average of over 2500g/tree and these genotypes can be selected further for evaluation for release to farmers. Within the backcrosses evaluated, BC06 had the highest yield. The hybrids ARH2 and ARH3 despite scoring high for oil, sucrose, and trigonelline poorly performed in yield and this could be attributed to poor fertility which causes poor development of the coffee beans.

The significant G x E effect for bean yield and bean grades and the lack of interaction for sensory traits and the biochemical attribute s was an indication that environmental variance contributed to a large extent in the determination of phenotypic variance. The evaluated coffee genotypes were stable for both cup quality and biochemical traits, meaning that the genotypic variance was the key determinant of the variation in these traits. evaluating genotypes in different environments is key in the determination of genotypic performance as shown by the significant G x E.

The resistance of coffee to CBD is important to ensure the high productivity of coffee resulting from the minimum cost of production. MAS is an important tool in any breeding programme in enhancing selection efficiency. the SAT 235 marker in this study identified genotypes with the

CK-1 gene linked to CBD resistance which is key in ensuring an efficient selection process. Except for the Arabica coffee (Caturra, SL28, and SL34), other genotypes (RAH3, ARH4, ARH5, ARH6, BC02, BCO3, BC04, BCO5, CV1, CV2, ARV, Batian, Ruiru 11) had the introgressed gene from HDT responsible for the CBD resistance. Genotypes such as Batian and Ruiru 11 were grouped in the same cluster since they share the same parents. The backcrosses being derivatives of the Arabusta hybrids were grouped in one cluster whereas the Caturra genotypes, SL34, and SL 28 were grouped as pure lines of Arabica coffee. Polymorphism derived from SSR markers was high in Arabusta hybrids (72%) when compared to Arabica (46.8%) indicating a broader genetic base in Arabusta coffee when compared to Arabica coffee. The wider variation among the is important for coffee improvement programmes. The genetic diversity results showed that the clustering of the genotypes was directly related to the clustering for the biochemical and cup quality.

The quality of coffee beans depend on bean size and cup quality is determined by the biochemical composition. The percentage of AA is important in defining the market prices of the coffee since this grade fetches higher prices followed closely by % AB bean grade. The weight of the coffee berry determined the grades since genotypes with heavy berries resulted in a higher percentage of both AA and AB bean grades. The Arabusta hybrids ARH1, ARH4, and ARH6 on average had more than 80% of both the AA and AB beans, comparing well with Ruiru11, Batian, and SL28. All the Arabusta hybrids including ARV scored more than 80% on the total score for sensory traits thus qualifying for the specialty markets. The total physical count of the percentage of pea berries was high in Arabusta coffee when compared to Arabica coffee. Pea berries are known to have better roast than the normal beans and thus its quality is expected to be high. Different countries sell pea berries in specialty markets and therefore the Arabusta coffee can be produced easily for

such a market. This would imply that the farmers would then get premium prices for the coffee sold. This implies that the specific hybridization was successful in the introgression of high-quality traits from Arabica to Robusta coffee.

The variability of the coffee genotypes for both quality and biochemical attributes is an indicator of genetic diversity. The genotypes were grouped into different groups based on quality and biochemical attributes creating an interrelationship between the two traits. Genotypes that were grouped based on high levels of sucrose, oil, trigonelline were grouped for high cup-quality. Whereas those that grouped based on chlorogenic acids and caffeine were grouped for low cup- quality. Breeding for high levels of sucrose, oil, and trigonelline, the caffeine, and chlorogenic acids will lead to producing varieties that have low caffeine and chlorogenic levels. Genotypes that had high levels of caffeine and chlorogenic acids including Robusta, CV1, and CV2 scored below 80% on the total score for quality. On average Arabusta hybrids and backcrosses compared well with the Arabica coffee and outperforming the Robusta coffee. This indicates that there was an improvement in quality. The biochemical composition is critical in defining the final cup quality.

7.3 Recommendations

1. The Arabusta hybrids that scored over 2500g/tree in yield are promising candidates commercial production since they scored high in cup quality of more than 80%. These hybrids need to be evaluated further under National Performance Trials (NPTs) including the coastal regions to be able to release a variety specific to each environment.
2. The grading system that was earlier developed and is currently being used is specific to Arabica coffee. From the study, it was found out that Arabusta coffee has more pea berries

when compared to Arabica coffee. To target the specialty market for pea berries, it is important to develop a new grading system that will work well with the Arabusta coffee hybrids.

3. Robusta coffee has been one of the major coffee species cultivated in Busia and Siaya and due to its limitations on cup quality, its cultivation and marketing have been declining over the last 30 years. The discovery of better performing Arabusta hybrids could be a substitute for Robusta. This study was only carried out in two environments and this could limit evaluation for the G X E interactions hence the potential of the genotypes could not be maximized thus the need to evaluate them in more environments.
4. Further backcrossing needs to be done between BC06 and SL28 to develop an Arabica coffee variety that is disease resistant and has high cup quality.
5. Busia and Siaya counties border Uganda where Robusta coffee is the major species of cultivated coffee. Coffee Wilt Disease (CWD) caused by *Giberrella xylarioides* is one of the major diseases affecting Robusta coffee in Uganda. During this study, coffee genotypes were not evaluated for resistance against CWD although no signs nor symptoms were observed in the field. Further evaluation for resistance to CWD needs to be carried out to evaluate their performance.

REFERENCES

Abadiga, A. A. (2010). Assessment of coffee quality and its related problems in Jimma zone of Oromia regional state. MSc. Thesis, Jimma University, Ethiopia.

Abrar, S., Ali, M. and Solomon E. (2014). Processing method, variety and roasting duration effect on physical quality attributes of roasted Arabica coffee beans. *Sky Journal of Agricultural Research*, 3:5. 082 – 088.

Adepoju, A.F., Adenuga, O.O., Mapayi, E.F., Olaniyi O.O, Adepoju F.A (2017). Coffee: Botany, Distribution, Diversity, Chemical Composition and Its Management. *Journal of Agriculture and Veterinary Science* Volume 10, Issue 7 Ver. III, PP 57-62

Adugnaw, M., Nigussie, D. and Ali, M. (2015). Bean Quality Attributes of Arabica Coffees Grown in Ethiopia and the Potential for Discovering New Specialty Coffees. *East African Journal of Sciences* (2015) Volume 9 (2) 121-130

Aggarwal, K.R., Hendre, P.S., Varshney, R.K., Bhat, P.R., Krishnakumar, V. and Singh, L. (2007). Identification, characterization and utilization of EST-derived genic microsatellite markers for genome analyses of coffee and related species. *Theoretical and Applied Genetics* 114: 359–372.

Agwanda, C. O., Baradat. P. A. B., Eskes, C., Cilas, A., and Charrier, A. (2003). Selection for bean and liquor qualities within related hybrids of Arabica coffee in multilocal field trials. *Euphytica* 131: 1–14.

Agwanda, C. O., Lashermes, P., Rouslot, P., Combes, M. C., and Charrier, A. (1997). Identification of RAPD markers for resistance to Coffee berry disease, *Colletotrichum kahawae*, in Arabica coffee. *Euphytica,* 91. 241-248.

Agwanda, C.O. (1999). Flavour: an ideal selection criterion for the genetic improvement of liquor quality in Arabica coffee, in *the proceedings of 18th international scientific colloquium on coffee*. Helsinki, Finland, 383-389.

Aluka, P. (2013).Genetic and Phenotypic diversity of cultivated Robusta coffee (*Coffea canephora* Pierre) in Uganda and effect of environmental factors on quality. *PhD Thesis* University of Nairobi.

Aluka, P., Fabrice, D., Kahiu, N., Omaria, R,, Fourny, G., Leroy, T., Magali, D and Fabrice, P (2016). The Diversity of Green Bean Biochemical Compounds in Robusta Coffee (Coffea canephora Pierre ex A. Froehner) as Evaluated by Near Infrared Spectroscopy. Australian *Journal of Experimental Agriculture* 12(2): 1-13, DOI: 10.9734/AJEA/2016/23004

Alemseged Yilma (2018). Coffee Pea berry as a Seed Source for Production. Paper present on the 58th IACO Scientific Conference Hotel Nomad, Gabon 20-27.

Ameyu, M. (2017). Influence of harvesting and postharvest processing methods on the quality of Arabica coffee (Coffea arabica L.) in Eastern Ethiopia. *Journal of Food and Agriculture Science,* 7, 1–9.

Anaparti, A. M. (2013). Pharmacological Effect of Trigonelline. Retrieved February 27, 2013, from http://www.ehow.com/about 6789203 pharmacological-effect-trigonelline.html

Anim-Kwapong, E. and Adomako, B. (2010). Genetic and environmental correlations between bean yield and agronomic traits in *Coffea canephora*. *Journal of Plant Breeding Crop Science*., 2(4): 64-72.

Anthony, F., Combes, M.C., Astorga, C., Bertrand, B., Graziosi, G. and Lashermes, P. (2002). The origin of cultivated Coffea arabica L. varieties revealed by AFLP and SSR markers. *Theoretical Applied Genetics*. 104: 894-900.

Arega, Z., Fasil, A. and Hindorf, H. (2008). Occurrence of major coffee diseases in Afromontane rainforests on Ethiopia. In: Girma, A., Bayetta, B., Tesfaye. S., Endale, T. and Taye, K. (eds.). Coffee Diversity and Knowledge. EIAR, Addis Ababa, Ethiopia. pp. 261- 266.

Asfaw, E. (2008). Physical quality standards and grading systems of Ethiopian coffee in demand supply chain. In G. Adugna, B. Ballechwe, T. Shimber, E. Taye and T. Kufa eds. *Coffee Diversity and knowledge.* Ethiopian Institute of AgriculturalResearch, p.328-334

Assefa, K., Ketema, S., Tefera, H., Nguyen, H.T., Blum, A., Ayele, M., Bai, G., Simane, B. and Kefyalew, T. (1999). Diversity among germplasm lines of the Ethiopian cereal tef (Eragrostis tef (Zucc.) Trotter). *Euphytica,* 106, 87-97.

Association of Official Analytical Chemists (A0AC). (1995). Official methods of analysis of AOAC International (16th Ed.) Gaithersburg, MD, USA: AOAC International.

Avelino, J., Barboza, B., Araya, J. C., Fonseca, C., Davrieux, F. and Guyot, B. (2005). Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. *Journal of the Science of Food and Agriculture*, 85(11), 1869--1876.

Barbosa, J., Borem, F., Alves, H., Cirillo, M., Sartori, M., Ducatti, C (2014). Discrimination of Production Environments of Specialty Coffees by Means of Stable Isotopes and Discriminant Model. *Journal of Agricultural Science*, 6(5):50-55.

Barel, M. and Jacquet, M. (1994). Coffee quality: its causes, appreciation and improvement. *Plant Rech. Develop.* 1:5-13.

Batista L., and Chalfoun, S. (2014). Quality of coffee beans. Cocoa and Coffee Fermentation.ry perspective. *Food Research International* 62:315–325.

Baumann, T.W. (2006). Some thoughts on the physiology of caffeine in coffee - And a glimpse of metabolite profiling. *Brazilian Journal of Plant Physiology* 18(1)DOI: 10.1590/S1677-04202006000100017

Baye, T. (2002). Genotypic and phenotypic variability in *Vernonia galamensis* germplasm collected from eastern Ethiopia. *Journal of American Science*, Volume 139, Issue 2 pp. 161-168

Bayetta, B., Behailu, A., Fikadu, T., Ashenafi, A. and Tadesse, B. (2007). Genetic diversity and heterosis in Arabica coffee. In: Girma, A., Bayetta, B., Tesfaye, S., Endale, T. and Taye, K. (eds.). Coffee Diversity and Knowledge, Four Decades of Coffee Research and Development in Ethiopia. *Proceedings of National Workshop*, 14-17 August 2007, Addis Ababa (Ghion Hotel) Ethiopia. pp. 328-334

Bekele, M.E (2019). Review on the Management of Coffee Berry Disease (colletotrichum kahawae) in Ethiopia. *Journal of Food Science and Quality Management*. Vol. 76

Belachew, K and Teferi, D. (2016). A Review on the Status of Coffee Berry Disease (Colletotrichum kahawae) in Ethiopia. *Journal of Biology, Agriculture and Healthcare.* 2224-3208 Vol.6, No.19

Belay, A. (2011). Some biochemical compounds in coffee beans and methods developed for their analysis. *International Journal of the Physical Sciences*, 6(28), 6373-6378. http://dx.doi.org/10.5897/IJPS11.486

Belay, A., Ture, K., Redi, M. and Asfaw, A. (2008). Measurement of caffeine in coffee beans with UV-Vis spectrometer. *Food Chemistry*, 108, 310-315.

Belay, S., Mideksa, D., Gebrezgiabher, S. and Seifu, W. (2016). Factors affecting coffee (coffea arabica l.) quality in ehtiopia: a review. Journal of Multidisciplinary Scientific Research, 2016,4(1):27-33

Benowitz, N.L., Jacob, P. III, Mayan, H. and Denaro, C. (1995). Sympathomimetic effects of paraxanthine and caffeine in humans. *Clinical Pharmacology and Therapeutics* 58: 684–691.

Berthaud, J. (1976). Étude cytogenétique d'un haploide de C. arabica L. Café Cacao Thé, 20:91- 96

Berthaud, J. and Guillaumet, J.L. (1978) 'Les caféiers sauvages en Centrafrique' Cafe Cacao. *The*,22, 171-86.

Berthou, F. and Trouslot, P. (1977) 'L'analyse du polymorphisme enzymatique dans le genre Coffea: adaptation d'une méthode d'électrophorèse en skrie: premiers résultats', in 8th International Colloquium on the Chemistry of Coffee, ASIC, Paris, pp. 373-83.

Bertrand, B., E. Alpizar, L. Llara, R. Santacreo, M. Hidalgo, J.M. Quijano, P. Charmetant, C. Montagnon, F. Georget 1 and H. Etienne, (2010). Performance of Coffea arabica F1 Hybrids in Comparison with American Pure Line Varieties. pp. 484–495. Proceedings of the 23nd International Association for Coffee Science (ASIC), 3-8 October 2010, Bali,Indonesia

Bertrand, B., Guyot B., Anthony F., and Lashermes, P. (2003) Impact of introgression of Coffea canephora genes on the Arabica coffee cup quality. *Journal of Theoretical and Applied Genetics* 107:387–394

Bicchi, C.P., Binello, A.E., Pellegrino, G.M. and Vanni, A.C. (1995). Characterization of green and roasted coffees through the chlorogenic acid fraction by HPLC-UV and principal component analysis. *Journal of Agricultural and Food Chemistry* 43 (6) 1549–1555.

Bicho, N. C., Leit~ao, A. E., Ramalho, J. C., de Alvarenga, N. B., and Lidon, F. C. (2013a). Identification of chemical clusters discriminators of Arabica and Robusta green coffee. *International Journal of Food Properties*, 16(4), 895-904.

Bicho, N. C., Leit~ao, A. E., Ramalho, J. C., de Alvarenga, N. B., and Lidon, F. C. (2013b). Impact of roasting time on the sensory profile of Arabica and Robusta coffee. *Ecology of Food and Nutrition*, 52(2), 163-177.

Bicho, N. C., Leitao, A. E., Ramalho, J. C. and Lidon, F.C. (2011) Identification of chemical clusters discriminators of the roast degree in Arabica and Robusta coffee beans. *European Food Research and Technology*, vol. 233, no. 2, pp. 303–311.

Buffo, R.A and Freire, C.C. (2004). Coffee flavour: an overview. *Flavour Fragance. Journal*. 19: 99–104.

Campa, C., Doulbeau, S., Dussert, S., Hamon, S. and Noirot, M. (2005). Diversity in bean caffeine content among wild *Coffea* species: Evidence of a discontinuous distribution. *Food Chemistry,* 91:633–637.

Cantergiani, E., Brevar, H., Amado, R., Krebs, V., Feria-Morales, A. and Yeetizian, C. (1999), *Proceedings of 18th International Colloq Coffee.* Helsinki Finland

Caporaso, N., Whitworth, M.B., Grebby, S., and Fisk, I.D (2018). Non-destructive analysis of sucrose, caffeine and trigonelline on single green coffee beans by hyperspectral imaging. *Food Research International,* 106 (2018), pp. 192-2.

Capot, J.P, Depautex, and Durandeau, A. (1968). L'amelioration du caf'eier en Cote d'Ivoire. Duplication chromosomiqueet hybridization. Caf'e Cacao Th'e 12:114-126.

Capucho, A.S., Caixeta, E.T., Zambolim, E.M., Zambolim, L (2009). Heranc¸a da resisteˆncia do Hı´brido de Timor UFV 443-03 a` ferrugem-do-cafeeiro. Pesqui Agropecua´ria Bras 44:276–282. doi:10.1590/S0100-204X2009000300009

Carvalho, A. (1952) Taxonomia de Coffea arabica L. caracteres morfologicas dos haploids. Bragantia, 12: 201–212.

Carvalho, A. and Krug, C.A. (1949) 'Genetica de Coffea XII. Hereditarieade da côr Cuca0 Thé, 16,3-18' haploides', Bragantia, 12, 201-12 amarela da semente'. Erapantia. 9. 193-202.

Carvalho, A., Fenverda, F.P., Frahm-Leliveld, J.A., Medina, D.M., Mendes, A.J.T. and Monaco, L.C. (1969) 'Coffee (Coffea arabica L. and C. canephora Pierre ex Froehner)' in F.P. Ferwerda and F. Wit (eds). Outlines of Perennial Crop Breeding in the Tropics, Veenman & Zonen, Wageningen, pp. 189-241

Chahal, G.S. and Gosal, S.S. (2002) Principles and Procedures of Plant Breeding: Biotechnology and Conventional Approaches. *Alpha Science International*, UK, 604.

Charrier, A and Berthaud, J. (1985). "Botanical classification of coffee," in *Coffee, Botany, Biochemistry and Production of Beans and Beverage* , M. N. Clifford and K. C. Wilson, Eds., 13–47, Croom Helm, London, UK.

Charrier, A. and Eskes, A., (2004). Botany and Genetics of Coffee. In J. Wintgens (Ed.). *Coffee: growing, processing, sustainable production: A guidebook for growers, processors, traders, and researchers* (pp. 25-57). Wiley-VCH, Weinhein, Alemania

Cheng, B., Furtado, A., Smyth, H.H., and Henry, R.J. (2016). Influence of genotype and environment on coffee quality. *Trends in Food Science & Technology* 57 (2016) 20-30.

CIRAD, Centre de coopération International en Recherche en Agronomique pour le Développement (2003a). Analysis of caffeine in green coffee beans, Ref: CIR/CP:

CIRAD, Centre de coopération International en Recherche en Agronomique pour le Développement (2003b). Determination of trigonelline in green coffee beans, Ref: CIR/CP: 005

Clarke, R. J. and Macarae, R. (Eds.) (1985). Coffee: volume 1-Chemistry (pp. 1-7). England: Elsevier Applied Science Publishers.

Clifford, M. N. and K.C. Willson. (1985). Coffee: Botany, Biochemistry and Production of Beans and Beverages. Croom Helm, London and Sydney.

Clifford, M.N. (1985). Chemical and physical aspects of green coffee and coffee products. In: M. N. Clifford and K.C.Willson (Eds.). *Coffee Botany, Biochemistry, and Production of beans and beverage:* 305-374. Croom Helm, London.

Clifford, M.N. (1985). Chlorogenic acids. In : R.J. Clarke and R. Macrae (eds), Coffee, Elsevier Applied science London 1: 153-202.

Clifford, M.N., Williams, T. and Bridson,D. (1989). Chlorogenic acids and caffeine as possible taxonomic criteria in Coffea and Psilanthus. *Phytochemistry* 28: 829-838

Coffee Production Recommendation (CRF). (2016). KALRO-Coffee Research Institute 4th edition, 1st Revision

Coffee Research Foundation (CRF) (1976). Annual Report (1974/75), pp. 86-87.

Coffee Research Foundation (CRF) (1980). Annual Report (1978/79), pp. 72-73

Coffee Research Foundation(CRF) (2011). Coffee production recommendations. 4th ed., 11-12

Combes, M.C., Andrzejewski, S., Anthony, F., Bertrand, B., Rovelli, P., Graziosi, G. and Lashermes, P. (2000). Characterization of microsatellites loci in *Coffea arabica* and related coffee species. *Molecular Ecology* 9: 1178- 1190.

Condliffe, K., Kebuchi W., Love C. and Ruparell R. (2008). Kenya Coffee: A Cluster Analysis. In: Professor Michael Porter, Microeconomics of Competitiveness. Harvard Business School, pp. 2

Cullis, B.R., Jefferson, P., Thompson, R., and Smith, A.B. (2014) Factor analytic and reduced animal models for the investigation of additive genotype-by-environment interaction in

outcrossing plant species with application to a *Pinus radiata* breeding programme. *Theoretical Applied Genetics* 217:2193–2210

Curzi, D., Raimondi, V., Olper, A (2014). Quality upgrading, competition and trade policy: evidence from the agri-food sector. *European Review of Agricultural Economics*, 42(2):239-267

Cruz, C.D. (2008). Programa Genes: diversidade genética. Universidade Federal de Viçosa, Viçosa.

DaMatta, F.M., Ramalho J.D.C. (2006). Impacts of drought and temperature stress on coffee physiology and production: A review. *Brazilian Journal of Plant Physiology*., 18: 55-81.

Davis, A. P., Gole, T. W., Baena, S. & Moat, J. (2012). The Impact of climate change on indigenous arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PLoS One* 7: e47981

Davis, A.P. (2011). Psilanthus mannii, the type species of Psilanthus, transferred to Coffea. Nordic *Journal of Botany* 29: 471–472

Davis, A.P., Govaerts R., Bridson D.M., Stoffelen P. (2006). An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). *Botanical Journal Linnean Society* 152:465–512

De Maria, C.A.B., Trugo, L.C., Aquino Neto, F.R., Moreira, R.F.A. and Alviano, C.S. (1995). Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting. *Food Chemistry*, **55:**203–207.

Decasy, F., Avelino, J., Guyot, B., Perriot, J.I., Pineda, C. and Cilas, C. (2003) Quality of different Honduran coffees in relation to several environments. *Journal of food science* 68:2356- 2361

Dessalegn, Y.B, Maryke, O., Garry, H and Liezel. (2008). Genetic diversity and correlation of bean caffeine content with cup quality and green bean physical characteristics in coffee (Coffea arabica L.). *Journal of the Science of Food and Agriculture* . 88. 1726 - 1730.

Dias, R. C. E., Valderrama, P., Março, P. H., dos Santos Scholz, M. B., Edelmann, M., and Yeretzian, C. (2018). Quantitative assessment of specific defects in roasted ground coffee via infrared-photoacoustic spectroscopy. *Food Chemistry*, 255, 132–138.

Diniz, E.C.L., Ney S Sakiyama, Lashermes, P., Caixeta, E.T., Oliveira1,A.C.B., Zambolim, E.M., Loureiro,E.M., Pereira, A.A., Zambolim, L., (2005) Analysis of AFLP markers associated to the *Mex*-1 resistance locus in Icatu progenies. *Brazilian Society of Plant Breeding* 5:387-387.

Duarte, G.S., Pereira, A.A., Farah, A (2010). Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semidry and wet post-harvesting methods. *Food Chemistry* 118(3):85- 855.

Dyulgerova, B., and Valcheva, D. (2014). Heritability, Variance Components and Genetic Advance of Yield and Some Yield Related Traits in Barley Doubled Haploid Lines. *Turkish Journal of Agricultural and Natural Sciences*. Special Issue: 1

Esayas, A. (2005). Molecular genetic diversity study of forest coffee tree (*Coffea arabica* L.) populations in Ethiopia: Implications for conservation and breeding *PhD Thesis*. Swedish University of Agricultural Sciences, Alnarp.

Esquivel, P. and Jiménez, V.M. (2012) Functional Properties of Coffee and Coffee By-Products. *Food Research International*, 46, 488-495

Ewa, N. and Gra˙zyna, B. (2006) Evaluation of sensory attributes of coffee brews from Robusta coffee roasted under different conditions. *European Food Resource Technology* (2006) 224: 159–165

Falconer, D.S. (1989). Introduction to Quantitative Genetics. Longman Scientific and Techn.\ical.Jhony Wiley and Sons, Inc. New York 438p.

Farah, A, Depaulis T, Trugo LC, Martin PR (2005a). Effect of roasting on the formation of chlorogenic acid lactones, *Journal of agriculture and food chemistry.*, 53: 1505-1513.

Farah, A. (2012) in Y. Chu (Ed.), Coffee: emerging health effects and disease prevention, John Wiley & Sons, Blackwell Publishing Ltd (2012), pp. 21-58 London

Farah, A., Franca, A. S., Mendonca, J. C. F. and Oliveira, S. D. (2005b). Composition of green and roasted coffee of different cup qualities. *Lwt - Food Science and Technology*, 38, 709- 715.

Farah, A., Monteiro, M. C., Calado, V., Franca, A. S. and Trugo, L. C. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chemistry*, 98.373-380

Feria-Morales (2002). Examining the case of green coffee to illustrate the limitations of grading systems/expert tasters in sensory evaluation for quality control. *Food Quality and Preference* 13(6):355-367.

Findlay, C.J., Castura, J.C., Schlich, P., and Lesschaeve, I. (2006). Use of Feedback Calibration to reduce the training time for wine panels. *Food Quality and Preference*, 17, 266-276.

Flament, I. and Bessière-Thomas, Y. (2002). Coffee Flavour Chemistry. John Wiley and Sons Ltd New York. pp 410.

Folmer, B., Blank, I., and Hofmann, T. (2017). Chapter 17 – Crema—Formation, stabilization, and sensation. In F. Britta (Ed.). The craft and science of coffee (pp. 399–417). London: Academic Press

Food and Agriculture Organization (FAO). (1968b). World Coffee Survey Report. Food and Agriculture Organization of the United Nations, Rome FAO Macrae (Eds.), *Coffee Agronomy*; 1-41. Elsevier Applied Science, coffee constituents.

Franca, A. S., Oliveira, L. S., Mendonca, C. F. and Siva, X. A. (2005). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry*, 90, 89-94.

Fridell, G. (2014) Fair trade slippages and Vietnam gaps: the ideological fantasies of fair trade coffee, Third World Quarterly,35:7, 1179-1194

Gassert, W. L. (1979), Research on coffee berry disease in Ethiopia: Epidemiology and control. GTZ, Eschborn. 65 pp.

George, S.E., Ramalakshmi, K. and Rao, L.J.M. (2008). A Perception on Health Benefits of Coffee critical reviews in *food science and nutrition* 48(5): 464- 86.DOI: 10.1080/10408390701522445

Geromel, C., Ferreira, L., Davrieux, F., Guyot, B., Ribeyre, F., Santos-Scholz, M.B., … Marraccini, P. (2008). Effects of shade on the development and sugar metabolism of coffee

(Coffea arabica L.) fruits. *Plant Physiology and Biochemistry*, 46(5–6), 569–579. DOI: 10.1016/j.plaphy.2008.02.006

Getachew W, Sentayehu AL, Taye K, Tadesse B, (2013). Genetic Diversity Analysis of Some Ethiopian Specialty Coffee (Coffea arabica L.) Germplasm Accessions Based on Morphological Traits. *Time Journals of Agriculture and Veterinary Sciences*, 1 (4): 47-54.

Getachew, W., Sentayehu, Al., and Taye K., (2017). Genetic variability, heritability and genetic advance for quantitative traits in coffee (Coffea arabica L.) accessions in Ethiopia. African *Journal of Agricultural Research*. 12. 1824-1831. 10.5897/AJAR2016.12059.

Gichimu B.M**.**, Gichuru E.K., Mamati G.E. and Nyende A.B., (2014a). Biochemical Composition within *Coffea arabica* cv. Ruiru 11 and its Relationship with Cup Quality. *Journal of Food Research*; 3(3): 31-44.

Gichimu, B. M., and Omondi, C. O. (2010b). Morphological Characterization of Five Newly Developed Lines of Arabica Coffee as Compared to Commercial Cultivars in Kenya. *International Journal of Plant Breeding and Genetics, 4,* 238-246.

Gichimu, B.M and Phiri, N.A. (2010). Response of newly developed and introduced arabica coffee genotypes to coffee berry disease (*Colletotrichum kahawae*) in kenya Conference: *12th Biennial KARI Scientific Conference* At: KARI Headquarters Complex, Kaptagat Road, Loresho, Nairobi

Gichimu, B.M. and Omondi, C.O. (2010a). Early performance of five newly developed lines of Arabica Coffee under varying environment and spacing in Kenya. *Agriculture and Biology Journal of North America*, **1**: 32-39.

Gichimu, B.M., Gichuru E.K., Mamati G.E. and Nyende A.B (2013). Yield Selection within Coffea arabica cv. Ruiru 11. *American Journal of Experimental Agriculture*, 3(1): 76-88.

Gichimu, B.M., Gichuru E.K., Mamati GE, Nyende A.B (2012). Selection within *Coffea arabica* cv. Ruiru 11 for high cup quality. *African Journal of Food Science,* 6(18):456-464.

Gichimu, B.M., Gichuru, E.K., Mamati, G.E. and Nyende, A.B. (2014b). Occurrence of Ck-1 gene conferring resistance to Coffee Berry Disease in Coffea arabica cv. 55 Ruiru 11 and its parental genotypes. *Journal of Agricultural and Crop Research,* 2(3), 51-61

Gichuru, E.K. (2007). Characterization of genetic resistance to Coffee Berry Disease (Colletotrichum kahawae Waller and Bridge) in Arabica coffee (Coffea arabica L.) that is introgressed from Coffea canephora Pierre. *PhD Thesis*, University of Nairobi.

Gichuru, E.K., Agwanda C.O., Combes M.C., Mutitu E.W., Ngugi E.C.K., Bertrand B., and Lashermes P (2008) Identification of molecular markers linked to a gene conferring resistance to coffee berry disease (Colletotrichumkahawae) in Coffea arabica L. *Plant Pathology* 57:1117–1124

Gichuru, E.K., Ithiru J.M., Silva M.C., Pereira A.P. and Várzea V.M.P. (2012). Restructured Sampling Plan Enables the Characterization of More Virulence Genes of Hemileia vastatrix in Kenya. *Proceedings of the 24th International Conference on Coffee Science San José* (Costa Rica), 12th –16th November 2012. Pp. 588 – 592.

Gideon, K.T., Gachanja, P., Obere, A., (2013). Effects of Reforms on Productivity of Coffee in Kenya. *International Journal of Business and Social Science* Vol. 4 No. 15 [Special Issue – November 2013]

Gimase, J.M., Omondi, C.O., Kathurima, C.W., (2015). Coffee improvement by interspecific hybridization: A review. *Journal of Agricultural and Crop Research* Vol. 3(3), pp. 41-46.

Gimase, J.M., Thagana W.T., Kirubi D.T., Gichuru E.K., Kathurima C.W (2014b). Beverage quality and biochemical attributes of Arabusta Coffee (*C. arabica* L. x *C. Canephora* Pierre) and their parental genotypes. *African Journal of Food Science*. 8(9):456-464.

Gimase, J.M., Thagana, W.M., Kirubi, D.T., Gichuru, E.K. and Gichimu, B.M. (2014a). Genetic characterization of arabusta coffee hybrids and their parental genotypes using molecular markers. *Plant Cell Biotechnology and Molecular Biology* 15(1&2):31-42

Girma, A and Chala J. (2008). Resistance levels of Arabica coffee cultivars to coffee berry disease, coffee wilt and leaf rust diseases in Ethiopia. In: Sebil vol. 12. *Proceedings of the 12th Crop Science Society of Ethiopia* (CSSE), 22 – 24 May 2006, Addis Ababa, Ethiopia. Pp 92 – 103. 11.

Girma, A. (1995). The influence of climatic conditions on the resistance levels of released coffee types to coffee berry disease (CBD). In*: Proceedings of the 2nd annual conference of Crop Protection Society of Ethiopia* (CPSE): 26-27 April 1994. Addis Ababa, Ethiopia. CPSE, Addis Ababa, Ethiopia. Pp. 32.

Gomez, C.D.S., Hamon P., Hamon S., de Kochko A., Poncet V. (2009). Current genetic differentiation of *Coffea canephora* Pierre ex A. Froehn in the Guineo-Congolian African zone: cumulative impact of ancient climatic changes and recent human activities. BMC Evolutionary Biology.;9:167

Granati, E., V. Bisignano, D. Chiaretti, P. Crino and G.B. Polignano. (2003). Characterisation of Italian and exotic Lathyrus germplasm for quality traits. *Genetic Resources and Crop Evolution* 50:273-280

Grivet, L. and Noyer, J.L. (2003). Biochemical and molecular markers. In P. Hamon, M. Seguin, X. Perrier, J.C Glaszmann (eds). Genetic diversity of cultivated tropical plants. Science publishers, inc Plymouth, U.K. 1-29.

Grosch, W. (2001). Chemistry III: Volatile Compounds. In R.J. Clarke and Vitzthum O.G., (Eds.), Coffee Recent Developments, Blackwell Science Ltd cap.3, p.68-90

Hampson, C.R., Quamme, H.A., Hall, J.W., MacDonald, R.A., King, M.C. and Cliff, A. (2000). Sensory evaluation as a selection tool in apple breeding.*Euphytica* 111(2)

Herrera, J.C., Combes M.C., Anthony F, Charrier A., Lashermes P. (2002) Introgression into the allotetraploid coffee (Coffea arabica L.): segregation and recombination of the C. canephora genome in the tetraploid interspecific hybrid (C. arabica 9 C. canephora). *Journal of Theoretical and Applied Genetics 104:661 –668*

Hindorf, H. (1975). Colletotrichum occurring on Coffea arabica: A Review. J. Coffee Res. 5(3/3): 43-56.

Hollingsworth, R.G., Armstrong, J.W., Campbell, E. (2002). Caffeine as a repellent for slugs and snails. *Nature*, 417: 915–916

Hue, J., Zhu, J. and Xu, H.M. (2000). Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops. *Theoretical and Applied Genetics*. 101: 264–268.

Hue, T.T.M. (2005). Genetic Variation in Cultivated Coffee (*Coffea arabica* L.*)* Accessions in Northern New South Wales, Australia. *Masters Thesis*, Southern Cross University.

Hunter, E. A. and McEwan, J. (1998). Evaluation of an international ring trial for sensory profiling of hard cheese. *Food Quality and Preference*, **9:** 343-354

Illy, A., R. and Viani, (2005). Espresso Coffee: The Science of Quality (2nd ed.). London: Elsevier

International Coffee Organization (ICO). 2001. Available: http://www. ico. orgIPO. 2008.Intellectual Property Office.

ICO (2019a). International Coffee Organization, International coffee Council, 124th Session, country coffee profile, Kenya.

ICO (2019b). International Coffee Organization Coffee market report August.

International Standard, 5492 (ISO). (2008). Sensory analysis – Vocabulary, Second edition, published in Switzerland.

Jaetzold, R., Schmidt, H., Hornetz, B. and Shisanya, D. (2009). Farm management handbook of Kenya, Natural conditions and farm management information, West Kenya. Ministry of Agriculture, Kenya

Jansen., J. (1993). Generalized linear mixed models & their application in plant breeding research Eindhoven: Technische Universiteit Eindhoven DOI: 10.6100/IR395257

Joet, T., Laffargue, A., Salmoa, J and Dussert, S. (2009). Metabolic pathways in tropical dicotyledonous albuminous seeds: Coffea arabica as a case study. *New Phytologist* 182(1):146-62 DOI: 10.1111/j.1469-8137.2008. 02742.x

Johnson, R.A. and Wichern, D.W. (1988) Applied Multivariate Statistical Analysis. 2nd Edition, John Wiley & Sons Inc., New York

Kahiu, N. and Aluka, P. (2016). Sensory and Organoleptic Cup Attributes of Robusta Coffee (Coffea canephora Pierre ex A. Froehner). *Journal of Agricultural Studies* ISSN 2166- 0379 2016, Vol. 4, No. 1(1)

Kang, M.S. (2002). Genotype-environment interaction: Progress and prospects. In: M.S. Kang (Ed.), Quantitative genetics, genomics and plant breeding, CAB International: Wallingford: England, p.221-243.

Karanja, A. M. and Nyoro J. K. (2002), "Coffee Prices and Regulation and their Impact on Livelihoods of Rural Community in Kenya", Tegemeo Institute of Agricultural Policy and Development, Egerton University, Kenya

Kathurima, C. W., Kenji, G. M., Muhoho, S. M., Boulanger, R. Davrieux, F. (2010). Discrimination of *Coffea arabica* Hybrids of the Composite Cultivar Ruiru 11 by Sensorial Evaluation and Biochemical Characterization. *Advance Journal of Food Science and Technology,* 2(3), 148-154.

Kathurima, C.W. (2013). Characterization of coffee genotypes in Kenya by genetic, biochemical and beverage quality profiles (*PhD thesis*). Jomo Kenyatta University of Agriculture and Technology, Kenya

Kathurima, C.W., Gichimu, B.M., Kenji, G.M., Muhoho, S.M. and Boulanger, R (2009). Evaluation of beverage quality and green bean physical characteristics of selected Arabica coffee genotypes in Kenya. *Africa. Journal Food Science.* 3(11):365-371.

Kebede, M. and B. Bellachew, (2005). Genetic divergence of Harare coffee (*Coffee arabica* L.) germplasm accessions at pre-bearing stage. *Proceedings of the 20th International Conference on Coffee Science*, Oct. 11-15, Bangalore, India, pp: 1107-1112.

Kenneth Davids (2003). Home Coffee Roasting: Romance & Revival, Rev. updated ed., 76.

Knopp, S., Bytof, G., and Selmar, D. (2006). Influence of processing on the content of sugars in green Arabica coffee beans. *European Food Research and Technology*, 223(2), 195-201.

Kumar, A., Simmi, P., Naik, G. K., and Giridhar, P. (2015). RP-HPLC and transcript profile indicate increased leaf caffeine in *Coffea canephora* plants by light. *Journal of Biology and Earth Sciences*, 5(1), 1-9.

Ky, C. L., Guyot, B., Louarn, J., Harmon, S., and Noirot, M. (2001a). Trigonelline inheritance in the interspecific *Coffeapseudozanguebariae* x *C. liberica* var. Dewevrei cross. *Theoritical and Applied Geneicst,102*.630-634.

Ky, C. L., Louarn J., Guyot B., Dussert S., Hamon S. and Noirot M. (2001b). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild coffee Arabica and Canephora accessions. *Food Chemistry*, 75, 223-230.

Lashermes, P, Andrzejewski S, Bertrand B, Combes MC, Dussert S, Grasiozi G, Trouslot P. and Anthony, F. (2000a). Molecular analysis of introgressive breeding in coffee (Coffea arabica). *Theoritical and Applied Genetics* 100:139–146

Lashermes, P., Combes, M. C., Trouslot, P. and Charrier, A. (1997). Phylogenetic relationships of coffee-tree species (*Coffea Arabica* L.) as inferred from ITS sequences of nuclear ribosomal DNA. *Theoretical and Applied Genetics*, *94*, 947-955.

Lashermes, P., Combes, M.C., Ansaldi, C., Gichuru, E. and Noir S. (2011). Analysis of alien introgression in coffee tree (*Coffea arabica* L.) *Journal of Molecular Breeding,* 27: 223–232.

Lashermes, P., Combes, M.C., Robert, J., Trouslot, P., D'hont, A., Anthony, F. and Charrier, A (1999) Molecular characterization and origin of the Coffea arabica L. genome. *Molecular Genetics* 261:259–266

Lashermes, P., Paczek, V., Trouslot, P., Combes, M.C., Couturon, E. and Charrier, A. (2000b) Single-locus inheritance in the allotetraploid Coffea arabica L. and interspecific hybrid C. arabica 9 C. canephora. *Journal of Heredity* 91:81–85

Lattin, J., D. Carroll, and P. Green. (2002). Analyzing Multivariate Data. 1st ed. Duxbury Press

Leroy, T. Ribeyre, F. Bertrand, B. Charmetant, P. Dufour, M. Montagnon, C. Marraccini, P. and Pot, D. (2006). Genetics of coffee quality. *Brazilian journal of Plant Physiology* 18 (1): 229-242.

Lingle, T. R. (1996). The Coffee Brewing Handbook, (1996). 1st edition. Long Beach:

Lingle, T.R. (2001). The Cuppers Handbook. Systematic Guide to the Sensory Evaluation of Coffees flavor, Third edition pp71

Liu,C., Yang, Q., Linforth,R., Fisk, I.D., and Yang, N. (2019). Modifying Robusta coffee aroma by green bean chemical pre-treatment. *Food chemistry* Volume 272, 30 January 2019, Pages 251-257

Louarn, J. (1978) 'Diversitd compark des descendances de C. arabica obtenues en autoftcondation et en fhndation libre au Tonkoui', in A. Charrier (ed.), Etude de la Structure et de la Variabilité Genetique des Cafèiers. IFCC Bulletin no 14, pp. 75-8

Louarn, J. (1992) La fertilité des hybrides interspécifiquese les relations génomiques entre caféiers diploïdes d'origine africaine (Genre *Coffea* L. sous genre *Coffea*). Thèse ded'état, Université de Paris-Sud (Orsay), France, PhD thesis

Lu, M.Z., Wang, X.R. and Szmidt, A.E. (1997). Molecular properties of RAPDs in Pinus sylvestris (L) and their implications for genetic analysis. *Forest Genetics*, 4 (4); 227-234.

Mahe,´ L., Le Pierre`s, D., Combes, M.C. and Lashermes, P. (2007) Introgressive hybridizat ion between the allotetraploid Coffea arabica and one of its diploid ancestors, Coffea canephora, in an exceptional sympatric zone in New Caledonia. *Journal of Genomics* 50:316–324

Malau, S., Pandiangan S (2018). Morphological Variation in Arabica Coffee (Coffea arabica L.) Growing in North Sumatra Indonesia. Journal of Agronomy. Indonesia 46(3):314 -321 DOI: https://dx.doi.org/10.24831/jai.v46i3.19342

Manach, C, Scallbert, A., Morand, C., Remesy, C. and Jimcne, L. (2004). Ploy phenol food: source and bioavilability, *American Journal of Clinical Nutrition* 79: 727-747.

Mishra, K.M., Mallikarjuna, A., and Chandragupt, A. and Anil, K (2018). Molecular and physiological characterization of a natural interspecific coffee hybrid. Molecular and physiological characterization of a natural interspecific coffee hybrid. *Indian Journal of Plant Physiology* 23(4):810–821 https://doi.org/10.1007/s40502-018-0410-8

María, I.C., Michael,J.V., Pierluigi. B., Brian,M.G., Erich, G. and Ana,P.A. (2017). Identification of biochemical features of defective Coffea arabica L. beans. *Food Research International journal* vol 95 pg 59-67.

Marin, K., Pozrl, T., Zlatic, E. ad Plestenjak, A. (2008). A new Aroma Index to determine the Aroma quality of roasted and ground coffee during storage. *Food Technology and Biotechnology* 46 (4): 442 – 447

Marjanović-Jeromela, A., Nagl, N., Gvozdanović-Varga, J., Hristov, N., Kondić-Špika, A., and Marinković, M. V. R. (2011). Genotype by environment interaction for seed yield per plant in rapeseed using AMMI model. *Pesquisa Agropecuária Brasileira*, *46*(2), 174- 181. https://dx.doi.org/10.1590/S0100-204X2011000200009

Martens, M., (1999). A philosophy for sensory science, *Food Quality and Preference* **10** (1999), pp. 233–244.

Martin, M.J., Pablos F. and Gonzalez A.G. (1998b) Discrimination between Arabica and Robusta green coffee varieties according to their chemical composition. *Talanta* 46:1259 – 1264

Martin, R.P., Donald F. B, Stanley, W and Vincent, A. U. (1978) A comparison of mean separation techniques, *Journal of Statistical Computation and Simulation*, 7:1, 33-54.

Mazzafera, P. (1991*).* Trigonelline in coffee. *Phytochemistry* **3,0**:2309–2310.

Mburu, J.K. (2004), The current recommendations for the processing of high quality and safe coffee in Kenya. *20th International Conference on Coffee Science* Bangalore, India.

Meaza, D., Mesfin, K. and Girma, T., (2011). Additive main effects and multiplicative interaction analysis of coffee germplasms from southern Ethiopia. *Ethiopian Journal of Science* 34(1):63–70.

Mekonen, H.S (2009). Influence of Genotype, Location and Processing Methods On The Quality Of Coffee (Coffea Arabica L.). *Msc. Thesis* Hawassa University, Hawassa, Ethiopia

Mendes, A.J.T. and Bacchi, O. (1940). Observações citológicas em Coffea. V. Uma variedade haplóide (di-haplóide) de C. arabica L. *Journal of Agronomy* 3:183-206.

Mendes, L.C., Menezes, H.Z. and. Silva, M.A.A.P. (2001). Optimization of the roasting of Robusta coffee (C. canephora conillon) using acceptability tests and RSM. *Food Quality and Preference* 12: 153-162.

Minamisawa, M., Yoshida, S. and Takai, N. (2004). Determination of biologically active substances in roasted coffee using a diode-HPLC system, *Analytical science*., 20: 325-328.

Mishra, M. K, and Slater, A. (2012). Recent Advances in the Genetic Transformation of Coffee, Review Article. *Biotechnology Research International*, 2012, 17.

Missio, R.F., Caixeta, E.T., Zambolim, E.M., Pena, G.F., Ribeiro, A.P., Zambolim, L., Pereira, A.A. and Sakiyama N.S. (2009b). Assessment of EST- SSR markers for genetic analysis on coffee. *Bragantia*. 68(3): 573-581.

Missio, R.F., Caixeta, E.T., Zambolim, E.M., Pena, G.F., Zambolim, L., Dias, L.A.S. and Sakiyama, N.S. (2011). Genetic characterization of an elite coffee germplasm assessed by gSSR and EST-SSR markers. *Genetics and molecular research* 10(4):2366-2381.

Mohammadi, S. A. and Prasanna B. M. (2003). Analysis of Genetic Diversity in Crop Plants; Salient Statistical Tools and Considerations. *Crop Science*, 43: 1235–1248

Montagnon, C. (2000) Optimisation des gains genetiques dans le schema de selection rkurrente reciproque de *Cofea canephora* Pierre. *PhD thesis*, Ecole Nationale Superieure Agronomique de Montpellier, France.

Moreno, G., Moreno, E. and Cadena, G. (1995) Bean characteristics and cup quality of the Colombia variety (*Coffea arabica*) as judged by international tasting panels. In: *16th International Scientific Collo,quium on Coffee*. Kyoto, pp.574-583.

Motta, Ludymila Brandão, Soares, Taís Cristina Bastos, Ferrão, Maria Amélia Gava, Caixeta, Eveline Teixeira, Lorenzoni, Rodrigo Monte, & Souza Neto, José Dias de. (2014). Molecular characterization of arabica and Conilon coffee plants genotypes by SSR and ISSR markers. *Brazilian Archives of Biology and Technology*, *57*(5), 728- 735. https://dx.doi.org/10.1590/S1516-8913201402071

Moufida, S. and Marzouk, B. (2003). Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. *Phytochemistry* 62: 1283-1289.

Mulinge, S.K (1970). Development of coffee berry disease in relation to the stage of growth of the berry. *Annual Applied Biology*. 65:269-276.

Muller, R. A. (1984). Changes in susceptibility of coffee berries during their development, and consequences. In: Proceedings of the first regional workshop on coffee berry disease. 19- 23 July 1982, Addis Ababa, Ethiopia. pp 271-280.

Mumin, M.A., Akhter, K.F., Abedin, M.Z. and Hossain, M.Z. (2006). Determination and characterization of caffeine in tea coffee and soft drink by solid phase extraction and HPLC, *Malaysian Journal of Chemistry*., 8(1): 045- 051

Mureithi, P.L (2008).Coffee in Kenya: Some challenges for decent work. International Labour Office, Sectoral Activities Programme. – Geneva: ILO, 1 v. (Working Paper, No. 260)

Muschler, R.G. 2001. Shade improves quality in suboptimal coffee zone of Costa Rica. *Agroforestry Systems*, 51: 131-139.

Muvunyi, B.P., Sallah, P.Y.K., Dusengemungu, L.,and Zhang, J (2017). Assessment of Genetic Diversity of Coffee Accessions in Rwanda and Its Implication for Coffee Breeding. *American Journal of Plant Sciences,* 2017, 8, 2461-2473.

Mwangi, C. N., (1983). Coffee Growers' Handbook. Coffee Research Foundation,Kenya. pp128 .

Najafi, N.M, Hamid, A.S. and Afshin, R.K. (2003). Determination of caffeine in black tea by fourier transform IR spectrometery using multiple linear regression, *Microchemistry Journal*., 75: 151-158.

Narasimhaswamy, R.L. (1960). Arabica selection S.795: Its origin and performace – A study. Indian Coffee 24: 197-204.

Nei, M. (1987). Molecular evolutionary genetics. Columbia University Press, New York. Pgs 512

Niggeweg, R., Michael, A. J. and Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology*, 22(6), 746-754.

Nuhu, A.A. (2014). Bioactive Micronutrients in Coffee: Recent Analytical Approaches for Characterization and Quantification. ISRN Nutrition, 2014, 1–13. DOI: 10.1155/2014/384230

Nyoro, J. K. and Sprey, L. H., (1986). Introducing Ruiru 11 to estates and smallholders. Kenya Coffee 51: 7-28.

Odeny, D., Chemining'wa, G., & Shibairo, S. (2014). Beverage quality and biochemical components of shaded coffee. In Paper presented at the *25th international conference on coffee science, Colombia*.

Odeny, D.A, Chemining'wa, G.N., Shibairo, S.I. and Kathurima, C.W. (2016). Biochemical Components of Shaded Coffee under Different Management Levels. *Advance Journal of Food Science and Technology* 12(9): 519-526.

Oestreich-Janzen, S. (2010). Chemistry of coffee. *Comprehensive Natural Products*, II, 1085-1117.

Olika, K., Sentayehu, A., Taye, K and Weyessa, G. (2011). Variability of Quantitative Traits in Limmu Coffee (*Coffea arabica* L.) in Ethiopia. *International Journal of Agricultural Research, 6: 482-493*.485

Omingo, D. O., Omondi, C. O., Cheserek, J., Runo, S. and Okun, D. (2017). Diversity analysis of selected coffee genotypes using microsatellites and random amplified polymorphic DNA in *International Journal of Biotechnology and Food Science* Vol. 5(1), pp. 1-9, May 2017 Kenya.

Omondi, C. O., Ayiecho, P. O., Mwang'ombe, A. W., &Hindorf, H. (2001). Resistance of *Coffeaarabica*cv. Ruiru 11 tested with different isolates of *Colletotrichum kahawae*, the causal agent of Coffee berry disease. *Euphytica,121*.19–24.

Omondi, C.O. (2008). Coffee quality assessment: the case of two Kenyan cultivars, Ruiru 11 and SL28. p. 1307–1311. In Proceedings of the 22nd ASIC International Coffee Conference, Campinas, Brasil, Lausanne, Switzerland

Omondi, C.O. and Owour J.B.O (1992). The performance of interspecific crosses and Backcrosses involving Arabica and tetraploid Robusta coffee species. *Kenya coffee*, 57(666):1307-1312

Omondi, C.O., Gichimu, B., Cheserek, J., Gimase, J. (2016). Leveraging on germplasm acquisition for Arabica coffee improvement in Kenya. *Journal of Agricultural and Crop Research* Vol. 4(1), pp. 9-16

Opile, W.R. and Agwanda, C.O. (1993). Propagation and distribution of cultivar Ruiru 11: A review. *Kenya Coffee* 58:1496-1508

Osborne, D.R., and Voogt, P. (1978). Carbohydrates, In the Analysis of Nutrients in Foods, (pp 130-150). Academic Press Inc. London Ltd.

Owuor, J.B.O. (1988). An Assessment of the Cup Quality of the New Disease Resistant *Coffea arabica* cultivar Ruiru 11 in Kenya. *Acta Horticulture*. 224: 383-388

Perrois, C., Strickler, S. R., Mathieu, G., Lepelley, M., Bedon, L., Michaux, S., …, & Privat, I. (2015). Differential regulation of caffeine metabolism in Coffea arabica (Arabica) and Coffea canephora (Robusta). *Planta*, 241(1), 179-191.

Pestana, K.N., Capucho, A.S., Caixeta, E.T (2015) Inheritance study and linkage mapping of resistance loci to Hemileia vastatrix in Hı´brido de Timor UFV 443-03. *Tree Genet Genomes* 11:72. doi:10.1007/s11295-015-0903-9

Peterson, J., Harrison, H., Snook, M., and Jackson, D. (2005). Chlorogenic acid content in sweetpotato germplasm: Possible role in disease and pest resistance. *Allelopathy Journal*, 16(2), 239-249.

Rachilo, J.R and D.O. Michieka. (1991). *Reconnaissance soil survey of the Busia area*. Reconnaissance Soil Survey Report. No. R8. KARI/NARL. Kenya Soil Survey. Nairobi. Kenya.

Regina ,H.G., Priolli1 , Paulo.M., Walter, J.S., Milene, M., Maria I.Z., Luis, C.S.R., Paulo, B.G and Carlos, A.C. (2008). Caffeine inheritance in interspecific hybrids of Coffea arabica x Coffea canephora (Gentianales, Rubiaceae). Genetics and Molecular Biology, 31, 2, 498- 504

Reynier, J.F., Pernes, J. and Chaume, R. (1978) 'Diversitt observte sur les descendances issues de pollinisation libre au Tonkoui', in A. Charrier (ed.). Etude de la structure et de la variabilitt genttique des cafdiers. IFCC Bulletin no. 14, pp. 69-74

Riesenbierg, L.H., Baird, S.J.E., Gardner, K.A (2000). Hybridization, introgression and linkage evolution. *Plant Molecular Biology* 42: 205-224.

Risvik, E., Colwill, J. S., McEwan, J. and Lyon, D. H. (1992). Multivariate analysis of conventional profiling data: a comparison of a British and a Norwegian panel. *Journal of Sensory Studies*, **7**: 97-118.

Roche, D. (1995). Coffee Genetics and Quality. In the proceedings of the 16th International Scientific Colloquium on Coffee pp. 584-588. Kyoto.

Rodrigues, C. I., R. Maia, M. Miranda, M. Ribeirinho, J. M. F. Nogueira and C. Máguas. (2009). Stable isotope analysis for green coffee bean: a possible method for geographic origin discrimination. *Journal of food composition Analytical* 22:463- 471.

Sanz, C., Czerny, M., Cid, C. and Schieberle, P. (2002) Comparison of potent odorants in a filtered coffee brew and in an instant coffee beverage by aroma dilution analysis (AEDA). *European Food Research and Technology*, Berlin, v. 214, p. 299-302.

Schnabel, F., de Melo Virginio Filho, E., Xu, S., Fisk, I. D., Roupsard, O., and Haggar, J. (2017). Shade trees: A determinant to the relative success of organic versus conventional coffee production. *Agroforestry Systems*, 92, 1–15.

Silva, M.C., Varzea, V.M.P., Guerra-Guimarães, L., Azinheira, H.G., Fernandez, D., Petitot, S., Bertrand, B., Lashermes P. and Nicole, M. (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. *Brazilian Journal of Plant physiology* 18 (1): 119-147

Silvarola, M. B., Mazzafera, P. and Fazuoli, L. C. (2004) A naturally decaffeinated arabica coffee. *Nature*, 249, 826

Silveira, S.R., Ruas, P.M., Ruas, C.F., Sera, T., Carvalho, V.P. and Coelho, A.S.G. (2003). Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. Gen. Mol. Biol. 26(3): 329-336.

Simkin, A. J., Qian, T., Caillet, V., Michoux, F., Amor, M. B., Lin, C., …, and McCarthy, J. (2006). Oleosin gene family of Coffea canephora: Quantitative expression analysis of five

oleosin genes in developing and germinating coffee grain. *Journal of Plant Physiology*, 163(7), 691-708.

Singh, D.K. and Sahu, A. (2006). Spectrophotometer determination of caffeine and theoylline in pure alkaloids and its application in Pharmaceutical formulations, Analitical Biology Chemistry, 349: 176-180

Singh, R.K. and Chaudhary, B.D. (1985). Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, Pages: 300.

Sousa,T., Caixeta,T.E .,Alkimim,R.E., Baia˜o de Oliveira, C.A., Pereira,A.A., Zambolim,L., Sakiyama,S.N., (2017). Molecular markers useful to discriminate Coffea arabica cultivars with high genetic similarity. *Euphytica* (2017) 213:75 DOI 10.1007/s10681-017-1865-9.

Speer, K. and Ko¨lling-Speer, I. (2006). The lipid fraction of the coffee bean. *Brazilian Journal of Plant Physiology* **18**: 201–216.

Steiger, D.L., Nagai, C., Moore, P.H., Morden, C.W., Osgood, R.V. and Ming, R. (2002). AFLP analysis of genetic diversity within and among Coffea arabica cultivars. *Theoretical Applied Genetics*. 105: 209-215

Svilaas, A., Sakhi, A.K., Andersen, L.F., Svilaas, T., Strom, E.C., Jacobs, J.D.R., Ose, L. and Blomhoff, R. (2004). Intake of antioxidants in coffee; wine and vegetables are correlated with plasma carotenoids in human. *Journal of American Nutritional Science*, 134: 562- 567.

Tazeen, M., Nadia, K. and Farzana, N.N. (2009). Heritability, phenotypic correlation and path coefficient studies for some agronomic characters in synthetic elite lines of wheat. *Journal of Food, Agriculture and Environment.* 7(3&4): 278 - 282.

Tefera, F (2018). Variability and genotype by environment interaction for quantitative and qualitative traits of coffee (coffea arabica l.) hybrids in south western Ethiopia. PhD dissertation. Jimma University.

Teressa, A., Crouzillat, D., Petiard, V. and Brouhan, P. (2010). Genetic diversity of Arabica coffee (Coffea arabica L.) Collections. *European Journal of Applied Sciences and Technology*. 1(1): 63-79.

Tessema, A., Sentayehu, A., Taye, K., and Weyessa, G. (2011). Variability and association of quality and biochemical attributes in some promising *Coffea arabica* germplasm collection in Southwestern Ethiopia. *International Journal of Plant Breeding and Genetics*, 1-15.

Tolessa, K., Alemayehu, D., Belew D and Boeckx, P (2019). Biochemical composition of Ethiopian coffees (Coffea arabica L.) as influenced by variety and postharvest processing methods. *African Journal of Food Science* Vol. 13(2) pp. 48-56, DOI: 10.5897/AJFS2018.1770

Tolessa, K., Duchateau, L., Boeckx, P (2018). Analysis of coffee quality along the coffee value chain in Jimma zone, Ethiopia. *African Journal of Agricultural Research* 13(29):1468-1475.

Tran, H., Slade, L.L., Furtado, A., Smyth, H., and Henry R (2016). Advances in genomics for the improvement of quality in Coffee. *Journal of the Science of Food and Agriculture*, 96 (2016), pp. 3310-3312

Trugo, L. (2003). Analysis of coffee products. In L. C. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 1498-1506).

Tshilenge, P., Nkongolo K. K., Mehes M and Kalonji A. 1 (2009). Genetic variation in *Coffea canephora* L. (Var. Robusta) accessions from the founder gene pool evaluated with ISSR and RAPD. *African Journal of Biotechnology*. 8 (3):380-390

Upadhyay, R., & Mohan Rao, L. J. (2013). An outlook on chlorogenic acids occurrence, chemistry, technology, and biological activities. *Critical Reviews in Food Science and Nutrition* , 53(9), 968-984.

USDA (2014). World Markets and Trade. http://www.insidefutures.com/article/1341835/USDA Special Bulletin

Van der Vossen, H. A. M. and Walyaro, D. J. (1980). Breeding for resistance to coffee berry disease in Coffea arabica L. II. Inheritance of the resistance. *Euphytica* 29: 777 – 791

Van der Vossen, H.A.M. (1974) 'Plant Breeding', Coffee Reseurch Foundation of Kenya, Annual Report 1973-74, pp.40-51

Van der Vossen, H.A.M. (1985). Coffee Selection and Breeding. In: Clifford MN, Willson KC (Eds.) Coffee Botany, Biochemistry and Production of Beans and Beverage, Croom Helm, London pp. 49-96. Venables WN, Smith DM, R Development Core Team (2006). An introduction to R: A programming environment for data analysis and graphics. Version 2.5.1.

Van der Vossen, H.A.M. (2001). Coffee Breeding and Selection: Review of Achievements and Challenges. Proceedings of *19th International Colloquium* Association for Science and Information on Coffee, Trieste, Italy, 14-18 May 2001.

Van der Vossen, H.A.M., Cook, R.T.A and Murakaru, G.N.W. (1976) Breeding for resistance to coffee berry disease caused by *ColletotrichumcoVeanum* Noack (sensu Hindorf) in *CoVea arabica* L. I. Methods of preselection for resistance. *Euphytica* 25:733–745.

Variyar, P. S., Ahmad, R., Bhat, R., Niyas, N. and Sharma, A. (2003). Flavoring components of raw munsoned Arabica coffee and their changes during radiation process. *Journal of Agriculture and Food Chemistry*, 51, 7945-7950.

Verma, P.S. and Agarwal, V.K. (1982). Genetics. S. Chand and Co. Ltd., Ram Nagar, New Delhi P 555.

Vieira, E. S.N., Von Pinho Édila V. de R., Carvalho, M. G.G., Esselink D. G., and Vosman B. (2010). Development of microsatellite markers for identifying Brazilian *Coffea arabica* varieties. *Journal of Genetics Molecular Biology* 33(3):507-514 http://dx.doi.org/10.1590/S1415-47572010005000055.

Waitathu, S. (2010). Kenyas Coffee Industry shows dramatic growth. www. new-ag.info

Walyaro, D.J.A. (1983). Considerations in Breeding for Improved Yield and Quality in Arabica Coffee (Coffea arabica L.). A *PhD thesis*, Wageningen Agricultural University

Weising, K, Nybom, H, Wolff, K and Kahl, G. 2005. DNA Fingerprinting in Plants: Principles, Methods, and Applications. 2nd ed. CRC Press, Taylor and Francis Group.

Wen, X., Takenaka, M., Murota, M. and Homma, S. (2004). Antioxidative activity of a zinc chelating substances in coffee. *Journal of BioSciences, Biotechnology and Biochemistry*., 68(11): 2313-2318.

Williams, J.D., Sunarharum W., and Smyth H. (2014). Complexity of coffee flavor: A compositional and sensory perspective. *Food Research International*. 62. 315–325.DOI 10.1016/j.foodres.2014.02.030.

Wilson, A.J., Petracco, M. and Illy, E. (1997). Some preliminary investigations of oil biosynthesis in the coffee fruit and its subsequent re-distribution within green and roasted beans. In the Proceedings of 17th International Scientific Colloquium on Coffee, pp 92-99. Nairobi Kenya

Wintgens, J.N. (2004). Coffee: Growing, Processing, Sustainable Production. A guide book for growers, processors, traders and researchers. Weinheim 250-327

Worku M, De Meulenaer B, Duchateau L, Boeckx P (2018). Effect of altitude on biochemical composition and quality of green Arabica coffee beans can be affected by shade and postharvest processing method. Food Research International 105:278-285.

Wrigley, G. (1988) Coffee. Tropical Agriculture Series, London, John Wiley and Sons, Inc., New York.

Yan, W (2001). GGE biplot – a windows application for graphical analysis of multi environment trial data and other types of two-way data. *Agronomy Journal*, 93:1111-1118

Yigzaw, D. 2005. Assessment of cup quality, morphological, biochemical and molecular diversity of C. arabica L. genotypes of Ethiopia. *PhD thesis*, University Free State. p. 97

Yukawa, G.S (2004). Effects of coffee consumption on oxidatives susceptibility of low-density lipo proteins and serum lipid level on humans, *Journal of Biochemistry*, Moscow, 1: 70- 74.

Zhang, Q., Lian, H., Wang, W. and Chen, H. (2005). Separation of caffeine and theophylline in poly (dimethylsiloxane) microchannel electrophoresis with electrochemical detection, *Journal of chromatography Analysis*, 1098:172-176.

Zhu, H., Shako, H., Zhang, Z., Wang, W. and Yao, S. (2006). Laser flash photolysis study on antioxidant properties of hydroxycinnamic acid derivative, *Journal of Radiation Environment Biophysiology*, 45: 73-77.

APPENDICES

Appendix 1. Soil analysis report

KENYA AGRICULTURAL & LIVESTOCK RESEARCH ORGANIZATION
COFFEE RESEARCH INSTITUTE

TELEPHONE: 020 2176420, 020 2176427
CELL PHONE: 0733-333 060/0724-527 611

6252

When replying please quote

Our Ref:.....

DATE.....

SOIL CHEMICAL ANALYSIS RESULTS

INWARD REF.....
DATE SAMPLE RECEIVED ..... 13 — 3 — 2015
DATE SAMPLE REPORTED..... 4 — 5 — 2015
SAMPLES SENT BY: COFFEE BREEDING — FROM (PLACE)
MOI

Field designation	C.B.U AHIDE BUSIA		C.B.U SIAYA ATC							
Lab No.	1447	1448	1449	1450						
Depth	TOP	SUB	TOP	SUB						
Colour										
PH	4.5	4.5	4.9	4.7						
Hpm e%	2.40	2.50	0.20	1.15						
Na me%	0.12	0.19	0.15	0.14						
Kme%	0.19	0.09	0.51	0.12						
Ca me%	0.39	0.11	3.60	1.31						
Mg me%	1.71	1.41	2.39	1.85						
Mn m e%	0.54	0.35	0.67	0.28						
P ppm	44	38	44	38						
N%										
C%										
Ca +Mg / K	11.05	16.89	11.75	26.33						

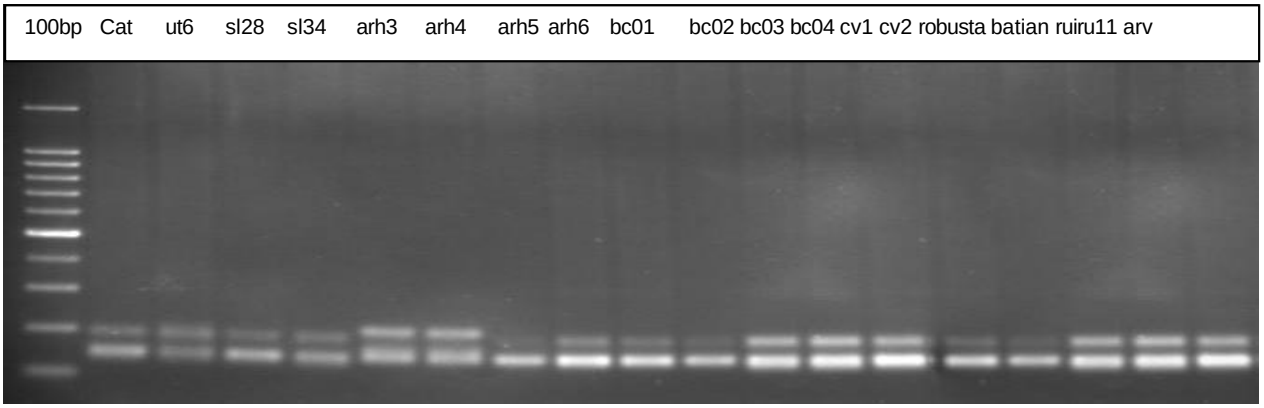
Remarks ... A1/a1, Al(V), B1/b1, C23/C2.1, D1/d1 magmax = 6759
B1/b1
AIC SIAYA = A2/a2, HP(II), C23/C2.1 D2/2 — no limming

All communications should be addressed to the Institute Director

CRI is ISO 9001:2008 Certified

Appendix 2. PCR amplified DNA fragments generated by polymorphic 19 primers

SAT 262



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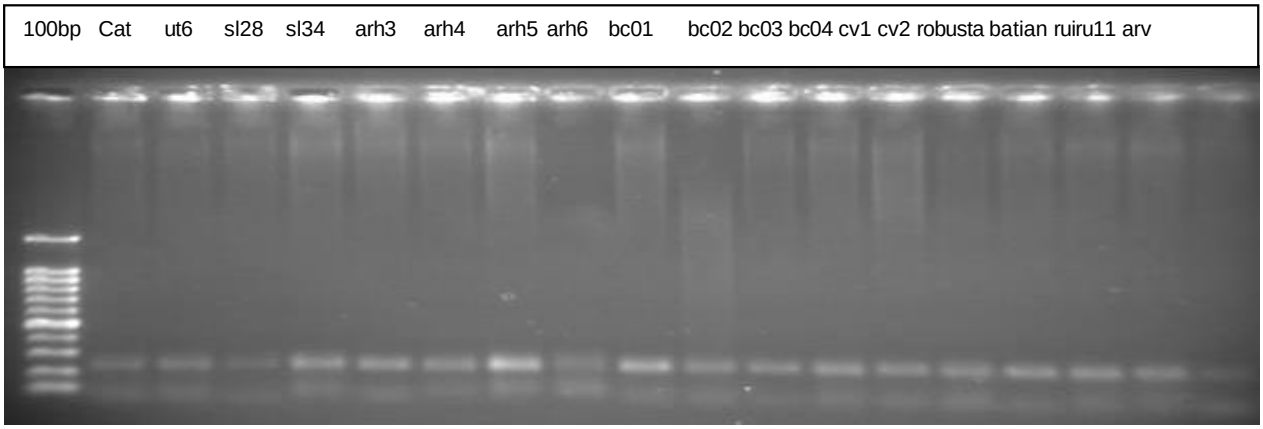

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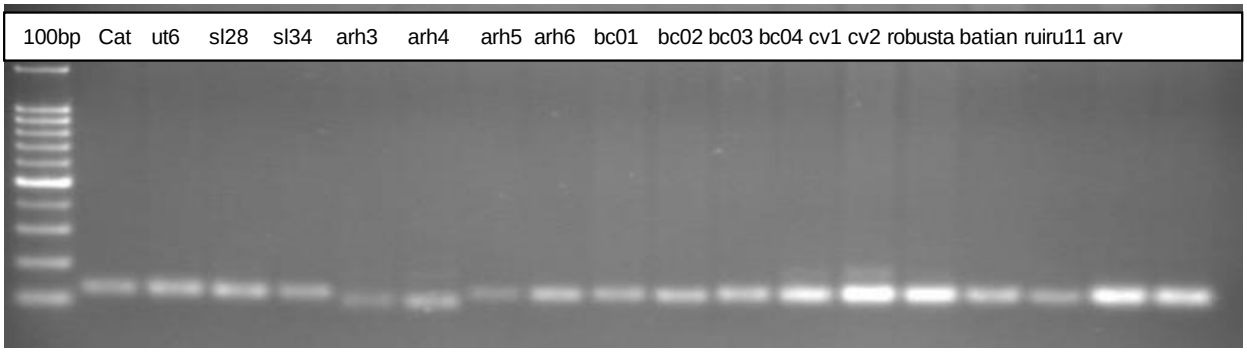

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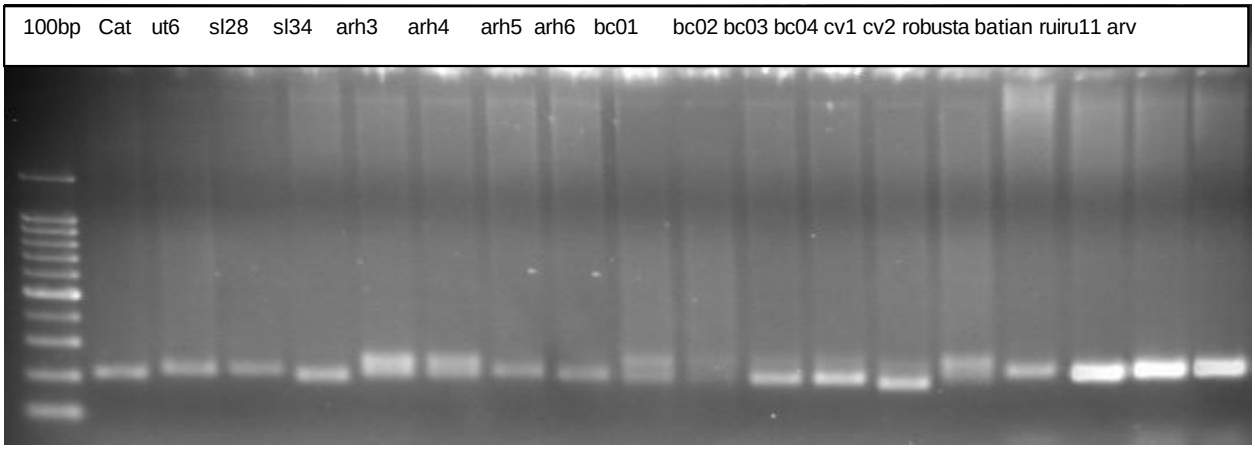

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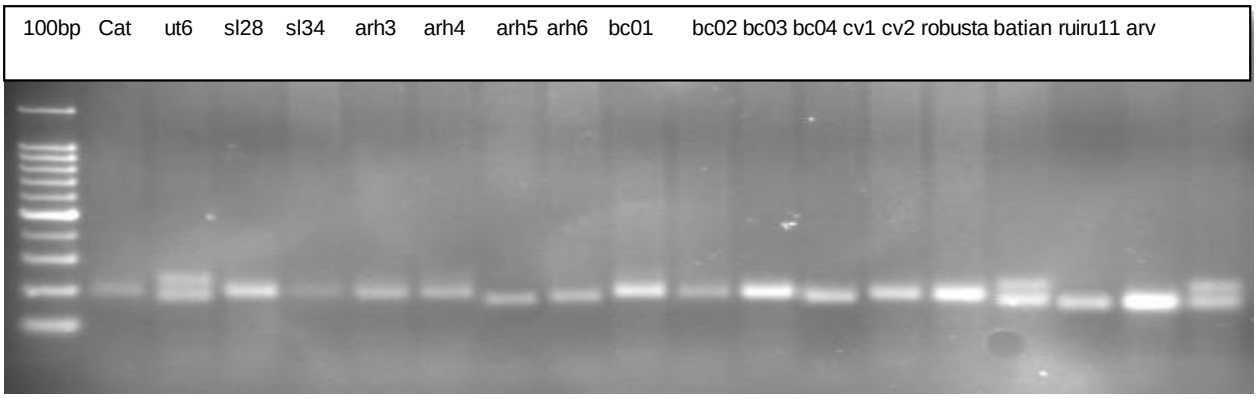

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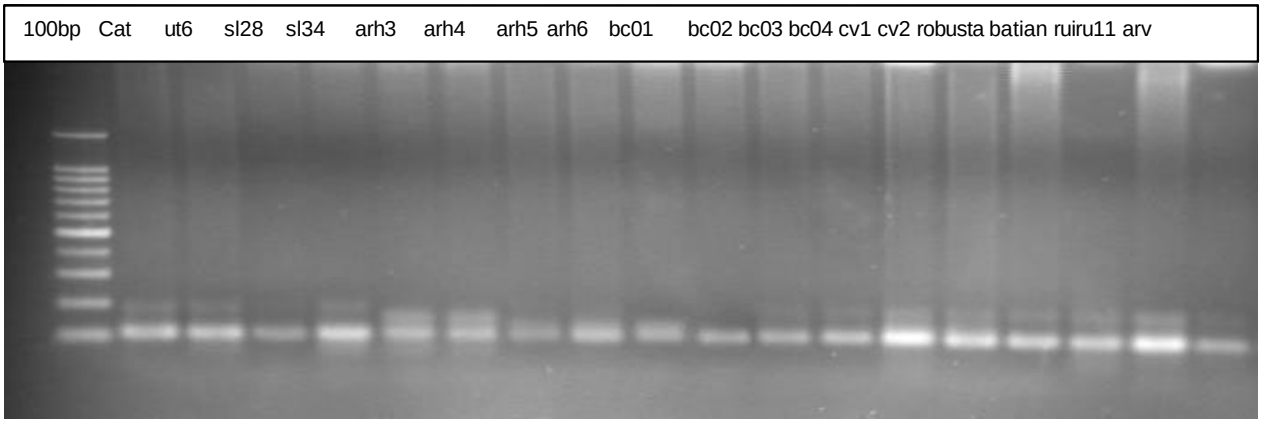

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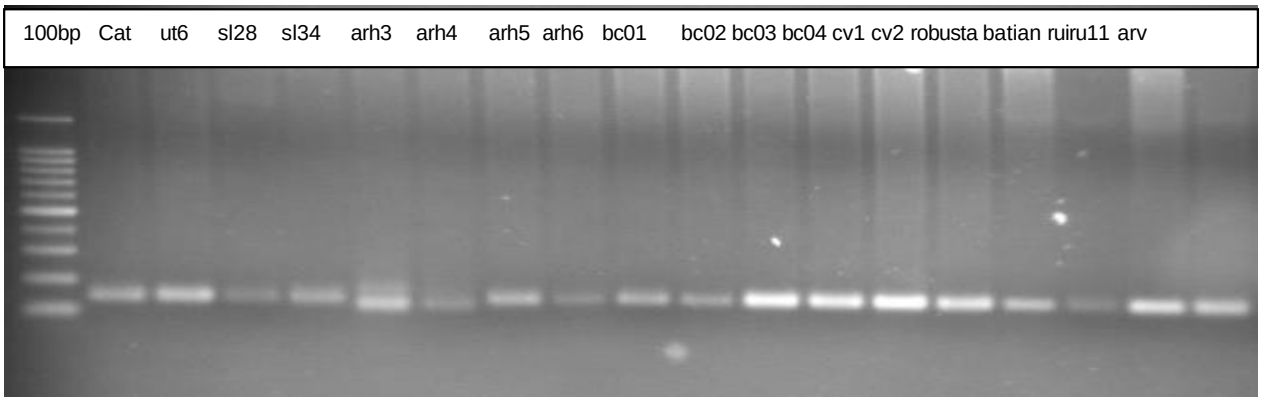

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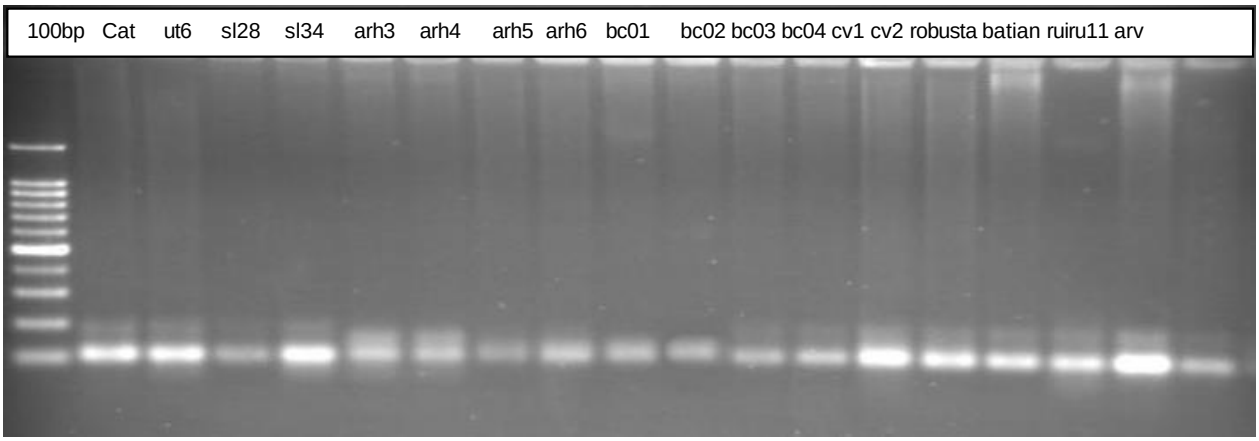

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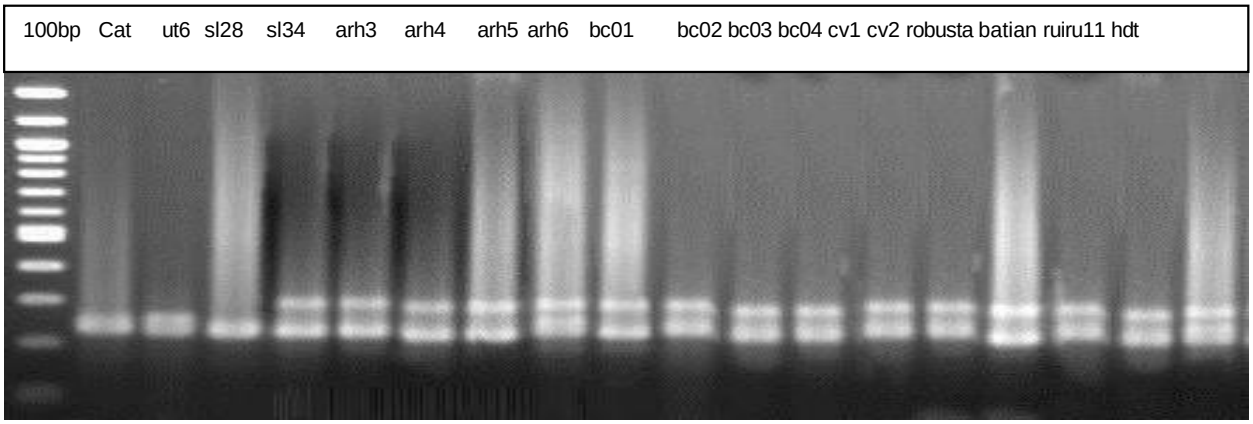

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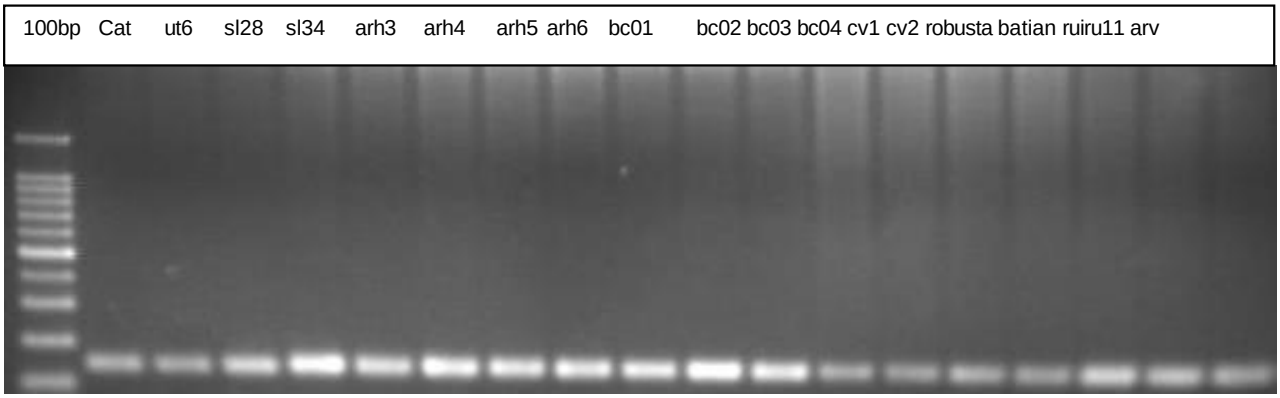

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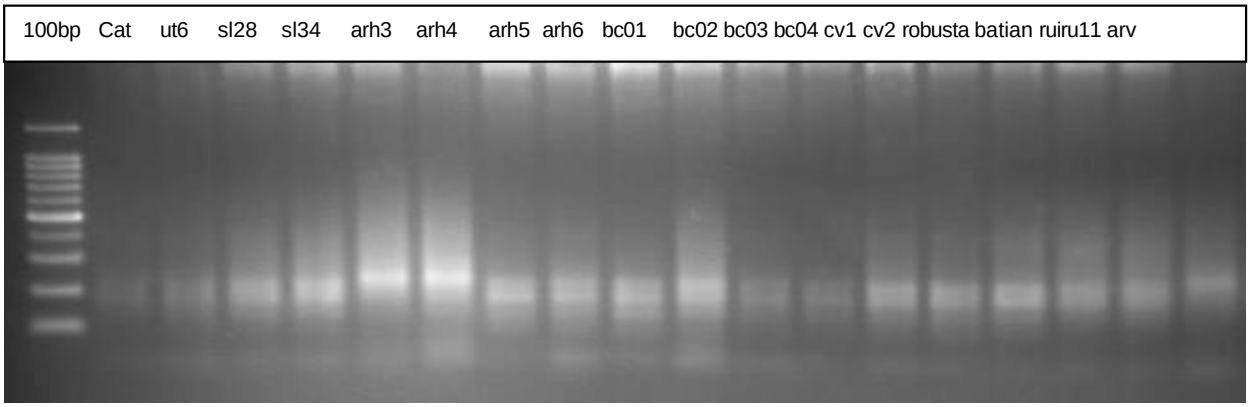

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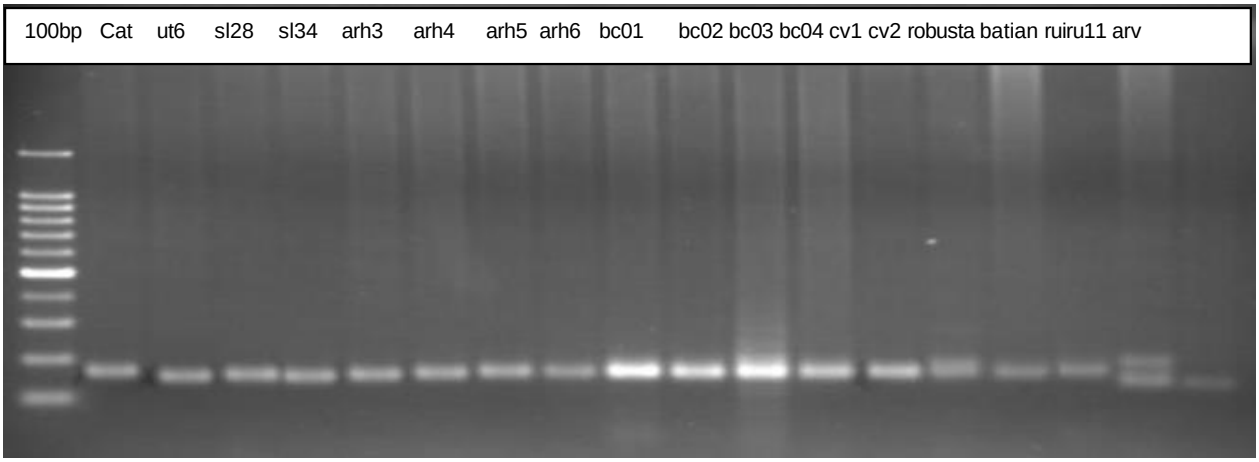

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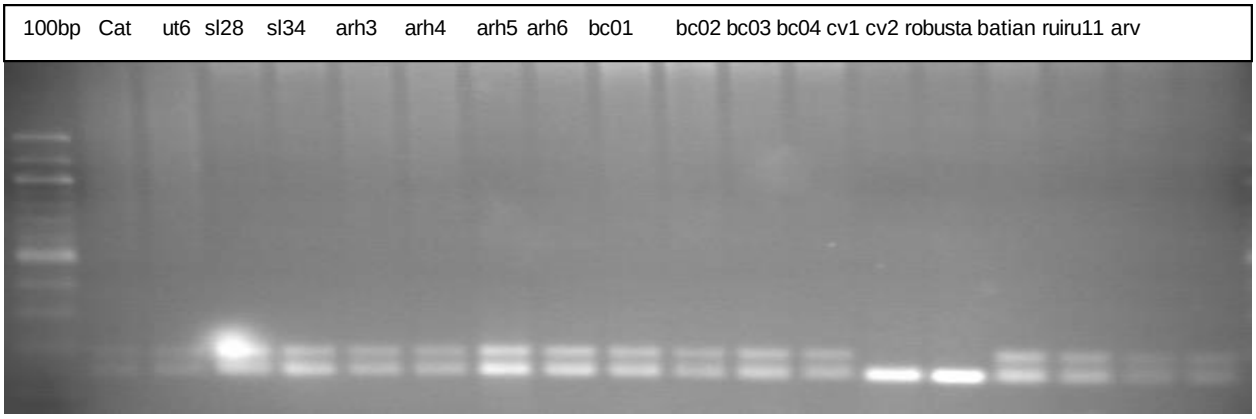
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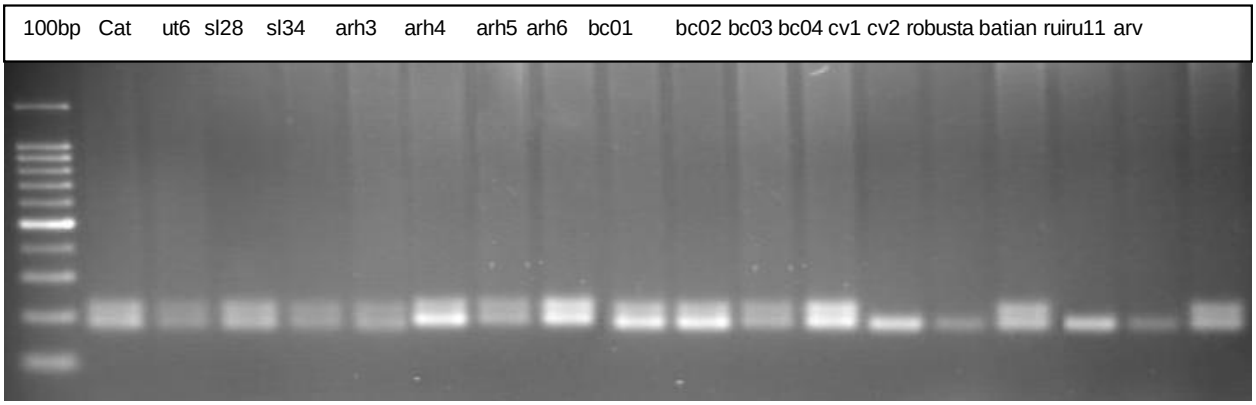
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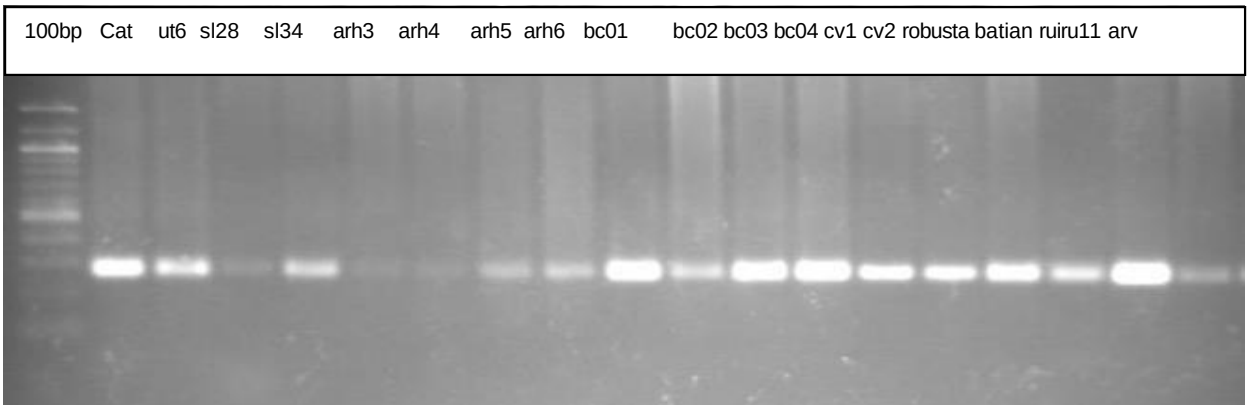
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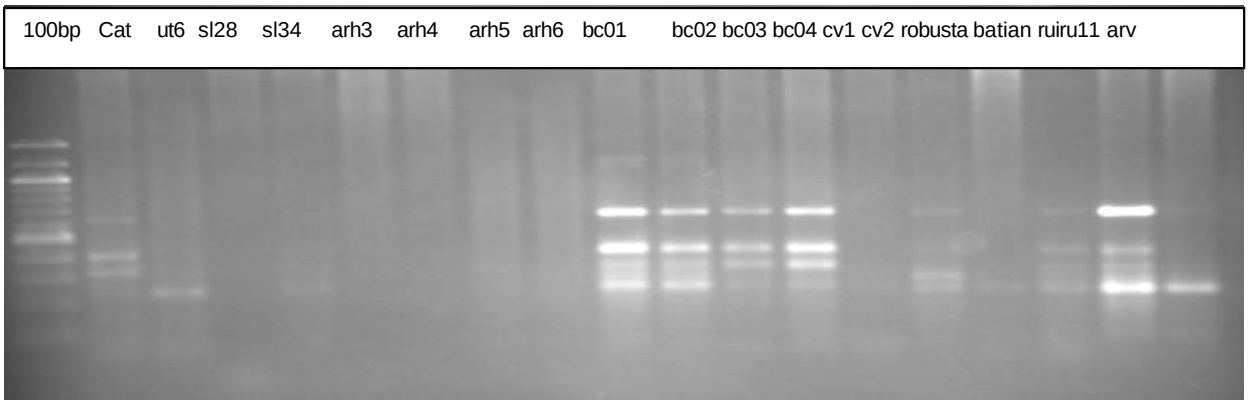
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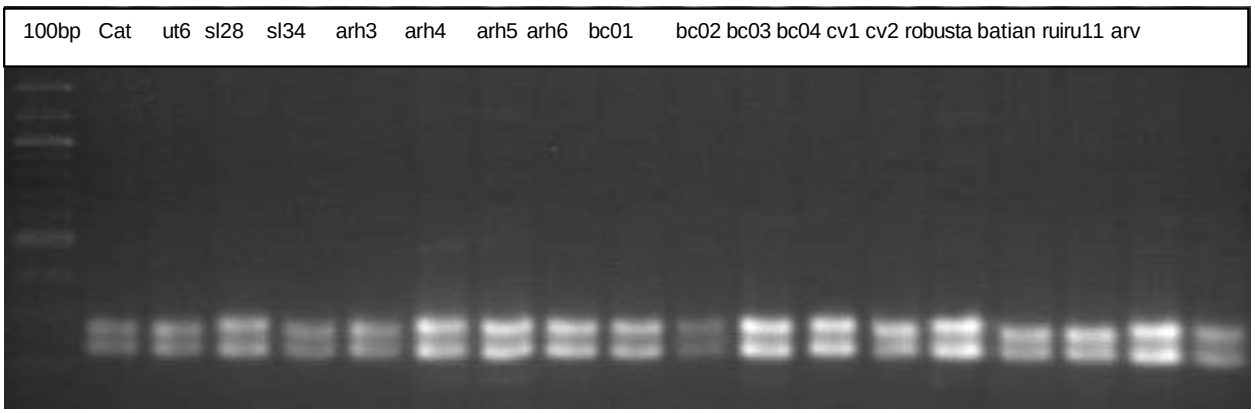
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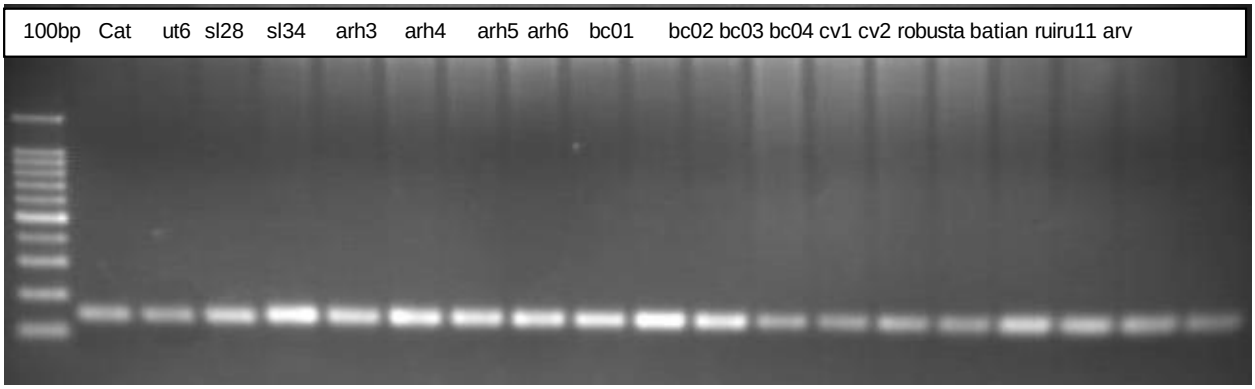
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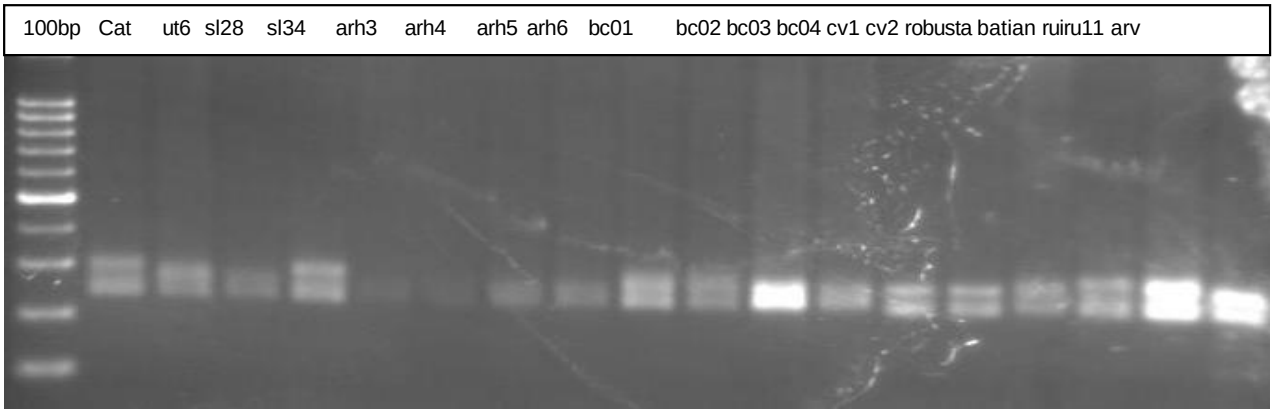
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